Thorough QT Study with Recommended and Supratherapeutic Doses of Tolterodine

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The objective of our study was to determine the QTc effects of tolterodine. A crossover-design thorough QT study of recommended (2 mg twice daily) and supratherapeutic (4 mg twice daily) doses of tolterodine, moxifloxacin (400 mg once daily), and placebo was performed. Electrocardiograms (ECGs) and pharmacokinetic samples were obtained on days 1–4; time-matched baseline ECGs were taken on day 0. Mean placebo-subtracted change from baseline Fridericia-corrected QT (QTcF) during peak drug exposure on day 4 was the primary end point. Mean QTcF prolongation of moxifloxacin was 8.9 ms (machine-read) and 19.3 ms (manual-read). At recommended and supratherapeutic tolterodine doses, mean QTcF prolongation was 1.2 and 5.6 ms (machine-read), respectively, and 5.0 and 11.8 ms (manual-read), respectively. The QTc effect of tolterodine was lower than moxifloxacin. No subject receiving tolterodine exceeded the clinically relevant thresholds of 500 ms absolute QTc or 60 ms change from baseline. In conclusion, tolterodine does not have a clinically significant effect on QT interval.

Tolterodine is an antimuscarinic agent approved for the treatment of overactive bladder.[1–3] The effects of tolterodine on cardiac ion channels have been evaluated *in vitro*. A thorough QT (TQT) study, reported here, was performed to determine any effect of tolterodine on cardiac repolarization.

Tolterodine is eliminated primarily by metabolism involving cytochrome P450 (CYP) 2D6 and 3A4.[4,5] CYP2D6 exhibits genetic polymorphism, and thus the metabolic disposition of tolterodine is different between subjects with normal CYP2D6 activity (extensive metabolizers (EMs)) and those with CYP2D6 deficiency (poor metabolizers (PMs)).[6,7] Metabolism of tolterodine by CYP2D6 in EMs results in the formation of an equipotent pharmacologically active metabolite, 5-hydroxy-methyltolterodine (DD01).[8,9] In PMs, however, CYP3A4 is involved in the formation of an inactive metabolite (**Figure 1**).[8] The unbound fraction of tolterodine and DD01 in serum is 3.7% and 36%, respectively.[7,10,11] Potent CYP3A4 inhibitors increase peak tolterodine exposure about twofold, requiring tolterodine dose reduction to 2 mg/day when used concomitantly.[8] Tolterodine is available as an immediate-release (IR) tablet (1 or 2 mg twice daily) and as an extended-release (ER) capsule (2 or 4 mg once daily).[10,11] The peak concentrations (C_{max}) of tolterodine and DD01 with ER capsules are approximately 61% and 67% lower, respectively, than with IR tablets.[12] For both IR and ER formulations, EMs have comparable C_{max} of tolterodine and DD01.[10,11] In PMs, DD01 is not formed and tolterodine C_{max} is six- to eightfold higher than in EMs.[10,11] However, because of the 10-fold lower unbound fraction of tolterodine, exposure to the active moiety (*i.e.*, sum of unbound tolterodine and DD01) is comparable between EMs and PMs, and no dose adjustment is necessary by CYP2D6 metabolizer status.[10,11]

A recent *in vitro* study of the effects of tolterodine on cardiac ion channels showed that it was a potent inhibitor of both human ether a-go-go-related gene (HERG) cardiac potassium and L-type calcium channels.[13] The HERG inhibitory concentration 50% (IC_{50}) value was reported to be 17 nM, with some inhibition evident at concentrations as low as 3 nM. In comparison, the steady-state C_{max} of unbound tolterodine at the therapeutic dose is much lower, averaging 0.31 nM in EMs and 1.33 nM in PMs after IR administration.[12] In the guinea pig ventricular myocytes model, even at concentrations far exceeding those associated with therapeutic exposures of tolterodine, there was a much smaller prolongation of action potential duration than that observed with the pure HERG/repolarizing cardiac potassium current (I_{kr}) antagonist dofetilide.[13]

Electrocardiogram (ECG) data from both healthy subjects and patients in clinical trials have not shown any clinically relevant QTc interval prolongation. Since the first approval in 1997, nearly 10 million patients have been exposed to

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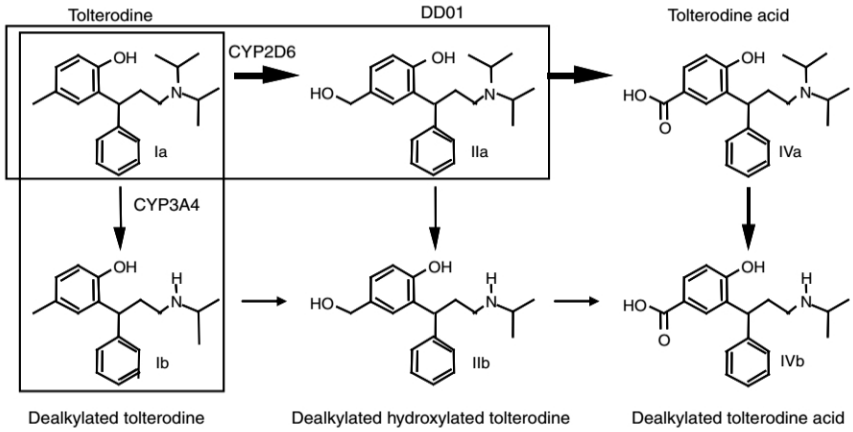

Figure 1 Metabolic pathways of tolterodine. In CYP2D6 EMs, tolterodine is preferentially metabolized to an active metabolite DD01, whereas in CYP2D6 PMs, it is metabolized to pharmacologically inactive dealkylated tolterodine via CYP3A4.

tolterodine (data on file). A review of all postmarketing cardiovascular events associated with tolterodine use has revealed no cases where tolterodine caused QT prolongation or *torsade de pointes* (TdP; data on file). A Prescription Event Monitoring study in the United Kingdom found no reports of TdP or sudden death in more than 14,000 patients receiving a prescription for tolterodine IR; only 17 cases (0.1%) of tachycardia or palpitations were possibly or probably related to tolterodine.[14] Four of 29 other arrhythmias were judged to be possibly or probably related to tolterodine use. None represented a serious arrhythmia, such as ventricular tachycardia or fibrillation.

Despite the lack of cardiac arrhythmic events potentially related to QT interval prolongation in the extensive clinical experience accumulated so far with tolterodine, considering positive results in the preclinical HERG assay, a TQT study was deemed to be important for a definitive assessment of the effects of this agent on the QT interval. With a few exceptions, regulatory agencies now require a TQT study, not only for drug candidates in clinical development, but also for marketed drugs.[15,16] The analysis and interpretation of the results of this TQT study took into account the International Conference on Harmonization (ICH) harmonized tripartite E14 guidelines.[15] In addition, we compared results using both machine- and manual-read interval data. Previous experience has shown that manual over-reading may introduce increased variability and bias into the ECG data sets.[17]

RESULTS

Subject demography

Forty-eight subjects (25 men and 23 women) entered the study and received $\geqslant 1$ dose of study medication. The study population was enriched in PMs (45%) compared with the natural frequency of about 7%.[18] Mean $\pm$ SD of the subjects' age, height, and body mass index were 36.7 ± 10.9 years, 1.71 ± 0.09 m, and 25.4 ± 3.5 kg/m^2, respectively. Approximately one-third of the subjects were 45–55 years of age. Three subjects discontinued from the study (one each because of adverse event, protocol deviation, and personal reasons).

Adverse events

Forty-eight adverse events were reported by 23 subjects given tolterodine 2 mg twice daily, 48 adverse events were reported by 25 subjects given tolterodine 4 mg twice daily, 31 adverse events were reported by 18 subjects given placebo, and 70 adverse events were reported by 25 subjects given moxifloxacin 400 mg once daily. The majority of adverse events reported were treatment related. The most frequent adverse events were headache, dry mouth, and nausea. One subject reported moderate chest pain and was noted to have ST-segment depression after administration of placebo and discontinued from the study.

Pharmacokinetics of tolterodine and moxifloxacin

Tolterodine was quickly absorbed with median time of C_{max} (t_{max}) of 1 h, regardless of dose and genotype. Tolterodine C_{max} and area under the concentration–time curve (AUC) increased proportionally with dose and were approximately 3–5 and 10 times, respectively, higher in PMs than in EMs (**Table 1**). Exposure of DD01 in PMs was 6–7 times less than EMs. Median t_{max} of DD01 was approximately 1 h regardless of dose. Moxifloxacin C_{max} was consistent with its published data in the package insert. The median t_{max} of moxifloxacin was 2 h. These pharmacokinetic data support the statistical analysis of QTc values at 1 and 2 h postdose for assessment of QTc prolongation at the time of maximum exposures of tolterodine and moxifloxacin, respectively.

The alpha$_1$-acid glycoprotein$_1$-acid glycoprotein (AAG) concentrations were generally similar across all four periods. The mean $\pm$ SD values of unbound fractions of tolterodine and DD01, calculated from the AAG concentrations in serum, were 0.026 ± 0.005 and 0.302 ± 0.044, respectively.

Table 1 Mean (SD) pharmacokinetic values of tolterodine, DD01, and moxifloxacin following single dose (day 1) and at steady state (day 4)

Analyte, study day	C_{max} (ng/ml)			AUC[a] (ng h/ml)		
	EMs	PMs	All	EMs	PMs	All
Tolterodine 2 mg b.i.d.						
Tolterodine, day 1	3.1 (2.4)	9.1 (3.7)	5.9 (4.3)		NC	
Tolterodine, day 4	3.4 (2.9)	15.8 (8.3)	9.1 (8.7)	12 (14)	128 (78)	65 (79)
DD01, day 1	2.5 (1.0)	0.2 (0.6)	1.5 (1.4)		NC	
DD01, day 4	2.8 (1.0)	0.3 (0.7)	1.7 (1.5)	13 (5.1)	2.0 (4.7)	8.4 (7.7)
Tolterodine 4 mg b.i.d.						
Tolterodine, day 1	6.1 (5.5)	20 (11)	12 (11)		NC	
Tolterodine, day 4	6.6 (5.7)	34 (19)	19 (19)	26 (31)	266 (166)	136 (166)
DD01, day 1	4.9 (2.1)	0.5 (1.1)	2.9 (2.8)	NC		
DD01, day 4	5.5 (1.7)	0.7 (1.4)	3.3 (2.8)	28 (8.4)	4.7 (8.6)	17 (15)
Moxifloxacin 400 mg q.d.						
Moxifloxacin, day 1	NA		3000 (718)	NA		NC
Moxifloxacin, day 4	NA		3610 (782)	NA		NC

AUC, area under the concentration–time curve; b.i.d., twice daily; EM, extensive metabolizer; NA, not applicable; moxifloxacin pharmacokinetics unaffected by CYP2D6 genotype; NC, not calculated; samples were collected and/or analyzed up to 4 h postdose; PM, poor metabolizer; q.d., once daily. [a]AUC calculated up to the time of last quantifiable concentration during the 12-h dosing interval.

Central tendency (mean) analysis of QTc effects of tolterodine and moxifloxacin

The time course of ΔΔ Fridericia-corrected QT (QTcF) values on day 4, by treatment, is shown in **Figures 2 and 3**, using the machine- and manual-read ECGs, respectively. The mean increase of heart rate associated with tolterodine 2 mg twice daily was 2.0, and 6.3 beats/min with tolterodine 4 mg twice daily. The change in heart rate with moxifloxacin was 0.5 beats/min. The results for ΔΔQTc at t_{max} (1 h for tolterodine, 2 h for moxifloxacin) are summarized in **Tables 2** and **3**. Mean ΔΔQTcF for tolterodine 2 mg twice daily was 13% (machine) and 26% (manual) of that after moxifloxacin. For 4 mg twice-daily tolterodine, ΔΔQTcF was 63% (machine) and 61% (manual) of that after moxifloxacin.

It is apparent from **Figures 2** and **3** that the maximum ΔΔQTcF, an ICH E14-recommended end point, occured for moxifloxacin at 4 h as opposed to the pharmacokinetic t_{max} of 2 h postdose; the mean (90% confidence interval (CI)) were 13.5 (9.9, 17.1) ms for machine-read ECGs and 22.4 (19.3, 27.1) ms for manual-read ECGs. For tolterodine, the time of maximum ΔΔQTcF effect coincided with its pharmacokinetic t_{max} of 1 h postdose, with the exception of manual-read ECGs at the 2 mg twice-daily dose, where the mean (95% CI) of the maximum ΔΔQTcF was 1.38 (−3.61, 6.37) ms at 3 h postdose.

The study sensitivity was confirmed because a mean QT prolongation effect in excess of 5 ms was seen with moxifloxacin. For each of the analyses, whether machine-read or manual-read, the QTc interval effects for both tolterodine doses were lower than with moxifloxacin.

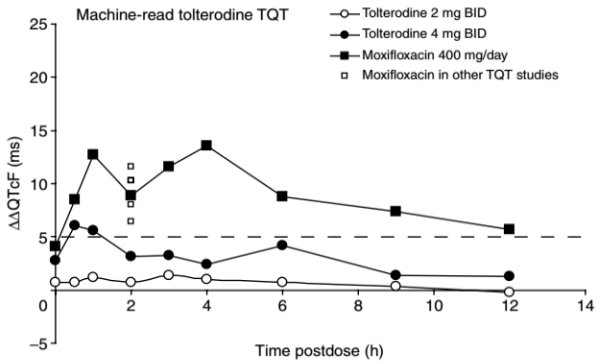

Figure 2 Placebo-subtracted time-matched change from baseline Fridericia QTc values (machine-read) on day 4. Dashed line shows the 5 ms threshold criterion for a negative TQT study. Moxifloxacin QTc effect from other trials is based on manual-read ECGs.

Categorical (outlier) analysis of QTc effects of tolterodine and moxifloxacin

None of the QTc intervals measured in this study exceeded 500 ms. One subject after moxifloxacin and no subject after tolterodine treatment had a QTc change from baseline >60 ms. Overall, the observed frequency of subjects with QTcF or population-corrected QT (QTcP) changes between 30 and 60 ms appeared to be lower for placebo ($\leqslant$2.1% for manual-read, 0% for machine-read analyses) and tolterodine

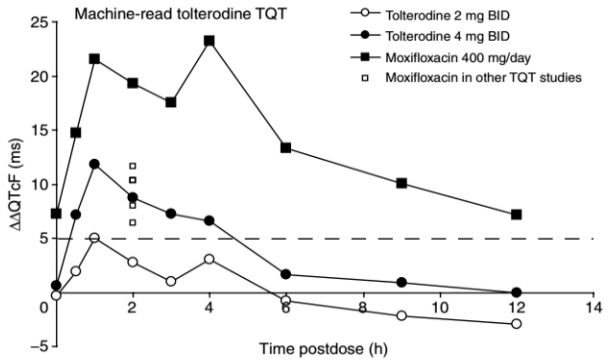

Figure 3 Placebo-subtracted time-matched change from baseline Fridericia QTc values (manual-read) on day 4. Dashed line shows the 5 ms threshold criterion for a negative TQT study. Moxifloxacin QTc effect from other trials is based on manual-read ECGs.

Table 2 Steady-state, placebo-adjusted change from baseline in machine-read QT/QTc values at t_{max} following tolterodine and moxifloxacin administration

	Tolterodine 2 mg b.i.d.[a]	Tolterodine 4 mg b.i.d.[a]	Moxifloxacin 400 mg q.d.[b]
QT (uncorrected)			
Point estimate	–2.9	–6.4	7.1
CI	(–10.5, 4.6)	(–14.0, 1.1)	(–0.0, 14.1)
SE for difference	*3.8*	*3.8*	*4.3*
QTcF (Fridericia)			
Point estimate	1.2	5.6	8.9
CI	(–3.0, 5.3)	(1.5, 9.8)	(4.8, 13.0)
SE for difference	*2.1*	*2.1*	*2.5*
QTcB (Bazett)			
Point estimate	3.2	11.9	9.6
CI	(–0.6, 7.0)	(8.1, 15.8)	(5.5, 13.7)
SE for difference	*1.9*	*1.9*	*2.5*
QTcP (population)		QTcP (population)	
Point estimate	2.0	8.3	9.3
CI	(–1.8, 5.8)	(4.5, 12.2)	(5.3, 13.2)
SE for difference	*1.9*	*1.9*	*2.4*

b.i.d., twice daily; CI, confidence interval; SE, standard error; q.d., once daily. [a]At t_{max} of 1 h; 95% CI. [b]At t_{max} of 2 h; 90% CI.

treatments (⩽16.7% for manual-read, ⩽6.3% for machine-read analyses) than moxifloxacin treatment (⩽45.7% for manual-read, ⩽23.9% for machine-read analyses). In the manual-read data set, there were no occurrences of QTcF >470 ms in women; QTc >450 ms occurred in one male subject after each of the three active treatments. In the machine-read data set, no women had QTcF >470 ms; among men, the occurrence of QTcF >450 ms was seen in one (4%), three (12%), and one (4%) subjects receiving tolterodine 2 mg twice daily, tolterodine 4 mg twice daily, and moxifloxacin 400 mg once daily, respectively.

Table 3 Steady-state, placebo-adjusted change from baseline in manual-read QT/QTc values at t_{max} following tolterodine and moxifloxacin administration

	Tolterodine 2 mg b.i.d.[a]	Tolterodine 4 mg b.i.d.[a]	Moxifloxacin 400 mg q.d.[b]
QT (uncorrected)			
Point estimate	1.1	–0.3	17.7
CI	(–5.9, 8.0)	(–7.2, 6.6)	(11.9, 23.4)
SE for difference	*3.0*	*3.0*	*3.5*
QTcF (Fridericia)			
Point estimate	5.0	11.8	19.3
CI	(0.3, 9.7)	(7.1, 16.6)	(15.5, 23.0)
SE for difference	*2.4*	*2.4*	*2.3*
QTcB (Bazett)			
Point estimate	6.9	18.3	19.9
CI	(1.9, 12.0)	(13.2, 23.3)	(15.5, 24.3)
SE for difference	*2.6*	*2.6*	*2.7*
QTcP (population)			
Point estimate	4.5	10.3	19.1
CI	(–0.4, 9.3)	(5.5, 15.1)	(15.3, 22.9)
SE for difference	*2.4*	*2.4*	*2.3*

b.i.d., twice daily; CI, confidence interval; SE, standard error; q.d., once daily. [a]At t_{max} of 1 h; 95% CI. [b]At t_{max} of 2 h; 90% CI.

The model-based QTcP increase for moxifloxacin was 10.8 ms, which is consistent with previously reported prolongation values.[19–23]. For tolterodine, this estimate was 7.9 ms for the supratherapeutic dose and 2.0 ms for the recommended dose. For both tolterodine and moxifloxacin, the model-predicted results agreed well with the point estimate from the statistical analysis of the machine-read data (**Table 2**).

DISCUSSION

According to the ICH E14 guidance, unless precluded by considerations of safety or tolerability owing to adverse effects, the QT interval prolongation of the study drug should be assessed at substantial multiples of the anticipated maximum therapeutic exposure.[15] Alternatively, if the concentrations of a drug can be increased by drug–drug or drug–food interactions, the QT assessment may be performed under conditions of maximum inhibition.[15] The 4 mg twice-daily dose of tolterodine was chosen to represent a worst-case scenario of supratherapeutic exposures to tolterodine, based on the twofold increase in tolterodine exposures in PMs

when used concomitantly with potent CYP3A4 inhibitors. It should be noted that such a scenario would be non-compliant with the prescribing information for tolterodine, which recommends a dose reduction in such circumstances.[10,11] Furthermore, the IR formulation of tolterodine was used in this TQT study, which gives peak concentrations approximately 1.5-fold higher than the more widely prescribed ER formulation.[12] Exposures much above those in PMs receiving tolterodine 4 mg twice daily would be difficult to tolerate owing to the dose-limiting anticholinergic side effects. Urinary retention was reported to be a major tolerability problem in healthy volunteers receiving 12.8 mg single and higher than 4 mg twice-daily doses.[3,4] Therefore, 4 mg twice daily was considered as an adequate and appropriate supratherapeutic dose of tolterodine for this study.

The E14 guidance recommends that all TQT trials should include a positive control that has mean QT/QTc interval prolongation of about 5 ms (*i.e.*, an effect that is close to the QT/QTc effect that represents the threshold of regulatory concern, around 5 ms).[15] Based on both the machine- and manual-read ECG data (**Tables 2** and **3**), the study sensitivity was confirmed because a >5 ms QT prolongation effect was seen with moxifloxacin. It was remarkable, however, that the QT effect of moxifloxacin was approximately twofold higher when using the manual-read QTc intervals than the machine-read intervals. The manual-read QTc changes of moxifloxacin, a widely used and well-characterized positive control, were considerably higher in this study than other TQT trials (shown in **Table 4** and plotted on both **Figures 2** and **3**).[19–23] Across several trials with both single and multiple doses of moxifloxacin, the mean QTc changes ranged from 6 to 13 ms, compared with the >19 ms manual-read mean change estimated in this study (**Figure 3**). The corresponding 90% CI was (15.5–23.0) and did not include the range of mean values reported in other studies. The moxifloxacin QTc effect measured by the machine-read ECG data was 8.9 ms (**Table 2**) and more consistent with previous studies.

The machine-read QT interval data from this study were deemed to be most appropriate for the basis of study conclusions. Although the exact reason(s) for the discrepancy in manual-read results remains unclear, several methodological issues were identified. Although this was a crossover study, the involvement of multiple readers could introduce subjectivity and non-standardized interval adjustment, increasing the overall variability of manual-read QTc values. The ECG tracings for the entire study were read over a relatively long period of 3 months, which could further add a within-reader component of variability. Additionally, the tangent method for determining the end of T-wave may be inappropriate. Combined, these logistical constraints, methodological issues, and a few operational inconsistencies (described in the Methods section) have the potential to affect the precision and accuracy of the manual-read QTc determinations. The potential for manual techniques to introduce bias is discussed further in a recent publication.[24]

A broad review of ECG tracings confirmed the absence of T-wave morphology changes. This supported the validity of using machine-read QT intervals in this healthy volunteer study. More than 14,000 ECGs were collected during the four treatment periods of the study. Among the machine-read ECGs, only six subjects showed nonspecific ST- and/or T-wave abnormalities at their screening visit but not at any of the measurement times during the four periods. Only one subject exhibited T-wave abnormality in a single ECG collected on the baseline day during moxifloxacin treatment period. The lack of any notable T-wave abnormalities was also confirmed by the central ECG readers' comments, where such observations were made in only a minimal number of ECGs (56) across more than 10,300 ECGs that were manually over-read.

The present regulatory determination of a negative TQT study is one where "the largest time-matched mean difference between the drug and placebo (baseline subtracted) for the QTc interval is about $\leqslant 5$ ms, with a one-sided 95% CI that excludes an effect >10 ms."[15] It is recognized that drugs that prolong the mean QT/QTc interval by about $\leqslant 5$ ms do not appear to cause TdP. Data on drugs that prolong the mean QT/QTc interval by >5 and <20 ms are inconclusive. In such cases, the clinical relevance of these modest changes may be best determined by careful examination of the clinical trial and postmarketing adverse events possibly related to QT/QTc interval prolongation, such as TdP, cardiac arrest, sudden cardiac death, and ventricular arrhythmias (*e.g.*, ventricular tachycardia and ventricular fibrillation). Drugs that have an average QT/QTc interval prolongation of >20 ms have an

Table 4 Mean (95% CI) changes in QTcF produced by moxifloxacin 400 mg in other TQT studies

TQT study drug	Moxifloxacin dose, regimen	Population sex (age, mean, or range)	Mean QTcF change (ms)[a]
Tolterodine	400 mg, q.d. $\times$ 4 days	Men/women (37 years)	19.3
Vardenafil[26]	400 mg, SD	Men (53 years)	7.7
Alfuzosin[25]	400 mg, SD	Men (27 years)	10.3
Solifenacin[23]	400 mg, SD	Women (51 years)	11.0
Trospium[24]	400 mg, q.d. $\times$ 5 days	Men/women (range, 18–45 years)	6.4
Darifenacin[27]	400 mg, q.d. $\times$ 6 days	Men/women (43 years)	11.6

CI, confidence interval; q.d., once daily. [a]Results are based on manual-read ECGs.

increased likelihood of being proarrhythmic. A basis of these interpretation guidelines is the survey of mean peak QTc prolongation by several drugs.[25]

Using the machine-read QTcF data, a clinically relevant 5-ms prolongation of QTcF could be ruled out; that is, a negative TQT study conclusion could be made for both the recommended and the supratherapeutic doses of tolterodine. The manual-read data indicated that a 5-ms QTcF prolongation could be ruled out for tolterodine at the recommended daily dose, but not at the supratherapeutic dose. It is noteworthy that none of the subjects, irrespective of their metabolic profile, exceeded the clinically relevant thresholds of 500 ms for absolute QTc or 60 ms for change from baseline, following both doses of tolterodine. The QTc interval changes following both recommended and supratherapeutic tolterodine doses were consistently smaller relative to the effect seen with the therapeutic dose of moxifloxacin (**Figures 2** and **3**).

Although the study-specified primary end point was the change from baseline QTcF at the pharmacokinetic t_{max} of tolterodine, the results showed that it was also coincident with the maximum change from baseline QTcF, the ICH E14-specified end point.[15] Therefore, the same study conclusions can be made whether using the pre-E14 study end point or that specified in the contemporary E14. The actual clinical impact of these QTc changes is unknown, but needs to be considered when prescribing tolterodine to patients taking certain drugs known to prolong QT interval, including Class IA or Class III antiarrhythmic medications, or to patients with congenital or documented acquired QT prolongation.

The relationship between QTc changes and the serum concentrations of tolterodine and its active metabolite was best described using a linear model. Based on this model, we can estimate the probable changes in QTc using the more widely prescribed ER formulation. The peak exposures of tolterodine and DD01 after dosing with the ER capsules are about 61% and 67%, respectively, compared with IR tablets, and thus any QTc effects of the ER capsules would also be expected to be proportionally smaller. The concentration–QTc modeling results also showed an exposure-related higher QTc effect in PMs than in EMs. Furthermore, QTc interval increases in PMs treated with tolterodine 2 mg twice daily were comparable to those observed in EMs receiving 4 mg twice daily.

The current understanding of cardiac safety in relation to the utility of QT prolongation as a biomarker for TdP and sudden cardiac death is poorly defined and is an area of continued investigation. Affinity for HERG channels *in vitro* is commonly used as a preclinical screen for a drug's likelihood to prolong QTc. The modest QTc changes reported here for tolterodine suggest that I_{kr}-binding affinity in the HERG assay may be an imperfect screening tool for determining the likelihood for clinical QT prolongation. If HERG channel results had been used as the sole criterion for continuing the development of tolterodine, it is possible that it would not have been tested in humans. However, other preclinical testing with tolterodine demonstrated a lack of effect on action potential duration. This TQT study, confirming the lack of clinically significant prolongation, is in agreement with the postmarketing cardiac safety assessment of tolterodine and supports the use of the latter type of analysis for the detection of safety signals, given the extensive clinical practice experience with tolterodine since 1997 (data on file). Broad searches within Pfizer's postmarketing database, as well as those maintained by the US Food and Drug Administration and World Health Organization, have not shown any signal of an association between tolterodine and QT prolongation/TdP in multiple sources searched. This includes the lack of any published case reports.

These non-clinical and clinical data for tolterodine demonstrate that drugs with high HERG affinity *in vitro*, do not necessarily produce clinically relevant QT prolongation or clinical cardiac events. Another example of a drug with high HERG affinity, but no cardiac safety liability, is verapamil.[26] It is possible that tolterodine and verapamil have activities at other ion channels, most notably calcium channel blockade, that mitigate any negative effects of HERG blockade; however, the precise mechanism(s) for such an effect have not been identified.[13,19]

In conclusion, this TQT study demonstrated that tolterodine does not have any clinically significant effect on the QTc interval. Compared with the therapeutic dose of moxifloxacin, the QTcF changes were up to one-fourth at the recommended dose and up to two-thirds at the supratherapeutic dose of tolterodine. Manual-read QTc data were more variable and the mean QTc change was consistently larger than machine data; however, this did not affect the aforementioned conclusions. The disparity between tolterodine's high affinity for HERG channels *in vitro* and its lack of clinical cardiac safety signals suggest caution in over-reliance on HERG assays as a sole preclinical screening tool for selecting drug candidates for further development.

METHODS

Study design. This was a positive- and placebo-controlled, multiple-dose, four-way crossover study conducted at two centers. The study was double-blinded with respect to tolterodine and placebo, with open-label moxifloxacin. This study evaluated the single-dose and steady-state QTc effects of the recommended (2 mg twice daily) and supratherapeutic (4 mg twice daily) doses of tolterodine IR and the positive control, moxifloxacin (400 mg once daily), each compared with placebo. Moxifloxacin was included as a positive control to confirm the sensitivity of the study to detect small QTc changes. Moxifloxacin is frequently used in TQT studies because it has a well-defined QT/QTc effect, usually about 6–12 ms.

At the screening visit, subjects underwent physical examination, assessment of previous medical history, laboratory screen, and CYP2D6 genotyping. Volunteers were admitted to the clinic in the evening 2 days before dosing on day 1 and remained in the clinic until the evening of day 4 in each period. Treatments were administered for 4 days (morning dose only on day 4), with a washout period of $\geqslant$5 days between periods. A follow-up visit was performed 5–7 days after period 4 ended. The mean elimination half-lives were 2.2 h (EM) and 9.6 h (PM) for tolterodine, 2.4 h (EM) for DD01, and 12 h for moxifloxacin. Therefore, the dosing and

washout periods were adequate for reaching steady state and for drug elimination between periods, respectively.

Subject population. Healthy adult men and women (aged 18–55 years) with a body mass index of 18–30 kg/m^2 were eligible. Screening blood samples were analyzed for CYP2D6 *3, *4, *6, *7, *8, and *10 alleles to determine their CYP2D6 genotype. Subjects with two or more non-functional alleles were identified as PMs. It was targeted to enroll 20–24 PMs. All subjects provided written informed consent. The study was conducted in accordance with Title 21 of the US Code of Federal Regulations, Good Clinical Practice guidelines, and the Declaration of Helsinki Principles.[27]

Sample size. To meet the primary objective, 43 completed subjects were more than adequate to provide 95% probability that the half-width (*i.e.*, margin of error) of the 95% CI for the true mean difference in change from baseline in QTcF between active treatment and placebo would not exceed 5 ms. To assess the sensitivity of the study, 43 subjects were required to complete treatment to provide 85% power to detect a 5-ms difference between moxifloxacin and placebo in change from baseline in QTcF, based on a one-sided paired *t*-test at the 5% significance level. Thus, the sample size of 48 subjects was adequate, assuming a dropout rate of 10%.

ECG acquisition. Single 12-lead ECGs were obtained at screening and follow-up visits. During each period, baseline and on-treatment ECGs were obtained in triplicate at prespecified time points, with the consecutive replicates about 2 min apart. All ECGs were performed after the subject had rested quietly for at least 10 min, with no food permitted 2 h before measurement. When the timing of an ECG coincided with blood collection, the ECG was obtained before the nominal time of blood collection.

ECGs were obtained predose on the mornings of days 1–4 in each period. At steady state, the ECGs were taken at 0 (within 1 h predose), 0.5, 1, 2, 3, 4, 6, 9, and 12 h postdose on day 4. After the first dose on day 1, the ECGs were taken just before and at 0.5, 1, 2, and 4 h after the morning dosing. Baseline ECGs were performed on day 0 at the same times of day as those on day 4.

All 12-lead ECGs were recorded on GE Marquette's MAC 1200® ECG recorders (GE Medical Systems, Milwaukee, WI) and the same ECG machine was used for all readings on a single subject. Ten seconds of all 12 leads of ECG data were simultaneously collected from each subject based on the specifics outlined in the protocol and stored in the MAC 1200®. All ECGs were digitally transferred to a dedicated MUSE system at the core ECG laboratory (Biomedical Systems, St Louis, MO).

All machine-read measurements for each ECG were calculated using the GE/Marquette 12SL program and were based on a "Global Median" beat, a superimposition of 12 median beats (one from each lead). The median beat within each lead was calculated with the following characteristics:

1. Beats of the same shape (within each lead) were combined into an accurate, representative cycle, thereby reducing the noise dramatically.
2. A "primary beat" was recognized as the group of beats with the most ECG information (*i.e.*, the beat most informative of normal conduction).
3. Middle (median) voltage of primary beats was taken for each sample (most informative of normal conduction with three or more samples).

Machine-read QT interval measurements were based on the earliest onset of the Q wave in any lead and the latest offset of the T wave in any lead. The 12SL algorithm uses the threshold method to identify the offset of the T wave. This algorithm includes the U wave in the QT measurement if the U wave merges with the T wave. Otherwise, the U wave is excluded from the QT measurement.

Manual over-read of QT intervals. The QT intervals of ECGs pertinent to the primary end point of the study, *i.e.*, those collected on study days 0 (baseline) and 4 (steady state) were measured in lead II using a validated on-screen method for manual over-read. A cardiac technician reviewed and adjusted, as needed, the computer-generated annotations. The technician used the median beat in lead II, therefore, not the earliest onset to latest offset across all 12 leads. A tangent line was placed on the maximum down-sloping part of a T-wave and the end of the T-wave was determined to be where the tangent line crossed the isoelectric line. In the presence of a U-wave, the technician was instructed to include the U-wave if it merged with the T-wave; if the U-wave was distinct and separate, it was excluded from the measurement.

Following the technician's review, a cardiologist reviewed and adjusted the interval measurements, provided an interpretation, and confirmed the ECG. Four technicians and three cardiologists were involved in this study. The same reader was to measure all ECGs from the same subject. In a few instances, in violation of protocol requirements and vendor standard operating procedures, more than one cardiologist was involved in reviewing a subject's entire set of ECG tracings. Although blinded to study treatment information, the cardiac technicians or cardiologists were not blinded to critical ECG information, such as subject ID, period ID, and date and time of ECG.

Heart rate correction of QT intervals. The QTc values were calculated using the Fridericia ($QTcF = QT/RR^{0.33}$), Bazett ($QTcB = QT/RR^{0.50}$), and study-specific population ($QTcP = QT/RR^{0.41}$) correction formulae, where RR = 60/HR. The exponent for RR in the study-specific population correction formula was determined from slope of the regression of ln(QT) on ln(RR). Based on QT–RR relationships in this study, the exponent of the population correction formula for the manual over-read data was 0.29, which compares well with the QTcF exponent of 0.33. Therefore, QTcP and QTcF results were expected to be comparable for the manual-read ECGs. For the machine-read data, the QTcP exponent was estimated to be 0.41, which is between QTcF (0.33) and QTcB (0.5) values.

The intent of QT correction methods is to make QT interval measurements independent of heart rate. Historically, QTcB has been the most commonly used, but it tends to overcorrect at high heart rates and hence is not an ideal correction under these circumstances.[28,29] As expected, tolterodine did cause a small increase in heart rate, further supporting the use of QTcF, which is less prone to overcorrection than QTcB.

Pharmacokinetic sampling and analysis. During each treatment, two sets of blood samples were obtained at the same time point as the ECGs. One set of samples was processed for serum and the other for plasma. Serum samples were assayed for tolterodine and DD01 by AvTech Laboratories (Kalamazoo, MI) using a validated liquid chromatographic/tandem mass spectrometric assay following a solid-phase extraction. The dynamic range of the assay was 0.100–60.0 ng/ml for tolterodine and 0.100–10.0 ng/ml for DD01. The plasma samples collected predose and up to 4 h postdose were assayed for moxifloxacin by PPD Development (Richmond, VA) using a validated high-pressure liquid chromatography method with ultraviolet absorbance detection following a liquid–liquid extraction. The assay had a dynamic range of 25.0–5000 ng/ml. A predose serum sample was obtained on day 1 of each period to assay the amount of AAG for the estimation of unbound fractions of tolterodine ($f_{u,tolterodine}$) and DD01 ($f_{u,DD01}$). Serum AAG was assayed by Quintiles (Uppsala, Sweden) using an immunoturbidimetric assay

utilizing a Hitachi 912 detection system. The dynamic range of the assay was 0.12–5.33 g/l. Unbound fractions were calculated from AAG concentrations as:[30]

$$f_{u,\text{tolterodine}} = 1/(1 + [2100 \times \text{AAG}/42])$$

$$f_{u,\text{DD01}} = 1/(1 + [130 \times \text{AAG}/42])$$

Individual concentration–time data for tolterodine, DD01, and moxifloxacin were analyzed by standard non-compartmental approaches using WinNonLin Enterprise, version 3.2 (Pharsight, Mountain View, CA). C_{max} and time of C_{max} (t_{max}) were obtained by direct observation of concentration–time data. AUC for tolterodine and DD01 were calculated using the linear-log trapezoidal rule from time zero to the time of last measurable concentration during the 12-h dosing interval on day 4.

Statistical analysis. The mean of triplicate QTc values at each measurement time was used for the categorical (outlier) and central tendency (mean effect) analyses. The statistical analyses for mean effect were based on a linear model with sequence, period, center, and treatment as fixed factors and subject (sequence) and within-subject error as random factors. Because two study sites were used, a term for "site" was included in the model. The covariance structure for within-subject-error assumed compound symmetry.

The primary end point was the mean placebo-subtracted change from baseline in QTcF (ΔΔQTcF) at tolterodine t_{max} on day 4. The time-matched mean changes from baseline QTc interval were also calculated at each postdose time point on day 4. These end points were also assessed using QTcB and QTcP in addition to QTcF. For each end point, the two-sided 95% CIs were calculated using the least squares means and appropriate standard errors. To verify the sensitivity of the study, time-matched changes from baseline in QTc after moxifloxacin and placebo were compared using a one-sided test at the nominal 0.05 level of significance; the same linear model as specified in the primary end point analysis.

For categorical analyses, the following cutoff values were used to report the number (percentage) of subjects who had increases in QTc from baseline of (1) $\geqslant$30 but $<$60 ms, and (2) $\geqslant$60 ms or absolute QTc values (1) $\geqslant$450 (men), (2)$\geqslant$470 (women), and (3)$\geqslant$500 ms.[16]

Concentration–QTc modeling. The concentration–QTc relationships for moxifloxacin and tolterodine were explored using machine-read, QTcP data. The model characterized the relationship between QTcP interval and concentration, accounting for placebo, baseline, and drug effects. For the concentration–QTc modeling, contributions were assumed from both tolterodine and DD01. There is a 10-fold difference in unbound fractions of tolterodine and DD01; therefore, the model used the sum of unbound serum concentrations along with a relative potency factor of I_{kr}-channel antagonism *in vivo* by tolterodine vs DD01. A similar model was fitted to QTcP interval vs moxifloxacin total plasma concentration data. Because there was no apparent nonlinearity when the data were graphically displayed, a population mixed effects modeling approach using linear models was employed to characterize the relationship between concentrations and the change from baseline QTc. The mixed effects modeling approach was implemented in NONMEM Version V Level 1.1 (Globomax LLC, Hanover, MD and NONMEM Project Group, UCSF, San Francisco, CA) using the first order conditional estimation method.

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CONFLICT OF INTEREST

Mr Anziano and Drs Glue, Malhotra, Mancuso, Sweeney, and Wicker are employees of Pfizer, Inc and hold stock options.

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