Investigation of potential mechanisms of sex differences in quinidine-induced Torsade de Pointes risk

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Brief title: Sex differences in quinidine-induced ECG changes

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Conflicts of interest: none

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Abstract

Introduction: The electrocardiographic index $T_{\text{peak}}-T_{\text{end}}$ has been proposed as a marker of dispersion of repolarization and may be a stronger predictor of torsade de pointes risk than QTc prolongation.

Methods and results: We assessed whether quinidine-induced $T_{\text{peak}}-T_{\text{end}}$ prolongation is greater in women than men. The relationship between QTc prolongation and quinidine concentration was greater in women than men (38 ± 10 vs. 28 ± 9 ms/ $\mu\text{g/ml}$, $p=0.02$), but there was no difference for $T_{\text{peak}}-T_{\text{end}}$ prolongation (39 ± 13 vs. 32 ± 13 ms/ $\mu\text{g/ml}$, $p=0.21$). There was a delay (hysteresis) between peak concentration and both maximum QTc and $T_{\text{peak}}-T_{\text{end}}$ prolongation and a trend toward higher serum quinidine concentration in men than women. Analysis controlling for hysteresis showed no sex difference for QTc (55 ± 18 vs. 43 ± 19 ms/ $\mu\text{g/ml}$, $p=0.14$), without changing the lack of sex difference with $T_{\text{peak}}-T_{\text{end}}$ (61 ± 22 vs. 55 ± 21 ms/ $\mu\text{g/ml}$, $p=0.49$).

Conclusions: Women do not have a greater quinidine-induced $T_{\text{peak}}-T_{\text{end}}$ prolongation than men. Sex differences in hysteresis and serum quinidine concentration in this study may have contributed to sex differences in quinidine-induced QTc prolongation.

Keywords: QTc prolongation; quinidine; torsade de pointes; sex differences; T wave notching

1 Introduction

2 Torsade de pointes (torsade) is a potentially fatal polymorphic ventricular tachycardia
3 characterized by a cyclic shifting of the QRS axis [1]. It is known that both cardiac and non-
4 cardiac drugs, including antiarrhythmics, antibiotics, antidepressants, antipsychotics and
5 antiemetics can cause torsade [2] and that women have a higher risk of developing drug-induced
6 torsade [3]. All drugs that have been removed from the market due to high risk of developing
7 torsade also had a blocking effect on the human *ether a gogo* related (hERG) potassium channel
8 [4].

9 The incidence of torsade is difficult to estimate because it is often a transient, rare event
10 that requires an ECG recording for diagnosis. However, many patients who experience syncope
11 or sudden cardiac death do not have an ECG recording at the time of the event making diagnosis
12 of torsade difficult if not impossible. In the U.S. >400,000 people die every year from sudden
13 cardiac death and 10 – 20 % have no evidence of structural heart disease [5]. As women 35 – 44
14 years old are a subgroup with an increased incidence of sudden cardiac death [5], it is a priority
15 to further understand the underlying mechanisms of sudden cardiac death due to torsade in
16 women.

17 Prior studies have suggested that the time from the peak to the end of the T wave ($T_{\text{peak}} -$
18 T_{end}) is a marker of dispersion of repolarization [6-8] and may be a stronger predictor of
19 arrhythmic risk than prolongation of the heart-rate corrected QT interval (QTc) alone [9]. In
20 addition, we previously showed that $T_{\text{peak}} - T_{\text{end}}$ is a specific marker of hERG potassium channel
21 block [10]. Quinidine was the first drug recognized to prolong QTc and cause torsade [11]. It has
22 previously been demonstrated that quinidine-induced QTc prolongation is greater in women than
23 men [12]; however that study did not examine possible sex differences in $T_{\text{peak}} - T_{\text{end}}$.

Determination of sex differences in the individual components of the T wave could provide insight into the mechanism of sex differences in torsade risk. The aim of this study was to test the hypothesis that quinidine-induced $T_{\text{peak}}-T_{\text{end}}$ prolongation is greater in women than men.

Methods

This study population has been previously described [12]. Briefly, 24 healthy nonsmoking volunteers (12 women, 12 men) between 18-35 years old participated in the study. The protocol was a randomized, single-blinded comparison of single doses of intravenous quinidine gluconate (4 mg/kg of base, Eli Lilly, Indianapolis, Indiana, USA) and matching intravenous placebo (saline). Quinidine gluconate (80 mg/ml) was diluted to a total volume of 20 ml with saline solution and infused over 20 minutes. Blood was collected through an intravenous catheter into plain vacutainer tubes from the opposite arm of the infusion at 28 nominal time points postdose. The samples were prepared for quantification of quinidine and 3-hydroxyquinidine and non-protein bound (free) concentrations were determined by ultrafiltration and high performance liquid chromatography, as previously described [12].

ECG acquisition and analysis

Digital 12-lead ECGs were recorded with a MacVu ECG machine (Marquette Electronics, Marquette, WI) immediately before the collection of each blood sample. There were 2 visits per subject, intravenous placebo and intravenous quinidine. Each visit had 28 nominal time points, with the last time point being 12 hours postdose, where ECGs were acquired for 10 seconds [12].

ECGs were analyzed independently from the prior analysis reported by Benton et al. [12]. Two independent ECG readers blinded to treatment and sex assessed each 10 second ECG using

semi-automated ECG analysis software [13]. Briefly, ECGs with missing leads or with excessive low- and high-frequency noise were discarded. QRS complexes were detected, baseline wander was corrected and a representative median beat was computed. Measurements were made in lead V5, as $T_{\text{peak}}-T_{\text{end}}$ in V5 has been described previously as a good predictor of torsade [9]. Of the 24 enrolled subjects, one subject was excluded from analysis due to a short PR interval of approximately 100 ms.

The majority of subjects developed T wave notching, also known as bifid waves or humps, after administration of quinidine. T wave notching includes both biphasic T waves and two positive peaks with a nadir in between. If there were two T peaks, T_{peak} was selected as the first peak maximum [6, 14]. If any maximum or minimum was in the form of a plateau, annotations were placed at the earliest point of the plateau. In order to minimize intra-observer variability, ECGs for each patient (56 ECGs each) were evaluated in one sitting and the ECGs were viewed in chronological order to better differentiate T wave notches versus T-U waves. Disagreements on a T wave being measureable, presence of a notch, or a difference of more than 5 ms in either T_{peak} or T_{end} were re-reviewed and adjudicated by an expert ECG reader.

QT intervals were heart rate corrected by the method of Frederica ($QT_C = QT / ((RR/1000 \text{ ms})^{1/3})$, where RR is in ms). Smetana et al. showed that $T_{\text{peak}}-T_{\text{end}}$ is rate dependent and differs between gender at shorter RR-intervals (faster heart rate) [15]. In our calculations for $T_{\text{peak}}-T_{\text{end}}$, we did not correct for heart rate, as the heart rate in this study was between 62-78 bpm and this range of heart rates is not associated with a significant change in the $T_{\text{peak}}-T_{\text{end}}$ interval [15].

Statistical analysis

The relationships between the serum quinidine concentration and change in QT_C and $T_{\text{peak}}-T_{\text{end}}$ were evaluated using two approaches: analysis of the direct relationship between

serum quinidine concentrations and ECG effect and using an effect-compartment model, using Nonmem 7.3 (ICON plc, Dublin, Ireland) [12, 16]. Prior work by Holford et al. [16], has shown that the delayed effect of quinidine can be modeled by using a sequential approach. In the first step of the sequential approach, a two-compartment model is fitted to the pharmacokinetic data alone, and afterwards the pharmacokinetic model is reapplied to the data with an added effect compartment. The effect compartment is then characterized by a parameter k_{eo} , which quantifies the clearance of drug from the effect compartment, and the equilibrium half life is $\log(2)/k_{eo}$. Unpaired Student's t-test was used to compare values between women and men and all values are reported using mean values $\pm$ SD. Statistical testing was performed using R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Results were considered significant if two-sided p-values were <0.05 .

Results

While women had a longer QTc than men at baseline, there was no difference in $T_{peak}-T_{end}$ (Table 1). Quinidine infusion resulted in substantial QTc prolongation and T wave morphology changes in both women and men (Table 1). Twenty of 23 (87%) subjects showed T wave notching after quinidine infusion (9 of 11 women vs. 11 of 12 men, $p=0.99$). Example ECGs in Figure 1 show QTc and $T_{peak}-T_{end}$ prolongation as well as notched T waves in two subjects.

Quinidine induced similar maximum placebo- and baseline-corrected QTc prolongation in women and men ([mean $\pm$ SD] 143 ± 44 ms; $p<0.001$ vs. 130 ± 36 ms, $p<0.001$; women vs. men $p=0.47$). The placebo- and baseline-corrected quinidine-induced prolongation in $T_{peak}-T_{end}$ was also similar in women when compared to men (140 ± 57 ms; $p<0.001$ vs. 147 ± 34

ms; $p<0.001$; women vs. men $p=0.73$). There were no significant differences between women and men in quinidine-induced maximum QTc (541 ± 40 vs. 510 ± 38 ms; $p=0.07$) or maximum $T_{\text{peak}}-T_{\text{end}}$ (216 ± 60 vs. 222 ± 37 ms; $p=0.76$). However there was a trend toward a lower maximum serum quinidine concentration in women compared to men (2.9 ± 0.7 vs. 3.7 ± 1.2 $\mu\text{g/ml}$; $p=0.07$).

Figure 2 shows scatterplots and regression lines comparing serum quinidine concentration to change in QTc and $T_{\text{peak}}-T_{\text{end}}$ for women (red) and men (blue). The slope describing the relationship between serum quinidine concentration and QTc prolongation was greater in women than in men (38 ± 10 ms/ $\mu\text{g/ml}$ vs. 28 ± 9 ms/ $\mu\text{g/ml}$; $p=0.02$) (Figure 2a). However, the slope for $T_{\text{peak}}-T_{\text{end}}$ was not different between women and men (39 ± 13 ms/ $\mu\text{g/ml}$ vs. 32 ± 13 ms/ $\mu\text{g/ml}$, $p=0.21$) (Figure 2b).

Further analysis revealed that when grouping the concentration-dependent effects by time point (Figure 3), differences between women and men occurred primarily in the first 20 minutes after quinidine infusion, when serum quinidine concentrations were higher in men than women. In men (blue), but not in women (red), there is an apparent delay (concentration-effect hysteresis) between reaching the maximum serum concentration and observing the maximum QTc and $T_{\text{peak}}-T_{\text{end}}$ effects, which is reflected by a wider loop in men (Figure 3). A prior intravenous quinidine study only in men demonstrated that an effect-compartment model is appropriate to model the delayed effect of intravenous quinidine [16]. Using this effect-compartment analysis controlling for hysteresis resulted in no sex difference for QTc (55 ± 18 ms/ $\mu\text{g/ml}$ vs. 43 ± 19 ms/ $\mu\text{g/ml}$, $p=0.14$), without changing the lack of sex difference for $T_{\text{peak}}-T_{\text{end}}$ (61 ± 22 ms/ $\mu\text{g/ml}$ vs. 55 ± 21 ms/ $\mu\text{g/ml}$, $p=0.49$). In the QTc model, the time to steady state in the effect compartment was 15.1 ± 10.4 min ($p<0.01$) in women and 18.8 ± 9.8 min ($p<0.001$) in men

(women vs. men $p=0.39$), which was similar with $T_{\text{peak}}-T_{\text{end}}$ (15.4 ± 9.2 min; $p<0.001$ vs. 19.4 ± 8.6 min; $p<0.001$; women vs. men $p=0.30$).

Discussion

Quinidine caused a prolongation of $T_{\text{peak}}-T_{\text{end}}$, which has been proposed as a marker of dispersion of repolarization in the heart [6-9]. However, there was no difference in $T_{\text{peak}}-T_{\text{end}}$ prolongation between women and men (Figure 2b). The majority of the subjects (20 of 23) developed T wave notching, and there was no difference between women and men in the occurrence of T wave notching. This suggests that quinidine-induced increase of $T_{\text{peak}}-T_{\text{end}}$ and T wave notching do not explain differences in torsade risk between women and men. However, we did observe a delay (concentration-effect hysteresis) between maximum serum quinidine concentration and maximum ECG effect. This trended toward greater delays and higher serum quinidine concentrations in men than women. One explanation for the observed concentration-effect hysteresis may be because quinidine blocks the hERG potassium channel in the intracellular space [17], whereby the delay could be due to the time required for quinidine to accumulate inside ventricular cells [16]. Sex differences in cardiac ion channel composition and distribution and protein binding in drug metabolism and membrane transport have been reported previously [18]. Whether sex differences in quinidine membrane transport contribute to the observed greater sensitivity of QTc prolongation to serum quinidine concentration in women deserves further study. Alternatively, the apparent sex difference may have been due to the higher serum quinidine concentrations observed in men in this study.

Effect of sex-hormones on cardiac electrophysiology

The QTc does not differ between women and men in childhood [19]. At the onset of puberty, the QTc interval shortens in males and then slowly increases in later years until women and men have equivalent QTc again sometime after 50 years of age [19]. This QTc shortening in men is likely due to the effects of testosterone [20, 21]. Preclinical studies in guinea pig ventricular myocytes have demonstrated that testosterone shortens action potential duration (and thus QTc) by decreasing L-type calcium current and increasing the slow potassium current (IKs) [22]. The decreased calcium current from testosterone may prevent early after depolarizations (the trigger for torsade) [23], while increased IKs current from testosterone increases repolarization reserve [24]. Both of these may contribute to the lower drug-induced torsade risk in men compared to women.

Testosterone is not the only sex hormone that affects the QTc interval. Preclinical studies have demonstrated that progesterone, like testosterone, shortens action potential duration by decreasing L-type calcium current and increasing the IKs current [25]. A previous study demonstrated that the ibutilide-induced QTc interval prolongation showed an inverse relationship with progesterone levels and the magnitude of the prolongation depended on the menstrual phase [26]. Therefore, the potential protective effects of progesterone against torsade in women might be masked by, and dependent on, the menstrual phase.

ECG Markers of Dispersion of Repolarization

Previous studies have suggested that $T_{\text{peak}}-T_{\text{end}}$ is a marker of dispersion of repolarization, and that it may be a better predictor of torsade than QTc [6-9]. Since women have a higher incidence of torsade than men [3], and quinidine is known to cause torsade, we hypothesized that

1 women would exhibit greater quinidine-induced $T_{\text{peak}}-T_{\text{end}}$ prolongation than men at similar drug
2 concentrations. However, no difference between women and men was identified between either
3 maximum $T_{\text{peak}}-T_{\text{end}}$ prolongation or the slope describing the relationship between serum
4 quinidine concentration and $T_{\text{peak}}-T_{\text{end}}$. While it is possible that women have a higher drug-
5 induced dispersion of repolarization, results of this study suggest that measuring drug-induced
6 changes in the $T_{\text{peak}}-T_{\text{end}}$ interval alone might not suffice to assess drug-induced changes in
7 dispersion of repolarization. This study did demonstrate that quinidine substantially prolongs
8 $T_{\text{peak}}-T_{\text{end}}$ in both women and men.

9 Previous studies have suggested that $Q-T_{\text{peak}}$ reflects the repolarization time in the region
10 with shorter repolarization time [7, 8]. While we did not assess $Q-T_{\text{peak}}$, results suggest that the
11 sex difference in $Q-T_{\text{peak}}$ ($=QT_c-(T_{\text{peak}}-T_{\text{end}})$) increased by ~15 ms after quinidine infusion (Table
12 1). On the other hand, hysteresis analysis (Figure 3) suggests that this increase may be explained
13 by concentration-effect delay. This approximation of $Q-T_{\text{peak}}$ must be interpreted with caution
14 because maximum effects on each interval happened at different time points in women than in
15 men (Figure 3). Sex-differences in drug-induced prolongation of $Q-T_{\text{peak}}$ or other markers of
16 early repolarization (e.g. $J-T_{\text{peak}}$) deserve further investigation.

17 A majority of the subjects (87%) developed notched T waves with quinidine and the
18 occurrence was similar in women and men. Previous studies in canine left ventricular wedge
19 models have demonstrated that the ventricular myocardium is not homogenous, but likely
20 comprised of three cell types, epicardial, endocardial and midmyocardial (M-cells), which
21 display electrical and functional differences [27]. Due to these differences, the response to drugs
22 differs between cell types. The T wave notching may be a representation of heterogeneity in the
23 myocardium that is accentuated by hERG potassium channel blocking drugs like quinidine.

Potential Mechanisms for Hysteresis

We observed that there was a delay between reaching maximum serum concentration and obtaining the maximum QTc and T_{peak}-T_{end} prolongation, and the wider counter-clockwise loops in men compared to women (Figure 3) suggested that a sex difference might be present. Sex-related differences in the activity of cell membrane transport of drugs have been reported for some drugs, though no such studies have been identified for quinidine [28]. Because quinidine acts as a blocker on the internal mouth of the hERG potassium channel [17], differences in cell membrane transport could cause sex differences in concentration-effect hysteresis. In a previous paper by Benton et al, they used the same dataset as in this study and showed that women had a significantly longer QTc prolongation than men after quinidine infusion [12]. The results of our analyses without using the effect compartment analysis were similar, but the magnitude of delay differed slightly between women and men and correction for concentration-effect hysteresis accounted for the differences in concentration-dependent QTc prolongation between sexes.

As shown in the analyses by Benton et al [12], women in this study had a trend toward a greater weight-adjusted clearance of quinidine (5.2 ± 1.1 versus 4.3 ± 1.6 ml/min/kg) and a slightly higher free fraction of the metabolite 3-hydroxyquinidine than men (0.53 ± 0.05 versus 0.47 ± 0.05 ; $p < 0.01$). Faster metabolism of quinidine by women may contribute to the lower serum concentrations of quinidine and higher quinidine metabolites in women than in men. However, it is unlikely that sex differences in drug metabolism fully explain the difference in hysteresis loops (Figure 3) early after drug administration. In this study, subjects received weight-based dosing of quinidine with men (on average 10 kg heavier than women) being administered a total higher quinidine dose than women over the same 20 minute infusion period. Thus, men were likely subjected to a more rapid change in drug concentration and a greater

disequilibrium between quinidine serum and intracellular concentration, where quinidine binds to the hERG potassium channel. This larger hysteresis effect with rapid changes in drug concentration was also observed in the prior study by Holford et al [16].

Limitations

The T wave morphology changes produced by quinidine make detection of the end of the T wave difficult. To address this difficulty, software was used to make automatic annotations, which were controlled, and if needed corrected, manually. As opposed to preclinical studies that invasively measure $T_{\text{peak}}-T_{\text{end}}$ directly on the myocardium, we measured $T_{\text{peak}}-T_{\text{end}}$ from lead V5. Thus, $T_{\text{peak}}-T_{\text{end}}$ is a more indirect measure of dispersion of repolarization, however this method has been used in prior clinical studies [9]. Women received their first infusion (quinidine or placebo) within 5 days after the first day of menstruation and received the second infusion 7-17 days later. Consequently, the variability in the phase of the menstrual cycle may have influenced ECG measurements [26]. The small sample size and retrospective nature of the findings related to concentration-effect hysteresis means that these observations should be further evaluated in prospective studies.

Conclusions

We found that both women and men had substantial QTc and $T_{\text{peak}}-T_{\text{end}}$ prolongation after quinidine infusion and almost all subjects developed T wave notching. Women had a greater slope than men in the relationship between serum quinidine concentration and QTc, but not $T_{\text{peak}}-T_{\text{end}}$. However, there was a delay between peak serum concentration and ECG effect, and analysis controlling for the concentration-effect hysteresis found no statistically significant difference in QTc or $T_{\text{peak}}-T_{\text{end}}$ prolongation between women and men. The observed

1 concentration-effect hysteresis may be because quinidine blocks the hERG potassium channel in
2 the intracellular space and the delay is due to the time required for quinidine to accumulate inside
3 ventricular cells. Potential sex differences in concentration-effect hysteresis should be considered
4 in future research on drug-induced torsade risk.

5 **Acknowldegments**

6 This study was supported by FDA's Critical Path Initiative, FDA's Office of Women's
7 Health and appointments to the Research Participation Program at the Center for Devices and
8 Radiological Health administered by the Oak Ridge Institute for Science and Education through
9 an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug
10 Administration. The mention of commercial products, their sources, or their use in connection
11 with material reported herein is not to be construed as either an actual or implied endorsement of
12 such products by the U.S. Department of Health and Human Services.

References

1. Dessertenne, F., *Ventricular tachycardia with 2 variable opposing foci*. Arch Mal Coeur Vaiss, 1966. **59**(2): 263-72.
2. CredibleMeds.org [Internet]. *QT Drugs List*. [updated 01/14/2015;cited 01/29/2015]; Available from: <https://www.crediblemeds.org/new-drug-list/>.
3. Makkar, R.R., B.S. Fromm, R.T. Steinman, M.D. Meissner, and M.H. Lehmann, *Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs*. JAMA, 1993. **270**(21): 2590-7.
4. Stockbridge, N., J. Morganroth, R.R. Shah, and C. Garnett, *Dealing with global safety issues : was the response to QT-liability of non-cardiac drugs well coordinated?* Drug Saf, 2013. **36**(3): 167-82.
5. Zheng, Z.J., J.B. Croft, W.H. Giles, and G.A. Mensah, *Sudden cardiac death in the United States, 1989 to 1998*. Circulation, 2001. **104**(18): 2158-63.
6. Emori, T. and C. Antzelevitch, *Cellular basis for complex T waves and arrhythmic activity following combined I(Kr) and I(Ks) block*. J Cardiovasc Electrophysiol, 2001. **12**(12): 1369-78.
7. Rautaharju, P.M., et al., *Electrocardiographic predictors of coronary heart disease and sudden cardiac deaths in men and women free from cardiovascular disease in the Atherosclerosis Risk in Communities study*. J Am Heart Assoc, 2013. **2**(3): e000061.
8. Rautaharju, P.M., et al., *Electrocardiographic repolarization-related predictors of coronary heart disease and sudden cardiac deaths in men and women with*

- cardiovascular disease in the Atherosclerosis Risk in Communities (ARIC) study. J Electrocardiol, 2015. **48**(1): 101-11.
9. Yamaguchi, M., et al., *T wave peak-to-end interval and QT dispersion in acquired long QT syndrome: a new index for arrhythmogenicity*. Clin Sci (Lond), 2003. **105**(6): 671-6.
10. Johannesen, L., et al., *Differentiating drug-induced multichannel block on the electrocardiogram: randomized study of dofetilide, quinidine, ranolazine, and verapamil*. Clin Pharmacol Ther, 2014. **96**(5): 549-58.
11. Selzer, A. and H.W. Wray, *Quinidine syncope. Paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias*. Circulation, 1964. **30**: 17-26.
12. Benton, R.E., M. Sale, D.A. Flockhart, and R.L. Woosley, *Greater quinidine-induced QTc interval prolongation in women*. Clin Pharmacol Ther, 2000. **67**(4): 413-8.
13. Vicente, J., L. Johannesen, L. Galeotti, and D.G. Strauss, *ECGlab: User friendly ECG/VCG analysis tool for research environments*. Comput Cardiol, 2013: 775-778.
14. Goldenberg, I., A.J. Moss, and W. Zareba, *QT interval: how to measure it and what is "normal"*. J Cardiovasc Electrophysiol, 2006. **17**(3): 333-6.
15. Smetana, P., V. Batchvarov, K. Hnatkova, A. John Camm, and M. Malik, *Sex differences in the rate dependence of the T wave descending limb*. Cardiovasc Res, 2003. **58**(3): 549-54.
16. Holford, N.H., P.E. Coates, T.W. Guentert, S. Riegelman, and L.B. Sheiner, *The effect of quinidine and its metabolites on the electrocardiogram and systolic time intervals: concentration--effect relationships*. Br J Clin Pharmacol, 1981. **11**(2): 187-95.

- 1 17. Yeola, S.W., T.C. Rich, V.N. Uebele, M.M. Tamkun, and D.J. Snyders, *Molecular*
2 *analysis of a binding site for quinidine in a human cardiac delayed rectifier K⁺ channel.*
3 *Role of S6 in antiarrhythmic drug binding.* Circ Res, 1996. **78**(6): 1105-14.
- 4 18. Gaborit, N., et al., *Gender-related differences in ion-channel and transporter subunit*
5 *expression in non-diseased human hearts.* J Mol Cell Cardiol, 2010. **49**(4): 639-46.
- 6 19. Rautaharju, P.M., et al., *Sex differences in the evolution of the electrocardiographic QT*
7 *interval with age.* Can J Cardiol, 1992. **8**(7): 690-5.
- 8 20. Bidoggia, H., et al., *Sex differences on the electrocardiographic pattern of cardiac*
9 *repolarization: Possible role of testosterone.* Am Heart J, 2000. **140**(4): 678-683.
- 10 21. Vicente, J., L. Johannesen, L. Galeotti, and D.G. Strauss, *Mechanisms of sex and age*
11 *differences in ventricular repolarization in humans.* American heart journal, 2014.
12 **168**(5): 749-756.
- 13 22. Bai, C.X., J. Kurokawa, M. Tamagawa, H. Nakaya, and T. Furukawa, *Nontranscriptional*
14 *Regulation of Cardiac Repolarization Currents by Testosterone.* Circulation, 2005.
15 **112**(12): 1701-1710.
- 16 23. January, C.T. and J.M. Riddle, *Early afterdepolarizations: mechanism of induction and*
17 *block. A role for L-type Ca²⁺ current.* Circ Res, 1989. **64**(5): 977-90.
- 18 24. Jonsson, M., M. Vos, G. Duker, S. Demolombe, and T. van Veen, *Gender disparity in*
19 *cardiac electrophysiology: implications for cardiac safety pharmacology.* Pharmacol
20 Ther, 2010. **127**(1): 9-18.
- 21 25. Nakamura, H., et al., *Progesterone Regulates Cardiac Repolarization Through a*
22 *Nongenomic Pathway: An In Vitro Patch-Clamp and Computational Modeling Study.*
23 Circulation, 2007. **116**(25): 2913-2922.

- 1 26. Rodriguez, I., M.J. Kilborn, X.K. Liu, J.C. Pezzullo, and R.L. Woosley, *Drug-induced*
2 *QT prolongation in women during the menstrual cycle*. JAMA, 2001. **285**(10): 1322-6.
- 3 27. Antzelevitch, C., *Cellular basis for the repolarization waves of the ECG*. Ann N Y Acad
4 Sci, 2006. **1080**: 268-81.
- 5 28. Meissner, K., et al., *Modulation of multidrug resistance P-glycoprotein 1 (ABCB1)*
6 *expression in human heart by hereditary polymorphisms*. Pharmacogenetics, 2004. **14**(6):
7 381-5.

Figure legends

Figure 1: The figure illustrates lead V5 from ECGs at 3 time points (baseline [black], 20 minutes post quinidine [00:20, blue], and 2 hours and 30 minutes post quinidine [02:30, red]) for 2 female subjects (A and B). The time points for QRS onset, T_{peak} and T_{end} , respectively, are denoted with vertical lines. At baseline the subjects have similar QTc and $T_{\text{peak}}-T_{\text{end}}$ durations. At 00:20 subject A developed T wave notching and showed a greater QTc and $T_{\text{peak}}-T_{\text{end}}$ prolongation due to T wave notching than subject B who did not develop T wave notching. At 02:30 the notching had disappeared and both subjects had similar QTc and $T_{\text{peak}}-T_{\text{end}}$ durations.

Figure 2: Women (red) and men (blue) mean baseline- and placebo-corrected serum quinidine concentration dependent changes (lines) and 95% confidence intervals (shaded areas) from the model predictions for (a) QTc and (b) $T_{\text{peak}}-T_{\text{end}}$. Dots correspond with the observed data. For QTc, a difference in slope between women and men was observed ($p=0.02$). No sex difference in slope was observed for $T_{\text{peak}}-T_{\text{end}}$ ($p=0.21$).

Figure 3: Relationship between mean serum quinidine concentration and mean prolongation in (a) QTc and (b) $T_{\text{peak}}-T_{\text{end}}$ for women (red) and men (blue) over time. The connected dots show the concentration for time points from 0-12 hours post dose for women and men. Time points at 5, 10, 20, and 60 minutes post dose are labeled. Men trended to reach higher maximum serum quinidine concentrations ($p=0.07$) with a trend toward a delay between maximum serum concentration and maximum ECG effect ($p=0.26$), which caused the appearance of a wide loop in men, but not in women. The maximum ECG effects were reached by 20-25 minutes (the fourth or fifth measurement points).

1 Tables

2 **Table 1.** Comparison between baseline characteristics and drug induced changes in men and
3 women

	Women n=11	Men n=12	P value
Baseline			
Age (years)	26 ± 8	30 ± 7	0.28
Height (m)	1.69 ± 0.06	1.79 ± 0.08	<0.01
Weight (kg)	65 ± 11	75 ± 13	0.047
BMI (kg/m ²)	23 ± 4	22 ± 3	0.57
Heart Rate (bpm)	64 ± 7	63 ± 10	0.58
QTc (ms)	405 ± 10	389 ± 17	<0.01
T _{peak} -T _{end} (ms)	77 ± 11	73 ± 4	0.10
After quinidine infusion			
Max Quinidine concentration (µg/ml)	2.9 ± 0.7	3.7 ± 1.2	0.07
Max Delta QTc (ms)	143 ± 44	130 ± 36	0.47
Max QTc (ms)	541 ± 40	510 ± 38	0.07
QTc slope (not hysteresis corrected) (ms/µg/ml)	38 ± 10	28 ± 9	0.02
QTc slope (hysteresis corrected) (ms/µg/ml)	55 ± 18	43 ± 19	0.14
Max Delta T _{peak} -T _{end} (ms)	140 ± 57	147 ± 34	0.73
Max T _{peak} -T _{end} (ms)	216 ± 60	222 ± 37	0.76
T _{peak} -T _{end} slope (not hysteresis corrected) (ms/µg/ml)	39 ± 13	32 ± 13	0.21
T _{peak} -T _{end} slope (hysteresis corrected) (ms/µg/ml)	61 ± 22	55 ± 21	0.49

4 Values are presented as mean ± SD.

1 **Figures**

2 *Figure 1*

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2 **Figure 2**

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2 **Figure 3**

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