Clinical Pharmacology & Therapeutics

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

Lars Johannesen, Jose Vicente, Jay Mason, Carlos Sanabria, Kristin Waite-Labott, Mira Hong, Ping Guo, John Lin, Jens Stampe Sørensen, Loriano Galeotti, Jeffry A Florian, Martin Ugander, Norman Stockbridge, David G. Strauss

Cite this article as: Lars Johannesen, Jose Vicente, Jay Mason, Carlos Sanabria, Kristin Waite-Labott, Mira Hong, Ping Guo, John Lin, Jens Stampe Sørensen, Loriano Galeotti, Jeffry A Florian, Martin Ugander, Norman Stockbridge, David G. Strauss, Differentiating Drug-Induced Multi-channel Block on the Electrocardiogram: Randomized Study of Dofetilide, Quinidine, Ranolazine and Verapamil, *Clinical Pharmacology & Therapeutics* accepted article preview online 23 July 2014; doi:10.1038/clpt.2014.155

Received 05 May 2014; accepted 09 July 2014; Accepted article preview online 23 July 2014

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

Lars Johannesen[1,2], Jose Vicente[1,3], Jay Mason[4], Carlos Sanabria[4], Kristin Waite-Labott[4], Mira Hong[5], Ping Guo[5], John Lin[5], Jens Stampe Sørensen[6], Loriano Galeotti[1], Jeffry A Florian[6], Martin Ugander[1,2], Norman Stockbridge[7], David G. Strauss[1,2]

[1] Office of Science and Engineering Laboratories, CDRH, US FDA, Silver Spring, MD, USA

[2] Department of Clinical Physiology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

[3] BSICoS Group, Aragón Institute of Engineering Research (I3A), IIS Aragón, University of Zaragoza, Zaragoza, Aragon, Spain

[4] Spaulding Clinical Research, West Bend, WI, USA

[5] Frontage Laboratories, Inc, Exton, PA, USA

[6] Division of Pharmacometrics, Office of Clinical Pharmacology, Office of Translational Sciences, CDER, US FDA, Silver Spring, MD, USA

[7] Division of Cardiovascular and Renal Products, Office of New Drugs, CDER, US FDA , Silver Spring, MD, USA

Corresponding author:

David G. Strauss, MD, PhD

U.S. Food and Drug Administration

10903 New Hampshire Avenue, WO62-1126

Silver Spring, MD, 20993, USA

E-mail: david.strauss@fda.hhs.gov

Telephone: 301-796-6323

Fax: 301-796-9927

Number of references: 59

Number of figures: 6

Number of tables: 1

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

Abstract

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 multi-channel 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 both early and late repolarization, while additional inward current block (calcium or late sodium) preferentially shortens early repolarization. Characterization of multi-channel drug effects on human cardiac repolarization is possible and may improve the utility of the electrocardiogram in the assessment of drug-related cardiac electrophysiology.

Introduction

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-a-go-go*-related gene (hERG) potassium channel (an outward current) and prolong the QT interval on the electrocardiogram (ECG).[1, 2] In response, the United States Food and Drug Administration (FDA) requires almost all new drugs to undergo "thorough QT" studies.[3] A positive thorough QT study does not preclude the 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 with a low risk of torsade.[5] This is likely because blocking inward currents can prevent early after depolarizations, 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 electrocardiogram (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 consistent with these inward currents being active during the early repolarization phase of the action potential (phase 2).[13] However, the prior study[13] was limited by the fact that preclinical ion channel data were not available for all drugs and the risk of torsade de pointes was not known for the drugs studied.

Thus, we designed a prospective randomized controlled clinical trial, funded by 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 with calcium and sodium channel block. 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 blocker[14] 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 the hERG potassium channel, but also block 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 compared to multi-channel block. We

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.

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 body mass index of 23.0±2.6 kg/m^2; see table 1 for baseline characteristics. All completed the study except for 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: 1.0 to 4.0 h) for dofetilide and 2.0 h (0.5 to 4.0 h) for quinidine, and with similar half-lives of (mean±SD) 7.2±1.1h (dofetilide) and 7.8±1.5h (quinidine). The maximum concentrations for dofetilide and quinidine were 2.7±0.3 ng/mL and 1.8±0.4 µg/mL, respectively. Ranolazine peaked later at 4.0 h (1.0 to 14 h) with a concentration of 2.3±1.4 µg/mL and had a half-life of 7.5±4.0 h. Lastly, verapamil peaked at 1.0 h (0.5 to 2.0 h) with a plasma concentration of 130.4±75.8 ng/mL and had a half-life of 10.4±3.2h.

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

Dofetilide prolonged the heart rate corrected global QT interval (QTc) interval by 78.9 ms (95% confidence interval [CI]: 71.9 to 85.9 ms, p<0.001; Figure 3a), with equal prolongation of the heart rate corrected J-T_{peak} (J-T_{peak}c: 39.1 [32.5 to 45.8] ms, p<0.001) and T_{peak}-T_{end} (40.0 [33.0 to 46.9] ms, p<0.001). Similarly, the concentration-dependent analysis showed that the QTc prolongation by dofetilide equally affected J-T_{peak}c and T_{peak}-T_{end} (J-T_{peak}c: 14.0 [10.9 to 17.2] ms per ng/mL, p<0.001, T_{peak}-T_{end}: 14.5 [11.0 to 17.9] ms per ng/mL, p<0.001; p=0.87 for J-T_{peak}c vs. T_{peak}-T_{end}; Figure 4a). These findings support the notion that hERG potassium channel block prolongs both J-T_{peak}c and T_{peak}-T_{end}.

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 78.3 ms (71.2 to 85.4 ms, p<0.001; Figure 3b); however, quinidine was more strongly associated with prolongation of T_{peak}-T_{end} than J-T_{peak}c (J-T_{peak}c: 29.9 [23.1 to 36.6] ms, p<0.001; T_{peak}-T_{end}: 49.8 [42.8 to 56.8] ms, p<0.001; Figure 3b). Similarly, the concentration-dependent analysis showed a stronger relationship for T_{peak}-T_{end} compared to J-T_{peak}c (J-T_{peak}c: 11.9 [3.7 to 20.1] ms per µg/mL, p=0.008; T_{peak}-T_{end}: 29.9 [19.2 to 40.7] ms per µg/mL; p<0.001; p=0.026 for J-T_{peak}c 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_{peak}c 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 12.5 ms (5.5 to 19.5 ms, p<0.001; Figure 3c), primarily via prolonged T_{peak}-T_{end} (J-T_{peak}c: 3.4 [-3.3 to 10.0] ms, p=0.32, T_{peak}-T_{end}: 8.8 [1.9 to 15.8] ms, p=0.013; Figure 3c). A similar observation was seen in the concentration-dependent analysis where ranolazine concentrations were associated with an increase in T_{peak}-T_{end} (4.4 [2.6 to 6.2] ms per µg/mL, p=0.001; Figure 4c), however there was no association with J-T_{peak}c (p=0.42) (p<0.001 for J-T_{peak}c vs. T_{peak}-T_{end}; Figure 4c). These findings suggest that late sodium current block primarily reduces the J-T_{peak}c 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 (5.3 [-1.7 to 12.3] ms p=0.14; Figure 2d), J-T_{peak}c (-2.4 [-9.1 to 4.2] ms, p=0.48; Figure 2d) or T_{peak}-T_{end} (4.8 [-2.2 to 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_{peak}c 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_{peak}c 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 a large PR prolongation (32.1 [26.7 to 37.4] ms, p<0.001), which was concentration dependent (0.2 [0.2 to 0.3] ms per ng/mL, p<0.001). Quinidine, which blocks the calcium channel,[14] trended toward PR prolongation (5.1 [-0.3 to 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] Ranolazine exhibits minimal calcium channel block and produced a small amount of PR prolongation (6.5 [1.1 to 11.8] ms, p=0.018 and 1.1 [0.1 to 2.0] ms per µg/mL, p=0.033).[19] Dofetilide is not associated with calcium channel block and produced no PR prolongation.[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 only expected to block the sodium current 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 has weak peak sodium current block,[19] and was only associated with slight QRS prolongation (2.7 [0.5 to 4.9] ms, p=0.018); however, there was no correlation between plasma levels and QRS. 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 to 4.8] ms, p=0.020); however, there was no relationship between concentration and QRS (p=0.64).

Lastly, both verapamil and quinidine caused an increase in heart rate of 9.4 bpm ([6.4 to 12.4], p<0.001) and 9.8 bpm ([6.8 to 12.9], p<0.001), respectively. In both cases, there was a relationship between plasma concentration and heart rate (verapamil: 0.07 [0.05 to 0.09] bpm per ng/mL, p<0.001; quinidine: 4.7 [3.4 to 6.1] bpm per µg/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 to 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_{peak}c) and late repolarization (global T_{peak}-T_{end}), a pure hERG potassium channel blocking drug with high torsade risk can be differentiated from multi-channel blocking drugs that also block inward currents during repolarization (calcium or late sodium). Detecting additional calcium or late sodium current block is of importance, as both the calcium and sodium current support early after depolarizations, which can trigger torsade de pointes.[6-8] Thus, being able to detect multi-channel 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_{peak}c and T_{peak}-T_{end}, while additional calcium and late sodium current block (quinidine, ranolazine and verapamil) preferentially shorten J-T_{peak}c. Of note, the pure hERG potassium channel blocker dofetilide and quinidine caused equal QTc prolongation. Despite the comparable QTc prolongation, dofetilide and quinidine were associated with different effects on J-T_{peak}c and T_{peak}-T_{end}, suggesting that the QTc interval cannot differentiate pure hERG potassium channel block from multi-channel 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 multi-channel block, whereas evaluation of J-T_{peak} and T_{peak}-T_{end} intervals provide

insight into multi-channel block of relevance for cardiac safety evaluation. These findings suggest that future studies should consider reporting the ratio of the changes in J-T_{peak}c and T_{peak}-T_{end}, in addition to reporting the changes.

It is notable that dofetilide and quinidine caused substantial QTc prolongation (~78 ms) in the present study. These results are consistent those from prior single dose studies of dofetilide and quinidine. Coz et al. reported a slope of dofetilide concentration vs. QTc of 31 ms per ng/mL,[23] compared to 29 ms per ng/mL in our study. Benton et al. reported a slope of quinidine concentration vs. QTc of 29 ms per µg/mL in men and 42 ms per µg/mL in women,[24] compared to 43 ms per µg/mL in our study. However, a separate study of dofetilide 750 µg twice daily for four 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_{peak}-T_{end} prolongation with verapamil suggests that when calcium channel block is stronger than hERG potassium channel block that it may also attenuate the effect of hERG potassium channel block on T_{peak}-T_{end}. These findings with verapamil are consistent with those in the rabbit heart where changes in QT and T_{peak}-T_{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] Thus, PR prolongation is not always present with calcium channel block, and other markers such as J-T_{peak}c and T_{peak}-T_{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 and flattens and widens the T-wave. This is in contrast with 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, while hypercalcemia creates the equivalent of calcium channel block. Similarly, genetic abnormalities in the hERG potassium channel (congenital long QT type 2) causes 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 between 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 where the number of quinidine-induced arrhythmias was greater at lower rather than higher quinidine concentrations,[17, 18] which produces more calcium and late sodium current block.

The development of torsade requires an electrical trigger and then 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 after depolarizations, 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 after depolarizations (the trigger for torsade).

The T_{peak}-T_{end} interval, as measured in a precordial lead, has been proposed as a measure of transmural dispersion.[36] The relationship between transmural dispersion and T_{peak}-T_{end} is subject to controversy as it has been proposed that T_{peak}-T_{end} instead reflects total dispersion and not transmural dispersion.[37, 38] T_{peak}-T_{end} has, however, been shown to be prolonged by hERG potassium channel block and be longer in LQT2 patients (abnormalities in the hERG potassium channel) compared to LQT1 (abnormalities in the slow potassium channel) and LQT3 (abnormalities in the sodium channel).[13, 39] In this study, we quantified T_{peak}-T_{end} globally on the vector magnitude derived lead, which is likely more consistent than measuring T_{peak}-T_{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, anti-malarial, anti-viral, 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, where 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 congenital long QT type 3 patients (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 long QT type 2 patients (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 where 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 co-administering an inward current blocker with a hERG potassium channel blocker could offset 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 increase 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 as 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} interval 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 only likely to influence results 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, as J-T_{peak}c, T_{peak}-T_{end} and QTc prolongation were strongly associated with quinidine-concentration, and the effects of quinidine on T_{peak}-T_{end} was almost twice that of J-T_{peak}c. For verapamil, it is possible that an individualized correction for heart rate could cause different results. Lastly, 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}c) 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. In contrast, only evaluating the QTc interval does not detect multi-channel block. This clinical study investigating the ECG signatures of multi-channel 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] where 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 evaluating the risk for drug-induced torsade de pointes.

Methods

Clinical Study Design

We conducted a randomized controlled five-way single-dose cross-over clinical trial in 22 healthy volunteers (11 females) at a phase 1 clinical research unit (Spaulding Clinical, West Bend, Wisconsin, USA). The study was approved by the U.S. 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), quinidine sulfate (400 mg, quinidine sulfate, Watson Pharma Inc), ranolazine (1500 mg, Ranexa, Gilead Sciences) and verapamil hydrochloride (120 mg, verapamil hydrochloride, Mylan Pharmaceuticals).

The inclusion criteria required subjects to be of general good health as determined by a physician, without a history of heart disease, unexplained syncope or family history of long QT syndrome, be 18 to 35 years of age, weigh at least 50 kg, have a body mass index of 18 to 27 kg/m^2 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-hour 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-hour 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, Wisconsin) using the Mason-Likar[52] electrode configuration was connected to each subject. The continuous ECG recordings were made at 1000 Hz and with an amplitude resolution of 1 μV. From the continuous recording, three replicate 10-second ECGs (pre and post-dose) were extracted at 16 predefined time-points (pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 14, 24 hours post-dose) during which the subjects were resting in a supine position for 10 minutes. 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 spectroscopy (LC-MS/MS) method by Frontage Laboratories (Exton, Philadelphia, PA, USA).

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-LLC, New York City, NY, USA).[49] This resulted in a total of 48 ECGs per subject per treatment period and 5280 planned ECGs in total, which were all evaluated including computerized interval annotations on high-resolution images by the ECG reader blinded to treatment and time. The same ECG reader evaluated all ECGs from the same subject and determined the 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 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 (see 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.

Of note, globally measured T_{peak}-T_{end} is different compared to 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 measureable, presence of a notch or a difference of more than 5 ms in either the 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}). The J-T_{peak} was corrected for heart rate using a coefficient obtained from a previous analysis of pooled subjects from 34 thorough QT studies[13] ($J\text{-}T_{peak}c = J\text{-}T_{peak}/RR^{0.58}$ with RR in seconds) and QT was corrected with Fridericia's correction[58] ($QTc = QT/RR^{1/3}$ 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 the 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, North Carolina, USA). The change from baseline for each ECG biomarker (e.g., the average QTc, T_{peak}-T_{end}) by time-point was the dependent variable, where

baseline was defined as the average pre-dose value. Sequence, period, time, treatment and an interaction between treatment and time were included as fixed effects, and subject 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.

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 pro-arrhythmic 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}c (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}c.

What this study adds to our knowledge?

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

How this might change clinical pharmacology and therapeutics?

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

Acknowledgements

We would like to 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 FDA. The opinions presented here are those of the authors. No official support or endorsement by 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 FDA. This project was supported by FDA's Critical Path Initiative and Office of Women's Health.

Disclosures

JWM, KWL and CS are employees of Spaulding Clinical Research and MH, PG and JL are employees of Frontage Laboratories Inc., which are contract research organizations.

Author Contributions

DGS, LJ, JV, JWM, CS, KWL, MH, PG, JSS, LG, JL, JAF, MU, and NS wrote the manuscript

DGS, LJ, JV, JWM, LG, JAF, and NS designed the research

LJ, JV, JWM, CS, KWL, MH, PG, and JL performed the research

DGS, LJ, JV, JWM, CS, KWL, MH PG, JSS, LG, JL, JAF, MU, and NS analyzed the data

References

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**, 167-82 (2013).

2 Trudea, M.C., Warmke, J.W., Ganetzky, B. & Robertson, G.A. HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* **269**, 92-5 (1995).

3 *The International Conference on Harmonization 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/RegulatoryInformation/Guidances/ucm129357.pdf (2005). Accessed March 14 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., Klingenheben, T. & Singh, B.N. Amiodarone-associated Proarrhythmic Effects: A Review with Special Reference to Torsade de Pointes Tachycardia. *Ann Intern Med* **121**, 529-35 (1994).

6 January, C.T. & Riddle, J.M. Early afterdepolarizations: mechanism of induction and block: a role for L-type Ca++ current. *Circ Res* **64**, 977-90 (1989).

7 Wu, L. *et al.* Augmentation of late sodium current unmasks the proarrhythmic effects of amiodarone. *Cardiovasc Res* **77**, 481-88 (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**, 300-7 (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**, 557-61 (2008).

10 Sarapa, N., Mortara, J.L., Brown, B.D., Isola, L. & Badilini, F. Quantitative performance of E-Scribe warehouse in detecting quality issues with digital annotated ECG data from healthy subjects. *J Clin Pharmacol* **48**, 538-46 (2008).

11 Couderc, J.P. Measurement and regulation of cardiac ventricular repolarization: from the QT interval to repolarization morphology. *Phil Trans R Soc A* **367**, 1283-99 (2009).

12 Malik, M. Drug-induced changes in the T-wave morphology. *Drug Saf* **32**, 613-7 (2009).

13 Johannesen, L. *et al.* Improving the Assessment of Heart Toxicity for All New Drugs through Translational Regulatory Science. *Clin Pharmacol Ther* **95**, 501-8 (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**, 1088-93 (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**, 1124-52 (1999).

18 Wu, L. *et al.* Role of late sodium current in modulating the proarrhythmic and antiarrhythmic effects of quinidine. *Heart Rhythm* **5**, 1726-34 (2008).

19 Antzelevitch, C. *et al.* Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. *Circulation* **110**, 904-10 (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 Schmiedebergs Arch Pharmacol* **294**, 169-77 (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**, 481-9 (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**, 260-73 (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**, 533-42 (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**, 413-8 (2000).

25 Demolis, 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**, 1592-9 (1996).

26 Pfizer Laboratories Division of Pfizer Inc. *Tikosyn (Dofetilide) Capsule [package insert]*. (2011). Accessed 2014 June 9.

27 Liu, T. *et al*. Differentiating electrophysiological effects and cardiac safety of drugs based on the electrocardiogram: A blinded validation *Heart Rhythm* **9**, 1706-15 (2012).

28 Marriott, H.J.L. *Practical Electrocardiography* (Williams & Williams: 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**, 782-9 (1997).

30 Scamps, F. & Carmeliet, E. Delayed K+ current and external K+ in single cardiac Punkinje cells. *Am J Physiol* **257**, C1086-92 (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**, 332-42 (2009).

32 Moss, A.J. *et al*. ECG T-Wave patterns in genetically distinct forms of the hereditary long QT syndrome. *Circulation* **92**, 2929-34 (1995).

33 Pfizer. *Risk Evaluation and Mitigation Strategy (REMS) Document*. http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM266277.pdf (2013). Accessed May 1 2014.

34 Yap, Y.G. & Camm, A.J. Drug induced QT prolongation and torsade de pointes. *Heart* **89**, 1363-72 (2003).

35 Antzelevitch, C. & Fish, J. Electrical heterogeneity within the ventricular wall. *Basic Res Cardiol* **96**, 517-27 (2001).

36 Antzelevitch, C. *et al.* Does Tpeak-Tend Provide an Index of Transmural Dispersion of Repolarization? *Heart Rhythm* **4**, 1114-9 (2007).

37 Opthof, T. *et al.* Dispersion of repolarization in canine ventricle and the electrocardiographic T wave: Tp-e interval does not reflect transmural dispersion. *Heart Rhythm* **4**, 341-8 (2007).

38 Xia, Y., Liang, Y., Kongstad, O., Holm, M., Olsson, B. & Yuan, S. Tpeak-Tend 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.* Tpeak Tend interval in long QT syndrome. *J Electrocardiol* **41**, 2008 (2008).

40 AZCERT. *CredibleMeds*. http://www.crediblemeds.org (2014). Accessed March 14 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**, 787-92 (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 blockade and to increases in heart rate. Implications for gene-specific therapy. *Circulation* **92**, 3381-6 (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**, 1289-93 (2008).

44 Gao, Y. *et al*. Inhibition of late sodium current by mexiletine: a novel pharmotherapeutical approach in timothy syndrome. *Circ Arrhythm Electrophysiol* **6**, 614-22 (2013).

45 Giardina, E.G. & Weschler, M.E. Low dose quinidine-mexiletine combination therapy versus quinidine monotherapy for treatment of ventricular arrhythmias. *J Am Coll Cardiol* **15**, 1138-45 (1990).

46 Duff, H.J., Mitchell, L.B., Mayari, D. & Wyse, D.G. Mexiletine-quinidine combination: electrophysiologic correlates of a favorable antiarrhythmic interaction in humans. *J Am Coll Cardiol* **10**, 1149-56 (1987).

47 Smetana, P., Batchvarov, V., Hnatkova, K., Camm, J.A. & Malik, M. Sex differences in the rate dependence of the T wave descending limb. *Cardiovasc Res* **58**, 549-54 (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**, 912-30 (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**, S22-9 (2009).

50 Sager, P.T., Gintant, G., Turner, J.T., 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 (2013).

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 (2013).

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**, 951-4 (2013).

54 Vicente, J., Johannesen, L., Galeotti, L. & Strauss, D.G. ECGlab: User friendly ECG/VCG analysis tool for research environments *Comput Cardiol* **40**, 775-8 (2013).

55 Guldenring, D. *et al.* Transformation of the Mason-Likar 12-lead electrocardiogram to the Frank vectorcardiogram. In Conf Proc IEEE Eng Med Biol Soc 2012 677-80.

56 Lepeschkin, E. & Surawicz, B. The measurement of the Q-T interval of the electrocardiogram. *Circulation* **6**, 378-88 (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 Fridericia, L.S. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Medica Scandinavica* **53**, 469-86 (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**, 13-8 (2008).

Table 1 – Baseline characteristics

	All subjects (N=22)
Demographic	
Age (years)	26.9 ± 5.5
Female	11 (50%)
Body mass index (kg/m^2)	23.0 ± 2.6
Vital Signs	
Systolic blood pressure (mmHg)	108.8 ± 9.1
Diastolic blood pressure (mmHg)	59.4 ± 6.6
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}c (ms)	224.4 ± 19.8
T_{peak}-T_{end} (ms)	73.1 ± 6.4
QTc (ms)	394.8 ± 17.1

Continuous variables are represented as mean ± SD

Figure legends

Figure 1: An illustration of a ventricular action potential (AP) and the corresponding surface electrocardiogram. Arrows pointing into the action potential are inward currents (calcium and late sodium) and arrows pointing out denote outward currents (hERG potassium). Blocking the calcium or late sodium current primarily shortens the early parts of repolarization ($J\text{-}T_{peak}$) whereas hERG potassium channel block prolongs both early ($J\text{-}T_{peak}$) and late repolarization ($T_{peak}\text{-}T_{end}$).[27]

Figure 2: Measured plasma concentrations (mean ± 95% confidence interval) for: dofetilide (**a**), quinidine (**b**), ranolazine (**c**) and verapamil (**d**). The x-axis in each plot is time post-dose in hours.

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

Figure 4: Drug-induced changes (mean ± 95% confidence interval) for the placebo-corrected change from baseline (ΔΔ) for QTc (gray), $J\text{-}T_{peak}c$ (orange) and $T_{peak}\text{-}T_{end}$ (blue) from model predictions from a linear-mixed effects model versus plasma concentrations for dofetilide (**a**), quinidine (**b**), ranolazine (**c**) and verapamil (**d**). For clarity, the observed data is not shown in this figure, but is in included in supplementary figure S1. 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.

Figure 5: Drug-induced changes in J-T_{peak}c and T_{peak}-T_{end} for a pure hERG potassium channel blocker on the left (**a**: dofetilide) and a hERG + late sodium current blocker (**b**: ranolazine) on the right. This zoomed plot on the concentrations of dofetilide that produces a comparable amount of QTc prolongation to ranolazine, shows the ability of J-T_{peak}c and T_{peak}-T_{end} to detect multi-channel block.

Figure 6: Method for assessment of the global T_{peak} and T_{end}. The 12-lead ECG 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, which involves locating the intersection between the line through the terminal descending part of the T-wave and the isoelectric line.

Figure 1

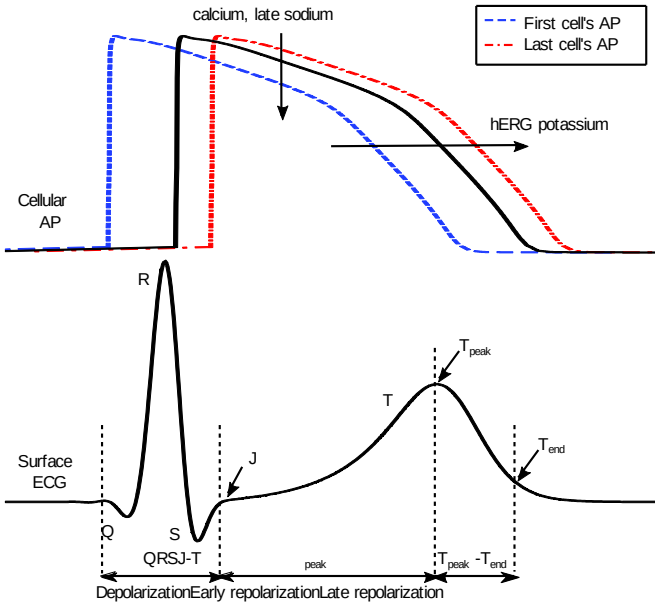

Figure2

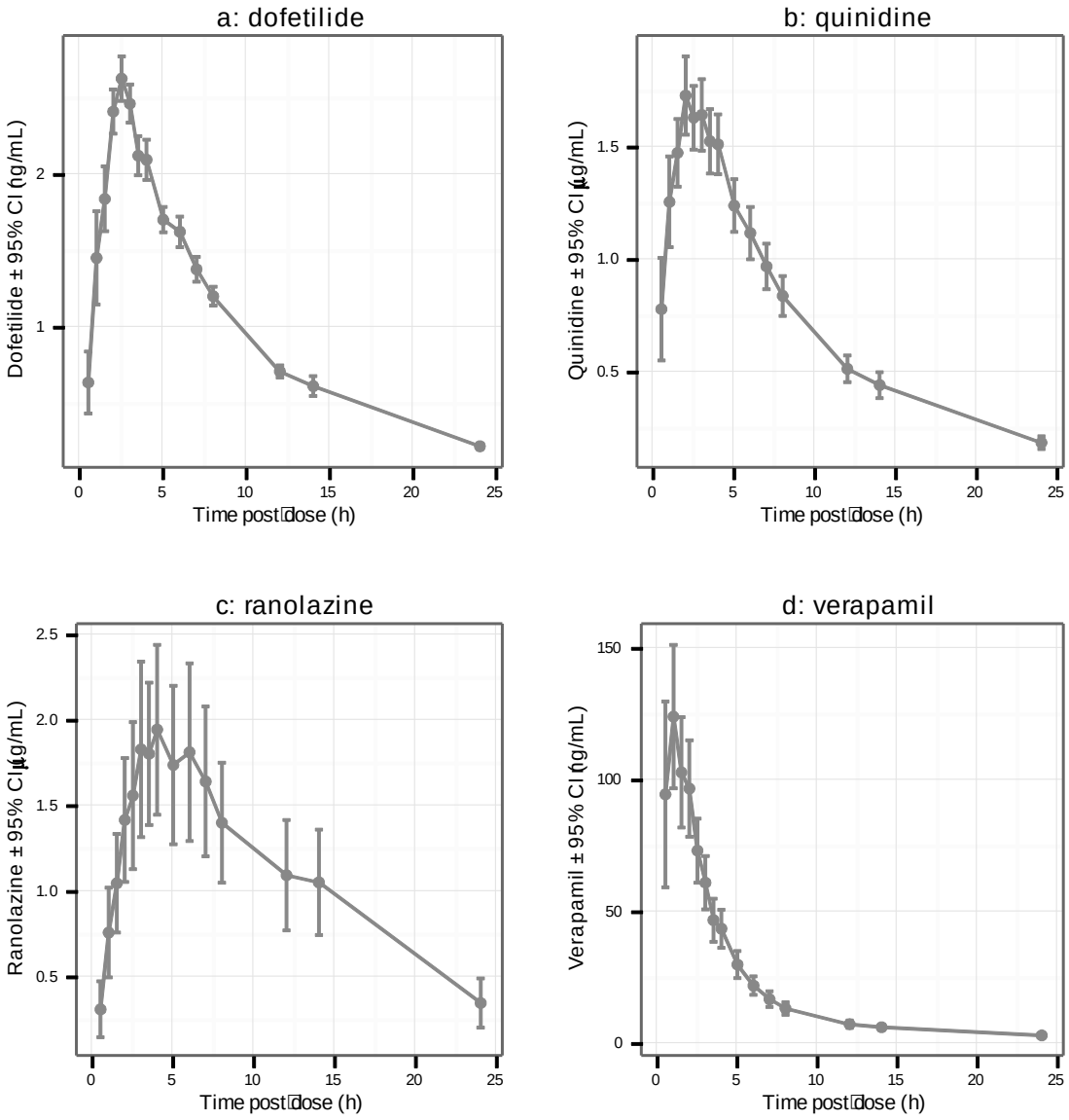

Figure 3

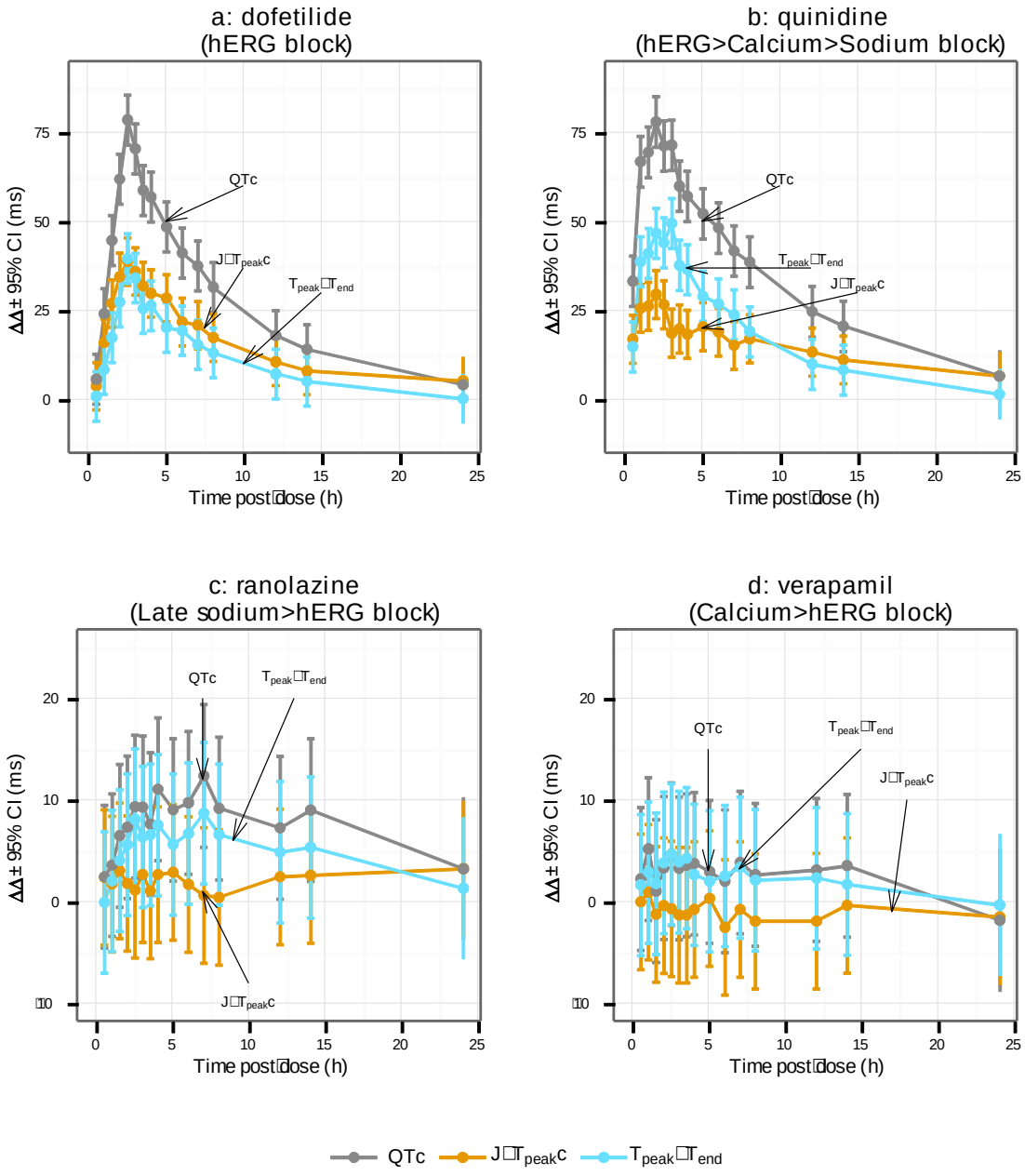

Figure 4

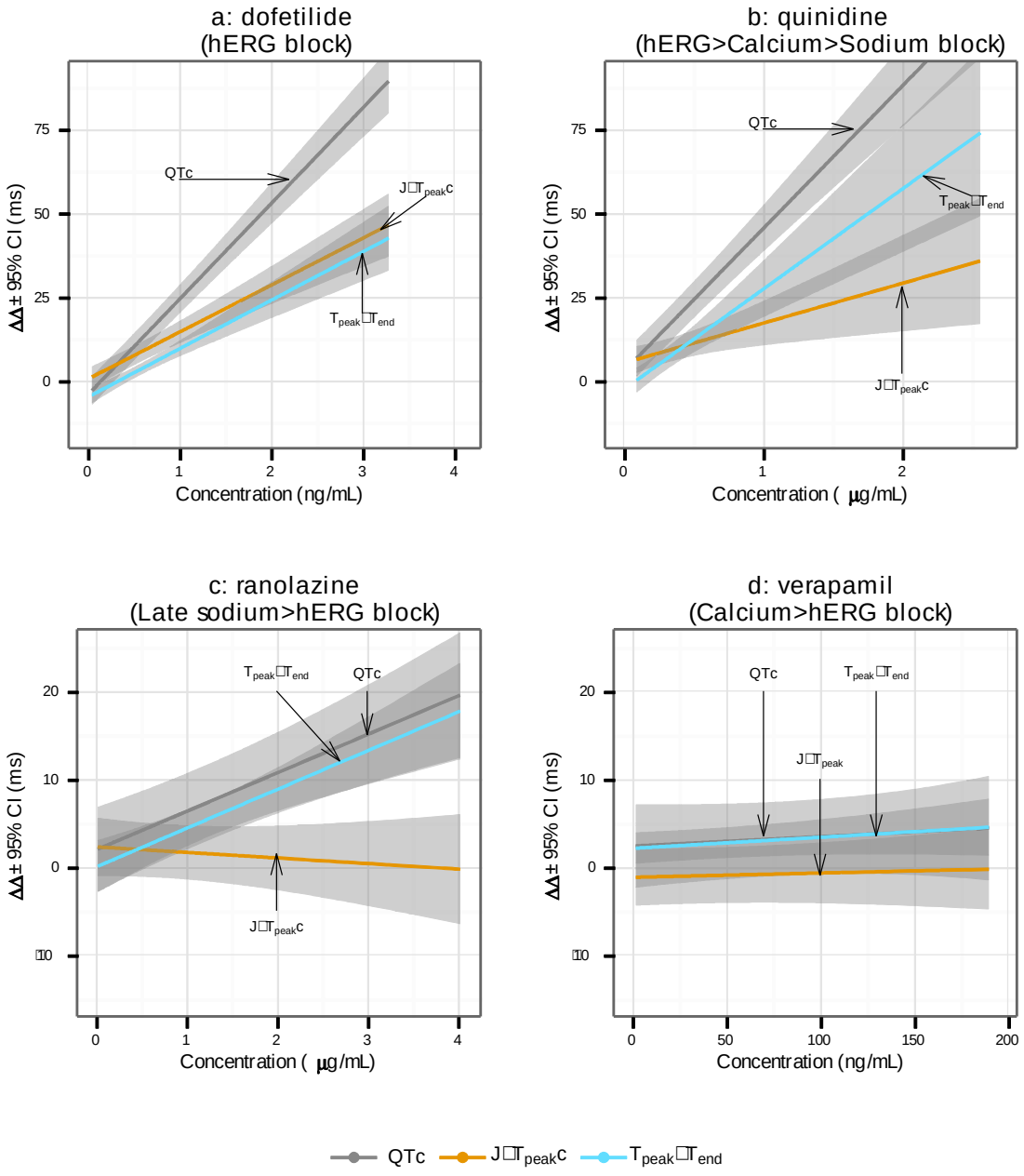

Figure 5

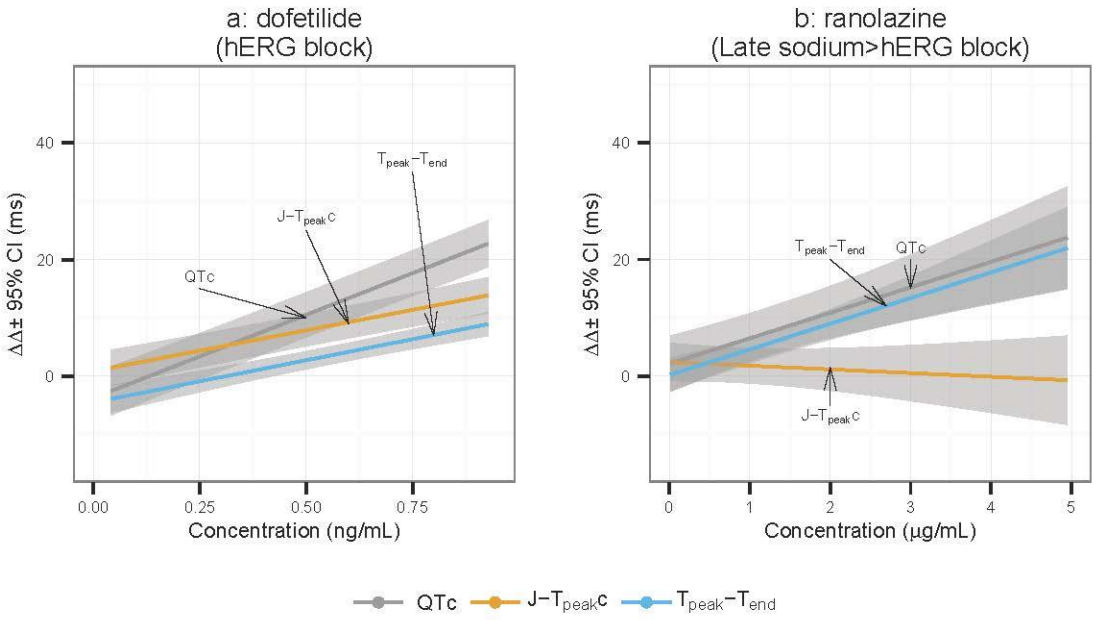

Figure 6