

Article Type: Clinical Trial

Accepted Article

Assessment of Multi-Ion Channel Block in a Phase-1 Randomized Study Design: Results of the CiPA Phase 1 ECG Biomarker Validation Study

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Conflicts of Interest

PT Sager has consulting agreements with Biomedical Systems, Charles River, and iCardiac. The other authors report no conflicts of interest.

Funding

This study was internally funded by the FDA/CDER Safety Research Interest Group

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cpt.1303

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Key words: drug-induced QT prolongation, torsade de pointes, multichannel block

Abstract

Balanced multi-ion channel-blocking drugs have low torsade risk because they block inward currents. The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative proposes to use an *in-silico* cardiomyocyte model to determine the presence of balanced block, and absence of J-Tpeakc prolongation would be expected for balanced blockers. This study included 3 balanced blockers in a 10-subject per drug parallel-design; lopinavir/ritonavir and verapamil met the primary endpoint of $\Delta\Delta J$ -Tpeakc upper bound <10 ms, while ranolazine did not (upper bounds of 8.8, 6.1, and 12.0 ms, respectively). A predominant hERG blocker (chloroquine) prolonged $\Delta\Delta QT_c$ and $\Delta\Delta J$ -Tpeakc by ≥ 10 ms. In a separate crossover design, diltiazem (calcium block) did not shorten dofetilide-induced ΔQT_c prolongation, but shortened ΔJ -Tpeakc and prolonged $\Delta T_{peak-Tend}$. Absence of J-Tpeakc prolongation appears consistent with balanced block, however, small sample size (10 subjects) may be insufficient to characterize concentration-response in some cases.

Introduction

Current regulatory guidelines from the International Council on Harmonisation (ICH) describe non-clinical (ICH S7B¹) and clinical strategies (ICH E14²) to assess the potential of a drug to prolong cardiac repolarization as well as its clinical proarrhythmic potential. No drugs with unexpected QT prolongation or torsade de pointes (torsade) risk have reached the market after the adoption of ICH S7B and E14 guidelines. However, there are drugs that block the potassium channel encoded by the human ether-à-go-go related gene (hERG) and prolong the heart rate corrected QT (QTc) interval but have low torsade risk (e.g., ranolazine, verapamil, amiodarone) because they block other inward currents (i.e., late sodium and/or L-type calcium currents). Block of these inward currents is antiarrhythmic by preventing early afterdepolarizations.³⁻⁹ ICH S7B and E14 discuss the importance of the drug's effects on other ion channels in addition to hERG and encourage interested parties to develop models for directly assessing drug-induced proarrhythmia of QTc prolonging drugs.^{1,2} In line with this, the goal of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) is to perform a mechanistic assessment of a drug's torsade risk.¹⁰

Under CiPA, the torsade risk of a drug will be determined by an *in silico* model that integrates the drug's effects on multiple ion channel currents of the human ventricular myocyte. CiPA's *in silico* model outputs a Torsade Metric Score called qNet,¹¹ which reflects the balance of inward and

outward currents throughout the action potential. Multichannel blocking drugs, that is, drugs that block hERG as well as late sodium and/or L-type calcium currents, will be considered balanced ion channel blockers (referred to as “balanced blockers”) if qNet predicts the drug to be low risk, and “predominant hERG blockers” otherwise (i.e., drugs that only block hERG or multichannel blockers predicted to be intermediate or high risk). For a balanced blocker, the CiPA initiative proposes to use electrocardiogram (ECG) analysis in early phase 1 clinical trials to determine if there is evidence of unexpected ion channel effects in humans compared to the preclinical data.¹² As described in a review of the CiPA initiative that included the rationale and design of this clinical study,¹³ prior work identified that the heart rate corrected J-Tpeakc (J-Tpeakc) interval could be used to differentiate predominant hERG blockers from balanced blockers. Specifically, predominant hERG blockers prolong QTc and J-Tpeakc, while balanced blockers prolong QTc with no J-Tpeakc prolongation.¹⁴⁻¹⁸

This study was designed in 2 parts with separate objectives.¹³ The primary objective of part 1 was to assess whether balanced ion channel-blocking drugs (ranolazine, verapamil and lopinavir/ritonavir) do not cause J-Tpeakc prolongation in a small sample size (10 subjects per drug and placebo) phase-1 type parallel study design. One predominant hERG-blocking drug (chloroquine) was included as a control. Balanced block was determined based on ion channel data available during study design,^{13, 19} and not based on qNet. Thus, drug categories may be different if based on data acquired with current CiPA protocols²⁰ and qNet. Prolongation of J-Tpeakc and QTc was defined using a concentration-response model as an upper bound of the predicted effect at maximum drug plasma concentration ≥ 10 ms. The primary objective of part 2 (crossover study) was to test the hypothesis that diltiazem (calcium channel block) can reduce the QTc prolongation by dofetilide (predominant hERG block) by shortening J-Tpeakc. Sample size and J-Tpeakc threshold were determined based on analyses from prior clinical studies.^{14, 15, 17, 18} and by resampling the data using similar methodology of Ferber et al.²¹ (see online supplement in Vicente et al.¹³ for details).

Results

Sixty healthy subjects (22 women) with a mean \pm SD age of 31.7 ± 8.7 years and body mass index of 25.8 ± 2.7 kg/m² were randomized with 50 subjects assigned to part 1 (parallel study) and 10 subjects assigned to part 2 (crossover study). No serious or unexpected treatment-related adverse events were observed. Two subjects were discontinued after receiving the second dose of diltiazem in part 2 and one subject withdrew consent prior to the second day of treatment in part 1. All other subjects completed the study. The online supplement includes full baseline characteristics, adverse events frequencies by treatment, CONSORT flow diagram as well as detailed results for multiple ECG biomarkers as described in the statistical analysis plan.¹³ The clinical data and raw annotated ECG waveforms are publicly available at PhysioNet's²² website (<https://physionet.org/physiobank/database/ecgcipa/>).

Part 1: parallel study

Administered drugs, their dosing times and their pharmacokinetic (PK) time profiles are shown in Figure 1. The observed geometric mean maximum drug plasma concentrations (C_{max}) after first dose on days 1 and 3 are shown in Table 1. As planned, subjects were exposed to a lower drug concentration on Day 1 and a higher drug concentration on Day 3.

Figure 2 shows time profiles of placebo corrected changes from baseline ($\Delta\Delta$) for $\Delta\Delta Q_{Tc}$ and $\Delta\Delta J-T_{peakc}$ for each treatment and time-point (see online supplement for $\Delta\Delta T_{peak-Tend}$ plots). Table 1 shows predictions for all ECG biomarkers at low and high concentrations on days 1 and 3, respectively, from concentration-response models with proper fit (i.e., linear relationship supported by observed data). Goodness of fit concentration-response plots are shown in Figure 3 and online supplement.

Ranolazine, lopinavir/ritonavir and verapamil

The pre-specified primary endpoint for the balanced ion channel-blocking drugs was that they would not prolong J-T_{peakc} defined by an upper bound of the two-sided 90% confidence interval of $\Delta\Delta J-T_{peakc} < 10$ ms at C_{max} on day 3. While not included in the primary endpoint, Q_{Tc} prolongation (i.e., upper bound of $\Delta\Delta Q_{Tc} \geq 10$ ms at C_{max} on day 3) was also expected because these drugs are known to block hERG.

The three balanced blockers had flat or negative mean $\Delta\Delta J-T_{peakc}$ slope and mean $\Delta\Delta J-T_{peakc}$ effects < 5 ms (Figure 3). The prespecified criterion was met with verapamil (mean [90% confidence interval] of -2.6 [-11.4 to 6.1] ms) and lopinavir/ritonavir (-2.9 [-14.6 to 8.8] ms), however the criterion was not met for ranolazine (1.2 [-9.5 to 12.0] ms).

Ranolazine prolonged Q_{Tc} by 14.1 [7.5 to 20.8] ms on day 1 and 28.2 [18.3 to 38.2] ms on day 3, while verapamil prolonged Q_{Tc} by 8.6 [4.4 to 12.9] ms on day 1 and 13.9 [10.0 to 17.8] ms on day 3 (Figure 3 and Table 1). Lopinavir/ritonavir prolonged Q_{Tc} by >20 ms on day 3, but exhibited non-linear concentration-dependent Q_{Tc} prolongation.

Chloroquine

Chloroquine was included as a non-balanced (predominant hERG) blocker drug. The pre-specified primary endpoint was that it would prolong $\Delta\Delta Q_{Tc}$ defined by an upper bound of the two-sided 90% confidence interval of $\Delta\Delta Q_{Tc} \geq 10$ ms at C_{max} on day 1. While not included in the primary endpoint, chloroquine was expected to prolong $\Delta\Delta J-T_{peakc}$.

Chloroquine met the prespecified criterion prolonging $\Delta\Delta Q_{Tc}$ by 30.7 [22.6 to 38.9] ms at C_{max} on day 1 (Figure 3). $\Delta\Delta J-T_{peakc}$ was prolonged as defined by it being ≥ 10 ms throughout the concentration range in the study (Figure 3), however the goodness of fit plot showed a poor fit of the concentration-J-T_{peakc} linear model with a large positive intercept (8.6 ms) and flat slope.

Part 2: crossover part - Dofetilide vs. dofetilide + diltiazem

The hypothesis for this part of the study was that diltiazem (L-type calcium channel block) can shorten the QTc prolongation caused by dofetilide (predominant hERG block) by shortening the J-Tpeakc interval. This was assessed in a crossover study design by subjects receiving dofetilide alone in one period, while in a second period having subjects receive diltiazem alone on days 1 and 2 and then diltiazem combined with dofetilide on day 3. Dosing schedule and pharmacokinetic results are shown in Figure 4. Diltiazem observed Cmax on day 3 was 385.2 ng/mL. Dofetilide Cmax concentrations on day 3 were 1.5 and 1.2 ng/mL for dofetilide alone and diltiazem + dofetilide treatment periods, respectively. When dofetilide was administered alone (Figure 5), it prolonged Δ QTc by prolonging both Δ J-Tpeakc and Δ Tpeak-Tend. When diltiazem was co-administered with dofetilide, diltiazem did not shorten Δ QTc (Figure 5.A and Figure 5.D), thus not meeting the primary endpoint. In the subsequent exploratory analysis, diltiazem shortened Δ J-Tpeakc by 11.5 [2.2 to 20.7] ms (Figure 5.B and Figure 5.E) and increased Δ Tpeak-Tend by 16.3 [4.8 to 27.7] ms (Figure 5.C and Figure 5.F).

PR and QRS effects for all study drugs

Chloroquine, verapamil, lopinavir/ritonavir and diltiazem prolonged Δ PR in a concentration-dependent fashion (Table 1). Goodness of fit plots supported a linear concentration- Δ PR relationship for chloroquine, which caused 14.8 [4.7 to 24.9] ms prolongation at Cmax on day 3. With verapamil, lopinavir/ritonavir and diltiazem, concentration-dependent Δ PR prolongation was linear at lower concentrations, but goodness of fit plots (see online supplement) suggested that it plateaued around 30 ms, which was the effect seen at Cmax on day 3. Ranolazine and dofetilide did not prolong PR.

Chloroquine prolonged QRS in a concentration dependent fashion, with Δ QRS of 9.9 [6.1 to 13.6] ms at Cmax on day 3 (Table 1). No QRS prolongation was observed with other drugs.

Discussion

Drug-induced torsade develops when there is an imbalance of inward and outward repolarization currents that triggers early afterdepolarizations. The CiPA initiative proposes to assess the effects of drugs on multiple ion channel currents (e.g., hERG, L-type calcium and late sodium) and integrate them in a mechanistic, *in silico* model of the human ventricular cardiomyocyte to predict torsade risk. The torsade risk metric (qNet) from the *in silico* model reflects the balance of inward and outward currents (i.e., how close a drug is to generating an early afterdepolarization, the trigger for torsade).¹¹ CiPA proposes to assess ECG data from early phase 1 clinical studies to determine if there are unexpected ion channel effects in humans compared to the results from the *in vitro* assays that inform the *in silico* model. For a balanced ion channel blocking drug (i.e., multichannel blocker that has a low torsade risk qNet), a lack of QTc prolongation would confirm that there are no unexpected ion channel effects. Moreover, if the balanced blocker causes QTc prolongation, a lack of J-Tpeakc prolongation could confirm presence of balanced ion channel effects. Thus, presence of J-Tpeakc prolongation would not be expected for balanced blockers.

Part 1

Drug categories were assigned based on ion channel data available during study design, and not on the qNet score from the *in silico* model.^{13, 19} The prespecified criterion to test the hypothesis for the balanced ion channel drugs in this study was that the upper bound of the two-sided 90% confidence interval of the predicted $\Delta\Delta J$ -Tpeakc using a concentration-response model would be <10 ms at Cmax on day 3. All three balanced ion channel drugs had flat or negative concentration-response relationships for $\Delta\Delta J$ -Tpeakc and the primary endpoint was met for verapamil (upper bound 6.1 ms) and for lopinavir/ritonavir (8.8 ms); however, it was missed for ranolazine (12.0 ms). Of note, with ranolazine this occurred when the QTc was prolonged by ~ 30 ms, whereas on day 1 the mean $\Delta\Delta QTc$ was ~ 14 ms and $\Delta\Delta J$ -Tpeakc upper bound was <10 ms (2.2 [-4.3 to 8.7] ms). One predominant hERG-blocking drug (chloroquine) that is associated with torsade^{23, 24} was included in the parallel part of the study as control. As hypothesized, chloroquine prolonged $\Delta\Delta QTc$ in a concentration-dependent fashion, meeting the primary endpoint with $\Delta\Delta QTc$ upper bound at Cmax on day 1 ≥ 10 ms. Chloroquine also prolonged $\Delta\Delta J$ -Tpeakc throughout the 3 days of the study as defined by an upper bound ≥ 10 ms, but the prolongation was not linearly related to concentration.

Concentration-Response Model Observations and Limitations

In the parallel (Part 1) of the study, concentration-response models for the primary endpoints had flat or negative J-Tpeakc slopes for ranolazine, verapamil and lopinavir/ritonavir, and a positive QTc slope for chloroquine, as expected. For the non-primary endpoints, it was observed that the lopinavir- $\Delta\Delta QTc$ relationship was non-linear and the chloroquine- $\Delta\Delta J$ -Tpeakc model had a positive intercept with a flat slope (Figure 3). With lopinavir, lower concentrations showed no QTc changes followed by a sharp increase of ~ 20 ms in mean $\Delta\Delta QTc$ at the higher concentrations on Day 3. This non-linear pattern was also present for $\Delta\Delta Tpeak$ -Tend (online supplement), and both are consistent with the time profile (Figure 2).

The positive intercept with a flat slope for the chloroquine- $\Delta\Delta J$ -Tpeakc linear model may have been due to not having enough data to characterize $\Delta\Delta J$ -Tpeakc response at low chloroquine concentrations (Figure 3). Positive intercepts have been reported before with small sample sizes (see Table 3 in Darpo et al.²⁵), and were observed in some other concentration-ECG relationships in this study (e.g., ranolazine-QTc, online supplement). In addition, multi-ion channel effects may play a more substantial role at higher chloroquine concentrations leading to a plateau of $\Delta\Delta J$ -Tpeakc prolongation. This hypothesis is supported by PR and QRS prolongation caused by chloroquine in this study (Table 1 and online supplement), which is consistent with prior observations.^{26, 27} In addition, chloroquine is known to accumulate in heart tissue, so the tissue concentration may have been substantially higher than the plasma concentration (e.g., up to ~ 150 fold),²⁸ leading to additional ion channel block. Lastly, QRS prolongation may be also explained by block of the inward rectifier potassium current (IK1) by chloroquine.^{19, 29, 30} Overall, the ECG findings for chloroquine may warrant further investigation.

Lastly, the variability of $\Delta\Delta J$ -Tpeakc measures observed at high ranolazine concentrations was due to heart-rate increase in two timepoints in two subjects. The observed variability could have been an artifact due to the use of previously developed population based heart-rate correction.¹⁴

Part 2

A prior study demonstrated that lidocaine and mexiletine (late sodium current blockers) shorten QTc prolongation by dofetilide by shortening J-Tpeakc.¹⁷ We hypothesized that diltiazem (L-type calcium channel blocker) would exert a similar effect. Diltiazem did not affect the slope of the dofetilide concentration- Δ QTc relationship (28.4 [21.9 to 34.9] ms per ng/mL with diltiazem and 29.2 [22.3 to 36.0] ms per ng/mL without), which were similar to those reported in our two prior studies (30.2 [26.9 to 33.5] ms per ng/mL and 27.9 [24.9 to 30.9] ms per ng/mL).^{15, 17} The reason for the lack of QTc shortening with diltiazem is not clear. Multiple nonclinical studies clearly demonstrate that L-type calcium channel block shortens action potential duration.³¹⁻³³ However, prior clinical studies combining diltiazem with quinidine and diltiazem with moxifloxacin also demonstrated a lack of QTc shortening by diltiazem.^{17, 34} This could be something specific to diltiazem, including potential secondary effects due to an autonomic response from diltiazem's effect on blood pressure or changes in ventricular loading from atrio-ventricular delay with mechanoelectrical feedback from stretch-activated ion channels.³⁵⁻³⁸

Diltiazem did shorten J-Tpeakc, but also increased Tpeak-Tend; which differs from what was observed for late sodium current block combined with predominant hERG block.¹⁷ Nevertheless, these findings were considered an exploratory analysis according to the statistical analysis plan.³⁹ Of note, verapamil and lopinavir/ritonavir, which block hERG and L-type calcium,¹⁹ did prolong QTc without prolonging J-Tpeakc, as expected (Figure 3). Thus, these specific drugs with a combination of L-type calcium and hERG block exhibited QTc prolongation without J-Tpeakc prolongation.

Summary of QTc and JTpeakc Effects with Comparison to Prior Studies

Figure 6 shows Δ QTc and Δ J-Tpeakc changes predicted from the linear-mixed effects models for balanced ion channel blockers (ranolazine and verapamil in this study along with ranolazine in a prior study) in comparison to predominant hERG blockers (dofetilide in two studies, quinidine and moxifloxacin). Concentration has been normalized in the X axis to facilitate comparison.^{15, 17}

Chloroquine and lopinavir/ritonavir are not shown because because poor fit of concentration-response linear models did not allow for reliable predictions of Δ J-Tpeakc for chloroquine and Δ QTc for lopinavir/ritonavir. At concentrations causing mean Δ QTc prolongations above 10 ms, the concentration-matched mean Δ J-Tpeakc changes remained close to zero or decreased for balanced blockers but increased proportionally to QTc for the predominant hERG blockers. The normalized concentration-response relationships are consistent across studies and drugs with balanced blockers prolonging QTc without prolonging J-Tpeakc, while predominant hERG blockers prolong QTc and J-Tpeakc.^{16, 18}

It is important to note that the presence vs. absence of J-Tpeakc prolongation interpreted in isolation alone does not necessarily portend the presence vs. absence of proarrhythmic risk. CiPA proposes that the ECG data should be interpreted with the nonclinical ion channel data and *in silico* Torsade Metric Score. In addition, moxifloxacin is a drug that is a predominant hERG blocker, however it has weak hERG block at standard clinical exposures and thus a substantially lower risk of torsade compared to dofetilide or quinidine.

Potential Implications of CiPA

ICH E14 states that it is inconclusive as to whether drugs that prolong QTc are proarrhythmic and that certain factors may modify the risk of QTc prolongation, including prolonging QTc up to a “plateau” value and that proarrhythmic risk might be influenced by other pharmacologic effects (e.g., other ion channel effects in addition to hERG block).² While ICH S7B and E14 guidelines state that the presence of QTc prolongation does not mean that a drug will cause arrhythmias and the presence of QTc prolongation should support planning for and interpretation of subsequent clinical studies, the way they have been used in practice has been to sometimes drop compounds or drugs from development, which may not always be appropriate.⁴⁰

One of the potential impacts of CiPA is to better inform when QTc prolonging drugs require intensive ECG monitoring in Phase 3 trials. ICH E14 describes a nuanced approach for ECG monitoring in late stage trials in the Q&A (see Figure 1 in Vicente et al.¹³). Briefly, the results can be divided into categories of whether the mean $\Delta\Delta\text{QTc}$ at therapeutic exposures is <10 ms, 10-20 ms or >20 ms. In the current ICH E14 Q&A, if the $\Delta\Delta\text{QTc}$ at maximal “worst case” therapeutic exposures is <10 ms, only routine ECG monitoring is recommended in late phase trials. Or, if higher exposures are only expected in a limited subset of patients, intensive ECG monitoring may only be required in that specific patient population. However, if the $\Delta\Delta\text{QTc}$ effect is 10-20 ms at therapeutic exposure, intensive ECGs are needed, and if $\Delta\Delta\text{QTc}$ is >20 ms, intensive ECGs and risk mitigation strategies are warranted.

It has been proposed that CiPA could impact clinical development for a drug with $\Delta\Delta\text{QTc}$ effect of 10-20 ms at worst-case therapeutic exposures, where a combined nonclinical and clinical integrated risk assessment should be taken into account to determine what level of ECG monitoring is appropriate in Phase 3. If the $\Delta\Delta\text{QTc}$ effect is >20 ms at therapeutic concentrations, it is anticipated that intensive ECG monitoring would still be required; however, the combined nonclinical and clinical data could be taken into account to better inform the type and extent of ECG monitoring and risk mitigation strategies. In addition, a goal of CiPA is to enable updating drug labels to be more informative about proarrhythmic risk, not just QT prolongation.

Conclusions

Results of this study suggest that concentration-response analysis of QTc and J-Tpeakc can differentiate QTc prolonging drugs with predominant hERG block from drugs that have balanced ion channel block. However, small sample sizes (10 subjects) may be insufficient to characterize concentration-response relationships for some multi-channel blocking drugs.

Methods

This study (clinicaltrials.gov number NCT03070470) was approved by the FDA Research Involving Human Subjects Committee and the local institutional review board. All subjects gave written informed consent and the study was performed at a Phase 1 clinic (Spaulding Clinical, West Bend, WI). The study rationale and design along with the protocol were previously published.¹³ The design is briefly summarized below.

Part 1 was a double-blind, randomized, placebo-controlled, 1-period parallel design to assess the effects of three balanced blockers (ranolazine, verapamil, and lopinavir/ritonavir combination), one predominant hERG blocker (chloroquine) and one placebo on the QTc and J-Tpeakc intervals in 50 healthy subjects. This parallel design is similar to early phase 1 studies and resulted in each study drug being administered to 10 subjects, and placebo to 10 subjects, in 3 consecutive days to achieve low and high drug plasma concentration levels on days 1 and 3, respectively. Administered drugs, their dosing times and their pharmacokinetic time profiles are shown in Figure 1.

Part 2 was a double-blind, randomized, 2-period crossover design in 10 subjects to assess whether diltiazem (calcium channel block) can reduce the QTc prolongation from dofetilide (hERG block) by shortening J-Tpeakc. In the dofetilide alone period, subjects received dofetilide on days 1 and 3. In the diltiazem + dofetilide period, subjects received diltiazem alone on days 1 and 2, and diltiazem + dofetilide on day 3. Dosing times and PK profiles are shown in Figure 4.

ECG assessment

Continuous ECG recordings were performed using the Mortara Surveyor system (Mortara, Milwaukee, WI) sampled at 500 Hz with an amplitude resolution of 2.5 μ V. From the continuous recording, three ECGs were extracted before the draw of each pharmacokinetic sample, based on heart rate stability and signal quality⁴¹ at 28 time-points during the treatment periods: -1 hour, -30 minutes, 0 (immediately before first oral dose on Day 1), 0.5, 2, 2.5, 3.5, 4.5, 5, 6, 8.5, 14, 24, 27.5, 32.5, 38, 48, 50, 50.5, 51.5, 52.5, 53, 54, 54.5, 55, 59, 61, and 73 hours after time 0 or the first oral dose on Day 1. The ECG extractions at -1 hour, -30 minutes, and time 0 (immediately before first oral dose on Day 1) were used for the baseline.¹³ Semi-automated measurements of the PR, QRS, J-Tpeak, Tpeak-Tend, and QT interval were performed using the derived vector magnitude lead in the median beat. Briefly, automatic annotations from CalECG (AMPS LLC, New York, NY) for global P onset, QRS on set and offset, and from a previously developed algorithm⁴² for Tpeak and Tend were generated. Next, two independent ECG readers blinded to treatment and time adjusted the measurements using high-resolution images using previously developed software.⁴³

None of the drugs caused an absolute mean $\Delta\Delta\text{HR} > 10$ beats per minute (bpm), so the QT interval and J-Tpeak intervals were corrected for heart rate with pre-specified population correction formulas.¹³ Specifically, the QT interval was corrected for heart rate using Fridericia's correction ($\text{QTc} = \text{QT}/(\text{RR}/1,000 \text{ ms})^{1/3}$, with RR in ms).⁴⁴ Similarly, the J-Tpeak interval was corrected for heart rate ($\text{J-Tpeakc} = \text{J-Tpeak}/(\text{RR}/1,000 \text{ ms})^{0.58}$, with RR in ms).¹⁴ No correction was performed for the Tpeak-Tend interval, as prior studies have shown a lack of rate dependence at resting heart rates.^{14, 45}

Pharmacokinetic sample analysis

Six validated liquid chromatography tandem mass spectrometric methods were used for pharmacokinetic sample analysis. The first method was the determination of dofetilide in K₂EDTA human plasma using deuterated dofetilide as the internal standard (IS) using simple methanol based protein precipitation. Reversed-phase ultra-performance liquid chromatographic (UPLC) separation was achieved with an Acquity UPLC C₁₈ column (2.1 x 100 mm, 1.8 micron). Mass spectrometric

detection was set at mass transitions of m/z 442.2→198.2. The second, third and fourth methods were developed for diltiazem, ranolazine and verapamil. All three analytes and their deuterated internal standards were chromatographed on Zorbax SB-C18 column (50 X 2.1 mm, 3.5 micron) after simple protein precipitation using acetonitrile. Mass spectrometric detections were set at m/z 415.3→178.0, m/z 428.3→279.0 and m/z 455.3→177.1 for diltiazem, ranolazine and verapamil, respectively. The fifth method, determination of chloroquine, and sixth method, simultaneous determination of lopinavir and ritonavir in K_2EDTA human plasma were developed. All three analytes and their deuterated internal standards were chromatographed on Phenomenex, Kinetex C_{18} column (50 x 2.1 mm, 1.7 micron). Mass spectrometric detections were set at mass transitions of m/z 320.2→247.2, m/z 629.0→155.2, m/z 722.0→268.1 for chloroquine, lopinavir and ritonavir, respectively.

Drug categories

Drug categories (i.e., balanced vs. predominant hERG block) were assigned based on ion channel data available during study design as previously described,^{13,19} and not on the qNet score from the *in silico* model. Ranolazine and verapamil are balanced blockers (similar potency for hERG and late sodium and for hERG and calcium, respectively). Lopinavir/ritonavir blocks hERG, calcium and late sodium at similar potencies and was included as a balanced blocker. Chloroquine has substantially more potent hERG block compared to calcium or late sodium and was labeled as a predominant hERG blocker. Drug categories may be different if based on qNet and new ion channel data acquired with current CIPA protocols.²⁰

Statistical analysis

The statistical analysis plan was previously published.¹³ Concentration-response modeling was performed following current best practices⁴⁶ with prespecified models. Linear-mixed effects models were used to characterize the relationship between drug concentration and ECG changes. The change from baseline (Δ) for each ECG biomarker (e.g., the average ΔQTc , ΔJ -Tpeakc) by time-point was the dependent variable, for which baseline was defined as the mean of the three predose ECG readings on Day 1 for each period. In part 1, we used linear mixed-effects models where ΔECG was the dependent variable (e.g., ΔQTc , ΔJ -Tpeakc) and drug concentration (set to 0 for placebo), nominal timepoint, treatment (coded 0 for placebo and 1 for active drug) were included as fixed effects and subject was included as random effect on intercept and drug concentration (i.e., allowing each subject to have his own concentration-response relationship). In part 2, we used a linear mixed-effects model where ΔECG was the dependent variable (e.g., ΔQTc , ΔJ -Tpeakc), dofetilide and diltiazem concentrations as well as dofetilide-diltiazem interaction were included as fixed effects, and subject was included as a random effect on intercept and dofetilide and diltiazem concentrations. All data before withdrawals or discontinuations were included in the analysis. All statistical analyses were performed in R 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

Study Highlights

What is the current knowledge on the topic?

Drug-induced hERG block and QTc prolongation are associated with torsade de pointes. However, there are QTc prolonging drugs that have low torsade risk because they have balanced block of inward currents (late sodium or L-type calcium) in addition to hERG.

What question did this study address?

Can concentration-response analysis of the J-Tpeakc interval be used in small sample size studies to confirm that balanced ion channel-blocking drugs do not prolong J-Tpeakc?

What does this study add to our knowledge?

A lack of J-Tpeakc prolongation can confirm balanced ion channel-block, however small sample size studies (10 subjects per drug) may be insufficient to characterize concentration-response relationships in some cases.

How might this change clinical pharmacology or translational science?

A comprehensive non-clinical CiPA assessment combined with assessment of the J-Tpeakc interval may be able to inform the intensity of ECG monitoring in phase 3 trials and drug labeling for mild-to-moderate QTc prolonging drugs.

Acknowledgements

This work was supported by the FDA Safety Research Interest Group and Critical Path Initiative in the Center for Drug Evaluation and Research, the FDA's Office of Women's Health, and by a research fellowship from the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the FDA. We would like to thank staff at Spaulding Clinical Research for executing the clinical study (NCT03070470).

Authors contributions

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Figures legends

Figure 1: Part 1 PK time profiles

Figure 1: Mean (dots) and standard error (error bars) of pharmacokinetic plasma concentration profiles for ranolazine, verapamil, lopinavir, ritonavir, and chloroquine per timepoint after first dose on day 1. Dashed vertical lines show the time of active treatment doses for ranolazine (1,500 mg after breakfast and in the evening), verapamil (120 mg immediate release after breakfast and in the afternoon, 240 mg extended release in the evening), lopinavir/ritonavir (lopinavir 800 mg/ritonavir 200 mg after breakfast and in the evening), and chloroquine (1,000 mg Day 1, 500 mg Day 2, and 1,000 mg Day 3, all before breakfast). Oral placebo was administered at dosing timepoints that had no active treatment dosing as well as throughout all the dosing timepoints in the placebo treatment arm (not shown).

Figure 2: Part 1 PD time profiles

Figure 2: Drug-induced changes (mean \pm 90% confidence interval) for the placebo-corrected change from baseline ($\Delta\Delta$) of QTc (black) and J-Tpeakc (orange) for (a) ranolazine, (b) verapamil, (c) lopinavir/ritonavir and (d) chloroquine. Horizontal dashed line corresponds with 0 ms. The y-axis range of each panel has been adjusted to enhance interpretation. See ECG analysis report in the online supplement for plots with full free-scale y-axis range including Tpeak-Tend.

Figure 3: Part 1 concentration-response plots

Figure 3: Predicted drug-induced placebo-corrected changes from baseline ($\Delta\Delta$) using concentration-response models for QTc (black) and J-Tpeakc (orange) for (a) ranolazine, (b) verapamil, (c) lopinavir/ritonavir and (d) chloroquine. The solid line with gray shaded area denotes the model-predicted mean placebo-adjusted $\Delta\Delta$ with 90% confidence interval (CI) as a function of concentration. Horizontal solid line with tick marks show the range of plasma concentrations divided into deciles. Vertical error bars denote the observed means and 90% CI for the $\Delta\Delta$ within each plasma concentration decile. Vertical dashed lines correspond with population average maximum concentration (Cmax) on days 1 (low Cmax) and 3 (high Cmax). Horizontal dashed and dotted lines correspond with 0 ms and 10 ms $\Delta\Delta$, respectively. The y-axis range of each panel has been adjusted to enhance interpretation. See ECG analysis report in the online supplement for plots with full free-scale y-axis range as well as other ECG measurements.

Figure 4: Part 2 PK time profiles

Figure 4: Mean (dots) and standard error (error bars) of pharmacokinetic plasma concentration profiles per timepoint after first dose on day 1 for dofetilide by itself (top panel) and for diltiazem + dofetilide combination (bottom panel). Bottom panel shows diltiazem concentration (right Y axis, green solid lines) and dofetilide (right Y axis, black dashed line). Dosing times were in the morning (after breakfast) and in the evening of days 1 and 2, and after breakfast in the morning of day 3. Dashed vertical lines show the time of active treatment doses for dofetilide (0.125 mg and 0.375 mg after breakfast on days 1 and 3, respectively, of dofetilide alone period, and 0.25 mg after breakfast on day 3 in the diltiazem + dofetilide period) and diltiazem (120 mg immediate release after breakfast on days 1 and 3, and 240 mg in the evening of days 1 and 2). Oral placebo was administered at dosing timepoints matching part 1 and that had no active treatment dosing (not shown).

Figure 5: Part 2 PD time-profiles and concentration-response linearity plots

Figure 5: Top row panels show drug-induced changes (mean \pm 90% confidence interval) for the change from baseline (Δ) of (a) QTc, (b) J-Tpeakc and (c) Tpeak-Tend on day 3 of dofetilide alone (black) and diltiazem + dofetilide (orange) treatments. The y-axis range of each panel has been adjusted to enhanced interpretation. Bottom row panels show exploratory plots with linear regression fit through all the data for dofetilide alone (black) and diltiazem + dofetilide (orange) for drug-induced changes from baseline (Δ) in (a) QTc, (b) J-Tpeakc, and (c) Tpeak-Tend. Note that there is no change in Δ QTc prolongation associated with dofetilide (hERG block) when diltiazem (calcium block) is coadministered. See online supplement for full time profiles as well as concentration-response plots of dofetilide (hERG block) vs. dofetilide + diltiazem (hERG + calcium) vs. dofetilide + mexiletine (hERG + late sodium).

Figure 6: $\Delta\Delta Q_{Tc}$ and $\Delta\Delta J\text{-Tpeakc}$ concentration-response for several predominant hERG and balanced ion channel blocking drugs

Figure 6: As concentration of drug increases, balanced blockers prolong $\Delta\Delta Q_{Tc}$ (top left) without prolonging $\Delta\Delta J\text{-Tpeakc}$ (bottom left), but predominant hERG blockers prolong both $\Delta\Delta Q_{Tc}$ (top right) and $\Delta\Delta J\text{-Tpeakc}$ (bottom right). Plots show mean (color lines) and 90% confidence intervals (shade areas) predictions for drug-induced baseline- and placebo-corrected changes in Q_{Tc} ($\Delta\Delta Q_{Tc}$, y axis, top panels) and $\Delta\Delta J\text{-Tpeakc}$ (y axis, bottom panels) vs. drug concentration (x axis) from concentration-response models. Before plotting, each drug concentration was normalized to the concentration that caused 20 ms $\Delta\Delta Q_{Tc}$ prolongation in its corresponding study. Balanced blockers shown include ranolazine (hERG + late sodium, blue), and verapamil (hERG + calcium, green). Predominant hERG blockers shown are dofetilide (gray), moxifloxacin (dark orange), and quinidine (light orange). Data from this study for multiple dosing of ranolazine (ranolazine-2) and verapamil (solid lines) and from two prior studies with single oral dose design (ranolazine-1, dofetilide-1, and quinidine from Johannesen et. al 2014,¹⁵ dashed lines; dofetilide-2 and moxifloxacin from Johannesen et al. 2016,¹⁷ dotted lines). Chloroquine and lopinavir/ritonavir not shown because because poor fit of concentration-response linear models did not allow for reliable predictions of $\Delta\Delta J\text{-Tpeakc}$ (chloroquine) and $\Delta\Delta Q_{Tc}$ (lopinavir/ritonavir). Black horizontal lines show 0 ms (dashed) and 10 ms (dotted) thresholds for Q_{Tc} (top panels) and $\Delta\Delta J\text{-Tpeakc}$ (bottom panels). Ranges of X and Y axes adjusted to to facilitate comparison.

Supplementary Materials

- HTML with Analysis Code
- Supplemental Material

Table 1: Part 1 drug-induced ECG predicted changes

Drug	Day	Cmax	$\Delta\Delta\text{QTc}$	$\Delta\Delta\text{J-Tpeakc}$	$\Delta\Delta\text{Tpeak-Tend}$	$\Delta\Delta\text{PR}$	$\Delta\Delta\text{QRS}$
Ranolazine	1	1191.2 ng/mL	14.1 [7.5 to 20.8] ms	2.2 [-4.3 to 8.7] ms	11.9 [5.9 to 17.9] ms	0.7 [-2.5 to 3.8] ms	-0.5 [-3.3 to 2.3] ms
	3	3046.3 ng/mL	28.2 [18.3 to 38.2] ms	1.2 [-9.5 to 12.0] ms	25.7 [14.7 to 36.7] ms	3.1 [-0.8 to 6.9] ms	-0.2 [-3.5 to 3.0] ms
Verapamil	1	101.0 ng/mL	8.6 [4.4 to 12.9] ms	3.6 [-2.4 to 9.7] ms	4.4 [0.6 to 8.2] ms	13.2 [7.2 to 19.2] ms	0.3 [-2.0 to 2.7] ms
	3	398.4 ng/mL	13.9 [10.0 to 17.8] ms	-2.6 [-11.4 to 6.1] ms	14.9 [9.1 to 20.6] ms	52.2 [27.9 to 76.6] ms**	1.8 [-1.7 to 5.4] ms
Lopinavir	1	10734.2 ng/mL	*	1.2 [-5.4 to 7.8] ms	*	14.8 [10.3 to 19.4] ms	*
	3	24351.5 ng/mL	*	-2.9 [-14.6 to 8.8] ms	*	33.5 [22.7 to 44.4] ms***	
Chloroquine	1	228.8 ng/mL	30.7 [22.6 to 38.9] ms	*	13.1 [8.4 to 17.9] ms	9.3 [2.8 to 15.8] ms	5.5 [3.0 to 8.0] ms
	3	404.9 ng/mL	50.5 [38.5 to 62.5] ms	*	24.9 [17.2 to 32.7] ms	14.8 [4.7 to 24.9] ms	9.9 [6.1 to 13.6] ms

Drug-induced placebo-corrected changes from baseline for QTc, J-Tpeakc, Tpeak-Tend, PR and QRS intervals at Cmax of days 1 and 3 for each treatment in Part 1. *: Values not reported because poor fit of concentration-response linear models did not allow for reliable predictions. **: Goodness of fit plots suggest linear models may overestimate $\Delta\Delta\text{PR}$ effects around Cmax (see text and online supplement for comparison with E-max and sigmoid models).











