Electrophysiological and ECG Effects of Perhexiline, A Mixed Cardiac Ion Channel Inhibitor, Evaluated in Non-clinical assays and in Healthy Subjects

Midei Mark G, MD[1], Darpo Borje, MD, PhD[2], Ayers, Greg[3], MD, PhD, Brown, Randy[2], Couderc, Jean-Philippe[2], Daly, William[4], Ferber, Georg, PhD,[5] Sager, Philip T, MD[6], Camm A John, MD[7]

1. Heart Metabolics, Dublin, IE*
2. ERT, Rochester, NY, USA
3. Heart Metabolics, Dublin, IE*
4. Heart Metabolics, Dublin, IE*
5. Statistik Georg Ferber GmbH, Riehen, Switzerland
6. Cardiovascular Research Institute and Dept. of Medicine, Stanford University, Palo Alto, CA, USA
7. Cardiovascular and Cell Sciences Research Institute, Division of Clinical Sciences, St George's University of London, London, UK

: At the time this work was performed

Short title: ECG effects of perhexiline

Corresponding author:
Borje Darpo, MD, PhD
Chief Scientific Officer Cardiac Safety,
ERT, 150 Allens Creek Road, Rochester, NY 14618, USA
Email: borje.darpo@telia.com
Telephone: +46 763 902 130
Draft December 18, 2018

Disclosures: At the time of the work, MM, GA and WD were employees of Heart Metabolics. BD is a consultant for and owns stock and is eligible for stock options in ERT. RB and JPC are employees of ERT. PS and JC were consultants for Heart Metabolics. The studies were sponsored by Heart Metabolics.

Access to data from this study may be raised with the corresponding author.

Abstract

Perhexiline has been used to treat hypertrophic cardiomyopathy. In addition to its effect on carnitine-palmitoyltransferase-1, it has mixed ion channel effects through inhibition of several cardiac ion currents.

Effects on cardiac ion channels expressed in mammalian cells were assayed using manual patch-clamp technique, action potential duration (APD) were measured in ventricular trabeculae of human donor hearts, and ECG effects were evaluated in healthy subjects in a thorough QT (TQT) study.

Perhexiline blocked several cardiac ion currents at concentrations within the therapeutic range (150 – 600 ng/mL) with IC_{50} for hCav1.2 ~ hERG < late hNav1.5. A significant APD shortening was observed in perhexiline treated cardiomyocytes. The TQT study was conducted with a pilot part in 9 subjects to evaluate a dosing schedule that would achieve therapeutic and supratherapeutic perhexiline plasma concentrations on Day 4 and 6, respectively. Guided by the results from the pilot, 104 subjects were enrolled in a parallel designed part with a nested cross-over comparison for the positive control. Perhexiline caused QTc prolongation, with a largest effect on ΔΔQTcF of 14.7 msec at therapeutic and 25.6 msec at supratherapeutic concentrations and a positive and statistically significant slope of the concentration-ΔΔQTcF relationship: 0.018 msec per ng/mL; 90% CI: 0.0119 to 0.0237. In contrast, the JTpeak interval was shortened with a negative concentration-JTpeak relationship, a pattern consistent with multichannel block. Further studies are needed to evaluate whether this results in a low proarrhythmic risk.

Word count: Abstract: 234; Text: 5146
Tables: 3 Figures: 3

Introduction

Perhexiline is a carnitine-palmitoyltransferase 1 (CPT-1) inhibitor which has been used to treat symptomatic hypertrophic cardiomyopathy (HCM) [1-3]. The drug was developed in the 1970's as an anti-ischemic drug for treatment of angina pectoris and has been used clinically for this indication in Australia and New Zealand for more than 30 years. Compared to other agents available at the time of its introduction, perhexiline had the unusual property of not inducing intolerable reductions in blood pressure or heart rate [4]. Inhibition of CPT-1 causes a change in preferred substrate for energy production in cardiac myocytes from fatty acids to glucose, an inherently more oxygen efficient process. This mechanism, in combination with its cardiac ion channel blocking properties, may reverse the impaired cardiac energetics present in HCM that are associated with abnormal myocyte function [2]. Recognizing the role of cellular ischemia in the pathophysiology of HCM and the high risk of pharmacologically-induced hemodynamic fluctuations, perhexiline has been used increasingly for this condition [4].

Perhexiline is known to have inhibitory effects on multiple cardiac ion channels [5,6], including the hERG channel [7]. Although it is known to prolong the QTc interval of the electrocardiogram (ECG), only a single case of torsades de pointes has been reported in over 40 years of clinical experience [8]. Others have reported on favorable suppression of cardiac arrhythmias [9-11]. In order to fully characterize the cardiac ion channel effects of perhexiline and their association to its electrophysiological properties, the effects of perhexiline on cardiac ion currents, action potential duration (APD) of human hearts, and on the ECG parameters were measured, including concentration (C)-QTc and C-JTpeak evaluation, in healthy human subjects.

Methods

Effects of Perhexiline on Cardiac Ion Channels

The effect of perhexiline on the following ion channels expressed in mammalian cells using manual patch-clamp techniques were examined (Charles River, Inc, Cleveland, Ohio):

1. The sodium channel (hNav1.5) responsible for peak and late INa in human heart, expressed in human embryonic kidney (HEK293) cells.
2. The L-type calcium channel (hCav1.2-β2-α2δ) responsible for ICa,L in the human heart, expressed Chinese hamster ovary (CHO) cells.
3. The potassium channel (hKvLQT1/hminK) responsible for the slow delayed rectifier current (IKs) in the human heart, expressed HEK293 cells.
4. The hERG (human ether-à-go-go-related gene) potassium channel current (a surrogate for IKr, the rapidly activating, delayed rectifier cardiac potassium current) expressed in HEK293 cells. This assay was conducted in compliance with Good Laboratory Practice (GLP).

A manual patch clamp technique at near-physiological temperature was used for all channels except Nav1.5, which was evaluated at room temperature. At least four (4) concentrations were selected to evaluate the concentration-response of each channel, except for hERG, which was evaluated at (1) one concentration. Each concentration was tested in at least three (3) cells ($n \geq 3$), with one exception, where a 10 µM concentration of perhexiline was applied to only two cells expressing Cav1.2 current. Since this was determined to be a saturating concentration, 4 lower concentrations were subsequently tested. Steady state was maintained for at least 30 seconds before applying the test article or the positive control. The peak current during test article and positive control application was monitored until a new steady state was achieved. A different procedure was followed for the hERG assay: The test article was applied for a period of approximately 5 minutes and then washed off. Monitoring of hERG current was then continued for up to 15 minutes to determine whether perhexiline's effect on hERG was attenuated. Samples of the test article formulation solutions collected from the outflow of the perfusion apparatus were analyzed for concentration verification during the hERG assay.

Effects of Perhexiline on Human Action Potential Duration

Trabeculae were harvested from 7 whole human donor hearts (5 from healthy subjects and 2 from subjects with left ventricular hypertrophy) procured by AnaBios (San Diego, California). Hearts were treated with a cardioplegic solution for transit. After ventricular trabeculae were prepared, continuous membrane potential monitoring was performed in the presence of oxygenated Tyrode's buffer.

Specimens were studied before and after exposure to 3 different concentrations of perhexiline: 0.72, 2.2, and 7.2 µM (200, 610 and 2 000 ng/mL). The following experimental parameters were varied to assess the effect of perhexiline under a variety of conditions: a) different vehicles/carriers were used: 3.5% human serum albumin, 0.3% human serum albumin or 0.1% DMSO; b) different drug exposure times were used, from 30 minutes to 4 hours; c) two different pacing frequencies were tested, 1Hz and 2Hz; and d) normal as well as pathological (left ventricular hypertrophy) hearts were used.

Dofetilide at a concentration of 100nM was used as a positive control after data was collected for the perhexiline exposures. Analysis included action potential duration at 30, 60 and 90 minutes ($APD_{30,60,90}$), resting membrane potential (RMP), maximum amplitude of action potential (APA), short term variability analysis (STV), and triangulation (APD_{90} -APD_{30}).

ECG effects in healthy subjects – the thorough QT (TQT) study

Regulatory approval was granted by the Therapeutic Goods Administration in Australia. Ethics Committee Approval was granted by the Belberry Human Research Ethics Committee. The study was conducted in accordance with the current version of the declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland; October 2000). The trial was conducted in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP). All subjects provided written informed consent to participate in the study prior to being screened.

The TQT study was conducted in compliance with ICH E14 guidance [12,13] and was double-blind, randomized, placebo-controlled, with parallel-group design and used a nested crossover comparison for the test of assay sensitivity with moxifloxacin [14]. The study was preceded by a small pilot phase, in which 9 subjects were dosed with perhexiline to evaluate whether the proposed dosing scheme resulted in the targeted plasma concentrations, i.e., 150 to 400 ng/mL after 4 days of dosing with the proposed schedule and somewhat above 600 ng/mL after 6 days. Subjects were randomly assigned 1:1 to one active group (Group 1) and one placebo/moxifloxacin group (Group 2). The target for the perhexiline dosing schedule was to achieve therapeutic plasma concentrations on Day 4 and supratherapeutic on Day 6. Perhexiline was given in a fixed dosing of 200 mg orally twice daily (BID) on Day 1 and 2, once daily on Day 3 and 4 and a single dose between 150 and 300 mg on Day 5, guided by a measurement of plasma concentration, and then a single dose of 400 mg on Day 6. In the active group, subjects received placebo for moxifloxacin on Day -1 and Day 7. In the control group, subjects received placebo for perhexiline on Day 1 through 6. In half of the group (n= 25), subjects received a single dose of 400 mg moxifloxacin on Day -1 and placebo for moxifloxacin on Day 7; in the other half (n= 25), placebo and moxifloxacin were administered in reversed order with moxifloxacin on Day 7.

Subjects were admitted to the clinical site (Nucleus Network; Melbourne, Australia) three days before dosing (Day -3) and stayed in-house until Day 8, after completion of safety procedures. Standard eligibility criteria for clinical pharmacology studies in healthy subjects were applied. Cytochrome P-450 2D6 (CYP2D6) poor metabolizer, and ultra-rapid metabolizers as determined via genotype were excluded from participation.

ECG Technique and Statistical Analyses

12-lead ECGs were extracted from continuous Holter recordings (M12R 12-lead digital ECG recorders, Global Instrumentation, Manlius, New York) on Day -2, -1, 4, 6 and 7 at predefined timepoints, intended to capture peak plasma levels of perhexiline and moxifloxacin and declining levels thereafter, with subjects supinely resting for at least 10 minutes. ECGs were extracted and measured at a central ECG laboratory (iCardiac Technologies/ERT, Inc., Rochester, NY). A median beat was computed from the vector magnitude lead (VM) (8, 13), derived from 12-lead ECGs with Mason-Likar lead configuration (14), transformed into a 3-lead orthogonal configuration (X, Y, and Z) by implementing the Guldenring procedure [15]. The magnitude of the vector described by these 3 leads was computed as the root of the sum of squares and finally a median beat was computed by

synchronizing all sinus beats within the 10-second replicate (by maximization of cross-correlation function on QRS complexes) [16]. Fiducial points were detected on each replicate median beat using iCOMPAS software and were manually adjudicated by the technician/cardiologist. Beats were defined as usable when they were both i) normal sinus beats, and ii) the correct placement of the fiducials of interest could be accurately determined. The ECG technicians were trained to use consistent rules in defining the peak of the T-wave. In case of biphasic T-wave, the T-wave peak was based on the peak of the major inflection of the T-wave (in absolute value). On each median beat, the technician reviewed the automated placement of the P on-set, QRS on-set and off-set, T apex, and T off-set. If the automated fiducial placement was determined to be within 10 milliseconds of the correct placement it was left unchanged; otherwise, the fiducial(s) were adjusted as needed. Subsequently, the JTpeak measurements were reviewed and adjudicated by a trained cardiologist. The QT interval was corrected for heart rate changes using the Fridericia formula (QTcF) and the J-Tpeak interval using a larger correction factor of 0.58 ($JTpeak\_c = JT \bullet RR^{-0.58}$), as suggested by Johannesen [17].

The primary endpoint in the statistical analysis was placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) with heart rate, PR, QRS, T-wave morphology and J-Tpeak and Tpeak-Tend as secondary endpoints. For the evaluation of ECG effect of perhexiline, baseline was time-matched values on Day -2. For the nested crossover comparison between placebo and moxifloxacin, baseline was the time-matched values on either Day -1 or Day 7. The primary analysis was performed separately for Day 4 and Day 6. It was based on a linear mixed effects model with change-from-baseline QTcF (ΔQTcF) as dependent variable, treatment (perhexiline and corresponding placebo), time (categorical), and treatment by time as factors. Subject was included in the model as random effect for the intercept. An unstructured covariance matrix was specified for the repeated measures at post dose timepoints within subject. A 2-sided 90% CI was calculated for the contrast in treatment QTc = "perhexiline – placebo". For J-Tpeak and Tpeak-Tend analysis, the same model as for ΔQTcF was used.

The analysis to show assay sensitivity was based on the change in QTc (primary endpoint) from time-matched baseline evaluated on moxifloxacin and matching placebo for Group 2. For each timepoint, a linear mixed effects model was fitted with treatment (moxifloxacin and corresponding placebo) and sequence (placebo-moxifloxacin or moxifloxacin-placebo) as fixed effects, time-matched baseline as a covariate, and subject as random effect for the intercept. For the timepoints 1, 2, 3, and 4 hours, the contrast in ΔQTc = "moxifloxacin – placebo" was tested against the 1-sided null hypothesis $\Delta\Delta QTc \leq 5$ msec at the 5% level. Multiplicity was controlled by using a Hochberg procedure [18]. If, following this procedure, QTc was significantly greater than 5 msec for at least 1 timepoint, assay sensitivity was considered to be shown.

The analysis of placebo-corrected ΔHR, ΔPR and ΔQRS was based on the same linear mixed effect model as for ΔΔQTcF and ΔΔJTpeak_c.

In the concentration-QTc analysis, a plot of standardized residuals versus fitted values was used to examine departure from assumptions of a linear model. The normal Q-Q plots of the random effects and the within-subject errors were used to investigate the normality of the random effects and the within-subject errors, respectively. A final assessment of the adequacy of the linear mixed effects model was provided by a goodness-of-fit plot (i.e., the observed concentration decile-ΔΔQTcF plot).

Via visual inspection of the goodness-of-fit plot, the assumption of linearity between ΔΔQTcF and plasma concentrations of perhexiline and how well the predicted ΔΔQTcF matched the observed data in the regions of interest were checked. Plots over ΔΔQTcF and perhexiline concentrations over time and hysteresis loops were used to evaluate presence of hysteresis, i.e., a relevant delay between peak plasma concentration and the largest QT effect. A linear mixed-effects models was applied to the data for the C-QTc analysis of the relationship between perhexiline plasma concentrations and ΔQTcF/JTpeak_c with separate analyses for each ECG parameter. The time-matched concentration of perhexiline was included in the model as a continuous covariate (0 for placebo), with centered baseline QTcF/JTpeak_c as an additional covariate, and treatment (active or placebo) and time as categorical factors; there was a random intercept and slope per subject. The degrees of freedom for the model estimates were determined by the Kenward Roger method. From the model, the slope and the treatment effect–specific intercept (defined as the difference between active and placebo) were estimated together with a 2-sided 90% CI. The predicted ΔΔQTcF/ΔΔJTpeak_c effect and 2-sided 90% CI at the geometric mean Cmax of perhexiline was calculated.

Results

Effects of Perhexiline on cardiac ion channels

Table 1 shows the perhexiline concentrations required to inhibit 50% of the respective cardiac ion channels (IC_{50}). Perhexiline inhibition of the L-type calcium current was near equipotent to the hERG inhibition (IC_{50} 247 vs. 167 ng/mL), whereas 50% inhibition of the late sodium current was achieved at 8-fold higher concentrations than hERG inhibition (IC_{50} 1359 ng/mL). In the GLP-compliant hERG assay, the perhexiline effect plateaued at 66% inhibition, with subsequent rapid but incomplete recovery upon washout to 48% inhibition, which remained for another 9 to 15 minutes. At the upper therapeutic concentration of 600 ng/mL, perhexiline is ~99% protein bound, which means that the ratio between IC_{50} and free therapeutic concentrations was ~28-fold for hERG and ~40-fold for the L-type calcium current.

Effects of Perhexiline on the Human Action Potential

Perhexiline at the studied concentrations up to 2 000 ng/mL had an overall shortening effect on APD and no effect on triangulation in contrast to dofetilide, administered as a control after data were obtained for perhexiline. Action potential results from a representative LVH specimen is displayed for the control condition, for perhexiline at various concentrations and for dofetilide in ***Figure 1***. Average percentage change obtained in five specimens derived from two of the LVH hearts for APD, APA, RMP maximum dV/dt, and for triangulation is shown in ***Table 2***. Under the conditions tested, a consistent shortening of the action potentials was observed. There were no increases in action potential triangulation, action potential instability or early after depolarizations. The shortening of the APD was associated with a small, not statistically significant increase of mean short-term variability (STV); 0.22 for control, 0.71, 0.45 and 0.43 for perhexiline 200, 610 and 2 000 ng/mL, respectively and 1.21 for 44 ng/mL dofetilide. In the experiments in which 44 ng/mL (100 nM) dofetilide was added as a positive control after the perhexiline challenge, the effect of dofetilide on APD_{90} was approximately 60% smaller than typically observed; only 32.9 ± 6.75% over baseline vs. 102% (data on-file, Anabios).

Effects of Perhexiline in the TQT Study

One-hundred-four subjects (63 males) were enrolled into the study and received at least one dose of study medication (52 in the perhexiline group and 26 each in the combined moxifloxacin/placebo groups). There were no apparent differences between the groups for demographic parameters of age, sex, race, body weight or BMI index. The majority (87%) of subjects were White with a mean (SD) age and body mass index of 25.5 ± 5.0 years and 23.3 ± 2.66 kg/m2, respectively.

Pharmacokinetics of perhexiline

The dosing schedule of perhexiline was successful in achieving a mean plasma level of perhexiline within the therapeutic range (150-400 ng/mL) on Day 4, and somewhat above the therapeutic range on Day 6. Mean Cmax was 279 ng/mL (90% CI: 240 to 323) on Day 4 and 678 ng/mL (90% CI: 621 to 740) on Day 6, with a median Tmax of 6.0 hours on both days (***Figure 2***).

Cardiodynamic effects

No significant changes in blood pressure or heart rate were seen with perhexiline on Day 4 or Day 6. The change-from-baseline heart rate (ΔHR) followed the same diurnal pattern in the perhexiline and placebo groups on Day 4, whereas a small separation was observed on Day 6 with mean ΔHR reaching 6.4 bpm and 8.5 bpm in subjects on perhexiline as compared to 1.5 bpm and 1.5 bpm among placebo subjects at 3 and 4 hours post-dosing (***Supplemental Figure S1***). The resulting mean placebo-corrected ΔHR (ΔΔHR) peaked at 6.9 bpm at 4 hours post-doing on Day 6 (***Supplemental Figure S2***).

Change-from-baseline QTcF (ΔQTcF) was larger at all timepoints in the perhexiline group as compared to placebo on both Day 4 and 6. On Day 4, mean ΔQTcF varied across post-dosing time points between 9.8 at 3 hours post-dose and 15.3 msec at 24 hours, whereas the placebo-response was slightly negative. On Day 6, the QT effect of perhexiline was larger than on Day 4 with a largest mean ΔQTcF of 26.7 msec at 24 hours post-dosing (***Figure 3A***). Mean placebo-corrected ΔQTcF (ΔΔQTcF) was between 10 and 15 msec for all of the time points on Day 4, with the largest value observed at the predose time point (14.7 msec). On Day 6, ΔΔQTcF was generally larger, starting with mean values near 20 msec and then increasing to a peak effect of 25.6 msec (90% CI: 20.47 to 30.70) at 24 hours post-dosing (***Supplementary Table S1***). Assay sensitivity was confirmed by the QT response after a single dose of 400 mg moxifloxacin with the largest mean ΔΔQTcF of 11.6 msec (90% CI: 8.53 to 14.65) at 4 hours and the lower bound of the 90% CI exceeding 5 msec at all 4 pre-specified timepoints: 7.00, 8.50, 6.73 and 8.53 msec at 1, 2, 3 and 4 hour post dosing.

Unlike the QTc interval, ΔJTpeak_c after dosing with perhexiline was negative or around zero at most time points, with the exception of the 24 hour time point on Day 6 with mean ΔJTpeak_c of 7.8 msec (90% CI: 4.47 to 11.06; ***Figure 3B***). Mean placebo-corrected ΔJTpeak_c (ΔΔJ-Tpeak_c) was below 10 msec on both Day 4 and 6, with the largest value at 2 hours (4.8 msec; 90% CI: 0.93 to 8.72) on Day 4 and at the predose time point (8.4 msec; 90% CI: −0.01 to 16.76) and at 24 hours post-dose on Day 6 (6.5 msec; 90% CI: 1.92 to 11.17; ***Supplementary Table S1***). To directly compare the effect of perhexiline on placebo-corrected ΔQTcF and ΔJTpeak_c, the profiles across post-dosing time points on Day 4 and 6 are displayed in the same graph in ***Figure 4***. In contrast, moxifloxacin with a smaller mean peak effect than perhexiline (11.6 msec) caused prolongation of the ΔΔJTpeak_c, that peaked at the same time point (4 hours post-dosing), amounting to 7.8 msec (90% CI: 4.75 to 10.80).

There were 4 (7.7%) subjects (3 on Day 4 and 1 on Day 6) in the perhexiline group with QTcF > 480 and < 500 ms msec at any post-dose time points vs. none in the placebo and no subject on perhexiline with QTcF > 500 msec vs. 1 (1.9%) in the placebo group. Five (9.6%) subjects in the perhexiline group exhibited ΔQTcF > 60 msec at total of 11 time points vs. 1 (1.9%) subject at 1 time point in the placebo group.

In the concentration-QTc analysis, plots over ΔΔQTcF and perhexiline concentrations over time (***Supplemental Figure S3***) and hysteresis loops did not indicate that the presence of hysteresis, i.e., a relevant delay between peak plasma concentration and the largest QT effect. The test for linearity demonstrated that a linear model would be appropriate, and the Goodness-of-fit plot (***Figure 5A***) showed that the model captured the observed data across all concentration deciles in an acceptable way. The slope of the C-QTc relationship was positive and statistically significant (0.018 msec per ng/mL; 90% CI: 0.0119 to 0.0237), with a large and statistically significant intercept (10.2 msec; 90% CI: 7.41 to 13.03). The slope of the C-JTpeak relationship was negative and not statistically significant: -0.004 msec per ng/mL (90% CI: -0.0105 to 0.0025) with an intercept of 2.7 msec (90% CI: -1.61 to 7.04). The Goodness-of-fit plot indicated that the model underestimated the effect of perhexiline on the ΔΔJTpeak_c in the highest concentration decile (***Figure 5B***). Using the linear C-QTc/JTpeak models, the effect on ΔΔQTcF at the geometric mean Cmax on Day 4 (279 ng/mL) and on Day 6 (678 ng/mL) can be predicted to 15.2 msec (90% CI: 12.67 to 17.67) and 22.3 msec (90% CI: 18.61 to 25.93), respectively. The effect on ΔΔJTpeak_c at the same perhexiline concentrations can be predicted to 1.6 msec (90% CI: -2.46 to 5.66) and 0.01 msec (-4.93 to 4.95).

Perhexiline exerted a small effect on the PR interval. The largest mean placebo-corrected ΔPR (ΔΔPR) reached 7.7 msec (90% CI: 5.08 to 10.27) at 3 hours on Day 4 and 9.8 msec (90% CI: 7.09 to 12.42) at 6 hours on Day 6. There were no subjects with a ΔPR >25% to a value >200 msec. Mean change-from-baseline QRS (ΔQRS) was somewhat larger on perhexiline on Day 6 as compared to placebo and the resulting mean placebo-corrected ΔQRS (ΔΔQRS) reached levels slightly above 2 msec at 4, 6 and 8 hours post-dose on this day with mean ΔΔQRS of 2.0, 2.1 and 2.4 msec (90% CI: 1.41 to 3.41), respectively. There were no subjects with ΔQRS >25% to a value >120 msec on any of the days.

Safety

The study drugs were safe and well tolerated; there were no arrhythmias or SAEs reported. Self-limited symptoms of headache, nausea, and dizziness occurred in 58% of perhexiline treated subjects and did not require discontinuation of study drug.

Discussion

Perhexiline has been used for decades to treat coronary ischemia and HCM in Australia without significant reports of pro-arrhythmias. The Australian package insert notes QT prolongation as a risk, but the level of this effect as not been well characterized. In this series of non-clinical and clinical studies, the effects of perhexiline on a variety of cardiac ion channels, on action potential parameters in human cardiomyocytes, and on ECG biomarkers in healthy subjects were investigated.

The objective of a TQT study in healthy subjects is to obtain a robust evaluation of a drug's potential effects on ECG parameters, with focus on the QTc interval; the study is therefore powered to

exclude a small (10 ms) effect on the QTc interval. Based on the results from the study, the level of ECG monitoring and in subsequent patient trials can be decided; if there are concerning effects on any of the studied ECG parameters, the results will also be used to exclude susceptible patients or to provide cautionary statements in the package insert once the drug is approved. It is widely agreed that a drug that causes more than 20 ms prolongation of the QTc interval may lead to proarrhythmias on the basis of delayed repolarization in susceptible patients, while there is an on-going debate as to which extent this also applies to drugs with only a mild effect, e.g., between 10 and 20 ms [13]. The QT interval seems to be an overly sensitive biomarker and there are examples of mildly QT prolonging drugs which do not appear to increase the risk of pro-arrhythmias. For drugs with only a mild effect on the QTc interval, it has also been proposed that lack of JTpeak prolongation, based on mixed ion channel effects, may mitigate the proarrhythmic risk of QT prolongation and thereby reduce or eliminate the need for ECG monitoring in patient trials [19-21]. With this series of non-clinical and clinical studies, an effort was made to better elucidate the relationships between ion channel effects and the effects on ECG biomarkers in humans, with focus on the QT and JTpeak intervals and to place the findings in context of proarrhythmic risk.

Assessment of perhexiline's effect on cardiac ion channels confirmed an inhibitory effect on the hERG channel at concentrations, which are clinically relevant. Additional ion channel effects were also revealed with inhibition of the L-type calcium channel with a similar IC_{50} value as hERG and sodium currents, including the late inward current, at somewhat higher concentrations. Perhexiline can therefore be categorized as a multi-channel blocking drug with equipotent effect on L-type calcium and hERG, a profile that has been suggested to infer low proarrhythmic risk [22,23]; A similar hERG/ L-type calcium IC50 relationship exists for verapamil which can cause QTc prolongation, and has been claimed to have a low risk of TdP [21].

In the in-vitro APD assay in human cardiomyocytes, no evidence of significant action potential prolongation, increased triangulation, action potential instability or EADs were seen. In several of the experiments, a small shortening of the action potential was observed. Although drugs that shorten the ventricular action potential can lead to beat-to-beat instability and proarrhythmias (e.g., flecainide [24], the shortening induced by perhexiline was not associated with a significant increase in short-term variability. Interestingly, in experiments in which dofetilide was added as a positive control after the perhexiline challenge, the prolongation of APD_{90} typically seen with dofetilide was reduced by 60%.

In an in-vivo toxicology study in equal numbers of male and female dogs, animals were dosed for 39 weeks in escalating dose cohorts of perhexiline (placebo n=8, 5 mg/kg/d n=8, 15 mg/kg/d n=8, and 45 mg/kg/d n=8). ECG measurements were recorded pretreatment (Days -8 to -5), and at 1 to 4 hours post-dose on Day 1, Week 13, 26, and 39. Changes of ECG parameters were very small across all dose groups, without indications of dose-dependent QTc prolongation or shortening (***Supplementary material***).

In contrast to the findings of mild APD shortening in human cardiomyocytes, the TQT study clearly demonstrated dose- and concentration related QTc prolongation at both therapeutic and somewhat supratherapeutic plasma concentrations. Mean $\Delta\Delta$QTcF was between 10 and 15 ms on Day 4 and between 18 and 25 ms on Day 6 and the concentration-QTc relationship was positive and statistically significant; 0.018 msec per ng/mL. The prolongation was more pronounced in the latter part (Tpeak-

Tend) of the T-wave and the JTpeak interval was only mildly affected: the largest mean ΔΔJTpeak_c value across all time points on both days was only 8 ms and the concentration-JTpeak relationship was very shallow, slightly negative and not statistically significant. Our findings thereby confirm that equipotent calcium and hERG inhibition may result in QTc prolongation without much of an effect on the first part of the T-wave, the JTpeak interval. This electrocardiographic pattern has been suggested to reduce or eliminate the proarrhythmic potential of pure hERG inhibition [19], which would be consistent with published clinical experience with perhexiline. It should however be acknowledged that mild QTc prolongation without JTpeak prolongation can also be observed with hERG blocking drugs that also affects the peak sodium current, seen as QRS widening [25]. The discrepancy of the findings in the in-vivo assay in human cardiomyocytes, in which QTc shortening was observed, with the results from the TQT study is difficult to understand, and it can be speculated that the distribution of cardiac ion channels was altered by the harvesting procedure, as compared to the beating heart. In this context, it can be noted that the current draft ICH S7B/E14 guidance document recommends standardized ion channel assays and in-vivo animal studies as primary non-clinical assays, with the potential to use in-vitro repolarization assays in follow-up studies [26].

The clinical experience with perhexiline comes from decades of use in Australia and New Zealand in patients with ischemic heart disease or HCM, i.e., in populations at increased risk of malignant ventricular arrhythmias [4]. The Australian regulatory authority, TGA, collects data on adverse events associated with prescribed drugs. While these data are subject to the usual problems associated with a voluntary reporting system, it provides some potentially useful information in the analysis of perhexiline's safety. The overwhelming majority of perhexiline toxicity occurs as a result of accumulation in patients who are genotypic poor metabolizers of CYP2D6, and the regular measurement of plasma levels is therefore recommended. If poor metabolizers are excluded from the clinical use of perhexiline, the attainment of supratherapeutic plasma levels is less likely. A total of 143 Adverse Drug Reports (ADRs) for perhexiline were filed between 1975 and 2011, none of which were reports of arrhythmias associated with its use. In this context, it is however, important to recognize the challenges and limitations associated with reporting of TdP, especially in a population with a high risk of polymorphic ventricular tachycardia and sudden death associated with coronary ischemia [27,28]. The Australian package insert notes QT prolongation as a risk, but the level of this effect as not been well characterized. This is apparently based on reports of QT prolongation with perhexiline and a single case report of TdP associated with perhexiline use [8]. In this case, a 69-year-old man with a history of aortic valve replacement and two prior coronary artery bypass graft (CABG) operations presented with a two-hour history of chest pain. He had been on maintenance perhexiline at therapeutic concentrations and a QTcF of 490 ms. During the initial 24 hours of hospitalization, QTcF increased to 540 ms and episodes of TdP were observed, which were abolished with over-drive pacing. Discontinuation of perhexiline was associated with no further episodes of torsades. In contrast, there are also reports on reduction of malignant ventricular arrhythmias with perhexiline in patients with ischemic heart disease [11]. In 4 separate publications during the 1970's, a reduction of ventricular arrhythmias associated with ischemic heart disease, mainly premature ventricular contractions, was reported [9,10,29,30]. Thus, despite convincing evidence of hERG channel inhibition at clinically relevant concentrations, TdP has been reported in only a single patient treated with perhexiline in decades of clinical usage (30). In WHO's database VigiAccess [31], which lists suspected adverse drug reactions collected by national drug authorities in over 110 participating countries, there are as of June 15, 2021 only 4 cases of cardiac arrest and one of ventricular

arrhythmia; none with the specific terms of TdP or QT prolongation . In territories where perhexiline is available, the risk of pro-arrhythmia appears to be low in patients who may benefit from perhexiline therapy, provided that plasma concentrations are monitored.

Although a TQT study remains the best clinical predictor of a drug's effect on the QT interval, its ability to predict the potential for pro-arrhythmia of a mixed cardiac ion channel blocker is less certain. It is therefore possible that the effects of hERG blockade may be mitigated by the inhibitory effects of perhexiline at another cardiac ion channel.

Conclusions

Perhexiline interacts with multiple cardiac ion channels resulting in a net lengthening of the QTcF interval clinically. In the TQT study, perhexiline caused prolongation of the QTc interval through lengthening of the latter part of the interval, the Tpeak-Tend subinterval, whereas the JTpeak subinterval was shortened. This pattern has been suggested to be indicative of mixed ion channel inhibition and to reduce the proarrhythmic risk of mildly QTc prolonging drugs, which would be consistent with decades of clinical experience with perhexiline.

Tables

Table 1. Perhexiline IC50 for cardiac ion channels using the manual patch clamp technique

Ion Channel	(+/-)-Perhexiline IC_{50} (ng/mL)	Cell Type
hERG (non-GLP)	1,139	HEK293
hERG (GLP)*	167	HEK293
Late hNav1.5	1,359	HEK293
hNav1.5 Tonic	1,324	HEK293
hNav1.5 Phasic	1,069	HEK293
hCav1.2	247	CHO
hKvLQT1/hminK	1,212	HEK293
hKv1.5	2,455	CHO
hKv4.3/hKChip2.2	5,874	HEK293
hKir2.1	>8,325	HEK293

**: GLP compliant; HEK293: Human embryonic kidney cells; CHO: Chinese hamster ovary cells*

Table 2: Effects of perhexiline on action potential parameters (mean change, %) for the 5 samples obtained from the two human donors with LVH

	APD_{30}	APD_{50}	APD_{60}	APD_{90}
Control	0.00 ±	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
200 ng/mL perhexiline	-3.33 ±	-3.36 ± 0.79	-3.14 ± 0.81	-0.72 ± 1.97
610 ng/mL perhexiline	-6.93 ±	-5.92 ± 2.44	-5.52 ± 2.31	-7.13 ± 3.50
2000 ng/mL perhexiline	-10.23 ±	-8.75 ± 2.31	-7.94 ± 2.17	-7.62 ± 2.57
44 ng/mL dofetilide	1.35 ±	11.57 ± 4.23	16.99 ± 4.75	32.87 ± 6.75
	APA	RMP	Max dV/dt	Triangulation
Control	0.00 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
200 ng/mL perhexiline	0.00 1.51	0.77 ± 1.32	55.08 ± 47.65	-1.34 ± 1.30
610 ng/mL perhexiline	-0.40 3.37	3.83 ± 1.74	22.84 ± 45.8	0.32 ± 2.29
2000 ng/mL perhexiline	-4.18 3.58	0.92 ± 2.62	-9.68 ± 20.19	2.01 ± 0.89
44 ng/mL dofetilide	-0.67 3.26	-0.10 ± 4.35	74.72 ± 66.3	85.11 ± 13.52

APD: Action Potential Duration at 30, 50, 60 and 90% repolarization; APA: Action Potential Amplitude; RMP: Resting Membrane Potential; Max dV/dt: Membrane potential

velocity; Triangulation: $APD_{90} - APD_{30}$

Figure legends

Figure 1

Representative action potential duration for LVH specimen stimulated at 1 Hz. Shortening of the APD was seen consistently at all concentrations of perhexiline compared to dofetilide administered as a control.

Figure 2

Plasma concentration profile of perhexiline and metabolites on Day 4 and 6.
Mean (ng/mL) and standard deviation
PEX: perhexiline; CIS: cishydroxyperhexiline; TRANS: transhydroxyperhexiline

Figure 3

Mean change from time-matched baseline QTcF (ΔQTcF, **Panel A**) and JTpeak_c (ΔJTpeak_c, **Panel B**) across time points on Day 4 and 6.
Mean and 90% CI from the linear mixed effects model.

Figure 4

Placebo-corrected change-from-baseline QTcF and JTpeak_c (ΔΔQTcF and ΔΔJTpeak_c, msec) across time points on Day 4 and Day 6.

Mean and 90% CI from the linear mixed effects model.

Figure 5

Goodness-of-fit graphs for the C-ΔΔQTcF (**Panel A**) and C-JTpeak_c (**Panel B**) analysis. The black line with the grey shaded area shows the mean predicted effect with 90% CI across the observed range of perhexiline plasma concentrations. The red filled circles with vertical bars denote the observed placebo-corrected mean ΔQTcF with 90% CI displayed within each plasma concentration decile. The horizontal red line with notches shows the range of perhexiline concentrations divided into deciles.

References

1. Horowitz JD, Chirkov YY. Perhexiline and hypertrophic cardiomyopathy: a new horizon for metabolic modulation. *Circulation.* 2010; 122: 1547-9.

2. Phan TT, Shivu GN, Choudhury A, et al. Multi-centre experience on the use of perhexiline in chronic heart failure and refractory angina: old drug, new hope. *Eur J Heart Fail.* 2009; 11: 881-6.

3. Lee L, Campbell R, Scheuermann-Freestone M, et al. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. *Circulation.* 2005; 112: 3280-8.

4. Ashrafian H, Horowitz JD, Frenneaux MP. Perhexiline. *Cardiovasc Drug Rev.* 2007; 25: 76-97.

5. Grima M, Velly J, Decker N, et al. Inhibitory effects of some cyclohexylaralkylamines related to perhexiline on sodium influx, binding of [3H]batrachotoxinin A 20-alpha-benzoate and [3H]nitrendipine and on guinea pig left atria contractions. *Eur J Pharmacol.* 1988; 147: 173-85.

6. Barry WH, Horowitz JD, Smith TW. Comparison of negative inotropic potency, reversibility, and effects on calcium influx of six calcium channel antagonists in cultured myocardial cells. *Br J Pharmacol.* 1985; 85: 51-9.

7. Walker BD, Valenzuela SM, Singleton CB, et al. Inhibition of HERG channels stably expressed in a mammalian cell line by the antianginal agent perhexiline maleate. *Br J Pharmacol.* 1999; 127: 243-51.

8. Kerr GD, Ingham G. Torsade de pointes associated with perhexiline maleate therapy. *Aust N Z J Med.* 1990; 20: 818-20.

9. Pickering TG, Goulding L. Supression of ventricular extrasystoles by perhexiline. *Br Heart J.* 1978; 40: 851-5.

10. Myburgh DP, Goldman AP. The anti-arrhythmic efficacy of perhexiline maleate, disopyramide and mexiletine in ventricular ectopic activity. *S Afr Med J.* 1978; 54: 1053-5.

11. Ten Eick RE, Singer DH. Proceedings: Effects of perhexiline on the electrophysiologic activity of mammalian heart. *Postgrad Med J.* 1973; 49: Suppl 3:32-43.

12. ICH E14: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Guideline.pdf, 2005. Accessed June 16, 2021

13. ICH E14 Questions & Answers (R3) December 10, 2015. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Q_As_R3__Step4. pdf. Accessed June 16, 2021

14. Hoch M, Darpo B, Brossard P, et al. Effect of ponesimod, a selective S1P1 receptor modulator, on the QT interval in healthy individuals. *Basic Clin Pharmacol Toxicol.* 2015; 116: 429-37.

15. Guldenring D, Finlay DD, Strauss DG, et al. Transformation of the Mason-Likar 12-lead electrocardiogram to the Frank vectorcardiogram. *Conf Proc IEEE Eng Med Biol Soc.* 2012; 2012: 677-80.

16. Couderc JP, Ma S, Page A, et al. An evaluation of multiple algorithms for the measurement of the heart rate corrected JTpeak interval. *J Electrocardiol.* 2017; 50: 769-775.

17. Johannesen L, Vicente J, Gray RA, et al. Improving the assessment of heart toxicity for all new drugs through translational regulatory science. *Clin Pharmacol Ther.* 2014; 95: 501-508.

18. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. *Stat. Med.* 1990; 9: 811-818.

19. Vicente J, Hosseini M, Johannesen L, et al. Electrocardiographic biomarkers to confirm drug's electrophysiological effects used for proarrhythmic risk prediction under CiPA. *J Electrocardiol.* 2017; 50: 808-813.

20. Vicente J, Zusterzeel R, Johannesen L, et al. Mechanistic Model-Informed Proarrhythmic Risk Assessment of Drugs: Review of the "CiPA" Initiative and Design of a Prospective Clinical Validation Study. *Clin Pharmacol Ther.* 2018; 103: 54-66.

21. Vicente J, Zusterzeel R, Johannesen L, et al. Assessment of Multi-Ion Channel Block in a Phase-1 Randomized Study Design:Results of the CiPA Phase 1 ECG Biomarker Validation Study. *Clin Pharmacol Ther.* 2018; 105: 943-953.

22. Martin RL, McDermott JS, Salmen HJ, et al. The utility of hERG and repolarization assays in evaluating delayed cardiac repolarization: influence of multi-channel block. *J Cardiovasc. Pharmacol.* 2004; 43: 369-379.

23. Vicente J, Strauss DG, Upreti VV, et al. The Potential Role of the J-Tpeak Interval in Proarrhythmic Cardiac Safety: Current State of the Science From the American College of Clinical Pharmacology and the Cardiac Safety Research Consortium. *J Clin Pharmacol.* 2019; 59: 909-914.

24. Fish FA, Gillette PC, Benson DW, Jr. Proarrhythmia, cardiac arrest and death in young patients receiving encainide and flecainide. The Pediatric Electrophysiology Group. *J Am Coll Cardiol.* 1991; 18: 356-65.

25. Darpo B, Benson C, Brown R, et al. Evaluation of the Effect of 5 QT-Positive Drugs on the JTpeak Interval - An Analysis of ECGs From the IQ-CSRC Study. *J Clin Pharmacol.* 2020; 60: 125-139.

26. ICH E14/S7B Implementation Working Group: Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential Questions and Answers. Draft version, August 2020. Available at: https://database.ich.org/sites/default/files/ICH_E14-S7B_QAs_Step2_2020_0827_0.pdf. Accessed June 16, 2021

27. Darpo B. Detection and reporting of drug-induced proarrhythmias: room for improvement. *Europace.* 2007; 9: iv23-iv36.

28. Sager PT. Key clinical considerations for demonstrating the utility of preclinical models to predict clinical drug-induced torsades de pointes. *Br J Pharmacol.* 2008; 154: 1544-1549.

29. Drake FT, Haring O, Singer DH, et al. Proceedings: Evaluation of anti-arrhythmic efficacy of perhexiline maleate in ambulatory patients by Holter monitoring. *Postgrad Med J.* 1973; 49: Suppl 3:52-63.

30. Sukerman M. Proceedings: Clinical evaluation of perhexiline maleate in the treatment of chronic cardiac arrhythmias of patients with coronary heart disease. *Postgrad Med J.* 1973; 49: Suppl 3:46-52.

31. World Health Organization UMC: Perhexiline: Reported Suspected Adverse Drug Reactions. Available at: http://www.vigiaccess.org; Accessed June 16, 2021

Figures

Figure 1

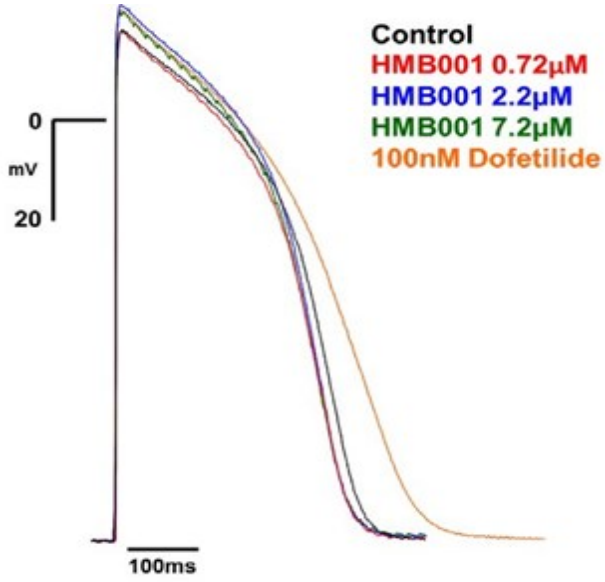

Figure 2

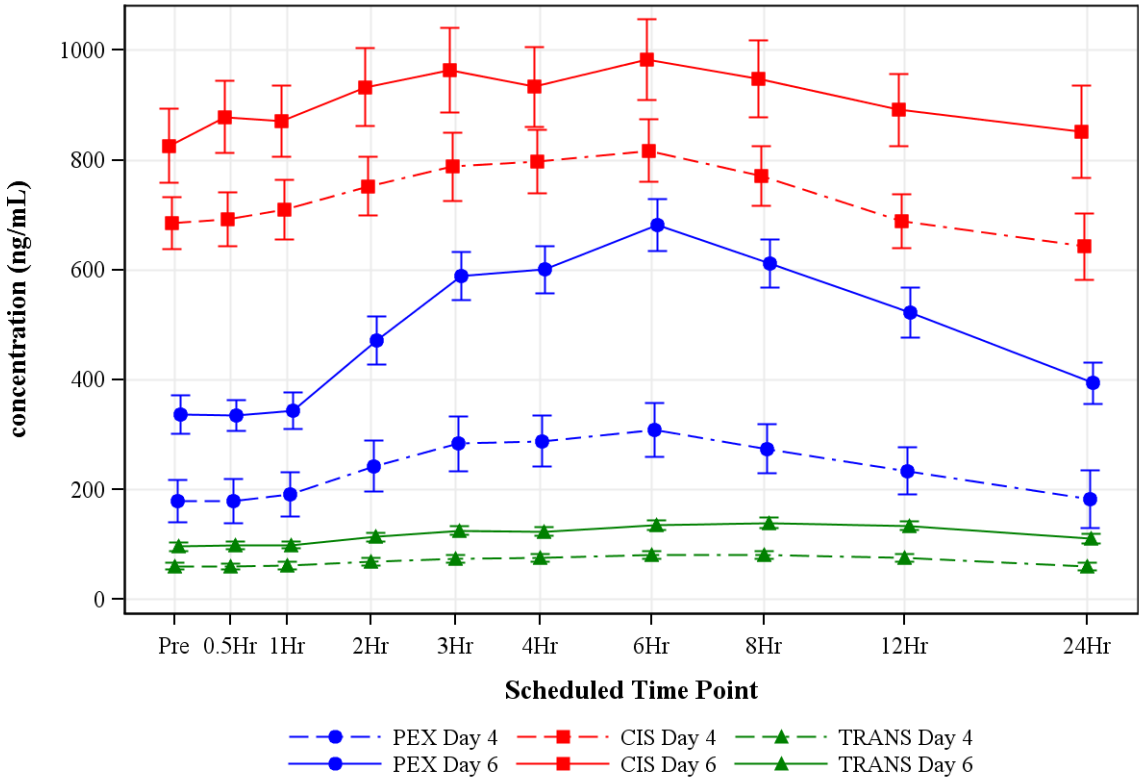

Figure 3, panel A

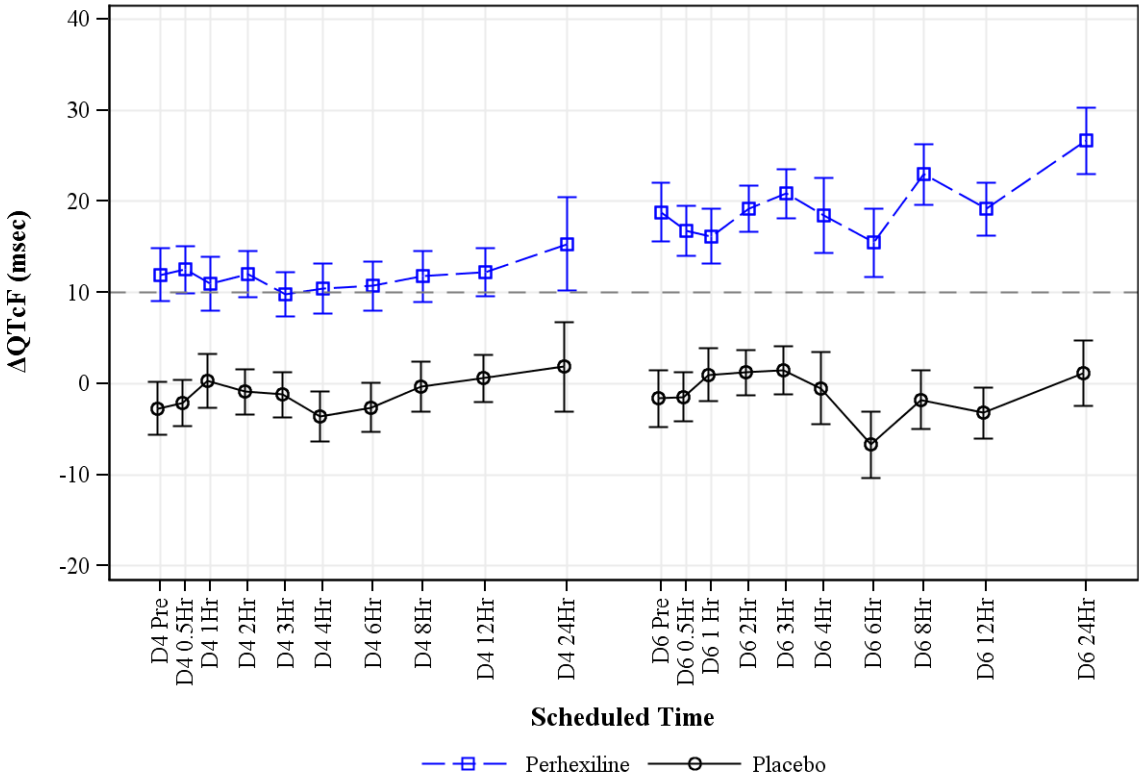

Figure 3, panel B

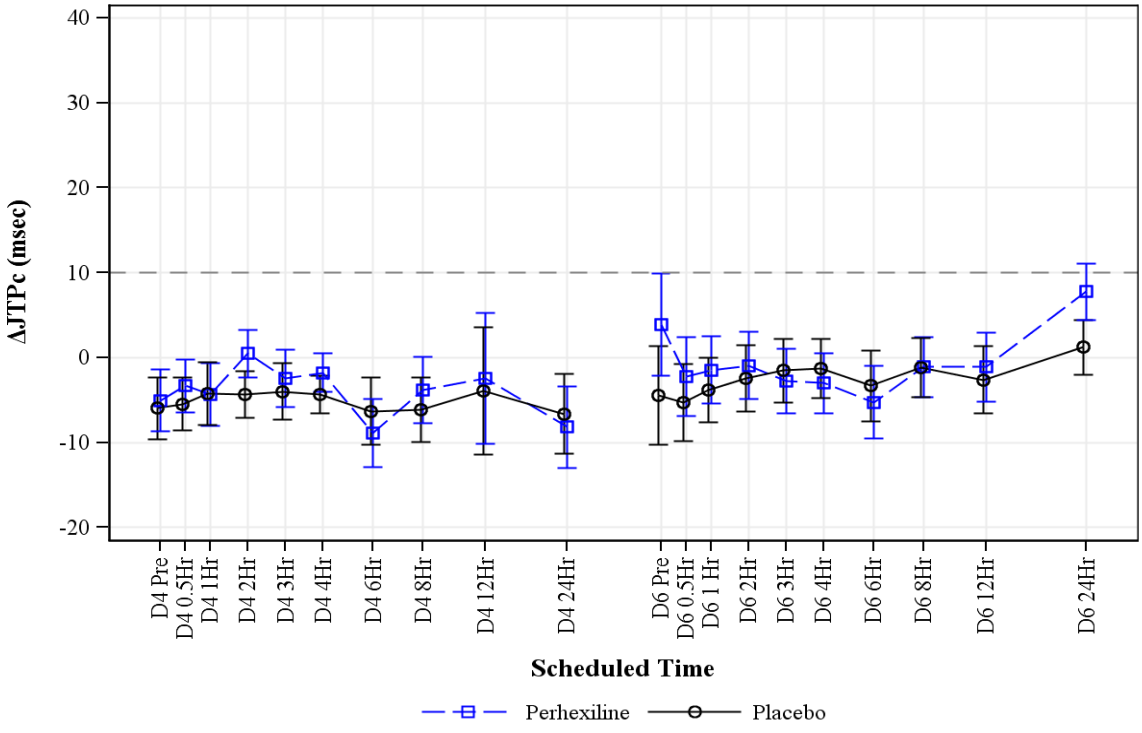

Figure 4

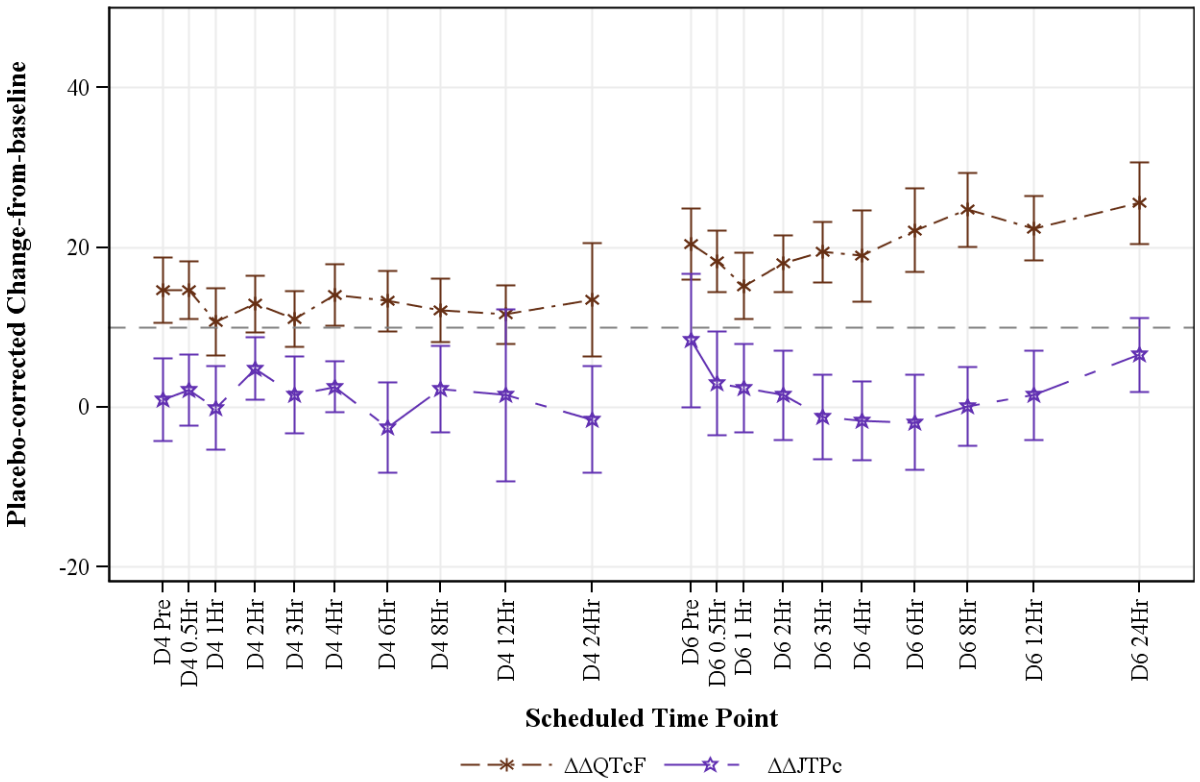

Figure 5 , panel A

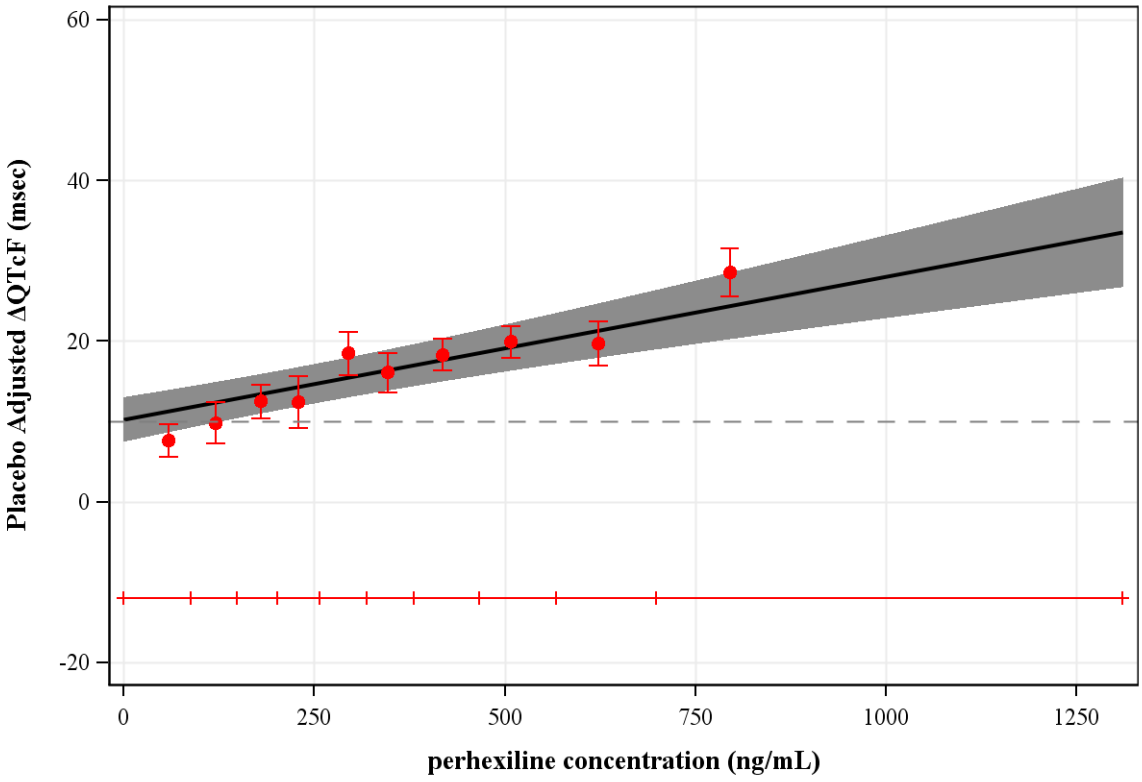

Figure 5, panel B