ORIGINAL ARTICLE

Effect of anagrelide on cardiac repolarization in healthy volunteers: a randomized, double-blind, placebo- and positive-controlled, thorough QT study

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Abstract

The aim of this study was to assess the effect of low and high therapeutic doses of anagrelide (0.5 and 2.5 mg) on cardiac repolarization, measured by the QTcNi (individual correction) and QTcF (Fridericia's correction), compared with placebo and moxifloxacin 400 mg (positive control) in healthy subjects. In this randomized, double-blind, crossover trial, 60 healthy volunteers were enrolled and randomized. Anagrelide 0.5 and 2.5 mg rapidly increased mean heart rate (HR) by 7.8 and 29.1 bpm respectively. For anagrelide 2.5 mg, the maximum time-matched change (one-sided 95% upper confidence bound) in mean QTcNi and QTcF was 13.0 msec (15.7 msec) and 10.0 msec (12.7 msec), respectively, at 1 h post dose. However, time-matched changes in QTcNi and QTcF quickly decreased to <6 msec by 2 h post dose, despite high stable HR and high plasma concentrations of anagrelide and its active metabolite at these times. For anagrelide 0.5 mg, the maximum time-matched change in mean QTcNi and QTcF was 7.0 msec (9.8 msec) and 5.0 msec (8.0 msec) respectively. No new safety concerns were reported. The increase in QTc interval met the definition for a positive thorough QT/QTc study only when HR was increasing rapidly, suggesting that the increased QTc may be related to the rapidly increasing HR rather than a direct effect of anagrelide plasma concentrations. However, the direct causal relationship of anagrelide on cardiac repolarization cannot be completely excluded, and caution is advised when treating patients with known risk factors for QT interval prolongation.

Abbreviations

AEs, adverse events; AUC, area under curve; BMI, body mass index; C_{max}, maximum plasma concentration; CARIM, Cardiovascular Research Institute Maastricht; CI, confidence interval; CL/F, oral clearance; CPU, clinical pharmacology unit; CV, co-efficient of variation; CYP1A2, cytochrome P450 1A2; ECG, electrocardiogram; HR, heart rate; HPLC-MS/MS, high-performance liquid chromatography tandem mass spectrometry; ICH, International Conference on Harmonisation; LSM, least squares mean; Med-DRA, Medical Dictionary for Regulatory Activities; MedDRA, Medical Dictionary for Regulatory Activities; PDE III, phosphodiesterase III HRherat rate; QTcB, Bazett-corrected QT; QTcF, Fridericia-corrected QT; QTc, HR-corrected QT; QTcNi, subject-specific QT; QTcV, QTc according to the Van de Water

formula; SD, standard deviation; SNP, single nucleotide polymorphism; TEAE, treatment-emergent AE; $t_{1/2}$, elimination half-life; t_{max}, maximal plasma level.

What is Already Known About this Subject

- Anagrelide is a phosphodiesterase III inhibitor with positive inotropic and vasodilation properties
- Postmarketing cases of torsades de pointes and ventricular tachycardia have been reported with anagrelide
- As all non-anti-arrhythmic drugs should be evaluated for their potential to prolong the QTc interval, this study was conducted in accordance with regulatory requirements

What this Study Adds

- Anagrelide demonstrated a moderate increase in corrected QT interval associated with a phosphodiesterase III-related rapid increase in heart rate in healthy subjects
- Results indicate that anagrelide may not significantly increase pro-arrhythmic risk
- Caution is advised when treating patients with known risk factors for QT interval prolongation

Introduction

Anagrelide is an orally active, quinazoline-derived, platelet-lowering agent that selectively targets megakaryocytes (Thiele et al. 2006). The precise mechanisms of action are not yet fully understood, but are believed to be mediated through reduced expression of GATA-1 and FOG-1 transcription factors and subsequent suppression of megakaryocytopoiesis (Ahluwalia et al. 2010). Anagrelide has been shown to effectively reduce platelet counts in patients with essential thrombocythemia (Harrison et al. 2005; Gisslinger et al. 2013) or thrombocytosis associated with myeloproliferative neoplasms (Steurer et al. 2004). Independently of this activity, anagrelide has been shown to potently inhibit cyclic adenosine monophosphate phosphodiesterase III (PDE III) (Gillespie 1988). PDE III inhibition is associated with vasodilatory, and positive inotropic and chronotropic effects, which may be responsible for the frequently reported palpitations, tachycardia, headache, and dizziness in patients with essential thrombocythemia (Birgegard et al. 2004; Mazzucconi et al. 2004; Harrison et al. 2005).

Anagrelide undergoes extensive first-pass metabolism via cytochrome P450 1A2 (CYP1A2) to form its active metabolite, 3-hydroxy-anagrelide, which is further metabolized to an inactive metabolite, RL603 (Wang et al. 2005; European Medicines Agency 2013). It is rapidly absorbed, reaching maximum plasma concentrations (C_{max}) within 0.5–1.5 h after administration (Besses et al. 2012; Martinez-Selles et al. 2013). Both anagrelide and 3-hydroxy-anagrelide are rapidly eliminated, with terminal half-lives ($t_{1/2}$) of approximately 1.7 and 3.1 h, respectively, in healthy volunteers (fasted state) (Martinez-Selles et al. 2013).

Results from preclinical evaluations suggest that anagrelide and its metabolites do not affect cardiac repolarization. In vitro, anagrelide did not significantly inhibit hERG tail current in HEK293 cells at concentrations up to 500 ng/mL ($>30\times$ clinical C_{max}) (K. McCulloch and A. G. B. Templeton, unpubl. data on file; Shire Development LLC, Lexington, MA). RL603 produced slow onset of hERG inhibition that was significant at concentrations above 300 ng/mL ($>180\times$ clinical C_{max}), but the concentration that inhibits 50% of the effect (IC_{50}) could not be estimated. The active metabolite, 3-hydroxy-anagrelide, was discovered late in the clinical development of anagrelide, and hERG evaluations were not performed for this metabolite. In dog cardiovascular assessments, anagrelide and 3-hydroxy-anagrelide both produced increased heart rate (HR), and reduced QT and HR-corrected QT (QTc) according to the Van de Water formula (QTcV) intervals (P. R. Attison and K. J. Baldouf, unpubl. data on file; Shire Development LLC, Lexington, MA), indicating that neither anagrelide nor 3-hydroxy-anagrelide cause QT prolongation in dogs. Similar findings were also observed in healthy human volunteers who received a single dose of anagrelide 1 mg; HR increased, and QRS and QT intervals decreased following anagrelide administration, but QTc interval did not change (Martinez-Selles et al. 2013).

The assessment of drug-induced changes in the QT and QTc interval has become a necessary component of drug development. This is because of the potential for new drugs with suspected or known effects on the heart to cause life-threatening delayed cardiac repolarization (manifested as QT prolongation) and torsades de pointes. Thus, the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline E14 (ICH E14) recommends companies to perform a thorough QT study to characterize the drug effect.

The present study was undertaken in accordance with regulatory requirements. The aim of the study was to assess the effect of low and high therapeutic single doses of anagrelide on cardiac repolarization, using the QT/QTc interval as a biomarker, in healthy subjects.

Materials and Methods

Objectives

The primary objective of the study was to evaluate the effect of low and high therapeutic single doses of anagrelide on the QT/QTc interval compared with placebo and the positive control moxifloxacin in healthy subjects. The secondary objectives were to examine the pharmacokinetic profile of anagrelide and 3-hydroxy-anagrelide, and explore the relationship between pharmacokinetic and pharmacodynamic effects, safety, and tolerability.

Trial design

This was a phase I, randomized, double-blind, placebo- and positive-controlled, four-period crossover trial conducted at a clinical pharmacology unit (CPU) in France (NCT01552928). The study included four treatment periods, with a 96-h washout period between treatments, during which subjects remained in the CPU for 16 days (15 nights). A follow-up clinical evaluation was performed 7 days (±2 days) after the last dose of investigational product. The study was conducted in accordance with the Declaration of Helsinki and ICH Good Clinical Practice guidelines, and according to local ethical and legal requirements. Prior to study initiation, the study protocol, protocol amendments, informed consent forms, and other documents were approved by the Independent Ethics Committee, Comité de Protection des Personnes Ile de France VIII (study identification code: 12 03 24); and regulatory agency, Agence Nationale de Sécurité du Médicament et des produits de santé (study identification code: A120221-27), formerly Agence Française de Sécurité Sanitaire du Médicament et des produits de santé. All participants provided written informed consent.

Participants

Eligible subjects were healthy volunteers aged 18–45 years; had a body mass index (BMI) of 18.5–30.0 kg/m^2; were willing to comply with contraceptive requirements of the protocol (both men and women); were not pregnant or lactating and were ≥90 days post-partum or nulliparous (women only); and were able to swallow a dose of investigational product. Subjects with a history of, or current baseline prolongation, of QT/QTc interval (e.g., repeated QTc or QT interval >450 msec for men and >470 msec for women) or history of additional risk factors for torsades de pointes, such as heart failure, hypokalemia, or family history of long QT syndrome, were excluded from the study.

Table 1. Randomized treatment sequences.

| Sequence | Day 1 | | | |
	Period 1	Period 2	Period 3	Period 4
1	Anagrelide 0.5 mg	Anagrelide 2.5 mg	Placebo	Moxifloxacin 400 mg
2	Anagrelide 2.5 mg	Moxifloxacin 400 mg	Anagrelide 0.5 mg	Placebo
3	Placebo	Anagrelide 0.5 mg	Moxifloxacin 400 mg	Anagrelide 2.5 mg
4	Moxifloxacin 400 mg	Placebo	Anagrelide 2.5 mg	Anagrelide 0.5 mg

Interventions

Subjects were randomized to one of the four treatment sequences (4 × 4 William's square design; Table 1) and received all of the four following treatments accordingly: anagrelide 0.5 mg (low therapeutic arm); anagrelide 2.5 mg (high therapeutic arm); moxifloxacin 400 mg (positive control); placebo.

In this study, the highest dose of anagrelide 2.5 mg represents a high therapeutic dose. Anagrelide doses >2.0 mg have not been administered to healthy subjects since the first-in-human study of anagrelide. In that first study, a single dose of anagrelide 5 mg produced clinically significant hypotension, orthostatic hypotension, and tachycardia related to PDE III inhibition in all nine healthy subjects who were administered anagrelide (Bristol Laboratories BLA-4162A Study No. 1, Study Report 1772, A. Glick, unpubl. data). Thus, anagrelide 2.5 mg was chosen to ensure subject safety and minimize withdrawals whilst providing clinically meaningful drug exposures to adequately assess the effect of anagrelide and its active metabolite on QTc prolongation.

A single dose of each investigational product was administered with 240 mL of water to subjects after an overnight fast of approximately 10 h on day 1 (day of dosing) of each period, and subjects continued to fast for approximately 4 h after dosing. Subjects were discharged on day 14 (i.e., 24 h after last dose in period 4). In order to reduce intersubject variability, all subjects ate the same lunch and dinner (both of low-to-moderate fat content) on day −1 (baseline day) and day 1 in each treatment period.

Assessments

Serial blood samples (6 mL) for pharmacokinetic evaluation were collected on day 1 at predose, and at the same time points as the electrocardiogram (ECG) recordings below. An additional blood sample was collected on day −1

for treatment periods 2, 3, and 4 for moxifloxacin analysis only to control for any unexpected residual plasma concentrations. Plasma samples were assayed using previously validated high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methods for anagrelide and 3-hydroxy-anagrelide at York Bioanalytical Solutions (York, UK, method described in [Besses et al. 2012]), or for moxifloxacin at Advion BioServices, Inc. (Ithica, NY).

A 12-lead ECG was recorded at screening; day −2 of period 1 only (admission); days −1 and 1 after dose administration in all study periods; day 2 of period 4 prior to discharge from the unit or at time of early termination; and at the follow-up visit. On day −1 and day 1 of each period, continuous ECG data were recorded using a Holter monitor from 60 min before the anticipated dose time on day 1 until approximately 24 h later. Three 10-second, 12-lead ECG waveforms were extracted when possible from the continuous ECG recording within the 60 min prior to dosing and up to 10 min before each of the following time points: 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, and 12 h post dose. To account for the anticipated increase in HR associated with the PDE III inhibitory effect of anagrelide, subjects participated in 10 min of light exercise (i.e., brisk walking) 1.5–2 h after the nominal dose time (corresponding to the time taken to achieve maximal anagrelide plasma levels [t_{max}]) during the baseline day. This was performed to provide a drug-free HR range of time-matched baseline ECG tracings for postdose comparisons.

Laboratory evaluations (biochemistry, hematology, and urinalysis) were performed by the CPU's local laboratory at screening, day −2 (period 1 only), day −1 (all periods), day 2 of period 4 or early termination, and at the follow-up visit. All laboratory evaluations occurred under fasting conditions except at screening and day −2 of period 1 as per protocol. Vital signs and physical examinations were performed throughout the treatment periods.

In compliance with the recommendations in the ICH E14, blood samples (8.5 mL) were collected predose on day 1 of period 1 for potential pharmacogenetic research of any outlier subjects. The DNA sample from one outlier subject was analyzed at the Department of Clinical Genetics at the Cardiovascular Research Institute Maastricht (CARIM), a clinical genetics laboratory accredited by the Coordination Committee for Quality Assurance in Health Care laboratories in the Netherlands; with validated screening protocols established for analysis of long QT syndrome genes. Analysis consisted of: bidirectional sequencing of coding regions (including intron-exon boundaries) of the long QT syndrome genes KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2, CAV3, and exon 8 of CACNA1C; and multiplex ligation-dependent probe amplification analysis of genes KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2.

Adverse events (AEs) were recorded throughout the study according to Medical Dictionary for Regulatory Activities (MedDRA) version 14.1.

Endpoints

The primary endpoint was QTcNi, defined as the QT interval corrected for heart rate using a subject-specific method. The QTcNi correction was derived from the individual subjects based on their index dataset. Each index dataset comprised the pooled day −1 ECG data from all four treatment periods plus the ECG data from day 1 of the placebo treatment (total 50 observations). Pharmacodynamic endpoints included changes from baseline (time-matched baseline from day −1 of the corresponding treatment period) in HR (and the corresponding RR interval), and the PR, QRS, QT, QTcNi, Fridericia-corrected QT (QTcF) and Bazett-corrected QT (QTcB) intervals. The pharmacokinetic profile of anagrelide and 3-hydroxy-anagrelide was assessed and the relationship between pharmacokinetic and pharmacodynamic parameters was explored. Safety and tolerability were assessed throughout the study by routine recording of ECGs, vital signs, laboratory safety assessments, physical examinations, and AEs.

Statistical methods

Pharmacokinetic parameters were determined from the plasma concentration-time data by non-compartmental analysis. Pharmacokinetic analyses were performed using WinNonlin Version 5.1.1 (Pharsight Corporation, Mountain View, CA). Plasma concentrations of anagrelide, 3-hydroxy-anagrelide, and moxifloxacin were determined using sensitive and specific, validated HPLC-MS/MS methods at York Bioanalytical Solutions (York, UK). For anagrelide and 3-hydroxy-anagrelide, the assay was linear over the range of 0.05–20 ng/mL with a lower limit of quantification of 0.05 ng/mL for both compounds. The interassay accuracy (%-bias) and precision (% co-efficient of variation [CV]) data for the quality control samples at 3 concentrations across all analytical batches were <10%, and all assay runs met the predefined acceptance criteria. For moxifloxacin, the assay was linear over the range of 25–5000 ng/mL with a lower limit of quantification of 25 ng/mL. The interassay accuracy (%-bias) and precision (% CV) data for the quality control samples at four concentrations across all analytical batches were <7%. All assay runs met the predefined acceptance criteria.

The sample size for this study was selected to be able to detect a true mean difference of 5 msec in the QTc interval (change from baseline) between the active and placebo groups with 90% power (and $k = 10$ time points), assuming a true standard deviation ≤6 msec. The threshold level of regulatory concern is around 5 msec as evidenced by an upper bound 95% one-sided confidence interval (CI) around the mean effect on QTc of 10 msec (European Medicines Agency 2005). For a 4×4 Williams crossover design consisting of four treatments and four periods in four pre-specified sequences and a total sample size of 48, 12 subjects were to be assigned to each treatment sequence. Approximately 60 subjects were to be enrolled to ensure 48 subjects completed the crossover design. This sample size would provide >90% power to show nonsuperiority (one-sided, 5% type I error) with an acceptance limit of 10 msec.

Time-matched analysis was used to determine and analyze the pharmacodynamic endpoints. The analysis examined the baseline-adjusted, largest time-matched drug/placebo difference in QTc intervals at 0.5–12 h post dose on day 1. The analysis aimed to determine if the upper boundary of the two-sided 90% CI for the largest time-matched baseline- and placebo-adjusted mean difference in QTcNi exceeded 10 msec. In addition, a repeated-measures analysis equalized variances across time points and yielded similar results. To demonstrate assay sensitivity, the lower boundary of the two-sided 90% CI for the positive control moxifloxacin exceeded 5 msec at ≥1 time points in the window of maximal concentrations of moxifloxacin (usually 1–5 h after administration); adjustments were made for multiple comparisons. The secondary objective comprised analysis of the pharmacodynamic variables at subject-specific t_{max} with an analysis of covariance framework using a mixed-effects model.

For 12-lead ECG data, baseline was defined as the mean of the three measurements on day −1 of each period. When triplicate readings were completed for a time point, the average of the three values was used to summarize continuous variables. If triplicate readings could not be made, the mean of the available values was used.

All demographic and baseline characteristic data, pharmacodynamic and safety data analyses were performed using SAS® Version 9.2 (SAS Institute, Cary, NC).

Results

Subjects

Between March 29 and July 25, 2012, 117 subjects were screened, of whom 60 (35 men and 25 women) were randomized to one of the four treatment sequence groups. All 60 subjects were included in the full analysis set, safety analysis set, and pharmacokinetic analysis set. Subject baseline characteristics are summarized in Table 2. The treatment sequence groups were generally balanced in terms of age, gender, race, ethnicity, weight, height, and BMI.

One subject did not complete the study and was withdrawn as a result of a treatment-emergent AE (TEAE) of ECG T-wave inversion after receiving anagrelide 2.5 mg in period 3, and thus did not receive treatment in period 4. The subject was, however, monitored until the follow-up visit.

The DNA sample from one subject who exhibited high baseline QTc values and a moderate increase from baseline in QTc underwent pharmacogenetic analysis. The sample was identified to be heterozygous for single nucleotide polymorphism (SNP) rs1805128 in *KCNE1*.

Pharmacokinetics

Following single-dose administration to fasted subjects, anagrelide was rapidly absorbed (mean t_{max} of 1.15–1.51 h) and subsequently eliminated with a mean $t_{1/2}$ of 1.39–1.69 h (Fig. 1). Similarly, 3-hydroxy-anagrelide was formed rapidly (mean t_{max} of 1.16–1.93 h) and was subsequently eliminated with a mean $t_{1/2}$ of 2.02–2.21 h (Fig. 1). For anagrelide, the geometric mean C_{max} was 55–75% higher and the mean area under the plasma concentration–time curve (AUC) was approximately 90% higher in women versus men. For 3-hydroxy-anagrelide, the mean C_{max} was 21–26% higher and the mean AUC

Table 2. Subject baseline characteristics (safety analysis set).

Characteristic	Total ($N = 60$)
Age, years	
Mean (SD)	29.2 (6.96)
Median (range)	27.0 (19–44)
Gender, *n* (%)	
Male	35 (58.3)
Female	25 (41.7)
Race, *n* (%)	
White	44 (73.30)
Black or African-American	13 (21.07)
Asian	2 (3.3)
American Indian or Alaska Native	1 (1.7)
Weight, kg	
Mean (SD)	71.2 (12.4)
Median (range)	71 (47.7–100.7)
BMI, kg/m²	
Mean (SD)	24.0 (2.8)
Median (range)	23.7 (19.1–30.0)

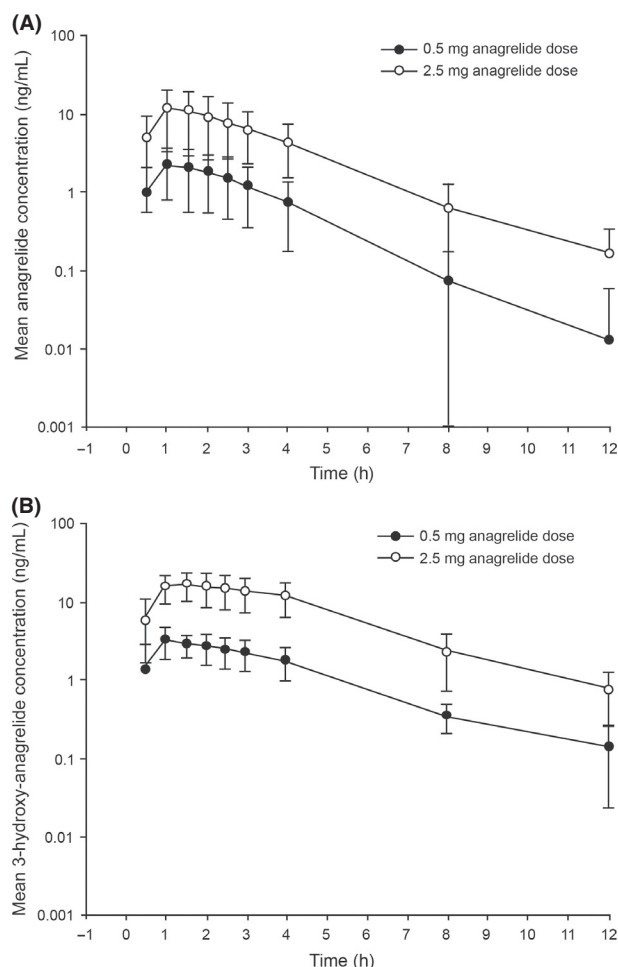BMI, body mass index; SD, standard deviation.

Figure 1. Mean ± SD plasma concentrations of anagrelide (A) and 3-hydroxy-anagrelide (B) in healthy subjects following administration of anagrelide (0.5 mg and 2.5 mg) (log scale) (pharmacokinetic analysis set). SD, standard deviation.

was approximately 45% higher in women versus men. Pharmacokinetic parameters of anagrelide and 3-hydroxy-anagrelide are summarized in Table 3. Pharmacokinetic exposure to anagrelide and 3-hydroxy-anagrelide increased approximately linearly in proportion with the fivefold increase in anagrelide dose in both men and women.

The anagrelide and 3-hydroxy-anagrelide exposures in the subject identified to be heterozygous for SNP rs1805128 in *KCNE1* were unremarkable for the anagrelide 0.5 mg dose, but the anagrelide exposures were higher than the average for the other subjects with the anagrelide 2.5 mg dose. The anagrelide and 3-hydroxy-anagrelide exposures in the subject who exhibited an inverted T-wave change were below the average for other subjects receiving this treatment.

Pharmacodynamics

Heart rate

Mean HR at 2 h postdose on day 1 was 57.9, 61.7, 65.8, and 87.3 bpm for placebo, moxifloxacin 400 mg, anagrelide 0.5 mg, and anagrelide 2.5 mg treatments respectively. The mean HR values after light exercise on day −1 corresponding to 2 h post dose ranged from 69.9 to 71.3 bpm. Time-matched differences from placebo in mean change from baseline HR are shown in Figure 2. Compared with placebo, anagrelide 0.5 mg increased mean HR at 1–8 h post dose (by 5.0–7.8 bpm from 1 to 4 h after administration). Anagrelide 2.5 mg rapidly increased HR 0.5–1.5 h after administration, with HR remaining elevated for up to 12 h. The largest increase of 29.1 bpm for anagrelide 2.5 mg and 7.8 bpm for anagrelide 0.5 mg occurred 2 h after administration. Women experienced greater increases in HR than men (maximum increase in mean ΔΔHR 36.6 vs. 24.9 bpm, respectively, for anagrelide 2.5 mg; data not shown), which remained elevated for a longer duration.

QTc interval

The QT versus RR regression slopes before (QT) and after subject-specific correction (QTcNi) for each individual subject are displayed in Figure 3A. Women had slightly higher QT regression slopes than men, although there is considerable overlap between the regression slopes in men and women. After correction, the regression slopes for QTcNi versus RR were almost zero for all subjects, demonstrating that the correction for HR worked well for the index dataset. However, a slight negative population correlation between RR and both QTcNi ($r = -0.15$) and QTcF ($r = -0.02$) was observed.

Time-matched differences in QTcNi and QTcF intervals compared with placebo are shown in Figures 3B and C respectively. After administration of anagrelide 0.5 mg, the maximum mean prolongation of QTcNi and QTcF did not exceed 7.0 msec and the one-sided 95% upper confidence bound was <10 msec at each observation time (Table 4). For anagrelide 2.5 mg, the mean increase was 13.0 and 10.8 msec at 1 and 1.5 h post dose for QTcNi, respectively, and 10.0 msec at 1 h post dose for QTcF. At these time points, one-sided 95% upper confidence bound exceeded 10 msec, but was <10 msec at all other observation times (Table 4). The expected QTcNi and QTcF prolongation after moxifloxacin administration was observed, with the one-sided 95% lower confidence bound >5 msec 1–8 h after administration, and the most pronounced effects observed 1–4 h post dose (Table 4). Women experienced higher maximum increase in mean

Table 3. Summary of pharmacokinetic parameters in healthy subjects receiving single-dose anagrelide 0.5 mg and anagrelide 2.5 mg (pharmacokinetic analysis set).

Treatment/Analyte	N	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng h/mL)	$t_{1/2}$ (h)	CL/F (L/h)
Mean ± SD (geometric mean)						
Anagrelide/0.5 mg						
Female	25*	3.64 ± 1.96 (3.17)	1.20 ± 0.55 (1.10)	9.81 ± 5.77 (8.60)	1.39 ± 0.33 (1.36)	64.7 ± 28.2 (58.1)
Male	34[†]	2.08 ± 0.98 (1.87)	1.15 ± 0.58 (1.03)	5.23 ± 1.89 (4.89)	1.54 ± 0.62 (1.46)	110.4 ± 48.0 (102.3)
Anagrelide/2.5 mg						
Female	25	16.38 ± 10.22 (13.72)	1.51 ± 0.56 (1.43)	53.08 ± 29.46 (46.07)	1.69 ± 0.43 (1.65)	61.9 ± 31.2 (54.3)
Male	35	10.59 ± 4.87 (9.61)	1.19 ± 0.47 (1.10)	27.76 ± 10.19 (26.17)	1.67 ± 0.37 (1.63)	100.9 ± 33.1 (95.6)
3-hydroxy-anagrelide/0.5 mg						
Female	25*	4.53 ± 1.41 (4.34)	1.34 ± 0.67 (1.20)	17.69 ± 4.26 (17.27)	2.05 ± 0.42 (2.02)	29.6 ± 6.0 (29.0)
Male	34	3.73 ± 1.26 (3.52)	1.16 ± 0.64 (1.03)	12.29 ± 3.13 (11.90)	2.21 ± 0.40 (2.18)	43.5 ± 11.6 (42.0)
3-hydroxy-anagrelide/2.5 mg						
Female	25	23.79 ± 7.95 (22.27)	1.93 ± 0.88 (1.77)	105.76 ± 33.18 (100.41)	2.02 ± 0.60 (1.95)	26.4 ± 10.3 (24.9)
Male	35	18.93 ± 6.02 (18.05)	1.30 ± 0.47 (1.22)	72.73 ± 19.71 (70.08)	2.12 ± 0.31 (2.10)	37.1 ± 10.6 (35.7)

$AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero to infinity; CL/F, oral clearance; C_{max}, maximum plasma concentration; SD, standard deviation; t_{max}, time to maximum plasma concentration; $t_{1/2}$, terminal half-life.
*N = 24 for $AUC_{0-\infty}$, $t_{1/2}$ and CL/F; [†]N = 33 for $AUC_{0-\infty}$, $t_{1/2}$ and CL/F.
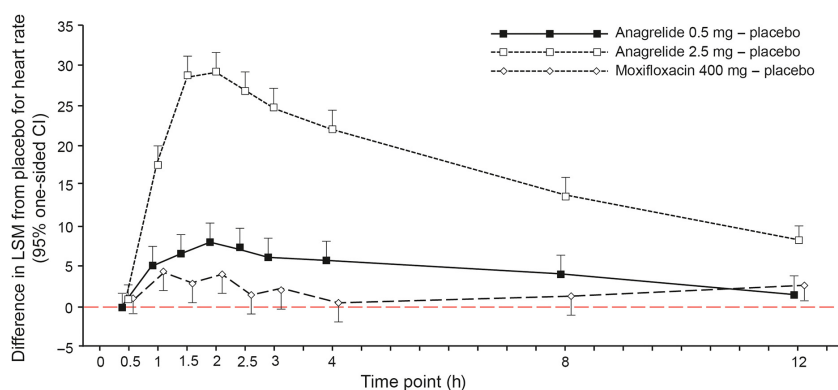

Figure 2. Twelve-lead electrocardiogram: time-matched analysis of the difference from placebo in least squares mean change from baseline in heart rate (bpm) in healthy subjects receiving a single dose of anagrelide (0.5 or 2.5 mg) or moxifloxacin 400 mg (full analysis set). Upper bars are the one-sided 95% upper confidence bound for mean changes from baseline between anagrelide and placebo, and the lower bars are the one-sided 95% lower confidence bound for mean changes from baseline between moxifloxacin and placebo. CI, confidence interval; LSM, least squares mean.

QTcNi than men (16.2 vs. 10.8 msec at 1 h after anagrelide 2.5 mg) and QTcF (11.6 vs. 8.8 msec at 1 h after anagrelide 2.5 mg). However, the increase in mean QTcNi and QTcF rapidly dissipated when the HR stabilized in both men and women.

A comparison of the change in mean ΔΔQTcNi and ΔΔQTcF to the change in mean ΔΔHR for anagrelide 2.5 mg demonstrated a pronounced clockwise hysteresis (Fig. 4). The HR and QTc both increased rapidly from 0.5 to 1.5 h after administration, but the HR continued to increase through 2 h after administration then slowly decreased over time as the QTc values decreased rapidly beyond the 1-h time point. The hysteresis between QT and HR (or RR) occurs when the HR changes rapidly,

and the QT can take up to 2 min to adjust to instantaneous changes in HR, making it difficult to accurately correct the QT interval while the HR is changing rapidly (Malik et al. 2008; Garnett et al. 2012). The continuous QT-RR hysteresis over 0.5–1.5 h kept the QT and RR out of sync. The anagrelide 0.5 mg dose showed less of a hysteresis in the relationship between ΔΔQTcNi and ΔΔQTcF versus ΔΔHR because the HR increased less rapidly for this treatment. Figure 4 shows the relationship between HR and QTc stabilizing between 2.5 and 12 h after administration when the HR was slowly decreasing back to baseline values (appears as a generally linear relationship with zero slope in Figure 4).
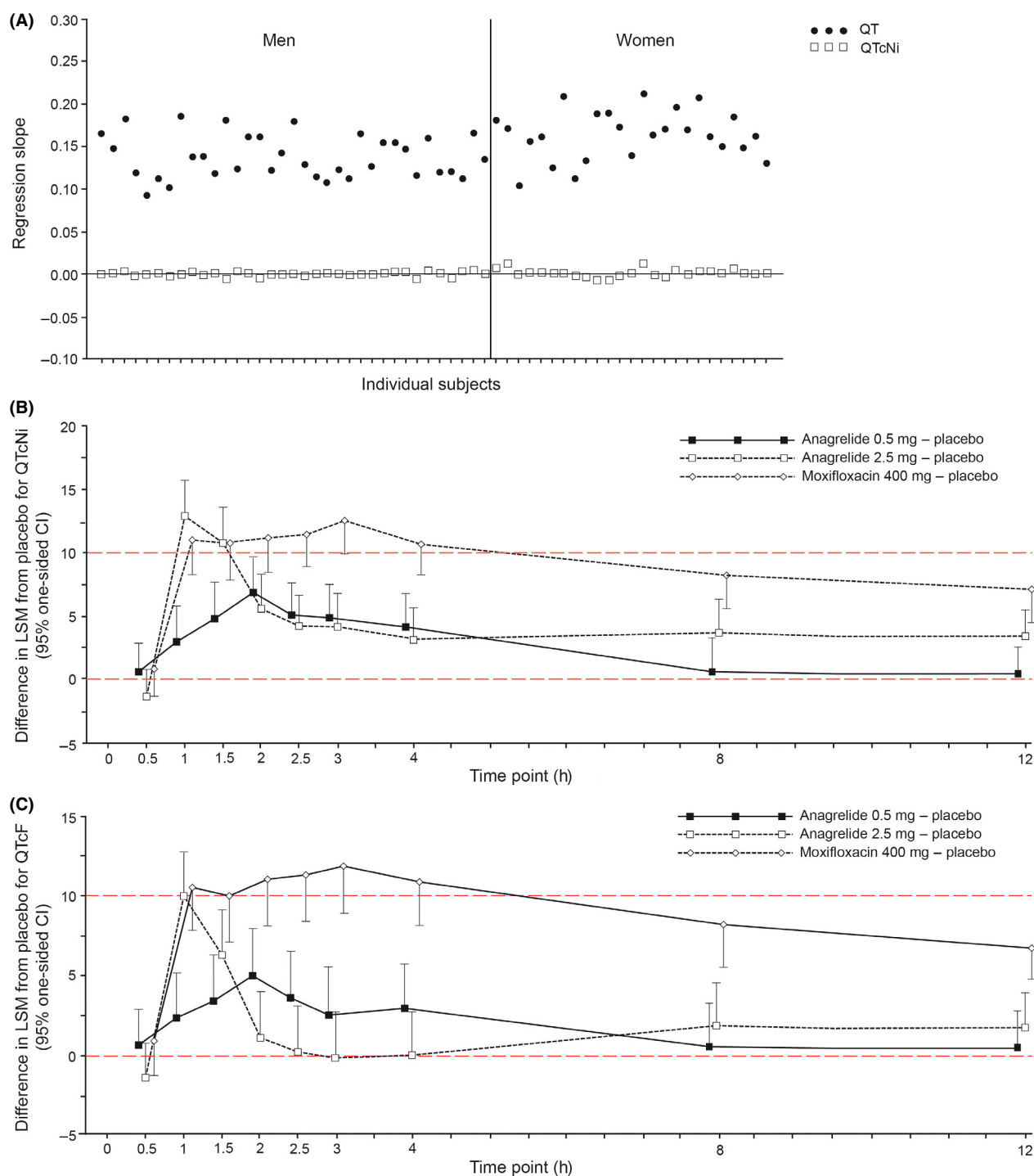
Figure 3. (A) Regression slopes of QT versus RR relationship for each individual subject before (QT) and after subject-specific correction (QTcNi) on day −1 and placebo day 1; (B and C) Time-matched analysis (double-delta method) of the difference from placebo in least squares mean change from baseline for QTcNi (msec; B) and QTcF (msec; C) in healthy subjects receiving a single dose of anagrelide (0.5 or 2.5 mg) or moxifloxacin 400 mg (full analysis set). Upper bars are the one-sided 95% upper confidence bound for mean changes from baseline between anagrelide and placebo, and the lower bars are the one-sided 95% lower confidence bound for mean changes from baseline between moxifloxacin and placebo. CI, confidence interval; LSM, least squares mean; QTcF, Fridericia-corrected QT interval; QTcNi, subject-specific-corrected QT interval.

Table 4. Mean change from baseline in heart rate (bpm), QTcNi (msec), and QTcF (msec); pairwise comparisons of anagrelide and moxifloxacin versus placebo from time-matched analyses by time point (full analysis set).

Day 1		Heart rate			QTcNi			QTcF		
			95% one-sided CI			95% one-sided CI			95% one-sided CI	
Treatment	N	Mean difference versus placebo	Lower	Upper	Mean difference versus placebo	Lower	Upper	Mean difference versus placebo	Lower	Upper
Hour 0.5										
Anagrelide 0.5 mg	49	−0.1	−1.7	1.5	0.7	−1.6	2.9	0.7	−1.6	2.9
Anagrelide 2.5 mg	54	1.1	−0.4	2.7	−1.3	−3.5	0.9	−1.4	−3.5	0.8
Moxifloxacin 400 mg	55	0.9	−0.7	2.4	0.9	−1.2	3.1	1.0	−1.2	3.1
Hour 1										
Anagrelide 0.5 mg	51	5.0	2.6	7.4	3.1	0.3	5.8	2.4	−0.4	5.2
Anagrelide 2.5 mg	55	17.7	15.3	20.1	13.0	10.3	15.7	10.0	7.3	12.7
Moxifloxacin 400 mg	53	4.3	1.8	6.7	11.1	8.3	13.8	10.6	7.8	13.3
Hour 1.5										
Anagrelide 0.5 mg	51	6.5	3.7	9.3	4.9	2.0	7.8	3.4	0.5	6.3
Anagrelide 2.5 mg	56	28.8	26.1	31.5	10.8	8.0	13.6	6.3	3.5	9.1
Moxifloxacin 400 mg	53	2.9	0.2	5.7	10.8	8.0	13.6	10.0	7.1	12.8
Hour 2										
Anagrelide 0.5 mg	50	7.8	5.3	10.4	7.0	4.2	9.8	5.0	2.1	8.0
Anagrelide 2.5 mg	54	29.1	26.6	31.6	5.6	2.9	8.4	1.1	−1.8	4.0
Moxifloxacin 400 mg	55	3.9	1.4	6.4	11.3	8.6	14.0	11.0	8.1	13.9
Hour 2.5										
Anagrelide 0.5 mg	51	7.3	4.9	9.7	5.2	2.7	7.7	3.6	0.6	6.6
Anagrelide 2.5 mg	54	26.9	24.5	29.3	4.3	1.8	6.8	0.2	−2.7	3.1
Moxifloxacin 400 mg	55	1.5	−0.9	3.8	11.5	9.0	14.0	11.3	8.4	14.2
Hour 3										
Anagrelide 0.5 mg	50	6.0	3.6	8.4	4.9	2.3	7.6	2.6	−0.4	5.5
Anagrelide 2.5 mg	56	24.9	22.5	27.2	4.2	1.6	6.8	−0.1	−3.0	2.8
Moxifloxacin 400 mg	53	1.9	−0.4	4.3	12.6	9.9	15.2	11.8	8.9	14.8
Hour 4										
Anagrelide 0.5 mg	50	5.8	3.3	8.3	4.2	1.7	6.8	3.0	0.2	5.8
Anagrelide 2.5 mg	55	22.1	19.6	24.5	3.2	0.8	5.7	0	−2.7	2.7
Moxifloxacin 400 mg	56	0.4	−2.0	2.8	10.8	8.4	13.3	10.9	8.2	13.6
Hour 8										
Anagrelide 0.5 mg	52	3.9	1.7	6.2	0.7	−1.9	3.3	0.6	−2.1	3.3
Anagrelide 2.5 mg	53	13.7	11.5	16.0	3.8	1.2	6.3	1.9	−0.8	4.6
Moxifloxacin 400 mg	56	1.2	−0.1	3.4	8.2	5.7	10.8	8.2	5.6	10.9
Hour 12										
Anagrelide 0.5 mg	51	1.4	−0.4	3.2	0.1	−2.4	2.5	0.2	−2.2	2.7
Anagrelide 2.5 mg	55	8.2	6.5	10.0	3.0	0.6	5.4	1.6	−0.8	4.0
Moxifloxacin 400 mg	53	2.6	2.8	4.4	7.2	4.8	9.6	6.7	4.3	9.1

CI, confidence interval; QTcF, Fridericia-corrected QT interval; QTcNi, subject-specific-corrected QT interval.

Compared with placebo, the PR interval decreased from 1 to 4 h after treatment, but more considerably after anagrelide 2.5 mg than for anagrelide 0.5 mg or moxifloxacin 400 mg. The reduction in PR interval was associated with increased HR and corresponding decreases in RR interval for anagrelide 2.5 mg. There were no significant differences in QRS interval changes following anagrelide or moxifloxacin treatment compared with placebo.

In the categorical analysis, no subject exhibited a QTcNi or QTcF change from baseline of ≥60 msec following any of the treatments. A QTcNi change from baseline of 30–59 msec was exhibited by 10 subjects following anagrelide 2.5 mg and one subject after moxifloxacin 400 mg. A similar pattern was observed for a QTcF change from baseline of 30–59 msec; five subjects following anagrelide 2.5 mg and one subject after moxifloxacin 400 mg. No subject in the anagrelide 0.5 mg or placebo groups experienced a QTcNi or QTcF change from baseline of 30–59 msec. A QTcNi value between 450 and 479 msec was exhibited by one subject following both doses of anagrelide and placebo, but not moxifloxacin
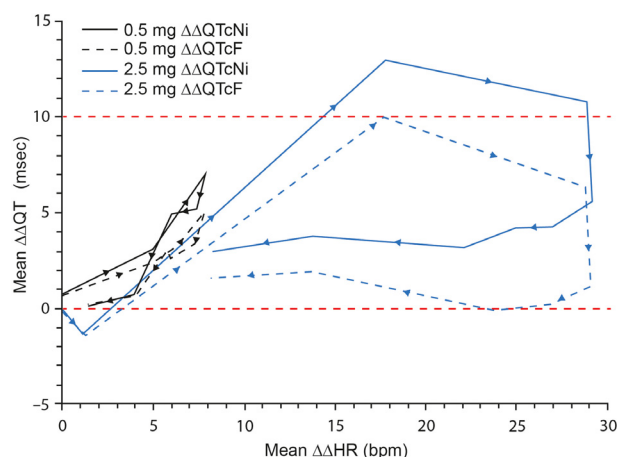
Figure 4. Hysteresis graph of the mean ΔΔQTcNi and ΔΔQTcF versus mean ΔΔHR in healthy subjects receiving anagrelide 2.5 mg (full analysis set). HR, heart rate; QTcF, Fridericia-corrected QT interval; QTcNi, subject-specific-corrected QT interval.

400 mg. Similarly, a QTcF value between 450 and 479 msec was exhibited by one subject after anagrelide 0.5 mg and placebo. No subject experienced a QTcNi or QTcF interval of ≥500 msec.

Pharmacokinetic/pharmacodynamic relationship

A comparison of the change in mean HR to the change in the mean plasma concentrations of anagrelide and 3-hydroxy-anagrelide demonstrates a counter-clockwise hysteresis (Fig. 5A and B). The hysteresis effect is more pronounced for anagrelide 2.5 mg versus anagrelide 0.5 mg. The mean anagrelide and 3-hydroxy-anagrelide plasma concentrations and mean HR both increase rapidly from 0.5 to 1.5 h after administration. Subsequently, the mean HR decreases more slowly than the mean plasma concentrations of anagrelide and 3-hydroxy-anagrelide. The changes in HR are believed to be associated with the plasma concentrations of the active metabolite, 3-hydroxy-anagrelide, because it is a more potent PDE III inhibitor than anagrelide and is present in slightly higher plasma concentrations than anagrelide. The counter-clockwise hysteresis between plasma concentrations and HR (Fig. 5A and B) combines with the clockwise hysteresis between HR and QTc (Fig. 4) to produce the generally clockwise hysteresis between plasma concentrations and QTc for anagrelide 2.5 mg (Fig. 5C and D). In Figure 5D, 3-hydroxy-anagrelide plasma concentrations above 14 ng/mL were associated with mean ΔΔQTcNi values ranging from 13.0 msec at 1 h to 4.2 msec at 3 h and mean ΔΔQTcF values ranging from 10.1 msec at 1 h to −0.1 msec at 3 h. This is because the mean

ΔΔQTcNi and ΔΔQTcF values decrease rapidly beyond 1 h post dose, despite the high plasma concentrations. Figure 6 shows the mean ΔΔQTcNi and ΔΔQTcF decreasing rapidly beyond 1 h post dose, despite the high stable HRs and high plasma concentrations of anagrelide and 3-hydroxy-anagrelide from 2 to 4 h after administration.

Interestingly, there was a counter-clockwise hysteresis between anagrelide and 3-hydroxy-anagrelide mean plasma concentrations and mean ΔΔQTc for anagrelide 0.5 mg (Fig. 5C and D), as the HR and QTc values increased more slowly than for anagrelide 2.5 mg (Fig. 3B and C).

Safety

AEs were generally mild and of short duration, with the majority being considered related to the investigational products by the principal investigator and/or coinvestigators. The proportion of subjects experiencing at least one TEAE was higher with anagrelide 2.5 mg (81.7%) compared with anagrelide 0.5 mg (22.0%), moxifloxacin 400 mg (11.7%), and placebo (6.7%). Headache was the most common TEAE with both anagrelide doses. Dizziness and nausea occurred in >20% of subjects receiving anagrelide 2.5 mg compared with <2% of subjects treated with moxifloxacin or placebo. Treatment-emergent tachycardia and dyspnea were only reported with anagrelide 2.5 mg and were more frequently reported in women than men. There were no deaths or serious AEs. As previously described, one subject was withdrawn from receiving further investigational product after experiencing ECG T-wave inversion following anagrelide 2.5 mg. This subject had previously exhibited nonspecific T-wave changes at 8 and 12 h after the nominal dose time on day −1 of period 1 before receiving any treatment in the study.

The investigational products had no apparent effects on hematology or clinical chemistry laboratory parameters. Anagrelide 0.5 mg had no clinically relevant effects on vital signs. In contrast, anagrelide 2.5 mg induced small reductions in systolic blood pressure 2.5 h post dose; reductions in diastolic blood pressure 1.5–2.5 h post dose; and marked increases in pulse 1.5–2.5 h post dose. Consistent with the results from the Holter monitoring, the proportion of subjects experiencing an elevated HR was higher with anagrelide 2.5 mg than anagrelide 0.5 mg, moxifloxacin, or placebo.

Discussion

Palpitations and tachycardia are the most frequently reported cardiovascular AEs observed with anagrelide therapy (Mazzucconi et al. 2004; Harrison et al. 2005; Euro-
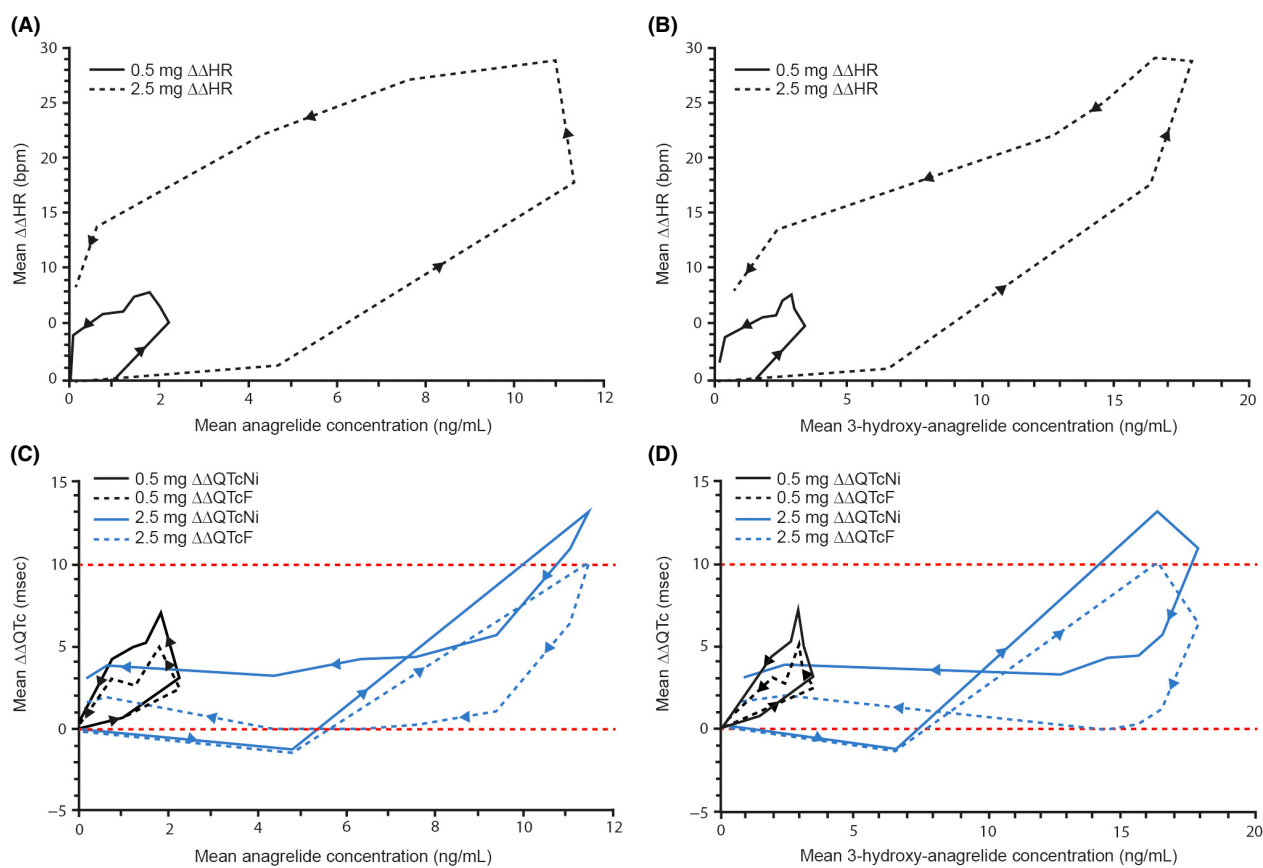
Figure 5. Hysteresis graphs of the mean ΔΔHR (A and B) and mean ΔΔQTcNi and ΔΔQTcF (C and D) versus mean plasma concentrations of anagrelide (A and C) and 3-hydroxy-anagrelide (B and D) in healthy subjects receiving anagrelide 0.5 and 2.5 mg (full analysis set). HR, heart rate; QTcF, Fridericia-corrected QT interval; QTcNi, subject-specific-corrected QT interval.

pean Medicines Agency 2013). Although infrequent, serious cardiovascular AEs, including angina, arterial hypertension, congestive heart failure, cardiomyopathy, cardiomegaly, arrhythmia, acute myocardial infarction, torsades de pointes, ventricular tachycardia, and pericardial effusion have also been reported with anagrelide use in patients with essential thrombocythemia (Anagrelide Study Group 1992; Harrison et al. 2005; Gugliotta et al. 2011; European Medicines Agency 2013). However, results from one study showed that cardiovascular AEs have little effect on treatment discontinuation rates (Gugliotta et al. 2011). The present thorough QT study was performed for the provision of comprehensive information regarding the potential of anagrelide to affect cardiac repolarization as measured by the QT/QTc interval.

The pharmacokinetics of anagrelide were as expected and showed that anagrelide was rapidly absorbed and rapidly metabolized to form 3-hydroxy-anagrelide after oral administration. The geometric mean C_{max} and AUC of anagrelide and 3-hydroxy-anagrelide were higher in women versus men, although no pharmacokinetic differences were

observed between men and women in a previous study (Martinez-Selles et al. 2013). However, results from this current study are consistent with the faster CYP1A2-mediated clearance observed in men versus women for olanzapine, clozapine, and riluzole (Schwartz 2003).

In this study, women demonstrated a higher maximum increase in mean ΔΔHR, QTcNi, and QTcF than men, which could be related to the higher C_{max} and AUC of anagrelide and 3-hydroxy-anagrelide in women. A similar phenomenon has been reported with mirabegron, which showed higher exposures and a greater increase in ΔΔHR in women versus men (Malik et al. 2012). However, the HR increases observed in our study were greater and occurred more rapidly than the mirabegron study, making it more difficult to account for the QT-RR hysteresis.

The rapid increase in HR at the anagrelide 2.5 mg dose produced a marked QT-HR clockwise hysteresis (or corresponding QT-RR counter-clockwise hysteresis). The increases in QTcNi and QTcF were greater when HR was increasing rapidly (0.5–1 h post dose) than during the slower HR increase (1–1.5 h post dose). The relationship
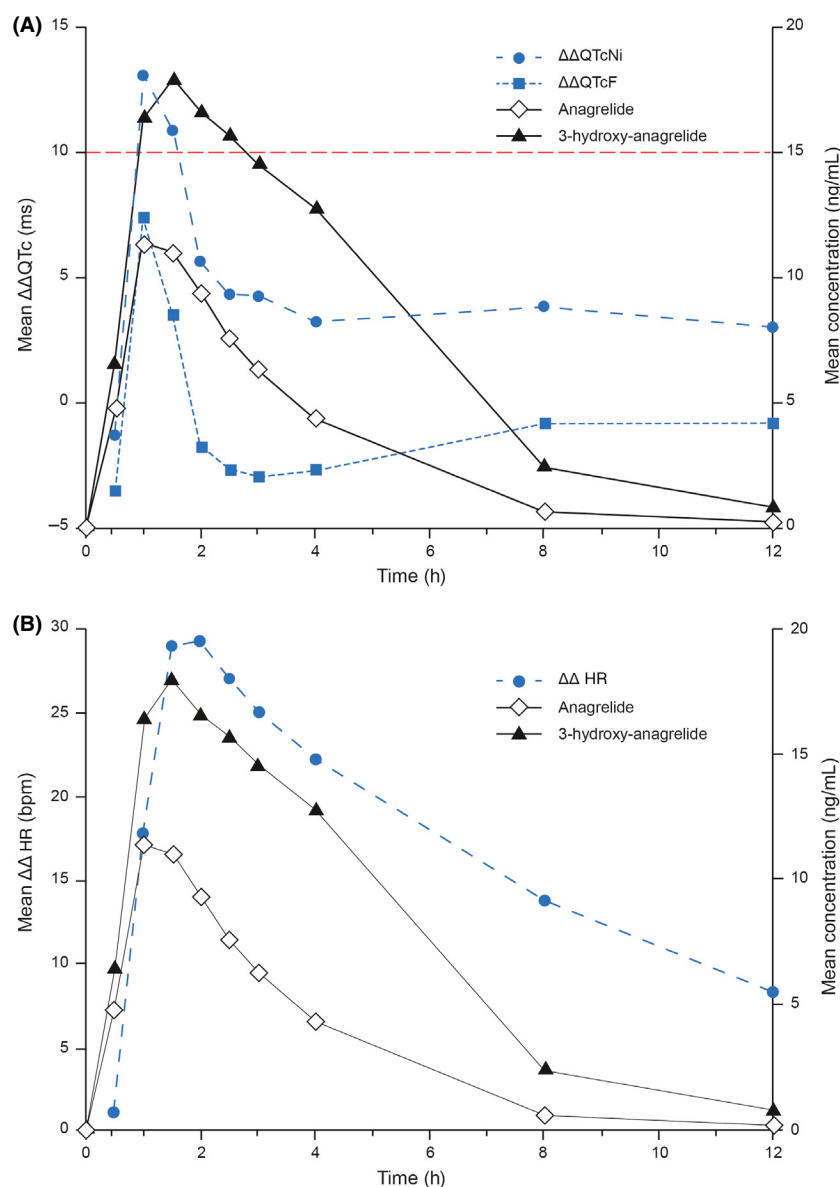
Figure 6. Mean plasma concentrations of anagrelide and 3-hydroxy-anagrelide plotted with the mean ΔΔQTcNi and ΔΔQTcF (A) and the mean ΔΔHR (B) in healthy subjects receiving high therapeutic dose of anagrelide (2.5 mg) (full analysis set). HR, heart rate; QTcF, Fridericia-corrected QT interval; QTcNi, subject-specific-corrected QT interval.

between HR and QTc rapidly stabilized when the HR was stable or slowly decreasing back to baseline values (2.5–12 h after administration).

Anagrelide 0.5 mg produced a maximum increase in mean QTcNi and QTcF of 7.0 msec, with the one-sided 95% upper confidence bound being <10 msec. In contrast, for anagrelide 2.5 mg, the maximum increase in mean QTcNi and QTcF was ≥10 msec and the one-sided 95% upper confidence bound was >10 msec for QTcNi at 1 and 1.5 h post dose and QTcF at 1 h post dose. The upper confidence bound of >10 msec meets the definition

of a positive thorough QT/QTc study according to ICH E14 (European Medicines Agency 2005). The increases in QTcNi and QTcF were more likely related to the rapid increase in HR from 0.5 to 1.5 h post dose and the corresponding QT-HR (or QT-RR) hysteresis that occurs when the HR increases or decreases rapidly (Malik et al. 2008; Garnett et al. 2012). This increase in both QTcNi and QTcF rapidly dissipated by 2 h post dose (after HR stabilized), despite the sustained high HRs and sustained high plasma concentrations of anagrelide and 3-hydroxy-anagrelide. Assay sensitivity was confirmed by the observa-

tion that moxifloxacin produced its expected prolongation of QTcNi and QTcF with one-sided 95% lower confidence bound >5 msec from 1 to 8 h after administration, meeting the definition of a positive control in the ICH E14 (European Medicines Agency 2005).

Generally, QTc increases of >20 msec are more concerning than smaller changes (Garnett et al. 2012). In this study, the maximum observed transient increases in QTcNi and QTcF ranged from 10 to 15 msec for anagrelide 2.5 mg. Additionally, pro-arrhythmic events related to delayed cardiac repolarization typically occur at low HRs, whereas high HRs are often considered arrhythmia-protective (Garnett et al. 2012). In this study, anagrelide 2.5 mg increased the observed QTcNi and QTcF only when HR was increasing rapidly (likely associated with the potent PDE III inhibition by 3-hydroxy-anagrelide), bringing the subject closer to tachycardia rather than bradycardia. Taken together, the moderate increase in QTc of 10 msec, the arrhythmia-protective nature of tachycardia, and the occurrence only during the time of rapidly increasing HR (with the associated QT-HR hysteresis complicating the correction of the QT interval) suggest that patients receiving high therapeutic doses of anagrelide may not be at a significantly higher risk of developing QTc-related arrhythmias. However, as torsades de pointes and ventricular tachycardia have been reported with anagrelide treatment, the direct causal relationship of anagrelide on cardiac repolarization cannot be completely excluded. Additionally, an increase in HR of ≥20 bpm may cause other cardiac-related AEs (Garnett et al. 2012).

This study included a period of light exercise on the baseline day before each treatment, but the increase in HR during the exercise period did not cover the range of high HR values (mean $\Delta\Delta$HR of +29.1 bpm) observed with anagrelide 2.5 mg. Single doses of anagrelide above 2.0 mg have not been administered to healthy volunteers since the first-in-man study, and the observed magnitude of the increased HR was not anticipated for the anagrelide 2.5 mg dose in this study. Therefore, the study design could have been improved by conducting a pilot study with anagrelide 2.5 mg to better understand the magnitude of HR increases at this dose. In addition, the method proposed by the Malik and Hnatkova groups (Hnatkova et al. 2010; Malik et al. 2012) could have been implemented, which involves a thorough baseline evaluation of the QT-RR relationship under a sequence of postural provocations in individual subjects at times of rapidly increasing HR. This test provides sufficient number of ECG intervals under rapidly increasing and decreasing HRs and at a wide range of slow to fast HRs to robustly define the QT-RR relationship, including the QT-RR hysteresis, in individual subjects.

In general, individually optimized HR corrections are regarded as being the most sensitive considering the inter-subject variability of the QT/RR interval (Malik et al. 2002), and the most suitable for thorough QT/QTc studies (European Medicines Agency 2005). As the increase in HR during the exercise period did not cover the range of high HR values observed with the anagrelide 2.5 mg treatment in this study, the baseline HR range may not have been broad enough to support derivation of the QTcNi correction for the anagrelide 2.5 mg treatment. In this scenario, the subject-specific correction QTcNi may be criticized (Garnett et al. 2012), although the analysis of QTcNi and QTcF produced generally consistent results in this study. In addition, the baseline values (following light exercise) were used along with the placebo data to derive the subject-specific correction slopes (Bi) for the QTcNi calculations. Since the placebo group did not undergo the same light exercise, including the placebo data in the derivation of the Bi slopes produced a slightly larger slope (median Bi 0.35) than if the placebo data were not included (median Bi 0.31) relative to Fridericia's fixed formula (0.33). Moreover, although QTc values correct for changes in HR, QTc does not take into account other confounding physiological factors that may have an effect on the QT interval, including autonomic tone, electrolyte changes, and metabolic state (Garnett et al. 2012). Thus, the optimal HR correction to use in thorough QT studies for compounds that rapidly modulate HR remains unclear.

The decreased PR interval is a well-known effect of increasing HR as demonstrated in healthy subjects during exercise (Carruthers et al. 1987). In this current study, the increased HR and reduced PR interval associated with anagrelide were similar to findings reported in a previous study in healthy subjects receiving anagrelide (Martinez-Selles et al. 2013). These cardiovascular effects may be attributed to the known potent PDE III inhibition (associated with positive inotrophy and vasodilation) of anagrelide and its active metabolite, resulting in frequently reported anagrelide-related palpitations (Martinez-Selles et al. 2013).

Both doses of anagrelide were generally well tolerated and AEs were consistent with the Summary of Product Characteristics, with no new safety signals identified (European Medicines Agency 2013). One subject was withdrawn from the study by the principal investigator and Shire medical monitor based on the observation of an inverted T-wave after receiving anagrelide 2.5 mg. However, this subject had previously exhibited nonspecific T-wave changes before receiving any treatment in the study. A different subject who exhibited high baseline QTc values and a moderate increase in QTc was identified to be heterozygous for SNP rs1805128 in *KCNE1*, a

variant shown to confer an altered delayed rectifier potassium current (IKr) functionality, inducing a reduction of >50% in IKr and an inability to adapt HR increases (Chen et al. 2009; Nof et al. 2011). This may explain the high baseline and moderate increase in QTc observed in this subject.

The use of a high therapeutic dose (anagrelide 2.5 mg) rather than a supratherapeutic dose, as recommended in the ICH E14, is a potential limitation of this study. Treatment with anagrelide begins with 1–2 mg/day, administered in divided doses. Anagrelide is slowly and carefully titrated based on platelet count response and tolerability. The maximum daily dose should not exceed 10 mg/day as divided doses with no more than 2.5 mg in any single dose (European Medicines Agency 2013; Food and Drug Administration 2014);. One study demonstrated that 95% of patients with essential thrombocythemia achieved platelet control with anagrelide doses ≤4 mg/day (Anagrelide Study Group 1992), suggesting that few patients receive the highest labeled therapeutic dose. Therefore, the exposure to anagrelide and 3-hydroxy-anagrelide following the anagrelide 2.5 mg dose in this study were above the exposures in the majority of patients with essential thrombocythemia being treated with anagrelide.

In summary, anagrelide demonstrated an increase in HR resulting in an increase in QTc interval in healthy subjects. However, this moderate increase in QTc occurred in the absence of bradycardia, and during times of rapidly increasing HR when it can be difficult to accurately measure and correct QT for HR. Furthermore, the effects on QTc rapidly ameliorated after the HR stabilized, despite the high plasma concentrations of anagrelide and its metabolite. Therefore, our findings suggest that anagrelide may not significantly increase pro-arrhythmic risk. However, the direct causal relationship of anagrelide on cardiac repolarization cannot be completely excluded. Thus, caution is advised when treating patients with known risk factors for QT interval prolongation, such as congenital long QT syndrome, a known history of acquired QTc prolongation, comedication with agents that can prolong QTc interval, and hypokalemia.

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Disclosures

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