

Differentiating Drug-Induced Multichannel Block on the Electrocardiogram: Randomized Study of Dofetilide, Quinidine, Ranolazine, and Verapamil

L Johannesen^{1,2}, J Vicente^{1,3}, JW Mason⁴, C Sanabria⁴, K Waite-Labott⁴, M Hong⁵, P Guo⁵, J Lin⁵, JS Sørensen⁶, L Galeotti¹, J Florian⁶, M Ugander^{1,2}, N Stockbridge⁷ and DG Strauss^{1,2}

Block of the hERG potassium channel and prolongation of the QT interval are predictors of drug-induced torsade de pointes. However, drugs that block the hERG potassium channel may also block other channels that mitigate torsade risk. We hypothesized that the electrocardiogram can differentiate the effects of multichannel drug block by separate analysis of early repolarization (global J–T_{peak}) and late repolarization (global T_{peak}–T_{end}). In this prospective randomized controlled clinical trial, 22 subjects received a pure hERG potassium channel blocker (dofetilide) and three drugs that block hERG and either calcium or late sodium currents (quinidine, ranolazine, and verapamil). The results show that hERG potassium channel block equally prolongs early and late repolarization, whereas additional inward current block (calcium or late sodium) preferentially shortens early repolarization. Characterization of multichannel drug effects on human cardiac repolarization is possible and may improve the utility of the electrocardiogram in the assessment of drug-related cardiac electrophysiology.

Fourteen drugs have been removed from the market worldwide because they increase the risk for torsade de pointes,¹ a ventricular arrhythmia that can cause sudden cardiac death. Drugs that increase the risk for torsade can be identified by assessing whether they block the human ether-à-go-go-related gene (hERG) potassium channel (an outward current) and prolong the QT interval on the electrocardiogram (ECG).^{1,2} In response, the US Food and Drug Administration (FDA) requires almost all new drugs to undergo “thorough QT” studies.³ A positive thorough QT study does not preclude a drug from regulatory approval but almost always increases the required cardiac safety evaluations during later stages of drug development.^{3,4} Because of the increased risk and cost of developing drugs that block the hERG potassium channel and/or prolong QT, some of these compounds are dropped from development, sometimes inappropriately.¹

Some drugs block the hERG potassium channel and prolong QT with minimal torsade risk because they also block calcium

and/or sodium channels (inward currents). The most notable example is amiodarone, which causes substantial QT prolongation but has a low risk of torsade.⁵ This is likely because blocking inward currents can prevent early afterdepolarizations, which trigger torsade.^{6–8}

Previous studies have focused on capturing changes in T-wave morphology,^{9–11} but so far these efforts have been focused primarily on detecting the presence of hERG potassium channel block.¹² Using data from 34 thorough QT studies, we demonstrated previously that multichannel block can be detected on the ECG and that not all QT prolongation is equal.¹³ That analysis suggested that hERG potassium channel block prolongs both early repolarization (J–T_{peak}: end of QRS to global peak of T-wave) and late repolarization (T_{peak}–T_{end}: global peak to end of T-wave), whereas calcium and late sodium current block preferentially shorten early repolarization (Figure 1). The preferential effect of calcium and late sodium currents on early repolarization is

¹Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, US Food and Drug Administration, Silver Spring, Maryland, USA;

²Department of Clinical Physiology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; ³BSCoS Group, Aragón Institute of Engineering Research (I3A), IIS Aragón, University of Zaragoza, Zaragoza, Aragón, Spain; ⁴Spaulding Clinical Research, West Bend, Wisconsin, USA; ⁵Frontage Laboratories, Exton, Pennsylvania, USA; ⁶Division of Pharmacometrics, Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, USA; ⁷Division of Cardiovascular and Renal Products, Office of New Drugs, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, USA. Correspondence: DG Strauss (david.strauss@fda.hhs.gov)

Received 5 May 2014; accepted 9 July 2014; advance online publication 17 September 2014. doi:10.1038/clpt.2014.155

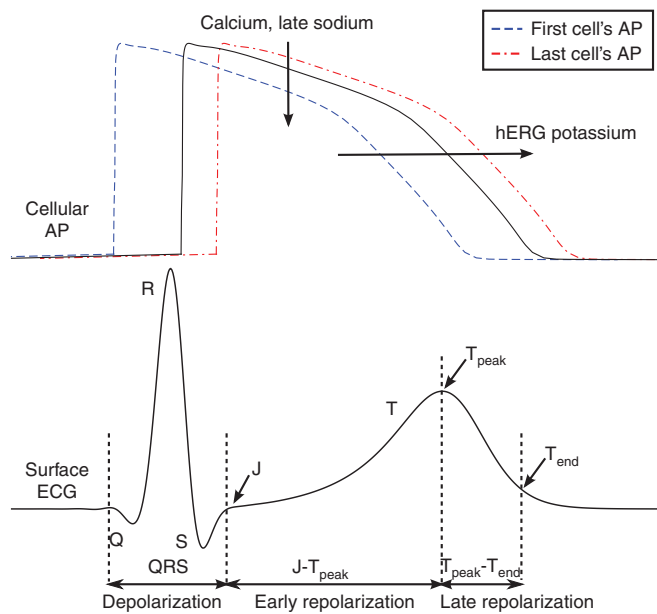


Figure 1 An illustration of a ventricular action potential (AP) and the corresponding surface electrocardiogram (ECG). Arrows pointing into the action potential are inward currents (calcium and late sodium) and arrows pointing out denote outward currents (human ether-à-go-go-related gene (hERG) potassium). Blocking the calcium or late sodium current primarily shortens the early parts of repolarization ($J-T_{peak}$), whereas hERG potassium channel block prolongs both early ($J-T_{peak}$) and late repolarization ($T_{peak}-T_{end}$).

consistent with these inward currents being active during the early repolarization phase of the action potential.¹³ However, the prior study¹³ was limited by the fact that *in vitro* ion channel data were not available for all drugs and the risk of torsade de pointes was not known for the drugs studied.

Therefore, we designed a prospective randomized controlled clinical trial, funded by the FDA's Critical Path Initiative, to assess the ECG effects of multiple marketed drugs that either block the hERG potassium channel alone or do so while also blocking calcium and sodium channels. The selected drugs included four strong hERG potassium channel blockers with varying degrees of sodium and calcium channel block: dofetilide, quinidine, ranolazine, and verapamil.

Dofetilide is a strong pure hERG potassium channel blocker¹⁴ with a high torsade risk.¹⁵ The second drug is quinidine, which is also a strong hERG potassium channel blocker, but in addition to blocking the hERG potassium channel it also blocks calcium and sodium channels at high concentrations,¹⁴ and torsade has been observed to occur more frequently at lower plasma quinidine concentrations.¹⁶⁻¹⁸ The last two drugs, ranolazine and verapamil, both block not only the hERG potassium channel but also the late sodium current (ranolazine) or L-type calcium channel (verapamil), likely explaining why they are both associated with a low risk of torsade.^{8,19}

Administration of these four drugs to the same subjects enables the characterization of ECG signatures of pure hERG potassium channel block as compared with multichannel block. We

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ The QT interval is a sensitive biomarker of drug-induced hERG potassium channel block and torsade de pointes risk; however, it is not specific. Characterization of the effects of additional inward current block (calcium or late sodium) on cardiac repolarization may improve risk assessment because inward current block can offset the proarrhythmic effects of hERG potassium channel block.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ This study tested the hypothesis that hERG potassium channel block prolongs both $J-T_{peak}$ (early repolarization) and $T_{peak}-T_{end}$ (late repolarization) intervals on the ECG, whereas the addition of calcium or late sodium current block preferentially shortens $J-T_{peak}$.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ This prospective clinical study demonstrated that pure hERG potassium channel block equally prolongs both $J-T_{peak}$ and $T_{peak}-T_{end}$, whereas additional inward current block (calcium or late sodium) preferentially shortens $J-T_{peak}$.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ Characterization of multichannel drug effects on human ECGs is possible and may change cardiac safety assessment by confirming comprehensive preclinical ion channel assessments and influence dosing strategies for drugs.

Table 1 Baseline characteristics

	All subjects (N = 22)
Demographic	
Age (years)	26.9 ± 5.5
Female	11 (50%)
Body mass index (kg/m ²)	23.1 ± 2.6
Vital signs	
Systolic blood pressure (mm Hg)	107.1 ± 8.5
Diastolic blood pressure (mm Hg)	59.7 ± 7.2
Heart rate (bpm)	56.8 ± 6.4
ECG	
PR interval (ms)	162.1 ± 21.6
QRS duration (ms)	97.4 ± 6.7
$J-T_{peak}$ (ms)	225.6 ± 19.8
$T_{peak}-T_{end}$ (ms)	73.1 ± 6.4
QTc (ms)	395.9 ± 17.1

Continuous variables are represented as mean ± SD. ECG, electrocardiogram.

hypothesized that hERG potassium channel block prolongs both the $J-T_{peak}$ and $T_{peak}-T_{end}$ intervals, whereas the addition of calcium or late sodium current block preferentially shortens the $J-T_{peak}$ interval.

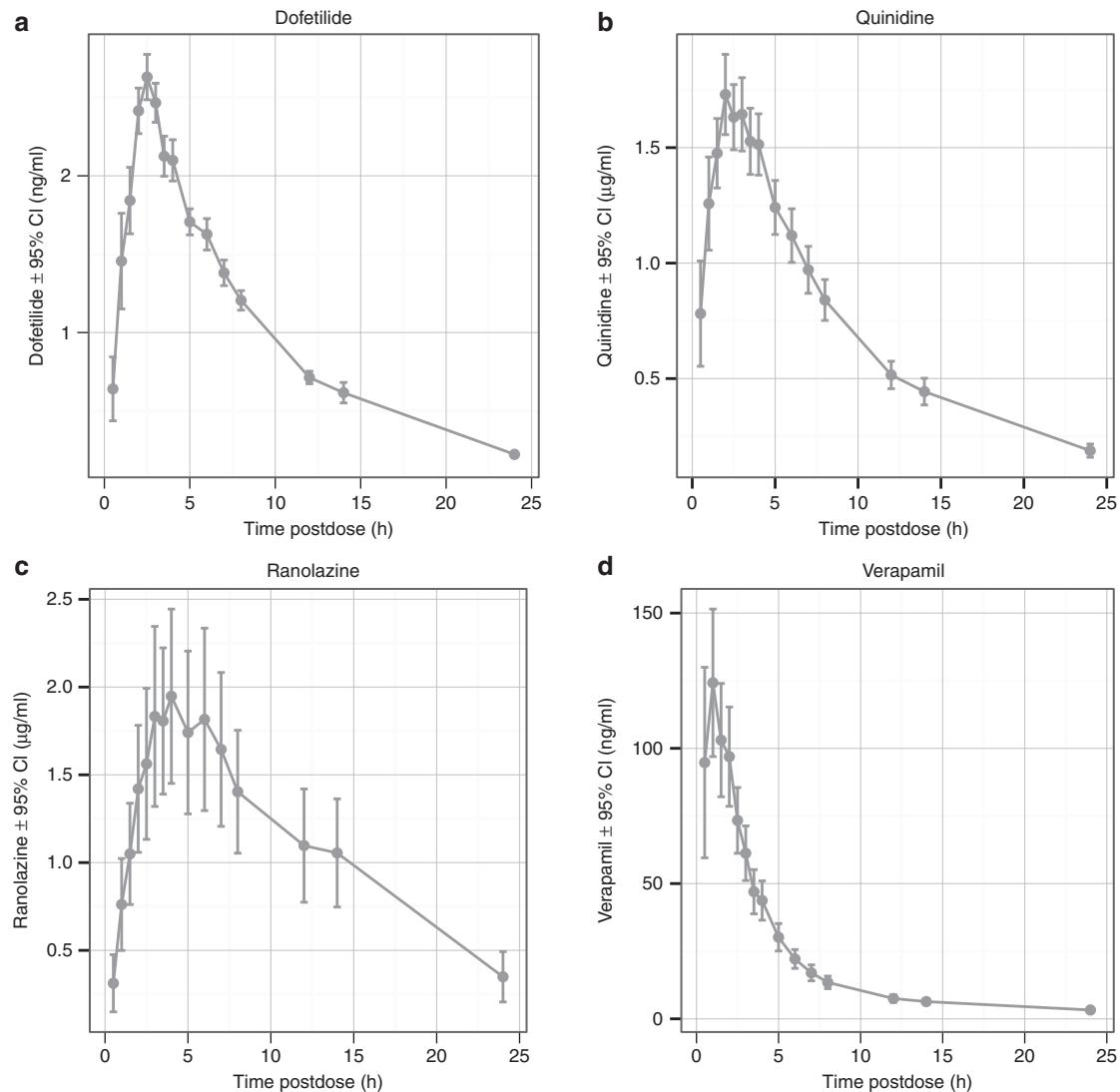


Figure 2 Measured plasma concentrations (mean \pm 95% confidence interval) for (a) dofetilide, (b) quinidine, (c) ranolazine, and (d) verapamil.

RESULTS

Twenty-two healthy subjects (11 females) participated in this randomized controlled clinical trial with a mean age of 26.9 ± 5.5 years and a mean body mass index of 23.1 ± 2.6 kg/m²; see [Table 1](#) for baseline characteristics. All completed the study, except one subject who withdrew prior to the last treatment period. There were no unexpected treatment-related adverse events.

Pharmacokinetic analysis

The results of the pharmacokinetic analysis are shown in [Figure 2](#) for each drug: dofetilide (a), quinidine (b), ranolazine (c), and verapamil (d). Dofetilide and quinidine exhibited similar pharmacokinetic profiles, with maximum concentration occurring at 2.5 h (range: 1–4.0 h) for dofetilide and at 2.0 h (0.5–4.0 h) for quinidine, and with similar half-lives: (mean \pm SD) 7.2 ± 1.1 h (dofetilide) and 7.8 ± 1.5 h (quinidine). The maximum concentrations for dofetilide and quinidine were 2.7 ± 0.3 ng/ml and 1.8 ± 0.4 μ g/ml, respectively. Ranolazine peaked later at 4.0 h

(1.0–14 h), with a concentration of 2.3 ± 1.4 μ g/ml, and had a half-life of 7.5 ± 4.0 h. Finally, verapamil peaked at 1.0 h (0.5–2.0 h), with a plasma concentration of 130.3 ± 75.8 ng/ml, and had a half-life of 10.4 ± 3.2 h.

Dofetilide: Pure hERG potassium channel block prolongs early and late repolarization equally

Dofetilide prolonged the heart rate–corrected global QT (QTc) interval by 79.3 ms (95% confidence interval (CI): 72.2–86.3 ms, $P < 0.001$; [Figure 3a](#)), with equal prolongation of the heart rate–corrected $J-T_{\text{peak}}$ ($J-T_{\text{peak}}^{\text{c}}$: 39.5 (32.8–46.2) ms, $P < 0.001$) and $T_{\text{peak}}-T_{\text{end}}$ (40.0 (33.0–46.9) ms, $P < 0.001$). Similarly, the concentration-dependent analysis showed that the QTc prolongation by dofetilide equally affected $J-T_{\text{peak}}^{\text{c}}$ and $T_{\text{peak}}-T_{\text{end}}$ ($J-T_{\text{peak}}^{\text{c}}$: 14.1 (11.0–17.3) ms per ng/ml, $P < 0.001$; $T_{\text{peak}}-T_{\text{end}}$: 14.5 (11.0–17.9) ms per ng/ml, $P < 0.001$; $P = 0.89$ for $J-T_{\text{peak}}^{\text{c}}$ vs. $T_{\text{peak}}-T_{\text{end}}$; [Figure 4a](#)). These findings support the notion that hERG potassium channel block prolongs both $J-T_{\text{peak}}^{\text{c}}$ and $T_{\text{peak}}-T_{\text{end}}$.

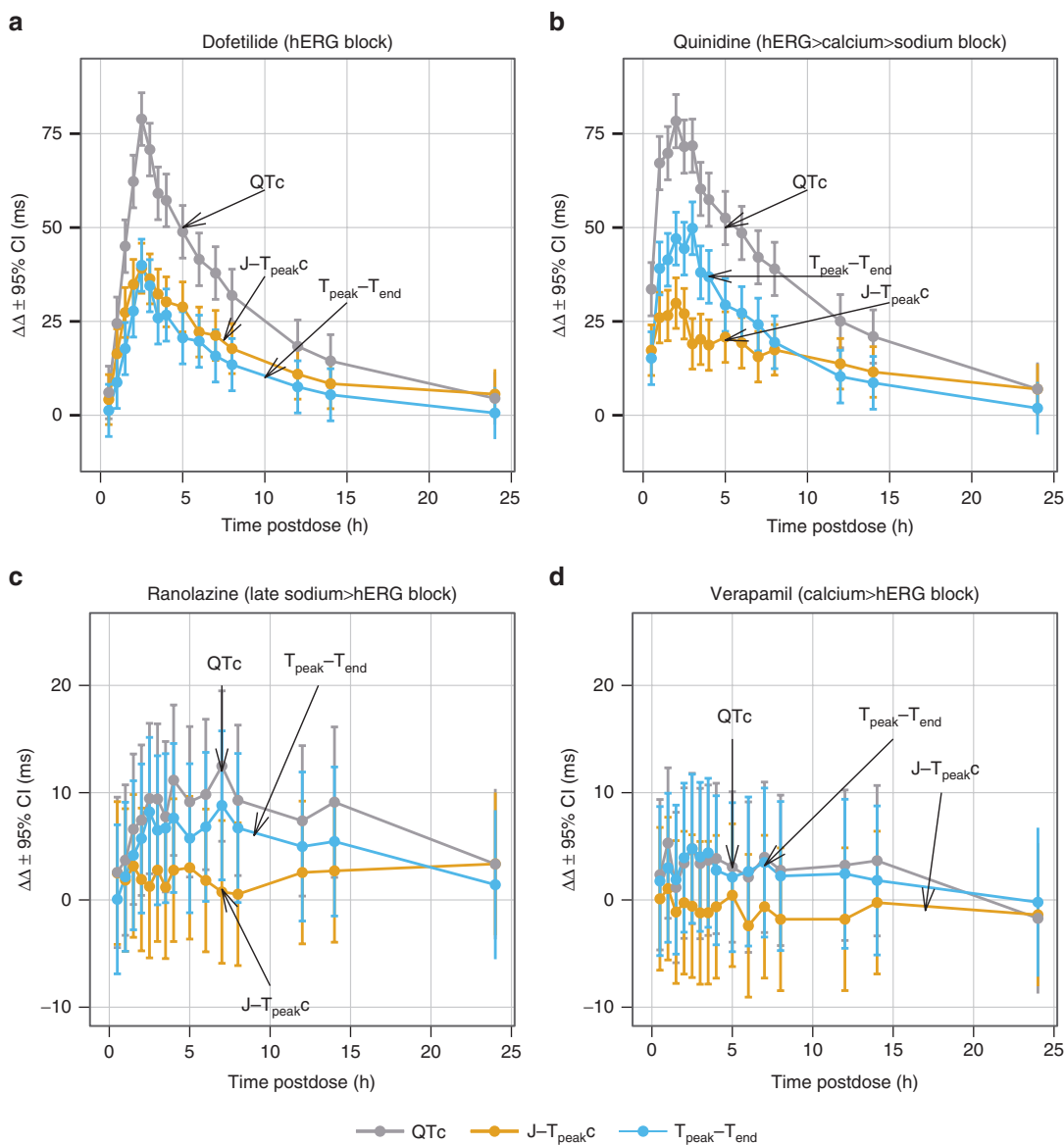


Figure 3 Drug-induced changes (mean \pm 95% confidence interval) for the placebo-corrected change from baseline ($\Delta\Delta$) of QTc (gray), J-T_{peakc} (orange), and T_{peak}-T_{end} (blue) for (a) dofetilide, (b) quinidine, (c) ranolazine, and (d) verapamil. The y-axis in each row of panels has been scaled to enhance interpretation.

Quinidine: strong hERG potassium channel block with additional calcium and sodium channel block prolongs late more than early repolarization

Similar to dofetilide, quinidine prolonged the global QTc by 78.1 ms (70.9–85.2 ms, $P < 0.001$; **Figure 3b**); however, quinidine was more strongly associated with prolongation of T_{peak}-T_{end} than of J-T_{peakc} (J-T_{peakc}: 29.1 (22.4–35.9) ms, $P < 0.001$; T_{peak}-T_{end}: 49.8 (42.8–56.8) ms, $P < 0.001$; **Figure 3b**). Similarly, the concentration-dependent analysis showed a stronger relationship for T_{peak}-T_{end} as compared with J-T_{peakc} (J-T_{peakc}: 11.6 (3.3–19.8) ms per $\mu\text{g/ml}$, $P = 0.008$; T_{peak}-T_{end}: 29.9 (19.2–40.7) ms per $\mu\text{g/ml}$; $P < 0.001$; $P = 0.025$ for J-T_{peakc} vs. T_{peak}-T_{end}; **Figure 4b**). The preferential prolongation of T_{peak}-T_{end} associated with quinidine is likely due to calcium channel block

reducing the J-T_{peakc} prolongation associated with hERG potassium channel block.

Ranolazine: late sodium current block opposes hERG effects on early repolarization

The global QTc prolongation associated with ranolazine was 12.6 ms (5.5–19.6 ms, $P < 0.001$; **Figure 3c**), primarily via prolonged T_{peak}-T_{end} (J-T_{peakc}: 3.3 (-3.4 to 9.9) ms, $P = 0.34$; T_{peak}-T_{end}: 8.8 (1.9–15.8) ms, $P = 0.013$; **Figure 3c**). A similar observation was seen in the concentration-dependent analysis, in which ranolazine concentrations were associated with an increase in T_{peak}-T_{end} (4.4 (2.6–6.2) ms per $\mu\text{g/ml}$, $P < 0.001$; **Figure 4c**); however, there was no association with J-T_{peakc} ($P = 0.42$) ($P < 0.001$ for J-T_{peakc} vs. T_{peak}-T_{end}; **Figure 4c**).

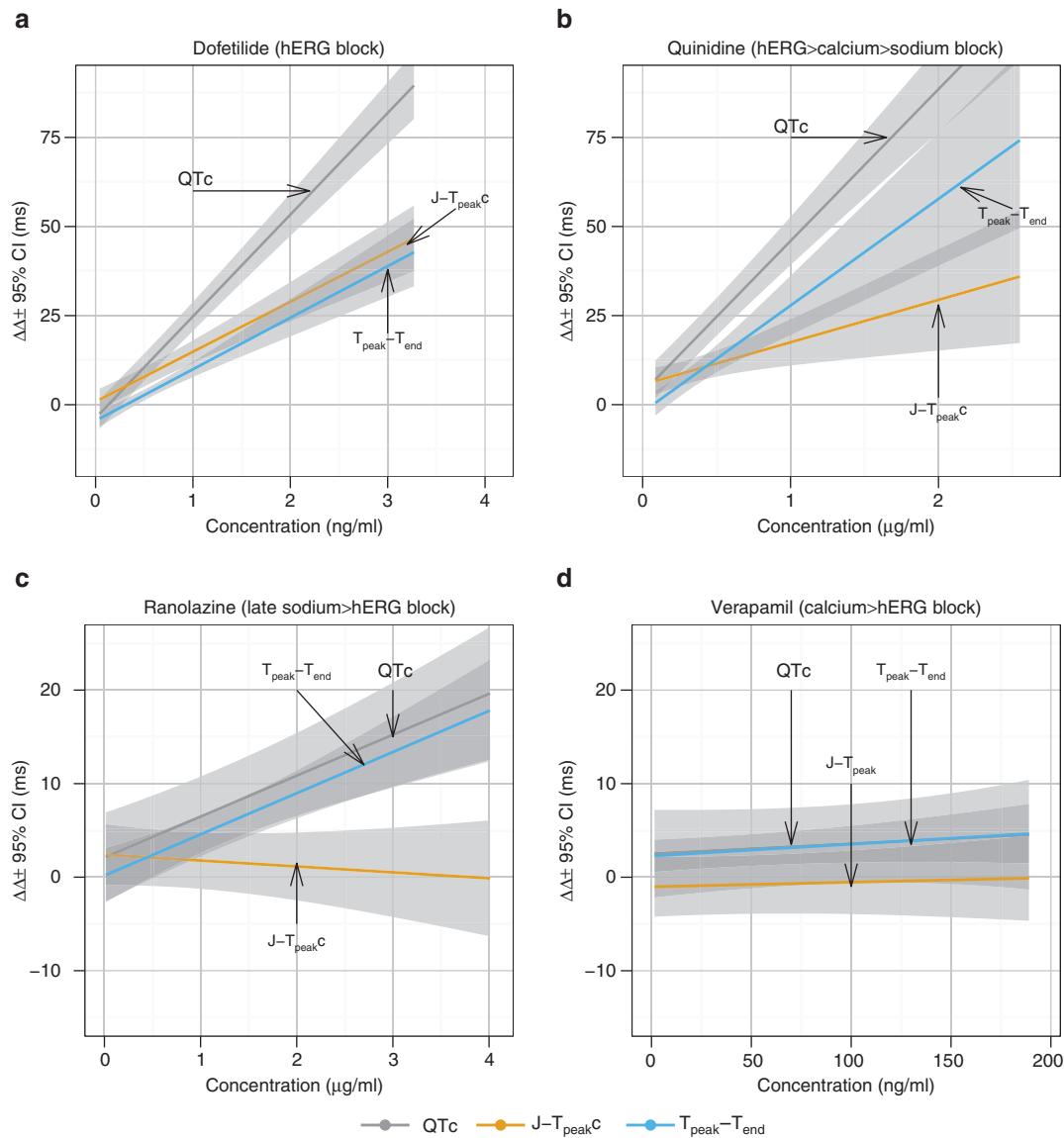


Figure 4 Drug-induced changes (mean ± 95% confidence interval) for the placebo-corrected change from baseline (ΔΔ) for QTc (gray), J-T_{peakc} (orange), and T_{peak}-T_{end} (blue) from model predictions vs. plasma concentrations for (a) dofetilide, (b) quinidine, (c) ranolazine, and (d) verapamil. For clarity, the observed data are not shown in this figure but are included in **Supplementary Figure S1** online. The range of the x-axis is 1.5 times the observed maximum concentration. The y-axis in each row of panels has been scaled to enhance interpretation. In each plot, the line represents the predicted mean effect of the linear model and the shaded area represents 95% confidence intervals.

These findings suggest that late sodium current block primarily reduces the J-T_{peakc} prolongation associated with hERG potassium channel block.

Verapamil: strong calcium block opposes effects of hERG block on early and late repolarization

Verapamil did not cause a significant change in global QTc (5.2 (-1.8-12.2) ms, *P* = 0.15; **Figure 2d**), J-T_{peakc} (-2.4 (-9.0-4.3) ms, *P* = 0.49; **Figure 2d**), or T_{peak}-T_{end} (4.8 (-2.2-11.7) ms, *P* = 0.18; **Figure 3d**). Similar observations were made in the concentration-dependent analysis (**Figure 3d**). The lack of prolongation of J-T_{peakc} and T_{peak}-T_{end} with verapamil suggests that strong calcium block may attenuate the effects of hERG potassium channel block on both J-T_{peakc} and T_{peak}-T_{end}.

Effect of drugs on PR, QRS, and heart rate

The PR interval is most commonly prolonged by slowing conduction through the atrioventricular node, where primarily calcium current regulates depolarization.²⁰ Verapamil, a strong calcium channel blocker, was related to PR prolongation (32.1 (26.7-37.4) ms, *P* < 0.001), which was concentration dependent (0.2 (0.2-0.3) ms per ng/ml, *P* < 0.001). Quinidine, which blocks the calcium channel,¹⁴ trended toward PR prolongation (5.1 (-0.3-10.5) ms, *P* = 0.065); however, there was no concentration dependence (*P* = 0.91). The lack of PR prolongation observed in this study with quinidine despite its calcium channel block is likely because of competing autonomic effects on atrioventricular conduction.²¹ Consistent with minimal calcium channel block, only a small amount of PR prolongation was observed

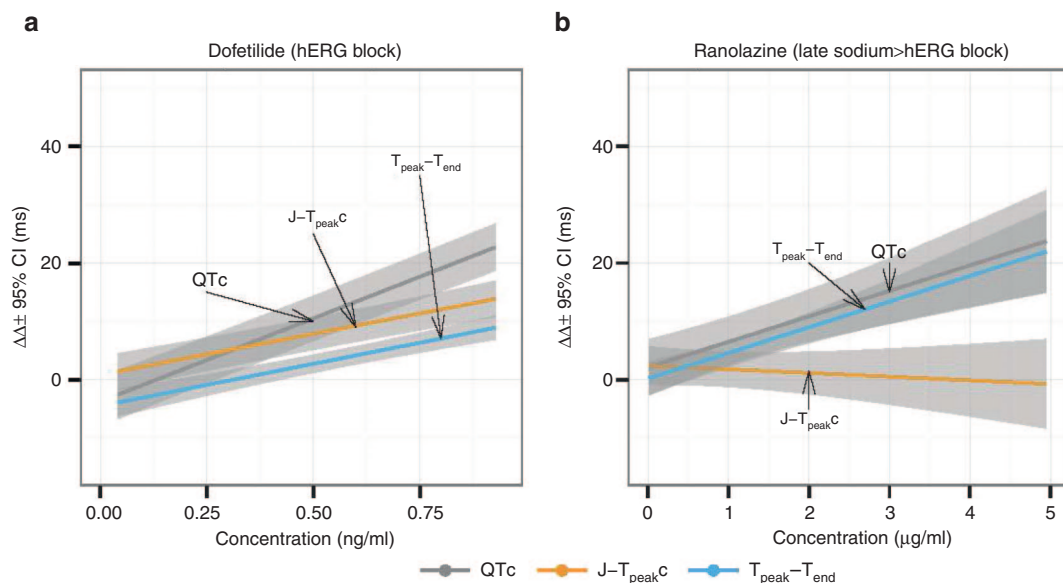


Figure 5 Drug-induced changes in $J-T_{peakc}$ and $T_{peak}-T_{end}$ for (a) a pure hERG potassium channel blocker (dofetilide) and (b) a hERG + late sodium current blocker (ranolazine). This zoomed plot of the concentrations of dofetilide that produce an amount of QTc prolongation comparable to that of ranolazine shows the ability of $J-T_{peakc}$ and $T_{peak}-T_{end}$ to detect multichannel block. hERG, human ether-à-go-go-related gene.

for ranolazine (6.5 (1.1–11.8) ms, $P = 0.018$ and 1.1 (0.1–2.0) ms per $\mu\text{g/ml}$, $P = 0.033$).¹⁹ No PR prolongation was observed for dofetilide, consistent with a lack of calcium channel block.¹⁴

Blocking the sodium current increases QRS duration by slowing ventricular conduction.²² Quinidine is a sodium channel blocker, but at the concentrations observed in this study, quinidine is expected to block the sodium current only minimally.¹⁴ Quinidine only trended toward QRS prolongation (2.1 (–0.2 to 4.3) ms, $P = 0.071$), and there was no correlation between plasma levels and QRS increase ($P = 0.95$). Ranolazine is also a sodium channel blocker, but it has weak peak sodium current block,¹⁹ and was associated with only a slight QRS prolongation (2.7 (0.5–4.9) ms, $P = 0.018$); however, there was no correlation between plasma levels and QRS ($P = 0.060$). Neither dofetilide nor verapamil is associated with sodium channel block.¹⁴ Dofetilide produced no QRS prolongation, and verapamil produced a slight QRS prolongation (2.6 (0.4–4.8) ms, $P = 0.020$); however, there was no positive relationship between concentration and QRS ($P = 0.39$).

Finally, verapamil and quinidine caused an increase in heart rate of 9.4 bpm ((6.4–12.4), $P < 0.001$) and 9.8 bpm ((6.8–12.9), $P < 0.001$), respectively. In both cases, there was a relationship between plasma concentration and heart rate (verapamil: 0.07 (0.05–0.09) bpm per ng/ml, $P < 0.001$; quinidine: 4.7 (3.4–6.1) bpm per $\mu\text{g/ml}$, $P < 0.001$). Dofetilide did not cause any change in heart rate ($P = 0.12$), and ranolazine increased heart rate slightly (4.2 (1.2–7.2) bpm, $P = 0.007$); however, there was no relationship between concentration and heart rate ($P = 0.22$).

DISCUSSION

This prospective randomized controlled clinical trial demonstrated that by separating the QT interval into early

repolarization (global $J-T_{peakc}$) and late repolarization (global $T_{peak}-T_{end}$), a pure hERG potassium channel-blocking drug with high torsade risk can be differentiated from multichannel-blocking drugs that also block inward currents (calcium or late sodium) during repolarization. Detecting additional calcium or late sodium current block is of importance because both the calcium and sodium current support early afterdepolarizations, which can trigger torsade de pointes.^{6–8} Thus, being able to detect multichannel effects using the ECG may have implications for the future of cardiac safety evaluation of drugs and clinical dosing strategies.

The results of this study demonstrate that pure hERG potassium channel block (dofetilide) equally prolongs $J-T_{peakc}$ and $T_{peak}-T_{end}$, whereas additional calcium and late sodium current block (quinidine, ranolazine, and verapamil) preferentially shorten $J-T_{peakc}$. Of note, the pure hERG potassium channel blocker dofetilide and the multichannel blocker quinidine caused equal QTc prolongation. Despite the comparable QTc prolongation, dofetilide and quinidine were associated with different effects on $J-T_{peakc}$ and $T_{peak}-T_{end}$, suggesting that the QTc interval cannot differentiate pure hERG potassium channel block from multichannel block. As an example of this, **Figure 5** shows a comparison between concentrations of dofetilide and ranolazine that produce equal amounts of QTc prolongation. From this figure, it is clear that the QTc interval cannot differentiate multichannel block, whereas evaluation of $J-T_{peakc}$ and $T_{peak}-T_{end}$ intervals provides insight into multichannel block that is of relevance for cardiac safety evaluation. These findings suggest that future studies should consider reporting the ratio of the changes in $J-T_{peakc}$ and $T_{peak}-T_{end}$.

It is notable that dofetilide and quinidine caused substantial QTc prolongation (~78 ms) in the present study. These results are consistent with those from prior single-dose studies of dofetilide

and quinidine. Coz *et al.*²³ reported a slope of dofetilide concentration vs. QTc of 31 ms per ng/ml, compared with 29 ms per ng/ml in our study. Benton *et al.*²⁴ reported a slope of quinidine concentration vs. QTc of 29 ms per µg/ml in men and 42 ms per µg/ml in women, compared with 43 ms per µg/ml in our study. However, a separate study of dofetilide 750 µg twice daily for 4 days only observed a QTc prolongation of ~60 ms.²⁵ This could be due to differences in measurement of the end of the T-wave, differences in study design, or decreased sensitivity to dofetilide over time as reported in the dofetilide label.²⁶

The lack of $T_{\text{peak}}-T_{\text{end}}$ prolongation with verapamil suggests that when calcium channel block is stronger than hERG potassium channel block, it may also attenuate the effect of hERG potassium channel block on $T_{\text{peak}}-T_{\text{end}}$. These findings with verapamil are consistent with those in the rabbit heart, in which changes in QT and $T_{\text{peak}}-T_{\text{end}}$ were not observed at concentrations similar to those observed in this study.²⁷ Previously, calcium channel block has been proposed to be detected by an increase in the PR interval,¹³ which was the case for verapamil in this study but not for quinidine. The reason for the lack of PR prolongation with quinidine is likely due to quinidine's competing autonomic effects on atrioventricular node conduction, as quinidine has been shown to prolong PR in heart transplant patients.²¹ Therefore, PR prolongation is not always present with calcium channel block, and other markers such as $J-T_{\text{peakc}}$ and $T_{\text{peak}}-T_{\text{end}}$ might prove more universally useful to detect the presence of calcium channel block.

Interestingly, these drug-induced ECG signature patterns have been observed previously with electrolyte abnormalities and with genetic abnormalities of ion channels. As early as the 1950s,²⁸ it was observed that hypokalemia prolongs the QT interval and flattens and widens the T-wave. This is in contrast to QT prolongation from hypocalcemia, which causes ST segment lengthening without T-wave changes.²⁸ Hypokalemic QT prolongation has been attributed to increasing the rate at which hERG potassium channels inactivate.^{29,30} Similarly, the rate of inactivation for calcium channels is dependent on calcium concentrations and has been shown to be the reason for hypercalcemia-induced action potential shortening.³¹ Thus, hypokalemia creates the equivalent of hERG potassium channel block, whereas hypercalcemia creates the equivalent of calcium channel block. Similarly, genetic abnormalities in the hERG potassium channel (congenital long QT type 2) cause flattening and widening of the T-wave, as with drug-induced hERG potassium channel block. Genetic abnormalities in the sodium channel (congenital long QT type 3) cause prolongation of the ST segment with a normal T-wave.³²

Therapeutic implications of multiple ion channel effects

With pure hERG potassium channel block, there is likely a direct relationship between increasing plasma drug concentration and torsade risk. For example, with dofetilide, there is a relationship among dose, QTc, and torsade risk.³³ However, with quinidine, torsade has been observed to occur more frequently at lower plasma quinidine concentrations.¹⁶ This has been confirmed in preclinical models in which the number of quinidine-induced

arrhythmias was greater at lower rather than higher quinidine concentrations,^{17,18} which is associated with more calcium and late sodium current block.

The development of torsade requires an electrical trigger and then a substrate to support re-entry.³⁴ Because of the differential expression of ion channels throughout the heart, hERG potassium channel block accentuates dispersion of repolarization, thus creating the substrate for re-entry.³⁵ The trigger for torsade is believed to be early afterdepolarizations, which can occur as a result of increased calcium or sodium current during repolarization.^{6,7} Thus, calcium and late sodium current block can prevent early afterdepolarizations (the trigger for torsade).

The $T_{\text{peak}}-T_{\text{end}}$ interval, as measured in a precordial lead, has been proposed as a measure of transmural dispersion of repolarization.³⁶ The relationship between transmural dispersion and $T_{\text{peak}}-T_{\text{end}}$ is subject to controversy as it has been proposed that $T_{\text{peak}}-T_{\text{end}}$ instead reflects total dispersion and not transmural dispersion.^{37,38} $T_{\text{peak}}-T_{\text{end}}$ has, however, been shown to be prolonged by hERG potassium channel block and to be longer in long QT2 patients (abnormalities in the hERG potassium channel) as compared with long QT1 (abnormalities in the slow potassium channel) and long QT3 (abnormalities in the sodium channel) patients.^{13,39} In this study, we quantified $T_{\text{peak}}-T_{\text{end}}$ globally on the vector magnitude derived lead, which is likely more consistent than measuring $T_{\text{peak}}-T_{\text{end}}$ in a single lead in the presence of complex T-wave patterns.

Many drugs remain on the market with a known torsade risk, including numerous antibiotics and antimalarial, antiviral, psychiatric, oncology, and cardiac drugs.⁴⁰ This raises the question of whether adding a late sodium- or calcium current-blocking drug to a hERG potassium channel-blocking drug could decrease torsade risk. This concept has been evaluated in canine studies, in which mexiletine (late sodium current blocker) decreased the torsade risk associated with sotalol (strong hERG potassium channel blocker).⁴¹ Mexiletine has also been evaluated as a potential "gene-specific" therapy for patients with congenital long QT syndrome. In patients with congenital long QT type 3 (abnormalities in the sodium channel), it has been shown that administering mexiletine shortened their QT intervals.⁴² Similar observations have been made with ranolazine.⁴³ In the mexiletine study, the investigators also evaluated the effects of mexiletine treatment in patients with long QT type 2 (abnormalities in the hERG potassium channel). They observed a numerical decrease in QTc that was not statistically significant; however, only six patients were studied. In addition, mexiletine has been shown to decrease the QTc interval in a subject with Timothy syndrome (abnormalities in the calcium channel).⁴⁴ Furthermore, the potential antiarrhythmic effect of mexiletine was evaluated in clinical studies in which mexiletine was administered in combination with quinidine. When mexiletine was administered on its own, there were no significant changes in QTc, but when mexiletine was administered to patients already receiving quinidine, there was a significant shortening of the quinidine-induced QTc prolongation.^{45,46} These studies suggest that coadministering an inward current blocker with a hERG potassium channel blocker could offset

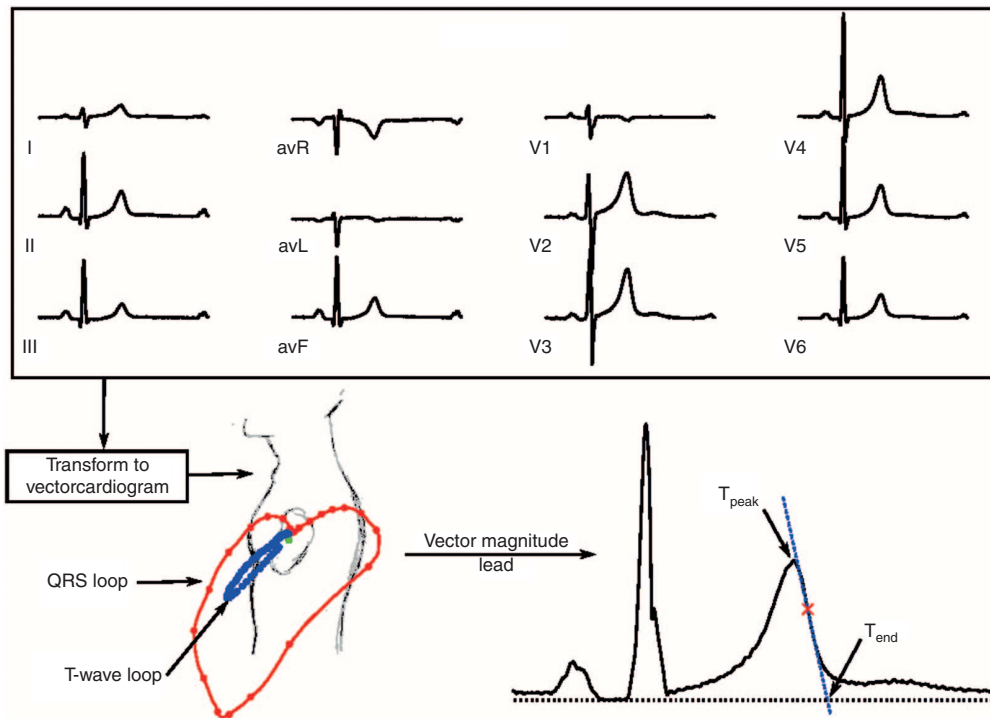


Figure 6 Method for assessment of the global T_{peak} and T_{end} . The 12-lead electrocardiogram is transformed to the vectorcardiogram via a published transformation matrix.⁵⁵ The peak and end of the T-wave are located in the vector magnitude lead. The end of the T-wave is determined using the tangent method,⁵⁶ which involves locating the intersection between the line through the terminal descending part of the T-wave and the isoelectric line.

the hERG potassium channel blocking effects, which should be evaluated in further studies.

Limitations

Two of the drugs (dofetilide and quinidine) are associated with massive QTc increases and T-wave changes, including notching, which can make T_{peak} and T_{end} determination difficult. However, there was strong agreement between two independent assessments of all ECGs and less than 5 ms difference in 98.6% of the ECGs. It should also be recognized that because the QT interval is subject to rate dependency and delayed adaptation to heart rate, it is likely that the $J-T_{peak}$ and $T_{peak}-T_{end}$ intervals are as well. Rate dependency for both the $J-T_{peak}$ and $T_{peak}-T_{end}$ intervals has been established previously, but the effect on $T_{peak}-T_{end}$ at near-resting heart rates is minimal,^{13,47} and thus the $T_{peak}-T_{end}$ interval was not corrected for heart rate.

It is possible that using individualized heart rate correction would lead to results different from those using population-based heart rate correction (i.e., the same correction factor for all subjects) as was done in this study for QT and $J-T_{peak}$. Different heart rate correction methods are likely to influence results only for drugs that significantly affect heart rate.⁴⁸ In our study, quinidine and verapamil increased heart rate by 10 and 9 bpm, respectively. For quinidine, it is unlikely that a different heart rate correction method would change the results because $J-T_{peak}$, $T_{peak}-T_{end}$, and QTc prolongation were strongly associated with quinidine concentration, and the effect of quinidine on $T_{peak}-T_{end}$ was almost twice that of its effect on $J-T_{peak}$. For verapamil, it is possible that an individualized correction

for heart rate could cause different results. Finally, the potential effects of delayed adaptation of repolarization to heart rate (hysteresis) were minimized by measuring the intervals in ECGs at stable heart rates.⁴⁹

Conclusion

This study supports the hypothesis that ECG measures of early repolarization (global $J-T_{peak}$) and late repolarization (global $T_{peak}-T_{end}$) can differentiate pure hERG potassium channel block associated with a high torsade risk from combined hERG potassium channel and inward current block (calcium or late sodium), which may lower torsade risk. By contrast, evaluating only the QTc interval does not detect multichannel block. This clinical study investigating the ECG signatures of multichannel block is one of three efforts studying potential approaches to improve the current regulatory paradigm of focusing almost exclusively on hERG potassium channel block and QTc. This includes a Comprehensive *in vitro* Proarrhythmia Assay (CiPA),⁵⁰ in which the effects of drugs on multiple ion channels would be assessed, and the use of detailed ECG collection in early clinical studies with exposure–response analysis.⁵¹ Ultimately, future approaches will likely use a more mechanistic approach to evaluate the risk for drug-induced torsade de pointes.

METHODS

Clinical study design. We conducted a randomized controlled five-way single-dose crossover clinical trial in 22 healthy volunteers (11 females) at a phase I clinical research unit (Spaulding Clinical, West Bend, WI). The study was approved by the US Food and Drug Administration

Research Involving Human Subjects Committee and the local institutional review board. All subjects gave written informed consent. The drugs evaluated in this study were dofetilide (500 µg, Tikosyn, Pfizer, New York, NY), quinidine sulfate (400 mg, Watson Pharma, Corona, CA), ranolazine (1,500 mg, Ranexa, Gilead, Foster City, CA), and verapamil hydrochloride (120 mg, Heritage Pharmaceuticals, Edison, NJ).

The inclusion criteria required subjects to be of general good health as determined by a physician, without a history of heart disease or unexplained syncope or a family history of long QT syndrome; to be 18–35 years of age, weigh at least 50 kg, and have a body mass index of 18–27 kg/m²; and to be able to read and understand the informed consent. In addition, subjects were excluded if they had more than 10 ectopic beats during a 3-h continuous ECG recording at screening.

In the morning of each treatment period, the subjects received one of the four drugs or placebo under fasting conditions. There was a 7-day washout period between each 24-h treatment period, so subjects received treatment on days 1, 9, 17, 25, and 33. Prior to dosing, a continuous 12-lead ECG recorder (Surveyor, Mortara Instrument, Milwaukee, WI) using the Mason–Likar⁵² electrode configuration was connected to each subject. The continuous ECG recordings were made at 500 Hz and with an amplitude resolution of 2.5 µV. From the continuous recording, three replicate 10-s ECGs (pre- and postdose) were extracted at 16 predefined time-points (predose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 14, 24 h postdose), during which the subjects were resting in a supine position for 10 min. After each ECG extraction time-point period, a blood sample was drawn for pharmacokinetic analysis. Plasma drug concentration was determined using a validated liquid chromatography with tandem mass spectrometry method by Frontage Laboratories (Exton, Philadelphia, PA).

ECG assessment. At each of the 16 time-points, three optimal ECGs were extracted with stable heart rates and maximum signal quality using Antares software (AMPS, New York, NY).⁴⁹ This resulted in a total of 48 ECGs per subject per treatment period and 5,280 planned ECGs in total, which were all evaluated with computerized interval annotations on high-resolution images by the ECG reader, who was blinded to treatment and time. The same ECG reader evaluated all ECGs from the same subject and determined P_{onset}, QRS_{onset}, and QRS_{offset} using lead II. To quantify the global repolarization intervals, two ECG readers blinded to treatment and time determined the global peak and end of the T-wave independently using previously developed software^{53,54} on the vector magnitude of the vectorcardiogram (obtained from the Mason–Likar 12-lead ECG by applying the Guldenring transformation matrix).⁵⁵ The end of the T-wave was determined using the tangent method,⁵⁶ which involves locating the intersection between the line through the terminal descending part of the T-wave and the isoelectric line (Figure 6). This approach of using the tangent method in the vector magnitude lead produces more consistent measurements of the QT interval.⁵⁷ Of note, the U-wave was not included in the measurement of the end of the T-wave.

Notably, globally measured T_{peak}–T_{end} is different as compared with T_{peak}–T_{end} measured in a precordial lead, which has been proposed as a measure of transmural dispersion.³⁶ The relationship between T_{peak}–T_{end} and transmural dispersion, is, however, subject to discussion.^{37,38} T_{peak}–T_{end} was measured globally, which is likely more consistent in the presence of complex T-wave patterns. T_{peak} was defined as the first discernable peak in the T-wave. Disagreements on a T-wave being measurable, presence of a notch, or a difference of more than 5 ms in either T_{peak} or T-wave_{offset} were re-reviewed and adjudicated by an expert ECG reader. This was the case for 73 ECGs (or ~1.4%). From the measured fiducial points, the PR, QRS, J–T_{peak} (QRS_{offset} to global T-wave peak), T_{peak}–T_{end} (global T_{peak} to T_{end}), and QT intervals were obtained (based on the global T_{end}). J–T_{peak} was corrected for heart rate using a coefficient obtained from a previous analysis of pooled subjects from 34 thorough QT studies¹³ (J–T_{peak}^c = J–T_{peak}/RR^{0.58} with RR in seconds), and QT was corrected with Fridericia's correction⁵⁸ (QTc = QT/RR^{1/3} with RR in seconds). Although heart rate dependency for T_{peak}–T_{end} has been reported,⁴⁷ rate correction was not performed for T_{peak}–T_{end}. Rate

correction was not done because previous studies, including a pooled analysis of subjects from 34 thorough QT studies, have shown that at resting heart rates T_{peak}–T_{end} exhibits minimal heart rate dependency.^{13,47}

Statistical analysis. The placebo-corrected change from baseline was computed using PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC). The change from baseline for each ECG biomarker (e.g., the average QTc, T_{peak}–T_{end}) by time-point was the dependent variable, for which baseline was defined as the average predose value. Sequence, period, time, treatment, and an interaction between treatment and time were included as fixed effects, and subject was included as a random effect. In addition, exposure–response analysis was performed with a linear mixed-effects model to evaluate the relationship between plasma drug concentrations and ECG measurements.⁵⁹ Differences in J–T_{peak}^c and T_{peak}–T_{end} for each drug were compared using a paired *t*-test in R 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria). *P* values <0.05 were considered statistically significant.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at <http://www.nature.com/cpt>

ACKNOWLEDGMENTS

We thank Jill Coker, FDA extramural clinical studies coordinator, and staff at Spaulding Clinical Research for executing the study. This project was supported in part by appointments to the Research Participation Programs at the Oak Ridge Institute for Science and Education through an interagency agreement between the Department of Energy and the FDA. The opinions presented here are those of the authors. No official support or endorsement by the FDA is intended nor should be inferred. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the FDA. This project was supported by the FDA's Critical Path Initiative and Office of Women's Health.

AUTHOR CONTRIBUTIONS

D.G.S., L.J., J.V., J.W.M., C.S., K.W.-L., M.H., P.G., J.S.S., L.G., J.L., J.F., M.U., and N.S. wrote the manuscript. D.G.S., L.J., J.V., J.W.M., L.G., J.F., and N.S. designed the study. D.G.S., L.J., J.V., J.W.M., C.S., K.W.-L., M.H., P.G., and J.L. performed the study. D.G.S., L.J., J.V., J.W.M., C.S., K.W.-L., M.H., P.G., J.S.S., L.G., J.L., J.F., M.U., and N.S. analyzed the data.

CONFLICT OF INTEREST

J.W.M., K.W.-L., and C.S. are employees of Spaulding Clinical Research, and M.H., P.G., and J.L. are employees of Frontage Laboratories, which are contract research organizations. The other authors declared no conflict of interest.

© 2014 American Society for Clinical Pharmacology and Therapeutics

1. Stockbridge, N., Morganroth, J., Shah, R.R. & Garnett, C. Dealing with global safety issues: was the response to QT-liability of non-cardiac drugs well coordinated? *Drug Saf.* **36**, 167–182 (2013).
2. Trudeau, M.C., Warmke, J.W., Ganetzky, B. & Robertson, G.A. HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* **269**, 92–95 (1995).
3. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance for industry E14 Clinical Evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. <<http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073153.pdf>> (2005). Accessed 14 March 2014.
4. Malik, M., Garnett, C.E. & Zhang, J. Thorough QT studies: questions and quandaries. *Drug Saf.* **33**, 1–14 (2010).
5. Hohnloser, S.H., Klingenhoben, T. & Singh, B.N. Amiodarone-associated proarrhythmic effects. A review with special reference to torsade de pointes tachycardia. *Ann. Intern. Med.* **121**, 529–535 (1994).
6. January, C.T. & Riddle, J.M. Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca²⁺ current. *Circ. Res.* **64**, 977–990 (1989).
7. Wu, L. et al. Augmentation of late sodium current unmasks the proarrhythmic effects of amiodarone. *Cardiovasc. Res.* **77**, 481–488 (2008).
8. Aiba, T. et al. Cellular and ionic mechanism for drug-induced long QT syndrome and effectiveness of verapamil. *J. Am. Coll. Cardiol.* **45**, 300–307 (2005).

9. Andersen, M.P., Xue, J.Q., Graff, C., Kanters, J.K., Toft, E. & Struijk, J.J. New descriptors of T-wave morphology are independent of heart rate. *J. Electrocardiol.* **41**, 557–561 (2008).
10. Acar, B., Yi, G., Hnatkova, K. & Malik, M. Spatial, temporal and wavefront direction characteristics of 12-lead T-wave morphology. *Med. Biol. Eng. Comput.* **37**, 574–584 (1999).
11. Couderc, J.P. Measurement and regulation of cardiac ventricular repolarization: from the QT interval to repolarization morphology. *Philos. Trans. A. Math. Phys. Eng. Sci.* **367**, 1283–1299 (2009).
12. Malik, M. Drug-induced changes in the T-wave morphology. *Drug Saf.* **32**, 613–617 (2009).
13. Johannesen, L. *et al.* Improving the assessment of heart toxicity for all new drugs through translational regulatory science. *Clin. Pharmacol. Ther.* **95**, 501–508 (2014).
14. Kramer, J. *et al.* MICE models: superior to the HERG model in predicting Torsade de Pointes. *Sci. Rep.* **3**, 2100 (2013).
15. Redfern, W.S. *et al.* Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* **58**, 32–45 (2003).
16. Roden, D.M., Woosley, R.L. & Primm, R.K. Incidence and clinical features of the quinidine-associated long QT syndrome: implications for patient care. *Am. Heart J.* **111**, 1088–1093 (1986).
17. Antzelevitch, C. *et al.* The M cell: its contribution to the ECG and to normal and abnormal electrical function of the heart. *J. Cardiovasc. Electrophysiol.* **10**, 1124–1152 (1999).
18. Wu, L. *et al.* Role of late sodium current in modulating the proarrhythmic and antiarrhythmic effects of quinidine. *Heart Rhythm* **5**, 1726–1734 (2008).
19. Antzelevitch, C. *et al.* Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. *Circulation* **110**, 904–910 (2004).
20. Narimatsu, A. & Taira, N. Effects of atrio-ventricular conduction of calcium-antagonistic coronary vasodilators, local anaesthetics and quinidine injected into the posterior and the anterior septal artery of the atrio-ventricular node preparation of the dog. *Naunyn. Schmiedeberg's Arch. Pharmacol.* **294**, 169–177 (1976).
21. Mason, J.W., Winkle, R.A., Rider, A.K., Stinson, E.B. & Harrison, D.C. The electrophysiological effects of quinidine in the transplanted human heart. *J. Clin. Invest.* **59**, 481–489 (1977).
22. Harmer, A.R., Valentin, J.P. & Pollard, C.E. On the relationship between block of the cardiac Na⁺ channel and drug-induced prolongation of the QRS complex. *Br. J. Pharmacol.* **164**, 260–273 (2011).
23. Le Coz, F., Funck-Brentano, C., Morell, T., Ghadanfar, M.M. & Jaillon, P. Pharmacokinetic and pharmacodynamic modeling of the effects of oral and intravenous administrations of dofetilide on ventricular repolarization. *Clin. Pharmacol. Ther.* **57**, 533–542 (1995).
24. Benton, R.E., Sale, M., Flockhart, D.A. & Woosley, R.L. Greater quinidine-induced QTc interval prolongation in women. *Clin. Pharmacol. Ther.* **67**, 413–418 (2000).
25. Démolis, J.L., Funck-Brentano, C., Ropers, J., Ghadanfar, M., Nichols, D.J. & Jaillon, P. Influence of dofetilide on QT-interval duration and dispersion at various heart rates during exercise in humans. *Circulation* **94**, 1592–1599 (1996).
26. Pfizer Laboratories Division of Pfizer. Tikosyn (Dofetilide) Capsule [package insert]. <http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/020931s007lbl.pdf> (2011). Accessed 8 August 2014.
27. Liu, T. *et al.* Differentiating electrophysiological effects and cardiac safety of drugs based on the electrocardiogram: a blinded validation. *Heart Rhythm* **9**, 1706–1715 (2012).
28. Marriott, H.J.L. *Practical Electrocardiography* (Williams & Wilkins, Baltimore, MD 1954).
29. Yang, T., Snyders, D.J. & Roden, D.M. Rapid inactivation determines the rectification and [K⁺]_o dependence of the rapid component of the delayed rectifier K⁺ current in cardiac cells. *Circ. Res.* **80**, 782–789 (1997).
30. Scamps, F. & Carmeliet, E. Delayed K⁺ current and external K⁺ in single cardiac Purkinje cells. *Am. J. Physiol.* **257**, C1086–C1092 (1989).
31. Grandi, E., Pasqualini, F.S., Pes, C., Corsi, C., Zaza, A. & Severi, S. Theoretical investigation of action potential duration dependence on extracellular Ca²⁺ in human cardiomyocytes. *J. Mol. Cell. Cardiol.* **46**, 332–342 (2009).
32. Moss, A.J. *et al.* ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. *Circulation* **92**, 2929–2934 (1995).
33. Pfizer. *Risk Evaluation and Mitigation Strategy (REMS) Document*. <<http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM266277.pdf>> (2013). Accessed 1 May 2014.
34. Yap, Y.G. & Camm, A.J. Drug induced QT prolongation and torsades de pointes. *Heart* **89**, 1363–1372 (2003).
35. Antzelevitch, C. & Fish, J. Electrical heterogeneity within the ventricular wall. *Basic Res. Cardiol.* **96**, 517–527 (2001).
36. Antzelevitch, C. *et al.* Does T_{peak}–T_{end} provide an index of transmural dispersion of repolarization? *Heart Rhythm* **4**, 1114–1119 (2007).
37. Opthof, T. *et al.* Dispersion of repolarization in canine ventricle and the electrocardiographic T wave: T_{pe-e} interval does not reflect transmural dispersion. *Heart Rhythm* **4**, 341–348 (2007).
38. Xia, Y., Liang, Y., Kongstad, O., Holm, M., Olsson, B. & Yuan, S. T_{peak}–T_{end} interval as an index of global dispersion of ventricular repolarization: evaluations using monophasic action potential mapping of the epi- and endocardium in swine. *J. Interv. Card. Electrophysiol.* **14**, 79–87 (2005).
39. Kanters, J.K. *et al.* T(peak)T(end) interval in long QT syndrome. *J. Electrocardiol.* **41**, 603–608 (2008).
40. AZCERT. *CredibleMeds*. <<http://www.crediblemeds.org>> (2014). Accessed 14 March 2014.
41. Chézalviel-Guilbert, F., Davy, J.M., Poirier, J.M. & Weissenburger, J. Mexiletine antagonizes effects of sotalol on QT interval duration and its proarrhythmic effects in a canine model of torsade de pointes. *J. Am. Coll. Cardiol.* **26**, 787–792 (1995).
42. Schwartz, P.K. *et al.* Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na⁺ channel block and to increases in heart rate. Implications for gene-specific therapy. *Circulation* **92**, 3381–3386 (1995).
43. Moss, A.J., Zareba, W., Schwarz, K.Q., Rosero, S., McNitt, S. & Robinson, J.L. Ranolazine shortens repolarization in patients with sustained inward sodium current due to type-3 long-QT syndrome. *J. Cardiovasc. Electrophysiol.* **19**, 1289–1293 (2008).
44. Gao, Y. *et al.* Inhibition of late sodium current by mexiletine: a novel pharmacotherapeutic approach in timothy syndrome. *Circ. Arrhythm. Electrophysiol.* **6**, 614–622 (2013).
45. Giardina, E.G. & Wechsler, M.E. Low dose quinidine–mexiletine combination therapy versus quinidine monotherapy for treatment of ventricular arrhythmias. *J. Am. Coll. Cardiol.* **15**, 1138–1145 (1990).
46. Duff, H.J., Mitchell, L.B., Manyari, D. & Wyse, D.G. Mexiletine–quinidine combination: electrophysiologic correlates of a favorable antiarrhythmic interaction in humans. *J. Am. Coll. Cardiol.* **10**, 1149–1156 (1987).
47. Smetana, P., Batchvarov, V., Hnatkova, K., John Camm, A. & Malik, M. Sex differences in the rate dependence of the T wave descending limb. *Cardiovasc. Res.* **58**, 549–554 (2003).
48. Garnett, C.E. *et al.* Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects. *Am. Heart J.* **163**, 912–930 (2012).
49. Badilini, F., Vaglio, M. & Sarapa, N. Automatic extraction of ECG strips from continuous 12-lead holter recordings for QT analysis at prescheduled versus optimized time points. *Ann. Noninvasive Electrocardiol.* **14** (suppl. 1), S22–S29 (2009).
50. Sager, P.T., Gintant, G., Turner, J.R., Pettit, S. & Stockbridge, N. Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. *Am. Heart J.* **167**, 292–300 (2014).
51. Darpo, B. *et al.* The IQ-CSRC prospective clinical Phase 1 study: “Can early QT assessment using exposure response analysis replace the thorough QT study?”. *Ann. Noninvasive Electrocardiol.* **19**, 70–81 (2014).
52. Mason, R.E. & Likar, I. A new system of multiple-lead exercise electrocardiography. *Am. Heart J.* **71**, 196–205 (1966).
53. Johannesen, L., Vicente, J., Galeotti, L. & Strauss, D.G. ECGlib: Library for processing electrocardiograms. *Comput Cardiol* **40**, 951–954 (2013).
54. Vicente, J., Johannesen, L., Galeotti, L. & Strauss, D.G. ECGlab: User friendly ECG/VCG analysis tool for research environments *Comput Cardiol* **40**, 775–778 (2013).
55. Guldenring, D. *et al.* Transformation of the Mason–Likar 12-lead electrocardiogram to the Frank vectorcardiogram. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2012**, 677–680 (2012).
56. Lepeschkin, E. & Surawicz, B. The measurement of the Q–T interval of the electrocardiogram. *Circulation* **6**, 378–388 (1952).
57. Diamant, U.B., Winbo, A., Stattin, E.L., Rydberg, A., Kesek, M. & Jensen, S.M. Two automatic QT algorithms compared with manual measurement in identification of long QT syndrome. *J. Electrocardiol.* **43**, 25–30 (2010).
58. Friderici, L.S. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Medica Scandinavica* **53**, 469–486 (1920).
59. Garnett, C.E. *et al.* Concentration–QT relationships play a key role in the evaluation of proarrhythmic risk during regulatory review. *J. Clin. Pharmacol.* **48**, 13–18 (2008).