The Journal of Clinical Pharmacology 2019, 00(0) 1–9

DOI: 10.1002/jcph.1534

Effects of the Fluoroquinolones Moxifloxacin and Levofloxacin on the QT Subintervals: Sex Differences in Ventricular Repolarization

Jörg Täubel, MD, FFPM[1,2], Krishna Prasad, MD, FRCP[3], Giuseppe Rosano, MD, PhD, FESC, FHFA[2,4], Georg Ferber, PhD[5], Helen Wibberley, MBBS[1], Samuel Thomas Cole, BM, BS[1], Leen Van Langenhoven, PhD[1], Sara Fernandes, PhD[1], Dilshat Djumanov, PhD[1], and Atsushi Sugiyama, MD, PhD[6]

Abstract

Women are associated with longer electrocardiographic QT intervals and increased proarrhythmic risks of QT-prolonging drugs. The purpose of this study was to characterize the differences in cardiac electrophysiology between moxifloxacin and levofloxacin in men and women and to assess the balance of inward and outward currents through the analysis of QT subintervals. Data from 2 TQT studies were used to investigate the impact of moxifloxacin (400 mg) and levofloxacin (1000 and 1500 mg) on QT subintervals using algorithms for measurement of J-T_{peak} and T_{peak}-T_{end} intervals. Concentration-effect analyses were performed to establish potential relationships between the ECG effects and the concentrations of the 2 fluoroquinolones. Moxifloxacin was shown to be a more potent prolonger of QT interval corrected by Fredericia (QTcF) and had a pronounced effect on J-T_{peak}c. Levofloxacin had little effect on J-T_{peak}c. For moxifloxacin, the concentration-effect modeling showed a greater effect for women on QTcF and J-T_{peak}c, whereas for levofloxacin the inverse was true: women had smaller QTcF and J-T_{peak}c effects. The different patterns in repolarization after administration of both drugs suggested a sex difference, which may be related to the combined I_{Ks} and I_{Kr} inhibitory properties of moxifloxacin versus I_{Kr} suppression only of levofloxacin. The equipotent inhibition of I_{Ks} and I_{Kr} appears to affect women more than men. Sex hormones are known to influence cardiac ion channel expression and differences in QT duration. Differences in I_{Kr} and I_{Ks} balances, influenced by sex hormones, may explain the results. These results support the impact of sex differences on the cardiac safety assessment of drugs.

Keywords

J-T_{peak}, T_{peak}-T_{end}, moxifloxacin, levofloxacin, ion channel effects, I_{Kr}, I_{Ks}

Distinct ion channels contribute to defining the morphology and duration of the cardiac action potential. To characterize drug proarrhythmic properties, it is of interest to explore which ionic currents play a significant role. It has been demonstrated that the balance of inward and outward currents can be detected in the ECG by analyzing the QT subintervals. I_{Kr} or *hERG*-encoded potassium channel blockade prolongs both early repolarization (J-T_{peak}) and late repolarization (T_{peak}-T_{end}), whereas multichannel blockers may shorten or have no effect on J-T_{peak},[1] depending on which channels are blocked and how potently as well as whether these channels facilitate depolarization or repolarization currents.

It is widely accepted that women are more prone to developing drug-induced arrhythmia.[2–4] Vicente et al reported sex- and age-specific measurements for all the QT subintervals in healthy subjects in 2014, demonstrating men to have a shorter rate-corrected QT interval (QTc) than women. Despite longer depolarization (QRS) and late repolarization (T_{peak}-T_{end}) phases, men have reduced early repolarization (J-T_{peak}) when compared with women, which summates to an overall shorter QTc.[5] This difference develops during puberty and diminishes with age. It is thought that sex hormones may play a role; simulated testosterone studies have shown that the male sex hormone affects both I_{CaL} and I_{Ks}, contributing to sex differences in

[1]Richmond Pharmacology Ltd, St George's, University of London, Cranmer Terrace, London, UK
[2]Cardiovascular and Cell Sciences Research Institute, St George's, University of London, London, UK
[3]Medicines and Healthcare Products Regulatory Agency, Department of Health and Social Care, London, UK
[4]Centre of Clinical and Experimental Medicine, IRCCS San Raffaele, Rome, Italy
[5]Statistik Georg Ferber GmbH, Cagliostrostrasse, Riehen, Switzerland
[6]Department of Pharmacology, Faculty of Medicine, Toho University, Ota-ku, Tokyo, Japan

Submitted for publication 29 July 2019; accepted 26 September 2019.

Corresponding Author:
Jörg Täubel, MD, Richmond Pharmacology Ltd, St George's, University of London, Cranmer Terrace SW17 0RE, London, United Kingdom
Email: j.taubel@richmondpharmacology.com

early repolarization.[4,5] Additionally, it has been suggested that endogenous testosterone (I_{Kr} and I_{Ks} upregulator) and progesterone (I_{Ks} upregulator) shorten the cardiac action potential. Endogenous estrogen (I_{Kr} and I_{Ks} downregulator) is thought to lengthen the cardiac action potential.[6] Studies of menopausal hormone therapy in the form of estrogen-alone therapy and estrogen plus progesterone therapy have suggested a counterbalancing effect of exogenous estrogen and progesterone on the QT. Specifically, estrogen-alone therapy lengthens the QT, whereas estrogen plus progesterone therapy has no effect.[7]

Fluoroquinolones are one of the most commonly prescribed class of antibiotics worldwide.[8] Prolongation of the QT interval is an adverse effect associated with the use of fluoroquinolones and has been the basis for their use as positive controls for thorough QT studies.[9,10] Fluoroquinolones prolong the QT interval by blocking voltage-gated potassium channels, especially the "rapid" component of the delayed rectifier current I_{Kr}, expressed by *hERG* (the human ether-à-go-go-related gene). However, the degree of QT interval prolongation appears to differ among fluoroquinolones. The overall risk of torsades de pointes (TdP) is small with the use of fluoroquinolones but has been documented in clinical studies and case reports.[11,12] Moxifloxacin has been used in the majority of TQT studies, and it is known to influence ventricular repolarization by inhibiting the I_{Kr} channel.[13,14] Oral moxifloxacin leads to an average QTc prolongation of 10-14 ms at a dose of 400 mg.[15–17] Levofloxacin, another fluoroquinolone, has also been shown to block hERG channels[13,18] and cause changes in the QTc interval.[19,20] The effect of supratherapeutic oral doses of levofloxacin on the QTc intervals of 4.73 and 7.12 ms was shown for 1000-mg and 1500-mg doses, respectively.[19]

Recently, Matsukura et al[21] indicated that moxifloxacin significantly prolonged both J-T_{peak}c and T_{peak}-T_{end}. Additionally, women were found to be more sensitive to overall QTc by Fredericia (QTcF) prolongation and (more specifically) to J-T_{peak} prolongation in a concentration-effect model analysis.

The purpose of the present study was to use combined data from 2 TQT studies comparing QTcF changes after supratherapeutic doses of levofloxacin (which is thought to primarily block hERG channels) and therapeutic doses of moxifloxacin (which has been shown to block both hERG and KvLQT1/mink).[1,14] The 2 studies were performed in the same year at the same clinical research unit using identical procedures for clinical conduct and ECG analysis. Both studies were balanced for sex, and the analyses performed in the moxifloxacin arm of each study were identical. This investigation characterizes the differences in early repolarization (J-T_{peak}) and late repolarization (T_{peak}-T_{end}) between moxifloxacin and levofloxacin and further defines observed sex differences in QTcF and its subintervals.

Materials and Methods

Study Design

Study 1 (EudraCT: 2006-006376-38) was approved by the local ethics committee (Covance Clinical Research Unit, Independent Ethics Committee, Leeds, UK) and the Medicines and Healthcare Products Regulatory Authority and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. This was a randomized, placebo-controlled, double-blinded, double-dummy, single-center, 4 × 4 crossover study. It consisted of 64 healthy, white volunteers (34 male and 30 female) who all provided written, informed consent before any study-specific procedures.

The study evaluated the effects of 2 single supratherapeutic oral doses of levofloxacin (1000 mg and 1500 mg Tavanic; Laboratoire Aventis, Groupe Sanofi-Aventis, Paris, France) and 1 single standard oral dose of moxifloxacin (400 mg Izilox; Bayer Pharma SAS, Puteaux, France) on the QTc intervals of healthy volunteers compared with a placebo group.[14] Each of the 4 periods consisted of 2 days: 1 placebo baseline day and 1 treatment day. These were separated by a 2-day washout period. The study design, ECG, and pharmacokinetic assessments were fully detailed by Taubel et al.[19]

Study 2 (EudraCT: 2006-002504-34) was approved by the local ethics committee (North London REC 3, Harrow, UK), and the Medicines and Healthcare Products Regulatory Authority and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. This was a randomized, placebo-controlled, positive-controlled, double-blinded, double-dummy, single-center, 3 × 3 crossover study. It consisted of 96 healthy, white volunteers (47 male and 49 female) who all provided written, informed consent before any study-specific procedures.

The study evaluated the effects of supratherapeutic repeated dosing of 4 g of once-daily strontium ranelate (Protelos; Les Laboratoires Servier, Neuilly-sur-Seine, France) for 15 days on the QTc interval of healthy volunteers. Eligible subjects were randomized to strontium ranelate, placebo, or moxifloxacin for the 3 treatment periods. Each treatment period consisted of 16 days: day 1 was the placebo baseline day at the unit; days 2-15 were out of the unit, and participants were on placebo or strontium ranelate. The final day (day 16) was spent at the unit, and the volunteers had either placebo, strontium ranelate, or moxifloxacin (Izilox; Bayer Pharma SAS, Puteaux, France). The treatment

periods were separated by 28-day washout periods. The methods used for ECG and pharmacokinetic assessments are described elsewhere.[22]

ECG Recording and Data Processing

Twelve-lead ECGs were recorded as described by Taubel et al.[19,22] Data were processed by the Department of Health Science and Technology of the Faculty of Medicine, University of Aalborg (Denmark). They used the commercially available GE Healthcare Marquette 12SL ECG analysis program and the US Food and Drug Administration 510(k)-cleared GE research package QT GuardPlus.[18,19] This software uses the simultaneous vector magnitude of all 12 leads to determine the onset and offset of the QRS complex as well as the offset of the T wave. The vector magnitude is a global single-lead representation of all 12 leads that did not display a biphasic (+/– or –/+) T wave. In cases with clear biphasic T waves in some of the leads, the vector magnitude representation may have displayed 2 obvious positive peaks in the T wave. In these cases the algorithm used the second peak of the T wave, the peak closer to the end. The end of the T wave is determined by the method of small windows.[23,24]

J-T_{peak} was heart-rate corrected using J-T_{peak}cJ.[25] At resting heart rates T_{peak}-T_{end} exhibits minimal heart-rate dependency, and, therefore, correction was not made.[26]

Statistical Analyses

The moxifloxacin ECG and pharmacokinetic data from studies 1 and 2 were combined. In total, 9315 ECGs were used in the moxifloxacin analysis: 1364 and 1741 triplicate ECGs from 61 subjects in the moxifloxacin treatment in study 1 and 72 subjects in the moxifloxacin treatment in study 2, respectively. To ensure consistency across the 2 studies, the values from the first day of each period (placebo baseline day) at the time points 0, 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 hours (common time points to the 2 studies) were averaged by subject and period to calculate baseline. The levofloxacin data were taken from study 1 only: 1385 triplicate ECGs from 62 subjects in the 1000-mg levofloxacin treatment and 1361 triplicate ECGs from 62 subjects in the 1500-mg levofloxacin treatment, resulting in a total of 8238 ECGs that were analyzed. The baseline-corrected variables (ΔQTcF, ΔJ-T_{peak}Cj, and ΔT_{peak}-T_{end}) were obtained by subtracting the baseline from the postdose value, by subject and time point within each period. The baseline and placebo-corrected variables were obtained by subtracting the baseline-corrected variable of the placebo period from the baseline-corrected variable of the drug administration period. All time points were used for these variables: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 8, 12, and 24 hours for study 1 and 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 hours for study 2.

Table 1. Subject Demographics

	Study 1	Study 2
Number of subjects enrolled	64	96
Age (y)	29 ± 7	27.7 ± 7.5
Sex (n)		
Male	34 (53%)	47 (49%)
Female	30 (47%)	49 (51%)
BMI (kg m^{-2})	24.1 ± 2.3	24.1 ± 2.8
Race (n)		
White	64 (100%)	96 (100%)

BMI indicates body mass index.

Mixed Models. The analysis follows the general statistical principles described by Garnett et al.[27] To ascertain whether the ECG markers differed between sex groups through the concentration profile, the analysis involved a mixed model for each outcome variable ($\Delta\Delta$QTcF, $\Delta\Delta$J-T_{peak}cJ, and $\Delta\Delta T_{peak}$-T_{end}), with sex and the interaction between concentration and sex as fixed effects. The random term included intercepts and concentration by subject.

The mixed models were fitted in SAS Enterprise Guide version: 7.1 (7.15 HF3 [7.100.5.6132]) with SAS version 9.4 (9.04.01M5P09132017) using the Restricted Maximum Likelihood method. For the degrees of freedom, the Kenward-Roger approach was used. All 2-sided confidence intervals are calculated using $\alpha = 0.1$. An unstructured covariance matrix was assumed for the random effects.

Results

Subject Demographics and Disposition

Subject demographics are presented by descriptive statistics in Table 1.

Three subjects from study 1 were withdrawn, 2 because of adverse events following treatment with moxifloxacin 400 mg (1 subject suffered from sustained supraventricular tachycardia, and the second suffered from anxiety). No serious or severe adverse events were observed.[14]

Twenty-six subjects were withdrawn in study 2. This was because of the very long duration of the trial due to a multiple-dose crossover design. One withdrew due to an adverse event after receiving moxifloxacin 400 mg (the subject experienced flu-like symptoms and was found pyrexial). Twenty-two participants were withdrawn for nonmedical reasons, and 3 were withdrawn due to noncompliance with protocol requirements (testing positive for drugs of abuse in subsequent treatment periods). There were no serious or severe adverse events in this study.

In the descriptive and statistical analysis described here, data from 61 subjects (28 women and 33 men) who received 400 mg moxifloxacin and data from 62 subjects who received levofloxacin (29 female and 33 male) have been included from study 1. From study 2, data from 72 subjects (35 female and 37 male) who received 400 mg moxifloxacin were included. The time course analysis data for moxifloxacin by study are presented in Supplemental Tables S1-S3. Overall, the point estimates were very similar between studies.

Time Course Analyses

The effects of moxifloxacin and levofloxacin on the ΔΔQTcF, ΔΔJ-T_{peak}cJ, and ΔΔT_{peak}-T_{end} are summarized in Figure 1. Generally, the effects on women were greater than those on men. This is unsurprising given the higher plasma concentrations due to demographic differences between sexes.

ΔΔ*QTcF*. The largest ΔΔQTcF with moxifloxacin was registered at 3.5 hours for both men (12.43 ms, 90%CI 10.56-14.31) and women (16.80 ms, 90%CI 13.88-19.72). Similarly, 1000 mg of levofloxacin produced the largest ΔΔQTcF at 2 hours for men (6.86 ms, 90%CI 5.11-8.62) and at 2.5 hours for women (7.51 ms, 90%CI 5.70-9.33). Following a 1500-mg dose, men showed the greatest ΔΔQTcF at 3 hours (9.91 ms, 90%CI 7.83-12.00), whereas women still showed the biggest difference at 3.5 hours (10.28 ms, 90%CI 8.16-12.40).

ΔΔ*J-T_{peak}cJ*. When moxifloxacin is administered, the highest ΔΔJ-T_{peak}cJ value observed in women was at 2 hours (10.86 ms, 90%CI 8.71-13.01). Men showed a smaller effect, and their highest value was displayed at 1 hour (7.21 ms, 90%CI 5.69-8.73).

When compared with moxifloxacin, the levofloxacin effect on ΔΔJ-T_{peak}cJ was short-lived, particularly in men, where values returned to baseline within 3-4 hours. In women, effects persisted for longer, returning to baseline by 8 hours. Notably, at the 8 hours' time point, women still showed a greater prolongation of ΔΔJ-T_{peak}cJ after a dose of moxifloxacin. The effects of 1000 mg levofloxacin on ΔΔJ-T_{peak}cJ were highest at 1.5 hours in women (3.86 ms, 90%CI 1.82-5.91) and at 2 hours in men (4.52 ms, 90%CI 3.17-5.88). The 1500-mg dose of levofloxacin led to an increase of 5.08 ms (90%CI 3.29-6.87) at 1.5 hours in men and 4.43 ms (90%CI 2.77-6.08) at 2 hours in women.

ΔΔ*T_{peak}-T_{end}*. The highest ΔΔT_{peak}-T_{end} values following administration of moxifloxacin were observed at 3.5 hours in both men (5.31 ms, 90%CI 4.12-6.49) and women (5.69 ms, 90%CI 3.89-7.50). The largest values with 1000 mg levofloxacin were at 3.5 hours for men (2.05 ms, 90%CI 1.12-2.98) and at 3 hours for women (4.42 ms, 90%CI 3.29-5.55). The 1500-mg levofloxacin cohort showed the same pattern with a maximum increase of 4.32 ms (90%CI 3.09-5.55) for men at 3.5 hours and 7.02 ms (90%CI 5.37-8.68) at 3 hours for women. The curves for ΔΔT_{peak}-T_{end} clearly separate after both doses of levofloxacin and remain elevated up to 4 hours.

Table 2. Summary of Intercepts and Slopes Obtained by Concentration-Effect Modeling

		Moxifloxacin					
		Slope (ms/[μg/mL])			Intercept (ms)		
		Estimate	90%CI		Estimate	90%CI	
ΔΔQTcF	Male	4.7030	4.0842	5.3217	1.9757	0.4349	3.5166
	Female	4.9893	4.4475	5.5310	3.1651	1.5514	4.7788
ΔΔJ-T_{peak}cJ	Male	3.3362	2.7835	3.8890	0.1106	−1.2909	1.5121
	Female	3.5921	3.1102	4.0740	1.8607	0.3927	3.3287
ΔΔT_{peak}-T_{end}	Male	1.2338	0.8640	1.6036	1.8283	0.9896	2.6671
	Female	1.3462	1.0304	1.6620	1.1385	0.2619	2.0152
		Levofloxacin					
		Slope (ms/[μg/mL])			Intercept (ms)		
		Estimate	90%CI		Estimate	90%CI	
ΔΔQTcF	Male	0.8636	0.7195	1.0076	−0.6135	−1.9374	0.7105
	Female	0.7115	0.5723	0.8507	−0.4185	−1.8261	0.9892
ΔΔJ-T_{peak}cJ	Male	0.3376	0.2205	0.4547	0.3337	−0.6236	1.2910
	Female	0.2047	0.09395	0.3155	−0.1555	−1.1717	0.8608
ΔΔT_{peak}-T_{end}	Male	0.3201	0.2376	0.4026	−0.7692	−1.5280	0.01044
	Female	0.4446	0.3670	0.5222	−0.6903	−1.4965	0.1160

Concentration-Effect Analysis

For each subject, the maximum concentration of the analytes moxifloxacin and levofloxacin was measured and used for calculating the overall and by-sex geometric means. The moxifloxacin geometric mean peak concentration (C_{max}) was 2.49 μg/mL (men 2.27; women 2.75). The levofloxacin geometric mean C_{max} was 11.37 μg/mL for men and 13.97 μg/mL for women (overall C_{max} 12.54 μg/mL).

The relationships between moxifloxacin and levofloxacin plasma concentrations and their respective predicted ΔΔQTcF, ΔΔJ-T_{peak}cJ, and ΔΔT_{peak}-T_{end} values are shown in Figure 2. The slopes and intercepts for all parameters are summarized in Table 2.

Moxifloxacin

ΔΔQTcF values increased with the concentration of moxifloxacin for both men and women. Women had almost consistently higher values for ΔΔQTcF compared with men across the concentration profile (Figure 2). The statistical model predicted ΔΔQTcF values at the overall C_{max} of 14.6 ms (90%CI 13.41-15.77). At their respective C_{max} levels, the ΔΔQTcF was 12.62 ms

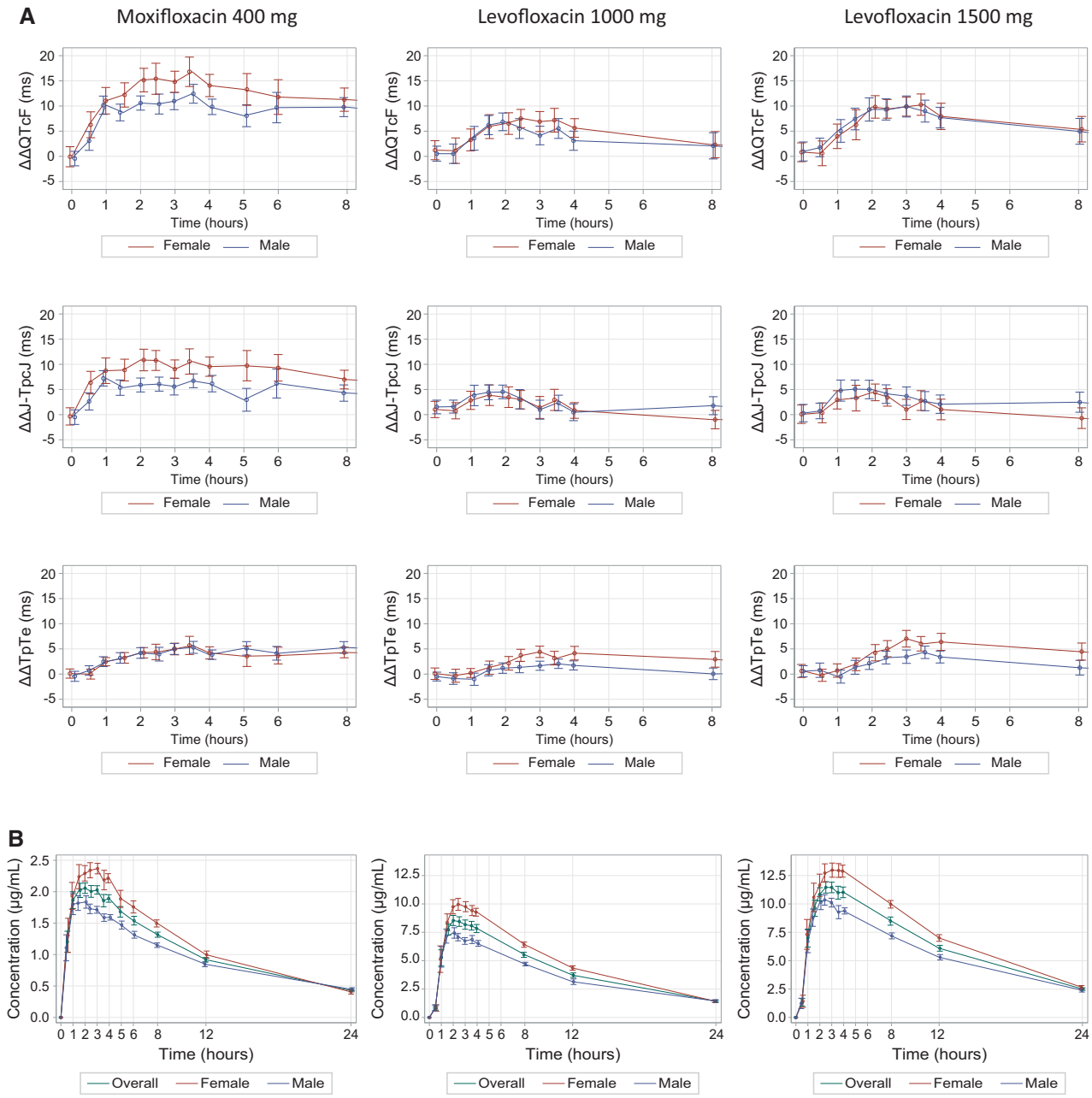

Figure 1. Time course of (A) ΔΔQTcF, ΔΔJ-T_{peak}cJ, and ΔΔT_{peak}-T_{end} and (B) plasma concentration following administration of 400 mg moxifloxacin and 1000 mg and 1500 mg levofloxacin. B, Vertical bars represent 2-sided 90%CIs of the mean. Te indicates T_{end}; Tp, T_{peak}.

(90%CI 11.02-14.21) for men and 16.9 ms (90%CI 15.17-18.63) for women.

There was a positive relationship between moxifloxacin plasma concentrations and the predicted ΔΔJ-T_{peak}cJ (Figure 2 and Table 2). The estimated ΔΔJ-T_{peak}cJ at C_{max} for men was 7.66 ms (90%CI 6.21-9.11) and 11.75 ms (90%CI 10.17-13.33) for women. The overall estimate at C_{max} was 9.56 ms (90%CI 8.49-10.63).

No difference was found for ΔΔT_{peak}-T_{end} with the 90%CI of both populations almost completely overlapping (Figure 2): at C_{max} men presented values of 4.62 ms (90%CI: 3.79; 5.46) whereas women presented slightly higher values: 4.85 ms (90%CI: 3.95; 5.74). The predicted ΔΔT_{peak}-T_{end} values at the overall C_{max} were 4.70 ms (90%CI: 4.08; 5.31).

The intercept and slope differences between men and women are shown in the Supplemental Table S4.

Levofloxacin

Levofloxacin elicited a smaller ΔΔQTcF and ΔΔJ-T_{peak}c than moxifloxacin. The ΔΔQTcF slope was

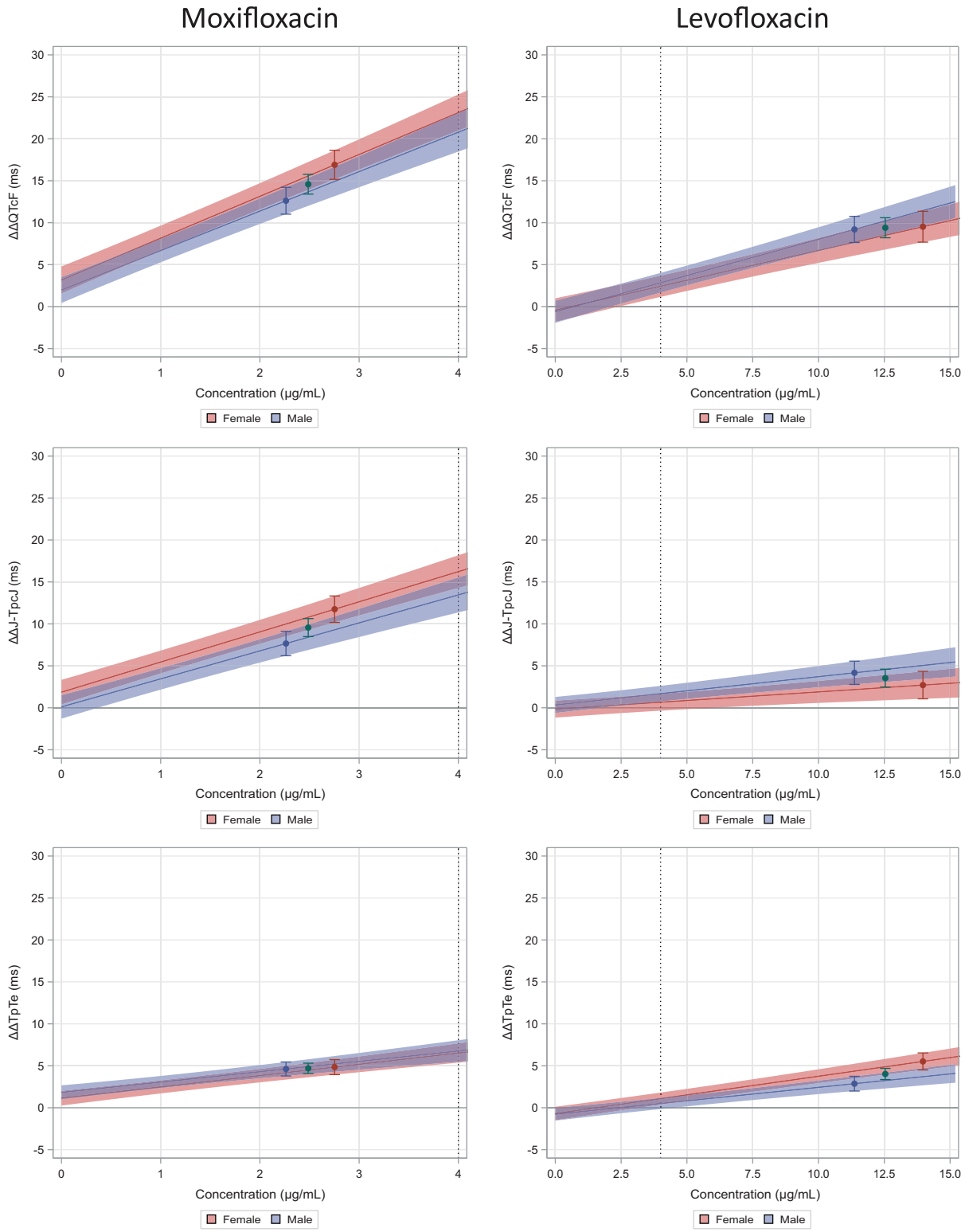

Figure 2. Relationship between moxifloxacin and levofloxacin plasma concentrations and $\Delta\Delta$QTcF, $\Delta\Delta$J-T_{peak}cJ, and $\Delta\Delta T_{peak}$-T_{end}. Regression lines with 2-sided 90% confidence regions are denoted by shaded areas. The means and whiskers show the predicted values for $\Delta\Delta$QTcF, $\Delta\Delta$J-T_{peak}cJ, and $\Delta\Delta T_{peak}$-T_{end} at C_{max} concentrations (the overall geometric mean of the individual C_{max} values and the geometric means of the individual C_{max} values by sex). C_{max} indicates peak concentration; Te, T_{end}; Tp, T_{peak}.

greater for men than for women (Table 2), which is the opposite of what is seen after moxifloxacin administration. Figure 2 shows that the ΔΔQTcF overlap zone of the 90% confidence regions is wider at smaller concentrations, indicating that sex-related differences in ΔΔQTcF seem to be more evident at higher concentrations. The concentration-effect analysis shows that the ΔΔQTcF value at the overall C_{max} was 9.40 ms (90%CI 8.21-10.59): 9.20 ms (90%CI 7.64-10.76) for men and 9.52 ms (90%CI 7.68-11.35) for women.

Almost consistently, men had slightly higher values of ΔΔJ-T_{peak}cJ than women across the concentration profile (Figure 2). Estimates for ΔΔJ-T_{peak}cJ at C_{max} were 4.17 ms for men (90%CI 2.78-5.56) and 2.70 ms for women (90%CI 1.07-4.34). The overall estimate was of 3.54 ms (90%CI 2.48-4.60). Again, this was the opposite of the effect seen with moxifloxacin.

In contrast to the previous parameters, women had greater ΔΔT_{peak}-T_{end} point estimates than men (Table 2). At C_{max} men presented estimates of 2.87 ms (90%CI 1.99-3.74), and women presented values of 5.52 ms (90%CI 4.51-6.53). The ΔΔT_{peak}-T_{end} value at the overall C_{max} was 4.03 ms (90%CI 3.36-4.69). Slope and intercept differences between men and women are shown in Supplemental Table S4.

Discussion

The literature indicates that of all the available fluoroquinolones, moxifloxacin carries the greatest risk of QT prolongation. As a result, it is advised that it should be used in caution in patients with predisposing factors for TdP.[28,29]

Women generally have a longer QTc than men[30] and an increased risk of drug-induced TdP.[31] These sex differences seem to be multifactorial and are still not very well understood.[32–35] Testosterone was reported to shorten the action potential duration in guinea pigs by decreasing the inward-depolarizing L-type calcium current ($I_{Ca\text{-}L}$) and increasing the outward-repolarizing "slow" delayed rectifier potassium current (I_{Ks}).[35] In healthy adult subjects the shorter QTc in men than in women was related to a shorter J-T_{peak} interval, a difference that diminished with age. The influence of sex hormones is also supported by findings showing similar QTc intervals at birth in male and female subjects.[36] The early repolarization changes in men were shown to be influenced more by the effect of testosterone on calcium currents than its effects on I_{Ks}.[5] Testosterone also diminished the proarrhythmic effects of the pure hERG blocker dofetilide in female rabbits.[30] Jonsson et al[4] have found that both I_{Ks} and I_{Kr} are influenced by sex hormones whereby estrogen reduces I_{Ks} and I_{Kr} expression, whereas progesterone enhances I_{Ks}. Testosterone, by contrast, enhances both I_{Kr} and I_{Ks}. This suggests that sex differences must be considered in thorough QT studies and that hormonal cycles may impact their results.The actions of sex hormones on cardiac ion channels are likely to contribute to the sex differences in cardiac repolarization processes and susceptibility of TdP.

In this study the assessment of ECG subintervals showed clear sex differences with moxifloxacin and levofloxacin. The QTcF prolongation of moxifloxacin in women was due to a prolongation of J-T_{peak}cJ of approximately 12 ms and a prolongation of T_{peak}-T_{end} of approximately 5 ms. Men registered smaller values of J-T_{peak}cJ (8 ms) and similar time-course values of T_{peak}-T_{end} (5 ms) when compared with women. This suggests that the greater effect in women on QTcF is due to their greater increase of J-T_{peak}cJ. Our results are well aligned with the previous work from Matsukura et al. In their study, women were also shown to be more sensitive than men to the moxifloxacin-induced J-T_{peak} prolongation and QTcF, whereas T_{peak}-T_{end} values were similar between the sex groups[21]. In women levofloxacin prolonged T_{peak}-T_{end} by 6 ms and J-T_{peak}cJ by 3 ms. Men presented higher J-T_{peak}cJ values and smaller T_{peak}-T_{end} values. With moxifloxacin, women demonstrated greater increase in QTcF and J-T_{peak}, whereas with levofloxacin, they demonstrated a greater increase in T_{peak}-T_{end}.

In summary, moxifloxacin showed a greater effect in women on the J-T_{peak}cJ interval, which accounted for most of their prolongation of QTcF. Levofloxacin showed a different pattern of effect as women had less effect on J-T_{peak}cJ, a more pronounced effect on T_{peak}-T_{end}, and the net effect on QTcF was a smaller effect in women.

The prolongation of J-T_{peak} seen for moxifloxacin and levofloxacin is in agreement with electrophysiology studies and indicates that drug-induced changes in T-wave morphology are directly related to the amount of hERG potassium channel block. Moxifloxacin was shown to be a more potent prolonger of QTcF and had a pronounced effect on J-T_{peak}c, consistent with its effects on both I_{Kr} and I_{Ks} channels, whereas levofloxacin had small and short-lived effect on J-T_{peak}c. Patch-clamp analyses suggested a roughly equipotent binding of moxifloxacin to I_{Kr} and I_{Ks} potassium channels.[1,14] In contrast, levofloxacin has been shown to have effects on I_{Kr} channels only at relatively high concentrations, and I_{Ks} was not a target for block by levofloxacin because high concentrations produced only modest reductions in I_{Ks}.[18]

In this study moxifloxacin and levofloxacin presented inverse sex-specific effects. The increase of early repolarization duration in women, measured as the heart rate corrected J-T_{peak} interval (where $I_{Ca\text{-}L}$, I_{Kr}, and late sodium current play a major role) was less

pronounced with levofloxacin than with moxifloxacin. Therefore, women seem to be more sensitive to a dual block, in this case to moxifloxacin, a recognized I_{Ks} and I_{Kr} inhibitor. In principle, considering that a larger prolonging effect of the J-T_{peak} interval may result in a greater risk of TdP, our data would suggest that therapeutic doses of moxifloxacin have a considerable higher risk of TdP in women than supratherapeutic doses of levofloxacin. These findings suggest that sex hormone–dependent differences in I_{Ks} may be involved in these apparent differences in QT subintervals between moxifloxacin and levofloxacin. Our results provide valuable insights into possible sex differences, the importance of female enrollment in cardiac assessments, and contribute to the estimated proarrhythmic potential of new chemical entities.

Limitations

This was a retrospective analysis, and the studies were not designed to explore the effects of moxifloxacin and levofloxacin on the QTc subintervals and the respective sex differences. However, the sample size utilized and the statistical analyses are sufficiently robust, and the results align well with previous published work by our research group and others.

In addition to the above outlined hormone effects, progesterone was shown to shorten action potential duration in guinea pigs mostly through inhibition of inward I_{Ca} and enhancement of I_{Ks}.[37] In women progesterone fluctuates through the menstrual cycle, and estrogen seems to have an opposing effect on cardiac repolarization.[38,39] This study does not explore the individual contributions of sex hormones and their effects in combination with moxifloxacin or levofloxacin on T-wave morphology.

The study did not record menstrual cycles and did not measure sex hormones. More studies considering hormonal fluctuations would be desirable.

Conflicts of Interest

Jörg Täubel, Helen Wibberley, Samuel Thomas Cole, Leen Van Langenhoven, Sara Fernandes, and Dilshat Djumanov 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.

References

1. Johannesen L, Vicente J, Mason JW, et al. Late sodium current block for drug-induced long QT syndrome: results from a prospective clinical trial. *Clin Pharmacol Ther*. 2016;99(2):214-223.
2. Rautaharju PM, Zhou SH, Wong S, et al. Sex differences in the evolution of the electrocardiographic QT interval with age. *Can J Cardiol.* 1992;8(7):690-695.
3. Bidoggia H, Maciel JP, Capalozza N, et al. Sex differences on the electrocardiographic pattern of cardiac repolarization: possible role of testosterone. *Am Heart J.* 2000;140(4):678-683.
4. Jonsson M, Vos M, Duker G, et al. Gender disparity in cardiac electrophysiology: implications for cardiac safety pharmacology. *Pharmacol Ther.* 2010;127(1):9-18.
5. Vicente J, Johannesen L, Galeotti L, Strauss DG. Mechanisms of sex and age differences in ventricular repolarization in humans. *Am Heart J*. 2014;168(5):749-756.
6. Pham TV, Sosunov EA, Anyukhovsky EP, Danilo P, Rosen MR. Testosterone diminishes the proarrhythmic effects of dofetilide in normal female rabbits. *Circulation.* 2002;106:2132-2136.
7. Larsen JA, Tung RH, Sadananda R, et al. Effects of hormone replacement therapy on QT interval. *Am J Cardiol.* 1998;82:993-995.
8. Owens RC Jr, Ambrose PG. Torsades de pointes associated with fluoroquinolones. *Pharmacotherapy*. 2002;22(5):663-668.
9. Owens RC Jr. Risk assessment for antimicrobial agent-induced QTc interval prolongation and torsades de pointes. *Pharmacotherapy.* 2001;21(3):301-319.
10. Rubinstein E, Camm J. Cardiotoxicity of fluoroquinolones. *J Antimicrob Chemother.* 2002;49(4):593-596.
11. Anderson ME, Mazur A, Yang T, et al. Potassium current antagonist properties and proarrhythmic consequences of quinolone antibiotics. *J Pharmacol Exp Ther.* 2001;296(3):806-810.
12. Frothingham R. Rates of torsades de pointes associated with ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin. *Pharmacotherapy.* 2001;21(12):1468-1472.
13. Alexandrou AJ, Duncan RS, Sullivan A, et al. Mechanism of hERG K^+ channel blockade by the fluoroquinolone antibiotic moxifloxacin. *Br J Pharmacol*. 2006;147(8):905-916.
14. Crumb WJ jr, Vicente J, Johannesen L, Strauss DG. An evaluation of 30 clinical drugs against the comprehensive in vitro proarrhythmia assay (CiPA) proposed ion channel panel. *J Pharmacol Toxicol Methods*. 2016;81:251-262.
15. Bloomfield D, Kost J, Ghosh K, et al. The effect of moxifloxacin on QTc and implications for the design of thorough QT studies. *Clin Pharmacol Ther*. 2008;84(4):475-480.
16. Florian JA, Tornøe CW, Brundage R, Parekh A, Garnett CE. Population pharmacokinetic and concentration-QTc models for moxifloxacin: pooled analysis of 20 thorough QT studies. *J Clin Pharmacol*. 2011;51(8):1152-1162.
17. Grosjean P, Urien S. Re-evaluation of moxifloxacin pharmacokinetics and their direct effect on the QT interval. *J Clin Pharmacol*. 2012;52(3):329-338.
18. Kang J, Wang L, Chen XL, Triggle DJ, Rampe D. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K^+ channel HERG. *Mol Pharmacol.* 2001;59(1):122-126.
19. Taubel J, Naseem A, Harada T, et al. Levofloxacin can be used effectively as a positive control in thorough QT/QTc studies in healthy volunteers. *Br J Clin Pharmacol.* 2010;69(4):391-400.
20. Sugiyama A, Fukuda R, Mori K, Fujita T, Kumagai Y. Effect of single 500 mg iv levofloxacin dose on QT interval in healthy subjects. *Jpn J Chemother.* 2009;57(2):106-114.
21. Matsukura S, Nakamura Y, Hoshiai K, et al. Effects of moxifloxacin on the proarrhythmic surrogate markers in healthy Filipino subjects: exposure-response modeling using ECG data of thorough QT/QTc study. *J Pharmacol Sci.* 2018;136(4):234-241.
22. Taubel J, Naseem A, Wang D, Arezina R, Lorch U, Camm AJ. Repeated supratherapeutic dosing of strontium ranelate over 15

days does not prolong QTc interval in healthy volunteers. *Br J Clin Pharmacol*. 2012;74(2):296-303.
23. Xue JX, Gao W, Chen Y, Han X. Study of repolarization heterogeneity and electrocardiographic morphology with a modeling approach. *J Electrocardiol.* 2008;41:581-587.
24. Xue JX. Robust QT interval estimation: from algorithm to validation. *Ann Noninvasive Electrocardiol.* 2009;14:S35-S41.
25. Johannesen L, Vicente J, Mason JW, et al. Differentiating drug-induced multichannel block on the electrocardiogram: randomized study of dofetilide, quinidine, ranolazine, and verapamil. *Clin Pharmacol Ther*. 2014;96(5):549-558.
26. Johannesen L, Vicente J, Gray RA, et al. Improving the assessment of heart toxicity for all new drugs through translational regulatory science. *Clin Pharmacol Ther.* 2014;95(5):501-508.
27. Garnett C, Bonate PL, Dang Q, et al. Scientific white paper on concentration-QTc modeling. *J Pharmacokinet Pharmacodyn*. 2018;45(3):383-397.
28. Briasoulis A, Agarwal V, Pierce WJ. QT prolongation and torsade de pointes induced by fluoroquinolones: infrequent side effects from commonly used medications. *Cardiology*. 2011;120(2):103-110.
29. Cho Y, Park HS Association of oral ciprofloxacin, levofloxacin, ofloxacin and moxifloxacin with the risk of serious ventricular arrhythmia: a nationwide cohort study in Korea. *BMJ Open.* 2018;8:e020974.
30. Pham TV, Rosen MR. Sex, hormones, and repolarization. *Cardiovasc Res.* 2002;53(3):740-751.
31. Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehman MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. *JAMA*. 1993;270(21): 2590-2597.
32. Rosano GM, Leonardo F, Sarrel PM, Beale CM, De Luca F, Collins P. Cyclical variation in paroxysmal supraventricular tachycardia in women. *Lancet*. 1996;347(9004):786-788.
33. Rodriguez I, Kilborn MJ, Liu XK, Pezzullo JC, Woosley RL. Drug-induced QT prolongation in women during the menstrual cycle. *JAMA.* 2001;285(10):1322-1326.
34. Furukawa T, Kurokawa J. Regulation of cardiac ion channels via non-genomic action of sex steroid hormones: implication for the gender difference in cardiac arrhythmias. *Pharmacol Ther*. 2007;115(1):106-115.
35. Bai CX, Kurokawa J, Tamagawa M, Nakaya H, Furukawa T. Nontranscriptional regulation of cardiac repolarization currents by testosterone. *Circulation*. 2005;112(12):1701-1710.
36. Stramba-Badiale M, Spagnolo D, Bosi G, Schwartz PJ. Are gender differences in QTc present at birth? MISNES Investigators. Multicenter Italian Study on Neonatal Electrocardiography and Sudden Infant Death Syndrome. *Am J Cardiol*. 1995;75(17):1277-1278.
37. Nakamura H, Kurokawa J, Bai CX, et al. Progesterone regulates cardiac repolarization through a nongenomic pathway: an in vitro patch-clamp and computational modeling study. *Circulation.* 2007;116(25):2913-2922.
38. Kurokawa J, Tamagawa M, Harada N, et al. Acute effects of estrogen on the guinea pig and human I_{Kr} channels and drug-induced prolongation of cardiac repolarization. *J Physiol*. 2008;586(12):2961-2973.
39. Kadish AH, Greenland P, Limacher MC, et al. Estrogen and progestin use and the QT interval in postmenopausal women. *Ann Noninvasive Electrocardiol.* 2004;9(4):366-374.

Supplemental Information

Additional supplemental information can be found by clicking the Supplements link in the PDF toolbar or the Supplemental Information section at the end of web-based version of this article.