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ORIGINAL RESEARCH ARTICLE

Cardiovascular Safety of Anagrelide in Healthy Subjects: Effects of Caffeine and Food Intake on Pharmacokinetics and Adverse Reactions

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Abstract

Background Essential thrombocythaemia (ET) is a rare clonal myeloproliferative disorder characterized by a sustained elevation in platelet count and megakaryocyte hyperplasia. Anagrelide is used in the treatment of ET, where it has been shown to reduce platelet count. Anagrelide is metabolized by cytochrome P450 (CYP) 1A2, and previous studies of the effect of food on the bioavailability and pharmacokinetics of anagrelide were conducted prior to the identification of the active metabolite, 3-hydroxyanagrelide.

Objectives The objectives of this study were to determine the effect of food and caffeine on the pharmacokinetics of anagrelide and its active metabolite, 3-hydroxyanagrelide, to monitor electrocardiogram (ECG) parameters following drug administration, and to document the relationship between palpitations, ECG changes and caffeine intake

Methods Thirty-five healthy subjects who received 1 mg of anagrelide following either a 10-h fast or within 30 min of a standardized breakfast, including two cups of coffee, were studied.

Results Time to maximum (peak) plasma concentration (C_{max}) of anagrelide was 4.0 h in the fed and 1.5 h in the fasted group ($p < 0.05$); similar results were observed for 3-hydroxyanagrelide. The mean C_{max} of anagrelide was 4.45 ± 2.32 ng/mL and 5.08 ± 2.99 ng/mL in the fed/caffeine and fasted groups, respectively; peak concentrations were higher for 3-hydroxyanagrelide in both the fed/caffeine and fasted groups. The most frequent adverse events (AEs) were headache (60 %) and palpitations (40 %). There were no serious AEs and all ECGs were normal, although significant reductions in PR interval, QRS length and QT interval were observed in both groups. Heart rate increased after anagrelide administration in both fed/caffeine and fasted states ($p < 0.01$); however, increased heart rate was significantly more frequent in the fed/caffeine state than in the fasted state ($p < 0.001$ for heart rate increase in the first hour after drug administration). There was a trend towards a greater heart rate increase in subjects reporting palpitations than in those without (mean heart rate ± SD at 1 h: 10.1 ± 6.4 vs. 8.0 ± 8.4 beats/min [$p = 0.35$]; at 4 h: 12.7 ± 7.5 vs. 9.1 ± 8.8 beats/min [$p = 0.10$], respectively).

Conclusion We conclude that food/caffeine delayed absorption of anagrelide. Anagrelide was generally well tolerated and had small effects on ECG parameters and heart rate. Caffeine may be implicated in a higher increase in heart rate and increased frequency of palpitations observed following administration of anagrelide with food/caffeine versus fasting.

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1 Introduction

Essential thrombocythaemia (ET) is a rare clonal myeloproliferative disorder [1] characterized by a sustained

elevation in platelet count (thrombocytosis) and megakaryocyte hyperplasia. Anagrelide is used in the treatment of ET, where it has been shown to reduce platelet count [2] and may, therefore, decrease the incidence of thrombohaemorrhagic complications [3].

In vitro studies have shown that anagrelide is metabolized by cytochrome P450 (CYP) 1A2 and produces the active metabolite 6,7-dichloro-3-hydroxy-1,5-dihydroimidazol[2,1-b] quinazolin-2-one (3-hydroxyanagrelide) [4, 5]. Caffeine is a known substrate of CYP1A2 [6] and the possible effects of the constituents of a normal high-fat breakfast with coffee on the rate and extent of absorption of anagrelide, as well as the rate and extent of formation of the active metabolite, was considered worthy of investigation.

Clinical experience has shown that palpitations are one of the most common treatment-emergent adverse events (AEs) associated with anagrelide use [7]. The palpitations are usually benign and non-arrhythmia-related and tend to abate over time, with lower incidences reported during long-term therapy compared with the initial treatment phase [8]. This is of particular importance with regard to 3-hydroxyanagrelide, as it has been shown to be a potent phosphodiesterase (PDE) III inhibitor. PDE inhibitors are associated with positive inotropy and vasodilation and may, therefore, result in tachycardia and palpitations [9]. In the present study, when assessing the safety and tolerability of anagrelide in the fed/caffeine and fasted state, it was considered of interest to include the monitoring of cardiac activity by means of heart rate, blood pressure and electrocardiogram (ECG) recordings. It was hoped that by including these assessments an insight into the relationship between anagrelide metabolism, palpitations, ECG changes, and food and caffeine intake would be afforded.

2 Methods

2.1 Subjects

Healthy Caucasian subjects aged between 18 and 40 years were eligible for inclusion. Subjects were required to be within 15 % of ideal weight for sex, height and body frame size according to the Metropolitan Life Tables. Subjects were required to be non–smokers (or have stopped smoking at least 6 weeks prior to study start). All subjects had negative HIV, hepatitis B surface antigen and hepatitis C antibody, and no clinically significant or relevant abnormalities of medical history, physical examination and ECG, or clinical or laboratory evaluation (haematology, biochemistry and urinalysis). All subjects provided written informed consent prior to entering the study.

2.2 Study Design

This was a phase I, open-label, randomized, two-way crossover study, which was approved by the ethics committee at Guy's Hospital, London, UK and appropriate local ethics committees, and conducted in accordance with the principles of the Declaration of Helsinki [10].

Subjects were restricted from strenuous exercise and consuming alcohol, grapefruit, caffeine or xanthine for 48 h prior to, and for the duration of, each study period. Subjects were randomized to one of two treatment sequences and stratified by sex: treatment in a fasted state followed by treatment in a fed state, or treatment in a fed state followed by treatment in a fasted state. The two treatment periods were separated by at least 3 days. Subjects received a single oral dose of anagrelide 1 mg either following a fast of at least 10 h or within 30 min of a standardized high-fat breakfast that contained 150, 250 and 500–600 cal from protein, carbohydrate and fat, respectively, as well as two cups of black coffee, each containing 58.8 mg of caffeine. Following dosing, subjects were not permitted to lie down for 4 h, and were discharged from the unit 24 h after dosing.

The primary objective of the study was to investigate the pharmacokinetics of anagrelide and its active metabolite 3-hydroxyanagrelide (particularly its rate of formation) following administration in fed/caffeine and fasted states. The secondary objectives included assessment of the safety and tolerability of anagrelide when administered in fed/caffeine or fasted states and the determination of the individual levels of CYP1A2 expression by measurement of the plasma caffeine:caffeine metabolite ratio and evaluation of its relationship to observed 3-hydroxyanagrelide exposure.

The study was conducted at Guy's Drug Research Unit in London, UK. The assessment of cardiovascular side effects and ECG analyses were performed in the Cardiology Department of Hospital Gregorio Marañón, Madrid, Spain.

2.3 Assessments

2.3.1 Pharmacokinetics

Blood samples for determination of plasma anagrelide and 3-hydroxyanagrelide concentrations were drawn pre-dose and then 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 8, 12, 16 and 24 h post-dose. The 4-h post-dose sample was also used for determination of individual expression levels of CYP1A2 by measuring the ratio between caffeine and its metabolite, paraxanthine. Plasma concentrations were determined by liquid/liquid extraction followed by reverse-phase high-performance liquid chromatography

and detection by tandem mass spectrometry. There were eight quality control samples used per test with concentration ranges of 0.05–20 ng/mL for anagrelide and 0.1–40 ng/mL for 3-hydroxyanagrelide, using a sample aliquot volume of 500 μL. The concentration levels were 0.15, 8, 17.5 and 35 ng/mL for anagrelide and 70 ng/mL for 3-hydroxyanagrelide (the highest concentration for each agent was diluted one in two before analysis). The upper limits of quantification of the analytical method were 20 ng/mL for anagrelide and 40 ng/mL for 3-hydroxyanagrelide. The lower limits of quantification were 0.05 ng/mL for anagrelide and 0.1 ng/mL for 3-hydroxyanagrelide.

Pharmacokinetic parameters were determined by non-compartmental analysis of the plasma concentration data using WinNonlin® Professional version 4.01 (Pharsight Corporation, Mountain View, CA, USA). The following pharmacokinetic parameters were determined: time to reach the maximum (peak) plasma concentration following drug administration (t_{max}), maximum (peak) plasma drug concentration (C_{max}), area under the plasma concentration-time curve from time zero to infinity (AUC_{∞}), apparent half-life of the terminal elimination phase ($t_{½z}$), lag time before the first measurable plasma concentration (t_{lag}) and apparent half-life of the absorption phase ($t_{½abs}$). Plasma concentration analysis was performed by York Bioanalytical Solutions, York, UK. Pharmacokinetic analyses were performed by Classic PK, Harpenden, UK.

2.3.2 Statistical Analysis

Statistical analysis was performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA).

Drug concentrations and pharmacokinetic parameters were summarized by arithmetic mean, standard deviation (SD), minimum, median, maximum, coefficient of variation (CV) and geometric mean. The influence of food on the pharmacokinetic parameters C_{max}, AUC_{∞} and $t_{½abs}$ for anagrelide and 3-hydroxyanagrelide was assessed by analysis of variance (ANOVA) for a two-way crossover design (WinNonlin). The sequence effect was tested using the subject-within-sequence effect, and all other effects were tested using the residual error of the model. All subjects for whom plasma concentration data were available in both the fasted and fed/caffeine states were included in the analysis. The parameters were log-transformed prior to analysis. Average bioequivalence was assessed according to whether or not the lower and upper 90 % confidence intervals (CIs) for the ratio of the geometric means lay within the range 0.80–1.25. The influence of food/caffeine on the parameters t_{lag} and t_{max} was assessed non-parametrically (WinNonlin) according to the procedure of Koch [11]. The significance of differences between fasted and fed/caffeine states was assessed at the 5 % level.

AEs were analysed according to study period (i.e., dietary status fed or fasted) and overall during the two periods of observation in the study. For cardiovascular parameters, means (±SD) are reported for continuous variables. Fisher's exact test or the Chi-squared tests were used to assess the significance of the differences between proportions. For the quantitative variables either Student's *t* test, the analysis of variance for a two-way crossover design (WinNonlin) or ANOVA (one-way and two-way, including time up to 4 h post-dose) repeated measures/Bonferroni were performed. All probability values are two-tailed.

2.3.3 Safety

Safety assessments conducted at screening and throughout the study included physical examination, vital signs, biochemistry, haematology and urinalysis. All AEs (related and unrelated, serious and non-serious) were recorded from the time the informed consent was signed until the end of treatment exposure using the Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. Assessment of intensity was determined according to the following definitions: mild—AE is easily tolerated and does not interfere with usual activity; moderate—AE interferes with daily activity, but the subject is still able to function; severe—AE is incapacitating and the subject is unable to work or complete usual activity. Serious AEs were defined as any untoward medical occurrence that resulted in death, or was life-threatening, or required hospitalization or prolongation of hospitalization, or resulted in persistent or significant disability/incapacity, or resulted in a congenital abnormality/birth defect.

ECG Safety A 12-lead ECG was performed at screening, check-in, pre-dose, 1, 2 and 4 h post-dose, and at discharge for each treatment period and follow-up. All traces were reviewed and any abnormalities, heart rate, PR interval, QRS length and QT intervals were recorded by an author (L.F.-G.) blinded to the study period, fasting state and presence of symptoms. Fridericia's correction was used to calculate corrected QT (QTc), $QTc = QT/RR^{0.33}$, as it is superior to Bazett's formula, particularly at low heart rates [12].

3 Results

3.1 Subjects

A total of 35 subjects were enrolled in the study (20 male, 15 female), of whom 34 completed both the fasted-fed/caffeine and the fed/caffeine-fasted treatment sequences. One female subject withdrew from the study for personal

Table 1 Subject characteristics

Characteristic	Treatment sequence		Total population ($n = 35$)
	Fasted—fed/caffeine ($n = 18$)	Fed/caffeine—fasted ($n = 17$)	
Sex, n			
Male	10	10	20
Female	8	7	15
Age, years			
Median	23	21	22
Range	19–38	18–28	18–38
Height, m			
Median	1.7	1.8	1.7
Range	1.6–1.8	1.6–1.8	1.6–1.8
Weight, kg			
Median	67.4	68.5	68.2
Range	57.5–77.5	50.8–81.8	50.8–81.8
BMI, kg/m^2			
Median	22	22	22
Range	20–25	20–27	20–27
Body frame size, n (%)			
Small	12 (67)	11 (65)	23 (66)
Medium	6 (33)	6 (35)	12 (34)
Large	0	0	0

BMI body mass index

reasons and so did not complete both sequences. Subjects' demographics are summarized in Table 1.

3.2 Pharmacokinetics of Anagrelide and its Metabolite

Pharmacokinetic parameters for anagrelide and 3-hydroxyanagrelide are presented in Table 2. Mean plasma concentration-time curves are shown in Fig. 1. Anagrelide was rapidly absorbed after oral administration, with peak plasma concentrations reached after a median time of 1.5 h in the fasting state. After food/caffeine, the median peak plasma concentration was significantly delayed to 4.0 h ($p < 0.05$) (Fig. 1). The mean C_{max} was 5.08 ng/mL (SD ± 2.99) in the fasted subjects and 4.45 ng/mL (SD ± 2.32) in the fed/caffeine group, while the mean total exposure to anagrelide was 13.15 ng·h/mL (SD ± 5.61) versus 15.88 ng·h/mL (SD ± 7.03) in the fasted and fed/caffeine states, respectively (Table 2). Bioequivalence analysis determined that exposure to anagrelide after food/caffeine was not equivalent to that in the fasted state (90 % CI values for C_{max} and AUC were 0.75, 1.01 and 1.11, 1.27 respectively). In addition, the t_{lag} before absorption was significantly increased in the presence of food/caffeine ($p < 0.05$). The elimination of anagrelide from plasma followed a mono-exponential pattern, with a mean $t_{½z}$ of 1.70 h (SD ± 0.86) in the fasted state and 1.85 h (SD ± 1.02) following food/caffeine.

The t_{max} of the metabolite 3-hydroxyanagrelide was similar to that of anagrelide, being reached at a median of 1.25 h in fasting conditions and 4.0 h after food/caffeine ($p < 0.05$). The peak concentrations were higher for the metabolite than for the parent compound; maximum concentration was 8.29 ng/mL (SD ± 4.85) in the fasted state and reduced to 5.93 ng/mL (SD ± 2.81) following food/caffeine. Bioequivalence analysis of C_{max} confirmed that this was not equivalent between the two states (90 % CI 0.62, 0.86). As with anagrelide, the t_{lag} before absorption was significantly increased in the fed state ($p < 0.05$). Total exposure to 3-hydroxyanagrelide was approximately 2.5 times greater than that for anagrelide but there was little change between fed and fasted states (29.67 ng·h/mL vs. 29.17 ng·h/mL [Fig. 1]), and these results were equivalent (90 % CI 0.95, 1.11). The plasma half-life of 3-hydroxyanagrelide was 3.11 h (SD ± 1.26) in the fasted state and 3.25 h (SD ± 1.60) after food/caffeine. For both anagrelide and 3-hydroxyanagrelide, there were small differences between males and females in mean values of pharmacokinetic parameters, although these were small in relation to the variation of the population as a whole. For anagrelide, the overall CV for C_{max} was 59 % and for AUC_{∞} was 43 %; for 3–hydroxyanagrelide, CV for C_{max} was 59 % and for AUC_{∞} it was 41 %.

3.3 CYP1A2 Status

Visual analysis of plots of anagrelide:3-hydroxyanagrelide ratios for C_{max}, AUC_{∞} and $t_{½z}$ versus the paraxanthine:caffeine ratio showed no evidence for a relationship between CYP1A2 status and exposure to 3-hydroxyanagrelide relative to that of anagrelide. Accordingly, no further evaluation was conducted.

3.4 Safety

Anagrelide was well tolerated during the study, with no serious or severe AEs (Table 3) and no withdrawals due to AEs. Overall AEs were reported by a similar number of subjects in the fasting and fed/caffeine states (23 vs. 19). Twenty-seven subjects (77 %) had at least one AE during the study and 26 subjects (74 %) had at least one treatment-related AE. In addition, there was one non-treatment-related AE of moderate severity: a case of urinary infection in a female subject subsequently treated with amoxicillin. The most frequently reported AEs were headache (21/35 subjects, 60 %), mild in 17/21 cases and moderate in 4/21; palpitations (14/35, 40 %), all mild; and dizziness or postural dizziness (9/35, 26 %), all mild. Only five

Table 2 Summary of pharmacokinetic parameters

Parameter	Anagrelide		3-hydroxyanagrelide	
	Fasted ($n = 35$)	Fed/caffeine ($n = 34$)	Fasted ($n = 35$)	Fed/caffeine ($n = 34$)
t_{max}, h [median (range)]	1.50 (0.50–6.00)	4.00 (0.75–6.00)	1.25 (0.50–6.00)	4.00 (1.75–6.00)
C_{max}, ng/mL [mean (SD, CV %)]	5.08 (2.99, 59 %)	4.45 (2.32, 52 %)	8.29 (4.85, 59 %)	5.93 (2.81, 47 %)
AUC_{last}, ng·h/mL [mean (SD, CV %)]	12.87 (5.56, 43 %)	15.23 (6.75, 44 %)	28.16 (11.76, 42 %)	27.93 (11.42, 41 %)
AUC_{∞}, ng·h/mL [mean (SD, CV %)]	13.15 (5.61, 43 %)	15.88 (7.03, 44 %)	29.17 (11.88, 41 %)	29.67 (11.74, 40 %)
$t_{½z}$, h [mean (SD, CV %)]	1.70 (0.86, 51 %)	1.85 (1.02, 55 %)	3.11 (1.26, 41 %)	3.25 (1.60, 49 %)
t_{lag}, h [mean (SD, CV %)]	0.34 (0.26, 76 %)	1.34 (0.89, 67 %)	0.33 (0.26, 80 %)	1.01 (0.99, 98 %)
$t_{½abs}$, h [mean (SD, CV %)]	0.43 (0.43, 51 %)	0.74 (0.31, 42 %)	0.78 (0.85, 110 %)	1.02 (0.53, 52 %)

AUC_{∞} area under the plasma concentration-time curve from time zero to infinity, AUC_{last} area under the plasma concentration-time curve from time zero to the time of the last measurable concentration, C_{max} maximum (peak) plasma drug concentration, *CV* coefficient of variation, *SD* standard deviation, $t_{½z}$ apparent elimination half-life, $t_{½abs}$ apparent absorption half-life, t_{lag} lag time before the first measurable plasma concentration, t_{max} time to reach C_{max}

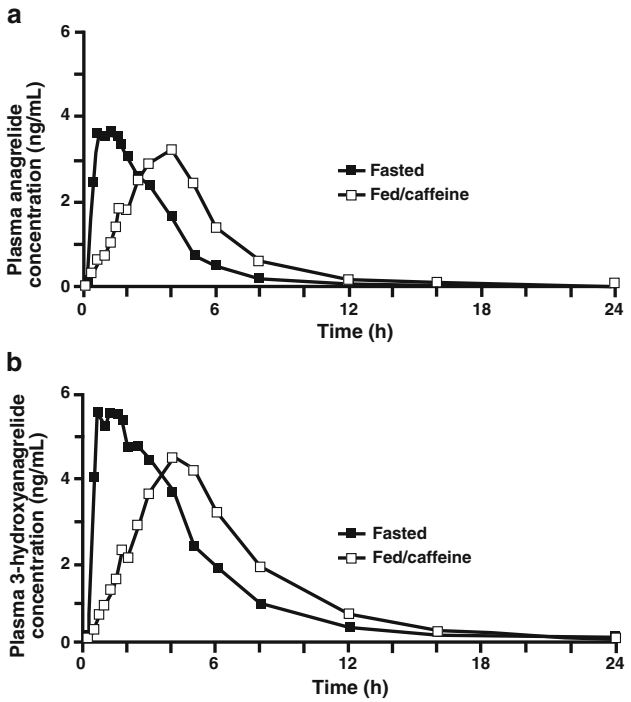

Fig. 1 Mean plasma concentration-time profiles of (**a**) anagrelide and (**b**) 3-hydroxyanagrelide, in healthy subjects following either a 10-h fast or within 30 min of a standardized breakfast, including two cups of coffee

treatment-related AEs were of moderate severity: four cases of headache and one of vomiting (the moderate vomiting occurred in a subject who also had moderate headache). These moderately severe events all occurred during the fasted treatment period. There was no evidence to suggest that they occurred in subjects with a particularly high plasma concentration of either anagrelide or 3-hydroxyanagrelide. There were no clinically significant changes in laboratory safety tests, vital signs or physical examination during the study.

3.4.1 Palpitations

Palpitations occurred in more individuals during the fed/caffeine period (12/34 subjects, 35 %) than during the fasted period, although the difference did not reach statistical significance (6/35, 17 %; $p = 0.09$). The mean duration of palpitations was 231 (SD ± 209) min. The mean duration of palpitations was higher in the fed/caffeine state than in the fasted state (252 ± [SD] 218 vs. 194 ± [SD] 204 min), but this difference was not significant ($p = 0.53$).

3.4.2 ECG

All ECGs performed were considered normal, including ECGs performed in subjects experiencing palpitations. Heart rate increased progressively after anagrelide administration ($p < 0.001$) with a mean peak increase (in fed/caffeine and fasted subjects) of 10 beats/min at 2 h and 8.5 beats/min at 1 h post-dose (Fig. 2). However, heart rate increase was higher in the fed/caffeine state than in the fasted state, although this difference was small and only significant in the first hour after anagrelide intake (mean increase of 12.2 SD ± 8.4 beats/min vs. 5.1 SD ± 4.7 beats/min; $p < 0.001$ [Student's *t* test]; Fig. 3). There was a trend towards a greater heart rate increase in subjects reporting palpitations than in those without them (mean ± SD at 1 h: 10.1 ± 6.4 vs. 8.0 ± 8.4 beats/min [$p = 0.35$]; at 2 h 10.3 ± 5.8 vs. 9.9 ± 8.5 beats/min [$p = 0.84$]; at 4 h 12.7 ± 7.5 vs. 9.1 ± 8.8 beats/min [$p = 0.10$], respectively). PR interval, QRS length and QT interval decreased following administration of anagrelide ($p < 0.001$), whereas there were no significant differences in QTc. Only QT interval change was influenced by the food/caffeine, with greater reductions in interval observed in the fed/caffeine state than in the fasted state (Fig. 3).

Table 3 Summary of adverse events (AEs)

Variable	Treatment group		
	Overall ($n = 35$)	Fasted ($n = 35$)	Fed/caffeine ($n = 34$)[a]
AEs after dosing (no.)	83	46	37
Mild	77	40	37
Moderate	6	6	0
Severe	0	0	0
Treatment-related AEs (no.)			
Mild	71	37	34
Moderate	5	5	0
Severe	0	0	0
Subjects with at least one AE [no. (%)]	27 (77)	23 (66)	19 (56)
Subjects with at least one treatment-related AE [no. (%)]	26 (74)	22 (63)	19 (56)
Subjects with AEs (MedDRA system organ class preferred term) [no. (%)]			
Cardiac disorders			
Palpitation	14 (40)	6 (17)	12 (35)
Gastrointestinal disorders			
Nausea	2 (6)	2 (6)	0
Nervous system disorders			
Dizziness	4 (11)	1 (3)	3 (9)
Dizziness, postural	5 (14)	2 (6)	3 (9)
Headache	21 (60)	19 (54)	8 (23)
Involuntary muscle contractions	2 (6)	1 (3)	1 (3)
Respiratory, thoracic and mediastinal disorders			
Epistaxis	2 (6)	2 (6)	1 (3)
Pharyngolaryngeal pain	4 (11)	2 (6)	2 (6)

MedDRA Medical Dictionary for Regulatory Activities

[a] One subject completed fasted study period only

4 Discussion

For the most part, the results of this study confirm previous findings on the pharmacokinetics of anagrelide and further demonstrate that anagrelide and its active metabolite, 3-hydroxyanagrelide, have a similar pharmacokinetic profile. In this study, anagrelide was shown to be rapidly absorbed after oral administration in fasted subjects, but absorption was significantly delayed and C_{max} was slightly reduced in subjects who had received food/caffeine. Furthermore, overall exposure to anagrelide differed between the fed and fasted states. Food is known to delay the absorption of anagrelide, but it has not been previously reported to influence overall exposure [13].

The recommended starting dosage of anagrelide, and the starting dose in the Medical Research Council Primary Thrombocythaemia-1 trial, is 0.5 mg twice daily [7]. It is recommended that the starting dose should be maintained for at least 1 week [13] and anagrelide-related palpitations have been found to generally occur during the first days or weeks [8]. Accordingly a dose of 1 mg was used in this study as representative of the recommended starting dose; however, in clinical practice, most patients receive higher maintenance doses [8].

The increased heart rate and reduced PR intervals known to occur following anagrelide administration may be attributed to the known inhibitory effects of anagrelide and its active metabolite on PDE III, leading to an increase in cyclic adenosine monophosphate, a second messenger of β-adrenergic receptors [14], and ultimately resulting in anagrelide-related palpitations.

The findings of the current study with regard to palpitations are analogous to a previous study comparing delayed- and immediate-release formulations of anagrelide, which found no difference between arms in the incidence of palpitations despite a lower frequency of AEs with the delayed-release formulation [4]. In our study, the median peak plasma concentration was significantly delayed by food intake. It is possible that this effect of food intake, similar to the delayed-release formulation, might reduce the incidence of AEs. On the other hand, the trend towards an increased incidence of palpitations in the fed/caffeine state versus the fasted state could be related to the ingestion of caffeine with the meal, although food and caffeine could present an opposite effect on the appearance of AEs. Regarding caffeine, the scientific literature is not conclusive on the relationship between caffeine and palpitations [15–20]. However, epidemiological studies have found that palpitations are significantly associated with coffee consumption [21, 22], with a relative risk of experiencing this symptom of 1.7 for people consuming 240 mg of caffeine per day compared with caffeine abstainers [22]. In contrast, a recent observational study suggests that regular coffee consumption is associated with reduced arrhythmia [23]. In this study, the increase in heart rate was slightly higher in the fed/caffeine group, who also tended to present with palpitations more frequently. The small increase in cardiac inotropism and vasodilation and decrease in peripheral vascular resistance may account for this adverse effect [24].

In the Medical Research Council Primary Thrombocythaemia-1 trial, with a median follow-up of 39 months, palpitations were the only cardiac event more common in the anagrelide–aspirin arm than in the hydroxycarbamide–aspirin arm (16.6 % vs. 1.7 %) [7]. However, even with this follow-up of over 3 years, arrhythmias were rare and had a similar incidence in each treatment group (<2 % in both cases) [7], confirming that the vast majority of anagrelide-related palpitations are benign. If an individual patient presents with anagrelide-related palpitations, an ECG examination during a period where symptoms are present

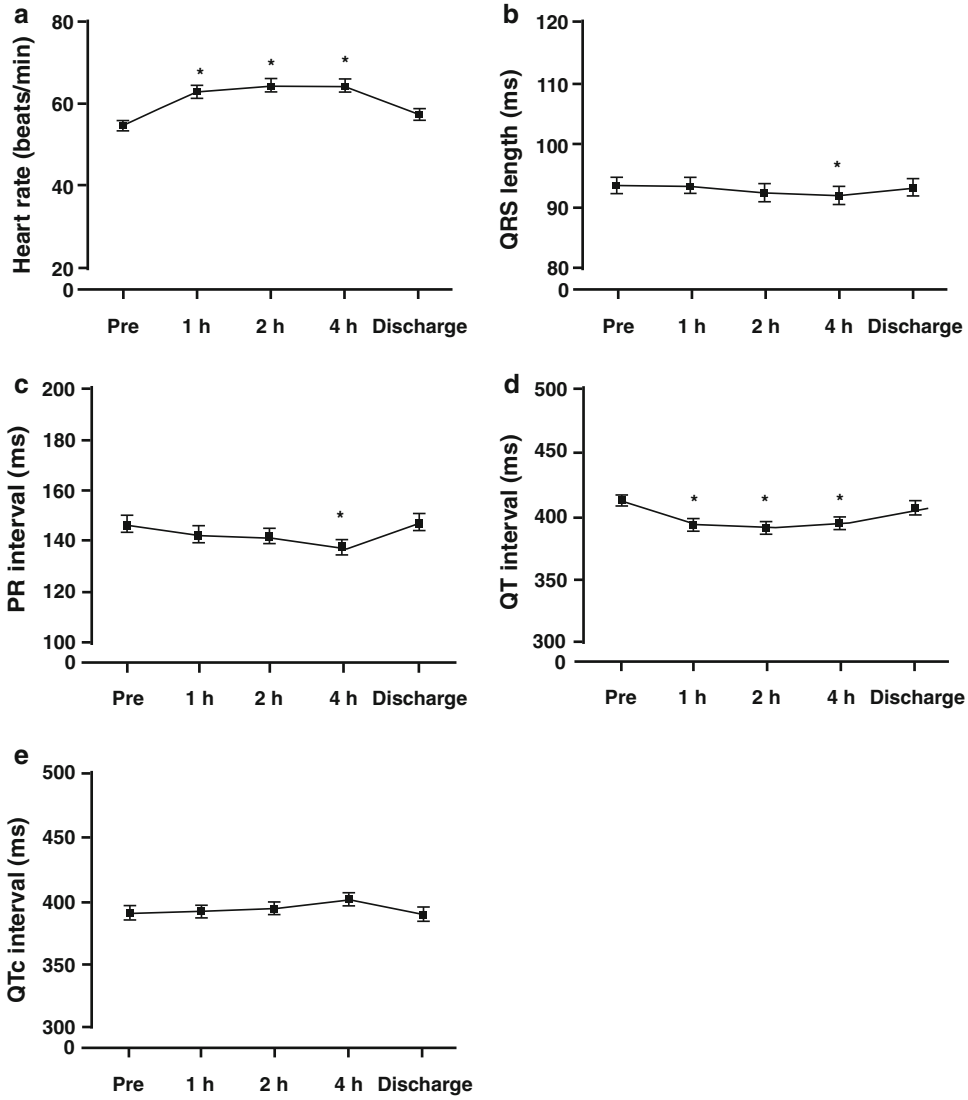

Fig. 2 Mean electrocardiogram (ECG) parameters before and after anagrelide administration: (**a**) heart rate, (**b**) QRS length, (**c**) PR interval, (**d**) QT interval and (**e**) corrected QT interval (QTc). *P* value for the ANOVA test was <0.001 in all cases except QRS ($p = 0.028$). * $p < 0.05$ for the comparison with pre-drug value (Bonferroni analysis). Error bars = 95 % confidence intervals

aids differentiation of benign from arrhythmic aetiology. Moreover, many of the anagrelide-related palpitations subside over time [8]. A study of a representative sample of the Norwegian working population ($n = 1475$) found that 10 % had experienced palpitations during the last month [25]. By comparison, the self-selected group that comprises healthy subjects was shown to complain less frequently of a range of symptoms, including palpitations, than a matched healthy general population outside the clinical research setting [26].

The incidence of palpitations is known to increase with age [27] but this is mainly due to arrhythmia-related palpitations [28] and, as previously stated, the majority of anagrelide-related palpitations are benign [7, 29]. However, the possibility that the effect of anagrelide on cardiac rhythm is more pronounced in elderly patients cannot be excluded.

The data suggest that further studies to better define the relationship between caffeine, anagrelide and palpitations are warranted. The present study was not specifically designed to test the effect of caffeine on AEs and only the combination of food and caffeine has been investigated. Although a standard breakfast was used, food and caffeine could have opposite effects regarding the appearance of AEs. However, our results show that, compared with anagrelide taken in the fasted state, intake with food and coffee produced lower and delayed peak concentrations in blood. Despite this, heart rate and ECG changes were more frequent in the fed/caffeine group. Until further data are acquired, a reduction in caffeine intake may prove to be a worthwhile strategy in patients receiving anagrelide who report palpitations. Other potential non-cardiac causes of palpitations, such as stress/anxiety, alcohol and over-the-counter or prescription medications should also be explored.

While the reduction in QRS length seen with anagrelide was minimal and did not exceed the normal range, QRS shortening has also been seen with other positive

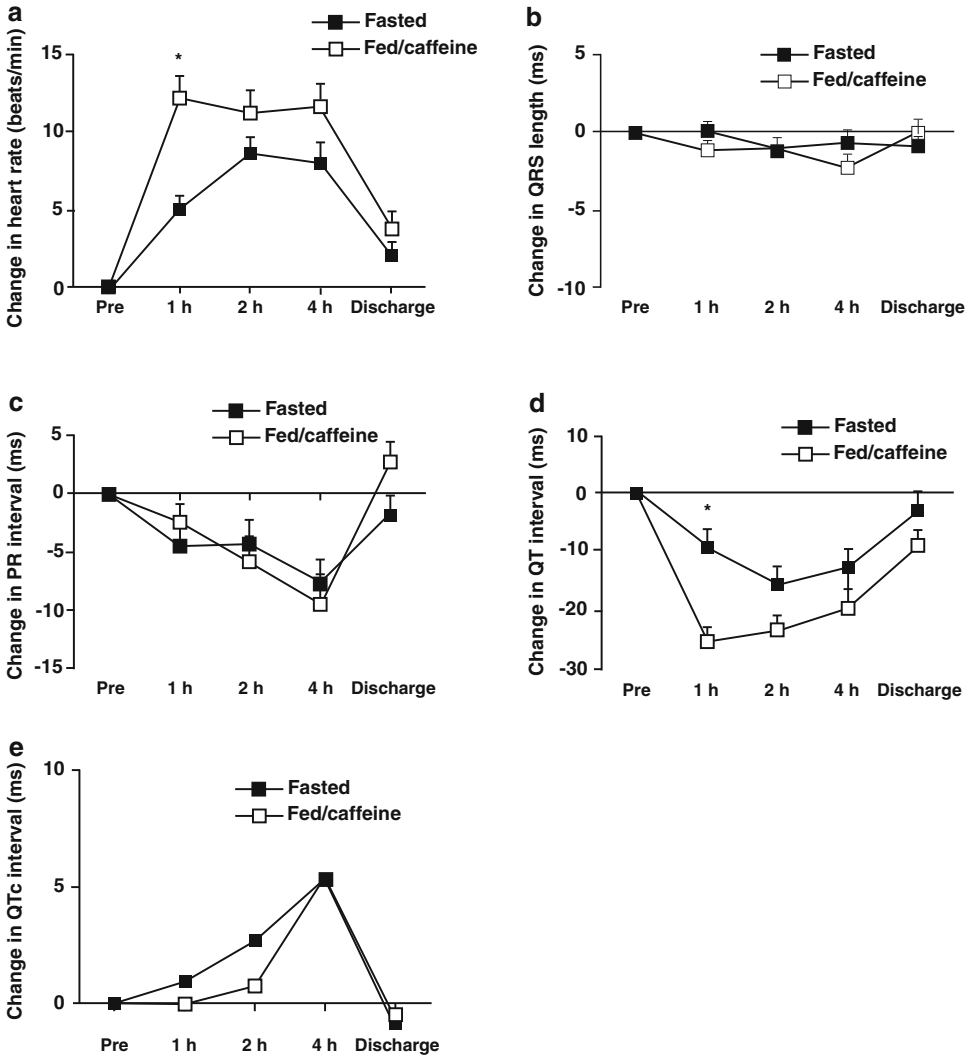

Fig. 3 Mean electrocardiogram (ECG) changes after anagrelide administration with respect to the fasted state: (**a**) heart rate, (**b**) QRS length, (**c**) PR interval, (**d**) QT interval and (**e**) corrected QT (QTc) interval. Two-way ANOVA included fasted state and time up to 4 h post-dose. In the case of heart rate and QT interval, the *p* value was <0.01 for the fasted state, time and interaction. In the cases of PR interval, QRS length and QTc interval, only time had a significant effect ($p < 0.01$). PR interval, QRS length and QT interval decreased following administration of anagrelide ($p < 0.001$), whereas there were no significant differences in QTc. * $p < 0.001$ for the comparison of fasted versus fed/caffeine state (Bonferroni analysis). Error bars = 95 % confidence intervals

ionotropic agents, such as catecholamines. This effect has been explained by greater synchrony in the onset of activation between different regions of the ventricle [30], suggesting that the effect seen with anagrelide may be intraventricular. The small reduction of QT interval observed with anagrelide has also been described with the catecholamine epinephrine in healthy subjects [31], and appears unlikely to be of clinical relevance. Moreover, QTc remained unchanged, suggesting that the reduction of QT interval was related to the increase in heart rate. All QT and QTc intervals, as well as all the other ECG parameters, were within the normal range (including ECGs performed in subjects reporting palpitations).

Case reports have been published regarding the association of anagrelide therapy with reversible high-output heart failure, atypical Takotsubo syndrome and reversible cardiomyopathy [32–36]. However, anagrelide has been used in patients with previous acute coronary syndromes [37]. Bearing in mind the ionotropic and chronotropic properties of this drug, it would seem prudent to assess and monitor, as appropriate, those patients receiving anagrelide who have cardiac disease, and to use it with caution in patients with severe cardiac disease. Although numbers were too small to permit any reliable comparison between the AE rates during the fed/caffeine and fasted periods, our results suggest that anagrelide tolerability, especially with regard to headaches, was slightly improved by taking it with food as, in general, AEs were more frequent during the fasted period than the fed/caffeine period. Corresponding to the higher peak concentration of anagrelide and 3-hydroxyanagrelide in fasted subjects, all events of moderate severity occurred during the fasted treatment period.

Our work is the first to systematically study ECG changes alongside pharmacokinetics in fed/caffeine and fasted subjects receiving anagrelide. These data also provide a potential explanation for palpitations experienced by patients taking anagrelide therapeutically, which were the most common side effect reported in some studies [2, 7].

5 Conclusion

Anagrelide is generally well tolerated in healthy subjects and has a small effect on ECG parameters and heart rate. The intake of food/caffeine affected the profile of anagrelide exposure. Caffeine was not shown to affect the metabolism of anagrelide by CYP1A2, although it may be implicated in the higher increase in heart rate and increased frequency of palpitations reported after food/caffeine intake.

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Conflict of interest Dr. Martínez-Sellés reports receiving lecture fees and consultancy fees from Shire Pharmaceuticals. Dr. Franklin reports being an employee of Shire Pharmaceuticals. Dr. Gama and Mr. Jones report being former employees of Shire Pharmaceuticals.

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