

Late Sodium Current Block for Drug-Induced Long QT Syndrome: Results From a Prospective Clinical Trial

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Drug-induced long QT syndrome has resulted in many drugs being withdrawn from the market. At the same time, the current regulatory paradigm for screening new drugs causing long QT syndrome is preventing drugs from reaching the market, sometimes inappropriately. In this study, we report the results of a first-of-a-kind clinical trial studying late sodium (mexiletine and lidocaine) and calcium (diltiazem) current blocking drugs to counteract the effects of hERG potassium channel blocking drugs (dofetilide and moxifloxacin). We demonstrate that both mexiletine and lidocaine substantially reduce heart-rate corrected QT (QTc) prolongation from dofetilide by 20 ms. Furthermore, all QTc shortening occurs in the heart-rate corrected J-T_{peak} (J-T_{peakc}) interval, the biomarker we identified as a sign of late sodium current block. This clinical trial demonstrates that late sodium blocking drugs can substantially reduce QTc prolongation from hERG potassium channel block and assessment of J-T_{peakc} may add value beyond only assessing QTc.

Study Highlights

• **WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?** hERG potassium channel blocking drugs that also block the late sodium current (e.g., ranolazine) have a low risk of torsade de pointes. A prior clinical trial demonstrated that ranolazine prolongs QTc and T_{peak}-T_{end} intervals, without J-T_{peakc} interval prolongation. • **WHAT QUESTION DOES THIS STUDY ADDRESS?** This study tested the hypothesis that two late sodium current blocking drugs (mexiletine and lidocaine) can shorten drug-induced QTc prolongation from dofetilide by shortening the J-T_{peakc} interval. • **WHAT THIS STUDY ADDS TO OUR KNOWLEDGE** This prospective clinical trial demonstrated that late sodium current block can substantially shorten drug-induced QTc prolongation. Furthermore, this study supports that the J-T_{peakc} interval is a biomarker of the balance between hERG potassium channel block (prolongs J-T_{peakc}) and late sodium current block (shortens J-T_{peakc}). • **HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS** Coadministration of late sodium current blocker can mitigate dofetilide-induced QTc prolongation and deserves further study in patients. In addition, the absence of J-T_{peakc} prolongation may be a sign of “benign” QTc prolongation.

Drug-induced QT prolongation increases the risk for torsade de pointes, a potentially fatal ventricular arrhythmia.¹ QT prolongation and increased risk for torsade de pointes have resulted in 14 drugs being removed from the market worldwide.² Furthermore, many drugs remain on the market with a known torsade de pointes risk, including numerous antibiotics, antimalarial, antiviral, psychiatric, oncology, and cardiac drugs.³ At the same time, the current regulatory paradigm for assessing drug effects on cardiac repolarization is preventing potentially effective medicines from reaching the market, sometimes inappropriately.² To address this, the US Food and Drug Administration (FDA) and multiple public-private partnerships are studying novel approaches

to assess the cardiac safety of new drugs with a Comprehensive *in vitro* Proarrhythmia Assay and in Phase 1 clinical trials.^{4,5} Essential to the novel approaches is a focus on understanding mechanisms by studying the effects of drugs on multiple cardiac ion channels, which can be either proarrhythmic or antiarrhythmic depending on the combination.⁶

Almost all drugs on the market that can cause torsade de pointes block the hERG potassium channel⁷ and prolong the QT interval of the electrocardiogram (ECG).⁸ However, some drugs block the hERG potassium channel and prolong QT with a minimal torsade de pointes risk (e.g., ranolazine,⁹ amiodarone¹⁰), likely because of additional block of inward currents,

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Received 14 May 2015; accepted 5 August 2015; advance online publication 11 August 2015. doi:10.1002/cpt.205

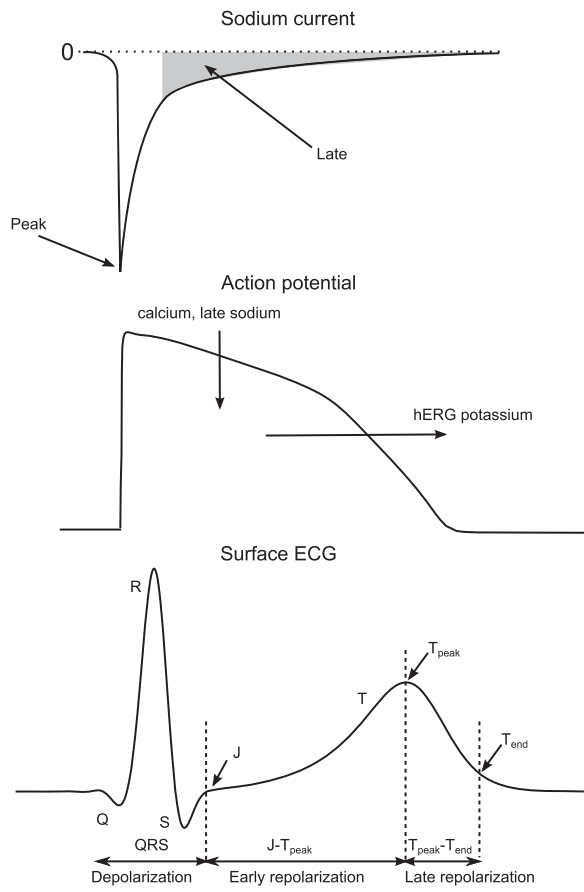


Figure 1 Late sodium current (shaded) correlates with the plateau of the action potential and early part of repolarization on the ECG, from J-point to peak of the T-wave.

such as the late sodium current or the L-type calcium current.¹¹ Preclinical studies have suggested that late sodium or calcium current block can shorten hERG potassium channel block-induced

action potential and QT prolongation and prevent torsade de pointes.^{12–15} However, species differences in cardiac ion channel expression exist and these preclinical observations have not yet been translated to drug-induced long QT syndrome in humans.¹⁶

In a prior retrospective analysis of 34 clinical trials and a prospective clinical trial of 4 individual drugs,^{17,18} we demonstrated that hERG potassium channel block prolongs both ECG early repolarization ($J-T_{peak}$, or corrected $J-T_{peak}$ ($J-T_{peakc}$) when corrected for heart rate) and late repolarization ($T_{peak}-T_{end}$), whereas additional late sodium or calcium current block shortens early repolarization ($J-T_{peakc}$; **Figure 1**). The prior studies were limited by the assessment of individual drugs in which the effects of combinations of drug-ion channel effects were inferred. Thus, we designed a first-of-a-kind Phase 1 clinical trial combined with a comprehensive preclinical assessment to assess the effects of drug combinations to dissect out the effects of single vs. multiple cardiac ion channel block.

The primary objective was to test the hypothesis that late sodium current blocking drugs (mexiletine or lidocaine) can attenuate the effect of hERG potassium current blocking drugs (dofetilide) on ventricular repolarization (QT or QTc when corrected for heart rate) by shortening $J-T_{peakc}$. The secondary objective was to assess the ability of a selective calcium current blocker (diltiazem) to reduce QTc prolongation associated with hERG potassium current block (moxifloxacin). In order to understand the mechanisms of our findings, we performed ion channel patch clamp experiments using overexpression cell lines and profiling of drug metabolites.

RESULTS

Clinical trial design

This Phase 1 clinical trial was designed as a pharmacokinetic-pharmacodynamic investigation of mexiletine combined with dofetilide, lidocaine combined with dofetilide, and diltiazem

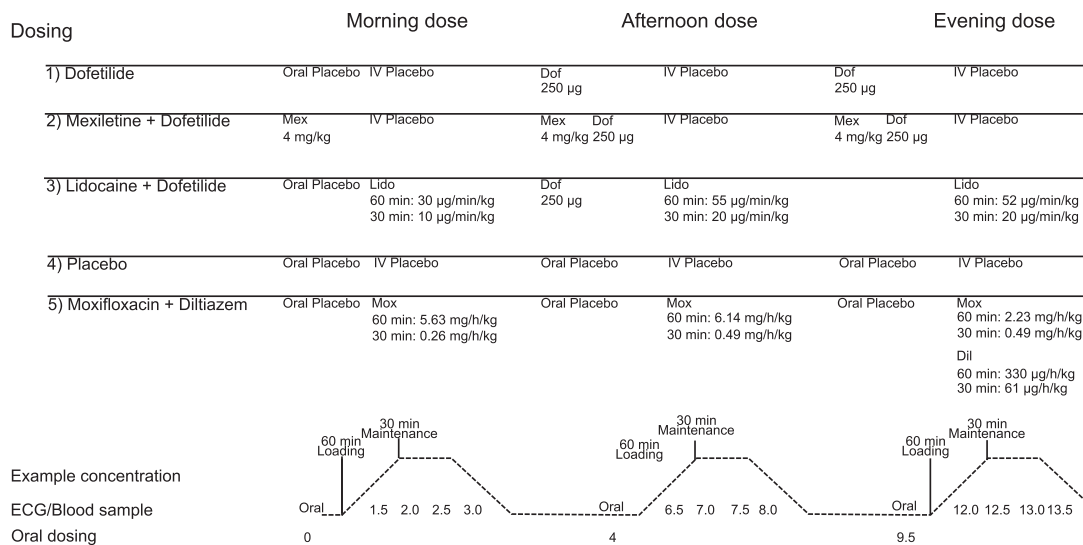


Figure 2 Morning, afternoon, and evening doses for each of the five treatment periods. Below the table, an illustration of the plasma drug level is shown to indicate when oral and intravenous dosing took place as well as when ECGs and plasma samples were taken (in hours after first oral dose). Dof, dofetilide; mex, mexiletine; lido, lidocaine; mox, moxifloxacin; dil, diltiazem.

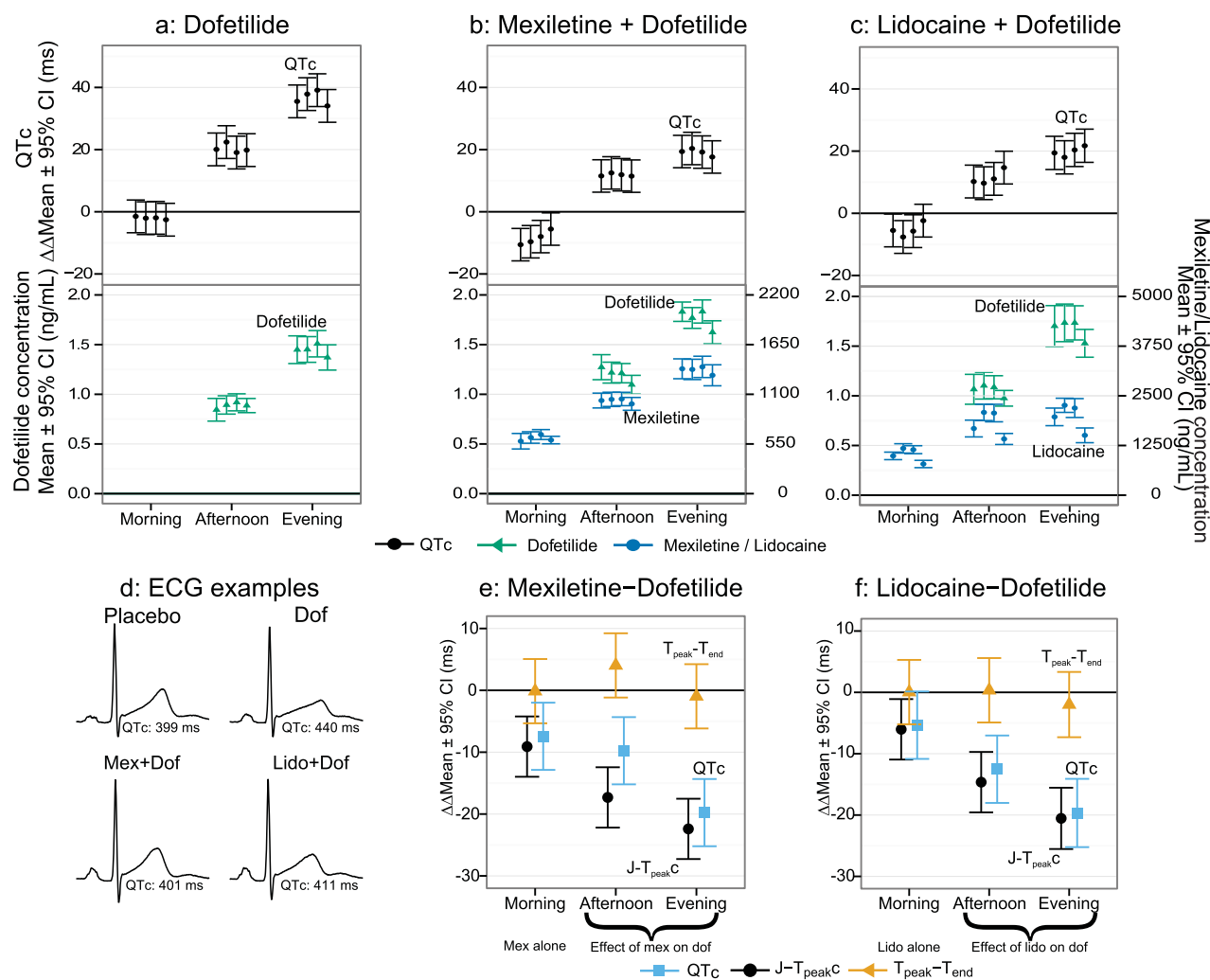


Figure 3 Changes shown in QTc for dofetilide alone (a), mexiletine combined with dofetilide (b), and lidocaine combined with dofetilide (c), while the lower part of the panels show the concentration of dofetilide (green) and mexiletine or lidocaine (blue) in their respective panels. The error bars represent 95% confidence intervals (CI). (d) Example ECGs from a subject during the evening dose of placebo, dofetilide, mexiletine combined with dofetilide, and lidocaine combined with dofetilide. The remaining bottom panels (e and f) show relative change compared to dofetilide alone for mexiletine combined with dofetilide (e) and lidocaine combined with dofetilide (f), where only the timepoint at the end of the loading dose is shown and points are shifted slightly for each interval to avoid overlap. Dof, dofetilide; mex, mexiletine; lido, lidocaine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

combined with moxifloxacin. The design was a five-period, randomized, crossover study with one week between treatment periods. In each treatment period, the subjects were dosed three times during the day (Figure 2) to allow for evaluation of the effects of low dose late sodium current block by itself, and of increasing levels of late sodium combined with hERG potassium channel block (see Figure 2 for dosing details). We were unable to combine diltiazem with dofetilide because of a pharmacokinetic interaction. Thus, we administered high-dose moxifloxacin (hERG potassium channel blocker) in the morning and afternoon doses and moxifloxacin combined with diltiazem in the evening dose.

The study included 22 healthy subjects (9 women) and their baseline characteristics are described in Table 1. The average age was (mean ± SD) 26.1 ± 4.9 years. The average weight was 69.9 ± 9.0 kg. Twenty subjects started the placebo treatment day, 20

the dofetilide alone day, 21 the mexiletine and dofetilide day, 19 the lidocaine and dofetilide day, and 20 the moxifloxacin and diltiazem day. No serious adverse events were observed, and the adverse events occurring at a frequency of >10% (dizziness, nausea, vomiting) are shown in Supplementary Table S1 by treatment day. There were three dropouts because of adverse events (Supplementary Figure S1).

Prolongation of QTc by dofetilide

Figure 3 shows the placebo and baseline corrected changes in the QTc interval along with plasma drug concentrations, whereas Figure 4 shows the concentration-dependent response. Dofetilide alone (Figure 3a) prolonged the QTc interval relative to placebo by 22.4 ms (P < 0.001) after the administration of the afternoon dose. After the evening dose of dofetilide, the QTc interval was prolonged by 39.1 ms (P < 0.001) and the plasma

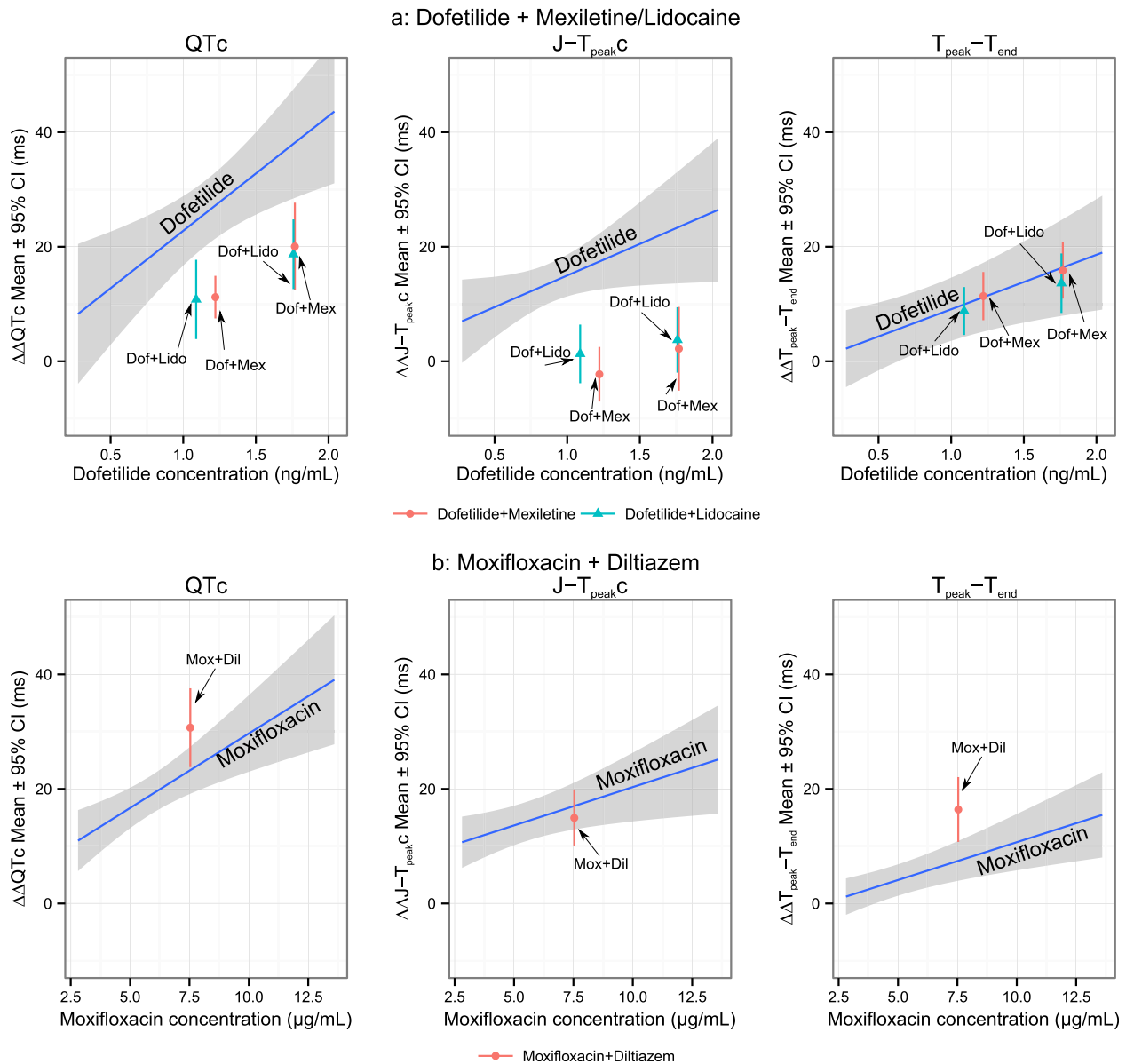


Figure 4 (a) The plasma drug concentration-dependent analysis for QTc, J-T_{peakc}, and T_{peak}-T_{end}, from left to right for dofetilide combined with mexiletine (red) or lidocaine (blue), and (b) for moxifloxacin and diltiazem. The solid line and shaded area reflects the fit of a linear model between drug concentration (dofetilide in panel a and moxifloxacin in panel b) and change in placebo and baseline-corrected change in each ECG interval. The points with error bars (95% confidence intervals (CIs)) reflect the maximum change when mexiletine is combined with dofetilide (red) or lidocaine is combined with dofetilide (blue) for panel a and moxifloxacin combined with diltiazem (red) in panel b. Dof, dofetilide; mex, mexiletine; lido, lidocaine; mox, moxifloxacin; dil, diltiazem. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

concentration was increased. Dofetilide prolonged both J-T_{peakc} and T_{peak}-T_{end} ($P < 0.001$ for all; **Figure 4a**). Based on our ion channel patch clamp experiments (**Figure 5a**), the dofetilide plasma concentration after the evening dose was associated with ~45% hERG potassium channel block.

Shortening of the QTc interval by mexiletine and lidocaine

The first dose of mexiletine alone shortened QTc by 10.6 ms ($P < 0.001$) relative to placebo (**Figure 3b**), whereas the first dose of lidocaine alone shortened QTc by 7.6 ms ($P = 0.005$; **Figure 3c**). Both mexiletine and lidocaine alone shortened the J-T_{peakc} interval, but not T_{peak}-T_{end} (**Figures 3e and 3f**).

The afternoon dose was either the combination of mexiletine and dofetilide, or lidocaine and dofetilide. When compared to the dofetilide alone day in the afternoon, both mexiletine (-9.8 ms; $P < 0.001$; **Figure 3e**) and lidocaine (-12.5 ms; $P < 0.001$; **Figure 3f**) shortened QTc when coadministered with dofetilide. This QTc shortening increased further after the evening dose, which was also associated with higher concentrations of mexiletine and lidocaine (**Figure 3e and 3f**). When compared to the dofetilide alone day in the evening, both mexiletine (-19.8 ms; 95% confidence interval: -25.2 to -14.3; $P < 0.001$) and lidocaine (-19.7 (-25.2 to -14.1) ms; $P < 0.001$) significantly shortened QTc after the evening dose (**Figures 3e and 3f**), which was the

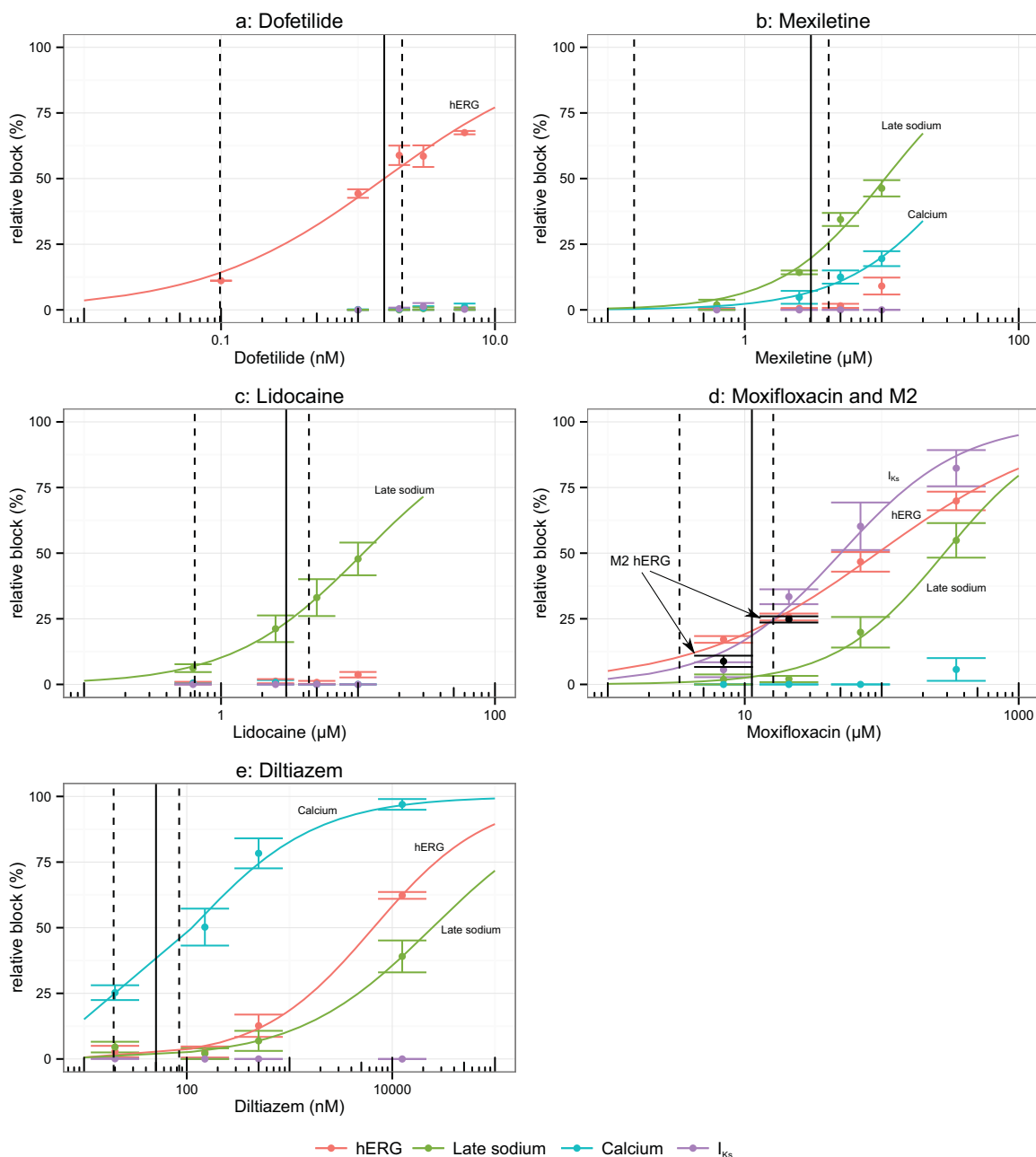


Figure 5 The results of the patch clamp experiments for dofetilide (a), mexiletine (b), lidocaine (c), moxifloxacin and M2 metabolite (d), and diltiazem (e) for hERG (red), late sodium (green), calcium (blue), and I_{Ks} (purple). The lines in each plot correspond to a fit between the measured relative reduction in current and drug concentration. The error bars denote \pm SE. The vertical dashed lines in each panel correspond to the range of observed clinical plasma concentrations, corrected for protein binding, and the solid line is the population average maximum plasma concentration in the clinical trial. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

prespecified primary endpoint of the study. An ECG example of the effects of mexiletine and lidocaine on drug-induced QTc prolongation is shown in **Figure 3d**. Our ion channel patch clamp experiments (**Figures 5b** and **5c**) suggest that both mexiletine and lidocaine have ~20% late sodium current block at the concentrations that caused ~20 ms shortening of QTc.

For both mexiletine and lidocaine, the shortening of the QTc interval was entirely of the J-T_{peak}c interval (**Figure 3e** and **3f**). Specifically, after the evening dose of mexiletine and dofetilide, the J-T_{peak}c interval was shortened (-23.2 ms; $P < 0.001$),

whereas no change in the T_{peak}-T_{end} interval was observed ($P = 0.56$; **Figure 3e**). Similarly, lidocaine shortened the J-T_{peak}c interval (-20.5 ms; $P < 0.001$) without changing the T_{peak}-T_{end} interval ($P = 0.13$; **Figure 3f**). Of note, the measured dofetilide concentrations were higher in the mexiletine-dofetilide arm compared to dofetilide alone (1.8 ± 0.3 vs. 1.5 ± 0.3 ng/mL; $P < 0.001$), whereas the dofetilide concentrations in the lidocaine-dofetilide arm trended toward greater dofetilide concentrations (1.8 ± 0.4 vs. 1.5 ± 0.3 ng/mL; $P = 0.068$). The findings of the time-dependent analysis are consistent with the

concentration-dependent analysis shown in **Figure 4**, where each panel shows the effect of increasing dofetilide concentration on QTc, J-T_{peakc}, and T_{peak}-T_{end} alone, compared to the changes when dofetilide was coadministered with mexiletine or lidocaine. This is similar to the exposure-response relationship for ranolazine, a drug that blocks the hERG potassium channel and the late sodium current. In our prior study, ranolazine caused concentration-dependent prolongation of QTc and T_{peak}-T_{end}, but not J-T_{peakc}.¹⁷ Thus, prolongation of QTc and T_{peak}-T_{end} without prolongation of J-T_{peakc} suggests the presence of multi-channel block, where the J-T_{peakc} interval is a balance of inward and outward current.

Effects of dofetilide, mexiletine, and lidocaine on heart rate and other ECG intervals are shown in **Supplementary Figure S2**. There were no changes in PR, QRS, or heart rate after administration of dofetilide alone (**Supplementary Figure S2**). Mexiletine and lidocaine were associated with small increases in heart rate (4.4 bpm; $P = 0.002$ and 3.1 bpm; $P = 0.033$, respectively). Notably, there was no effect of either drug on QRS duration, suggesting a lack of significant peak sodium current effects at resting heart rates.

Combined effects of moxifloxacin and diltiazem

The morning dose of moxifloxacin prolonged the QTc interval relative to placebo (20.2 ms; $P < 0.001$; **Supplementary Figure S3**). The afternoon dose further prolonged QTc relative to placebo (29.9 ms; $P < 0.001$) and was associated with a higher moxifloxacin plasma concentration, which exhibits ~20% hERG potassium channel block (**Figure 5d**). However, after the evening dose, QTc remained prolonged (31.3 ms; $P < 0.001$) despite a slightly lower plasma concentration of moxifloxacin and coadministration of diltiazem (**Supplementary Figure S3**), which was associated with ~35% calcium channel block (**Figure 5e**). Comparing the evening to the afternoon dose, QTc and T_{peak}-T_{end} were prolonged, whereas J-T_{peakc} was shortened (**Supplementary Figure S3**). To account for the higher moxifloxacin plasma concentration in the afternoon compared to the evening ($9.5 \pm 1.7 \mu\text{g/mL}$ vs. $8.0 \pm 1.7 \mu\text{g/mL}$; $P = 0.008$), we conducted a concentration-dependent analysis (**Figure 4b**). However, this analysis did not support diltiazem shortening moxifloxacin-induced QTc prolongation. Of note, diltiazem was associated with a ~20 ms PR prolongation (**Supplementary Figure S2**), suggesting that the plasma concentration of diltiazem was associated with sufficient calcium channel block to cause slowed conduction through the atrioventricular node. Interestingly, the results of our patch clamp analysis also suggest that moxifloxacin blocks the KvLQT1/minK potassium channel (I_{Ks} current; **Figure 5d**), the effect of which could be enhanced by the autonomic response resulting from the blood pressure drop associated with diltiazem administration.^{19,20} It is possible that this contributed to the lack of QTc shortening when diltiazem was added to moxifloxacin.

hERG potassium channel blocking effects of moxifloxacin metabolite

An alternative for the unexpected results from moxifloxacin-diltiazem could be due to the accumulation of a moxifloxacin

metabolite that exhibits hERG potassium channel block. The moxifloxacin glucuronide metabolite (M2) has been reported to reach plasma concentrations 40% of moxifloxacin and is only 5% protein bound, as opposed to 39% protein binding of moxifloxacin.²¹ We assessed the relative potency of the hERG potassium channel block of this metabolite and observed that the metabolite blocks hERG to a degree similar to that of the parent drug (**Figure 5d**). We profiled metabolites from five subjects and determined that the relative percentage of the M2 metabolite tended to increase during subsequent dosing (median: 14.1% vs. 20.9%; $n = 5$; $P = 0.06$). This suggests that the repeat intravenous moxifloxacin administration throughout the day led to an accumulation of an hERG potassium channel blocking moxifloxacin metabolite, thus confounding the diltiazem analysis.

DISCUSSION

This study highlights how a comprehensive assessment of drug effects on multiple cardiac ion channels through translational mechanistic-based approaches has the potential to improve the cardiac safety assessment of new drugs. In addition, this study provides an assessment in healthy volunteers of a therapy for drug-induced long QT syndrome and demonstrates that block of the late sodium current by mexiletine and lidocaine can have a substantial effect on cardiac repolarization in humans. Furthermore, this study provides substantial evidence that the J-T_{peakc} interval is a biomarker of the balance between hERG potassium channel block (prolongs J-T_{peakc}) and late sodium current block (shortens J-T_{peakc}). The combinations of dofetilide with mexiletine and dofetilide with lidocaine reproduced the ECG signature of ranolazine: QTc and T_{peak}-T_{end} prolongation with no net effect on the J-T_{peakc} interval. Thus, we hypothesize that this is a sign of “benign” QTc prolongation because of a balance of hERG and late sodium current block.

Interest in the late sodium current increased in the 1990s when it was discovered that patients with congenital long QT type 3 (LQT3) had prolonged QTc because of increased late sodium current.²² Two of the drugs initially tested were the Vaughan-Williams class Ib antiarrhythmics lidocaine and mexiletine.^{23,24} Although mexiletine and lidocaine block the peak sodium current at very high concentrations, at clinical concentrations, they preferentially block the late sodium current.^{25,26} This is consistent with the observations in this study where they produced 20 ms of QTc shortening (evidence for late sodium current block) while having no effect on QRS duration (evidence for absence of significant peak sodium current block).

In addition to showing antiarrhythmic efficacy in preclinical models of congenital LQT3,^{15,22} mexiletine was also shown to have antiarrhythmic efficacy in models of LQT2 (decreased hERG potassium current),²⁷ which is most relevant to drug-induced long QT syndrome. A clinical study of mexiletine found a statistically significant decrease in QTc in patients with LQT3 but not in patients with LQT2; however, only six patients with LQT2 were studied.²⁴ The present study demonstrates that both mexiletine and lidocaine can shorten QTc prolongation because of dofetilide, which is widely accepted to be a selective hERG potassium channel blocker.

A recent preclinical study found that chronic exposure to dofetilide for five or more hours may increase late sodium current.²⁸ In the present study, the primary ECG analysis was performed eight hours after the first dofetilide dose. It is therefore possible that part of the QTc shortening by mexiletine and lidocaine was from blocking an increase in the late sodium current induced by dofetilide. However, even before dofetilide administration, late sodium current block alone caused QTc shortening.

Although this is the first known clinical trial combining a late sodium current blocker with a selective hERG potassium channel blocker, small patient studies were performed in the 1980s combining mexiletine (Vaughan-Williams class Ib antiarrhythmic) with quinidine (Vaughan-Williams class Ia antiarrhythmic).^{27,29,30} Quinidine is a strong hERG potassium channel blocker, and with chronic therapy it was observed that mexiletine shortened the quinidine-induced QTc prolongation.²⁷ In addition, an *in vivo* study of chronic atrioventricular-blocked dogs demonstrated that mexiletine reduced sotalol-induced QTc prolongation and the number of torsade de pointes events.¹³ Specifically, torsade de pointes occurred in six of eight dogs receiving sotalol alone, but in only one of eight dogs receiving the combination of mexiletine and sotalol. Other preclinical studies have demonstrated that late sodium current block can prevent dispersion of repolarization (a substrate for torsade de pointes) and prevent early after depolarizations (a trigger for torsade de pointes) that is caused by drug-induced hERG potassium channel block.^{10,15} Although these data suggest that Vaughan-Williams class Ib antiarrhythmics, such as mexiletine or lidocaine, may reduce the risk for torsade de pointes associated with hERG potassium channel block, it should be noted that some preclinical studies suggest that lidocaine may increase conduction delay and heterogeneity in ischemic tissue.^{31–33}

Despite no known interaction between the pharmacokinetics of dofetilide and mexiletine, an increase in the observed maximum concentration of dofetilide after coadministration with mexiletine was observed. It is not known if this is a chance finding, as the study was not designed to evaluate this, but it highlights the importance of evaluating pharmacokinetic interactions when using a late sodium current blocker like mexiletine to counter drug-induced QTc prolongation.

We did not observe a reduction in QTc prolongation when diltiazem was administered with moxifloxacin. The addition of diltiazem was associated with a small shortening of J-T_{peakc}; however, there was also a small prolongation of T_{peak}-T_{end} and QTc. This finding was, in part, confounded by a decrease in the moxifloxacin concentration in the evening, but the concentration-dependent analysis showed a similar pattern. The lack of shortening of the J-T_{peakc} interval could potentially be due to the hERG potassium channel blocking moxifloxacin metabolite M2 accumulating during the day and reaching higher plasma levels when diltiazem was coadministered. Another possible explanation for the lack of QTc shortening with diltiazem is moxifloxacin-induced block of the I_{Ks} current. Although the effect of the I_{Ks} current is minimal in the absence of autonomic stimulation,²⁰ an autonomic response triggered by diltiazem reducing the blood pressure could increase the effect of I_{Ks} current block.¹⁹

Recently, there have been two efforts aimed at improving assessment of drug-induced proarrhythmic risk. A preclinical effort, the Comprehensive *in vitro* Proarrhythmia Assay, combines assessment of multiple cardiac ion channel currents using patch clamp studies integrated into *in silico* models and confirmed with experiments in induced pluripotent stem cell derived cardiomyocytes.⁴ The other effort focuses on assessment of QTc prolongation using pharmacokinetic-pharmacodynamic modeling in Phase I clinical studies.⁵ The findings of this clinical study, and our previous study,^{17,18} show that electrocardiographic biomarkers J-T_{peakc} and T_{peak}-T_{end} could be used to confirm multichannel effects, for example, prolongation of the QTc interval with minimal or no prolongation of J-T_{peakc} is likely an indicator of the presence of a multichannel block.

Limitations

Reducing drug-induced QTc prolongation does not necessarily equate to preventing drug-induced torsade de pointes. However, the combination of dofetilide and mexiletine or lidocaine recreates the ECG signature we observed in our prior clinical trial with ranolazine,¹⁷ a QTc prolonging drug with a low torsade de pointes risk. Future studies will be required to assess whether coadministration of a late sodium blocking drug with an hERG potassium channel blocker can reduce arrhythmic events, such as torsade de pointes without affecting therapeutic efficacy. The similar pharmacokinetic profiles among oral mexiletine, dofetilide, and sotalol suggest that this could be tested. In addition, new late sodium current blockers are in development.¹⁴

CONCLUSION

This study demonstrates that late sodium current block can reduce QTc prolongation associated with hERG potassium channel block. These findings suggest that coadministration of a late sodium current blocker can reduce drug-induced QTc prolongation in patients. Whether the reduction of drug-induced QTc prolongation in patients results in a lowered risk for torsade de pointes remains to be studied, but the minimal-to-no risk for torsade de pointes with ranolazine suggests that it might. This study also demonstrates that shortening of the J-T_{peakc} interval is an ECG sign of late sodium current block. J-T_{peakc} is the only biomarker that represents a balance of inward and outward ion channel currents.³⁴ Additional biomarkers beyond QTc (e.g., J-T_{peakc}) could be applied in Phase I clinical trials using concentration-response modeling *in lieu* of dedicated thorough QT studies.

METHODS

This study was approved by the FDA Research Involving Human Subjects Committee and the local institutional review board. All subjects gave written informed consent and the study was performed at a Phase I clinic (Spaulding Clinical, West Bend, WI).

Clinical trial design

The design was a prospective randomized crossover study with one week between treatment periods. It included typical inclusion and exclusion criteria for dedicated QT studies.³⁵ Healthy subjects between 18 and 35 years of age, weighing between 50 and 85 kg, and without any family history of cardiovascular disease or unexplained sudden cardiac death were

eligible for participation in the study. In addition, the subjects had to have <12 ventricular ectopic beats during a three hour continuous recording at screening, as well as a baseline QTc of <430 ms, using Fridericia correction.³⁶

In each of the five treatment periods, the subjects were dosed three times during the day (Figure 2). Dofetilide (Tikosyn, Pfizer, New York, NY) and mexiletine (Teva Pharmaceuticals USA, North Wales, PA) were administered orally immediately after meals, whereas lidocaine (B. Braun Medical, Bethlehem, PA) was administered intravenously (see Figure 2 for dosing details). The doses of mexiletine and lidocaine were chosen to target plasma levels from prior studies.^{23,27} Similarly, the dofetilide dose was chosen based on prior experience.¹⁷ Moxifloxacin doses were chosen to achieve QTc prolongation of at least 20 ms, while maintaining a total daily dose within what has been studied previously. Last, the diltiazem dose was chosen to target the highest dose in the FDA label.

Intravenous infusions were split into a 60-minute loading dose followed by a 30-minute maintenance dose. The primary pharmacokinetic samples were taken at the start and end of each of the three maintenance doses during the day, as these were expected to be associated with highest plasma concentrations of both the oral and intravenously administered drugs. Additional pharmacokinetic samples were taken at 30 and 120 minutes poststart of intravenous dosing during each of the three dosing periods during the day.

ECG measurement methodology

Continuous ECG recordings were performed using the Mortara Surveyor system (Mortara, Milwaukee, WI) sampled at 500 Hz with an amplitude resolution of 2.5 μV. From the continuous recording, three ECGs were extracted before the draw of each pharmacokinetic sample, based on heart rate stability and signal quality.³⁷

Semi-automated measurements of the QRS, J-T_{peak}, T_{peak}-T_{end}, and QT interval were performed using the derived vector magnitude lead, as previously described (see **Supplementary Methods**).¹⁷ The QT interval was corrected for heart rate using Fridericia’s correction ($QTc = QT / (RR/1,000 \text{ ms})^{1/3}$, with RR in ms).³⁶ Similarly, the J-T_{peak} interval was corrected for heart rate ($J-T_{peakC} = J-T_{peak} / (RR/1000 \text{ ms})^{0.58}$, with RR in ms).¹⁸ No correction was performed for the T_{peak}-T_{end} interval, as prior studies have shown a lack of rate dependence at resting heart rates.^{18,38} Additionally, the global PR interval was measured automatically (AMPS LLC, New York, NY).

Pharmacokinetic sample analysis

Quantification of drug concentration in plasma was performed using a validated liquid chromatography with tandem mass spectroscopy method by Frontage Laboratories (Exton, PA). In addition, the relative abundance of parent moxifloxacin to the M2 metabolite was determined for five subjects. The pharmacokinetic sample analysis is described in further detail in the **Supplementary Methods**.

Patch clamp experiments

Stably transfected hERG, Nav1.5, KvLQT1/minK (HEK-293) or Cav1.2 cells (CHO) from Cytocentrics Biosciences (Rostock, Germany) were used for whole-cell patch-clamp measurements to determine reduction in hERG, late sodium, L-type calcium, and I_{Ks} currents for all five drugs (dofetilide, lidocaine, mexiletine, moxifloxacin, and diltiazem); see **Supplementary Methods** for a more detailed description and **Supplementary Figure S4** for examples of current traces.³⁹ In addition, the effect of the moxifloxacin M2 metabolite on hERG was assessed. All currents were elicited using a ventricular action potential waveform (pacing rate = 0.1 Hz) at physiologic temperature (36 ± 1°C).

Statistical methods

The primary endpoint of the clinical trial was the reduction of dofetilide-induced prolonged QTc by either mexiletine or lidocaine using a linear mixed-effects model with time, treatment, sequence, and period

Table 1 Demographics

Demographics	All subjects (N = 22)
Age, years ^a	26.1 ± 4.9
Female	9 (41%)
Race	
White	10 (45%)
Black or African American	10 (45%)
American Indian/Alaska Native	1 (4.5%)
Asian	1 (4.5%)
Hispanic or Latino	2 (9%)
Weight, kg	69.9 ± 9.0
Vital signs	
Systolic blood pressure, mm Hg	109.5 ± 5.5
Diastolic blood pressure, mm Hg	60.2 ± 3.5
Heart rate, bpm	61.3 ± 6.7
ECG	
PR, ms	160.8 ± 19.1
QRS, ms	86.7 ± 8.5
J-T _{peakC} , ms	229.5 ± 19.0
T _{peak} -T _{end} , ms	81.9 ± 6.4
QTc, ms	397.8 ± 14.2

ECG, electrocardiogram; J-T_{peakC}, heart-rate corrected J-T_{peak}; QTc, heart-rate corrected QT.

^aContinuous variables are listed as mean ± SD.

as fixed effects, as well as an interaction between treatment and time, and a random intercept per subject. The analysis was performed using SAS 9.3 (SAS Institute, Cary, NC). The prespecified analysis only included the two center time points (60 and 90 minutes poststart of infusion) for the evening dose (highest expected plasma concentration of all drugs). All data before withdrawals was included in the analysis. For the secondary endpoint of diltiazem combined with moxifloxacin, a similar analysis was performed, except it involved comparing the evening timepoints (moxifloxacin and diltiazem) compared to the afternoon timepoints (moxifloxacin alone). Adjustment for multiple comparisons was performed according to the Bonferroni method (i.e., a significance level of 0.025 for the primary analysis). All other by-time analyses were carried out using a similar approach, but were not further adjusted for multiplicity, and should be interpreted with an appropriate level of caution.

In addition, a linear mixed-effects model was used to explore the concentration dependency of change in QTc, J-T_{peakC}, and T_{peak}-T_{end} with concentration as a fixed effect and subject as a random effect on intercept and concentration. Differences in maximum concentrations were evaluated using an independent *t* test with equal variance. Wilcoxon sign test was used to evaluate the difference between relative abundance of moxifloxacin M2 metabolite relative to the parent between the morning and evening dose.

Ion channel patch clamp results are presented as percentage of reduction of current amplitude, which was measured as current reduction after a steady-state effect had been reached in the presence of drug relative to current amplitude before drug was introduced (control). Each cell served as its own control.

Additional Supporting Information may be found in the online version of this article.

ACKNOWLEDGMENTS

We would like to thank staff at Spaulding Clinical Research for executing the clinical study (NCT02308748). 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.

SOURCE OF FUNDING

This project was supported by the FDA Critical Path Initiative, Office of Women's Health, and 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.

AUTHOR CONTRIBUTIONS

All authors have contributed as follows: protocol development (L.J., J.V., J.W.M., C.S., K.W.L., J.F., N.S., D.G.S.), data collection (C.E., C.S., K.W.L., M.H., J.L., P.G., A.M., J.W., W.J.C.), data analysis (L.J., J.V., J.F., D.G.S.), and preparing the manuscript (L.J., D.G.S.). All authors discussed the results and implications and commented on the manuscript.

CONFLICT OF INTEREST

J.W.M., K.W.L., C.S., and C.E. are employees of Spaulding Clinical Research, M.H., P.G., J.L., A.M., and J.W. are employees of Frontage Laboratories, and W.J.C. is an employee of Zenas Technologies LLC, which are contract research organizations.

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