JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 69–77

Received June 26, 2003
Accepted September 23, 2003
DOI 10.1211/0022357022520
ISSN 0022-3573

Evaluation of the impact of altered lipoprotein binding conditions on halofantrine induced QTc interval prolongation in an anaesthetized rabbit model

Michelle P. McIntosh, Andrew J. Batey, Susan J. Coker, Christopher J. H. Porter and William N. Charman

Abstract

Halofantrine has been observed to cause QT interval prolongation in susceptible patients and the effect has most commonly been observed after post-prandial administration. Halofantrine-induced QT prolongation occurs in conjunction with a significant increase in plasma halofantrine concentrations and an increase in halofantrine association with post-prandial plasma lipoproteins. The increased association of halofantrine with post-prandial lipoproteins is accompanied by a marked change in drug distribution between the different plasma lipoprotein fractions. This study was designed to evaluate the putative role of myocardium-based lipoprotein receptor-mediated uptake of lipoproteins as a possible contributing factor to the observed effect of halofantrine on QT intervals. The extent of QT interval prolongation following intravenous halofantrine administration (10 mg kg^{-1}) to normolipidaemic (fasted) or hyperlipidaemic (induced with Intralipid infusion) anaesthetized New Zealand White rabbits (n = 6) was determined, as was the distribution of halofantrine between the plasma lipoprotein classes. The results, however, were in contrast to the suggested hypothesis since the QT interval was reduced (and not increased) after halofantrine administration to hyperlipidaemic rabbits relative to fasted rabbits. Therefore, it is unlikely that lipoprotein-based uptake of halofantrine into the myocardium is a major contributor to the previously observed increase in QT prolongation after post-prandial administration of halofantrine.

Department of Pharmaceutics, Victorian College of Pharmacy, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia

Michelle P. McIntosh*, Christopher J. H. Porter, William N. Charman

Department of Pharmacology and Therapeutics, The University of Liverpool, Ashton Street, Liverpool, L69 3GE, UK

Andrew J. Batey†, Susan J. Coker

Correspondence: W. N. Charman, Victorian College of Pharmacy, Monash University (Parkville campus), 381 Royal Parade, Parkville, Victoria 3052, Australia. E-mail: bill.charman@vcp.monash.edu.au

Funding: This work was supported, in-part, by SmithKline Beecham Pharmaceuticals (UK).

Present address: *Center for Drug Delivery Research, University of Kansas, 2099 Constant Ave, Lawrence, Kansas 66047, USA; †Safety Pharmacology, GlaxoSmithKline Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR, UK.

Introduction

Halofantrine is a phenanthrenemethanol antimalarial drug effective in the treatment of sensitive and multi-drug resistant *Plasmodium falciparum* and *P. vivax* infections. Similar to some other antimalarial drugs, such as quinine, administration of high halofantrine dosages has been reported to cause QT interval prolongation in susceptible patients (Monlun et al 1993, 1995; Touze et al 1996). Prolongation of the QT interval of the electrocardiogram (ECG) is a serious adverse effect that can predispose patients to development of life-threatening cardiac arrhythmias such as polymorphic ventricular tachycardia (known as torsade de pointes). Clinical investigation of halofantrine-induced QT interval prolongation indicated that the effect was broadly dose-related (Karbwang et al 1993; Touze et al 1996). QT prolongation has also most commonly been observed when halofantrine is administered post-prandially (Nosten et al 1993; Monlun et al 1995) – an effect coincident with significantly enhanced oral bioavailability and an increase in the extent of halofantrine association with post-prandial plasma lipoproteins (Humberstone et al 1998a).

Halofantrine is a highly lipophilic drug with high affinity for plasma lipoproteins (Cenni et al 1995; Humberstone et al 1998a). Fifty percent of halofantrine in plasma is associated with plasma lipoproteins in fasted human plasma, and the association increases a further 18% in response to the post-prandial elevation of plasma lipoprotein concentrations (McIntosh et al 1999). The increased association of halofantrine with post-prandial lipoproteins is accompanied by a marked change in drug distribution

between lipoprotein fractions with a doubling in the amount of halofantrine in very-low-density lipoproteins and chylomicrons (Humberstone et al 1998a).

The increased association of halofantrine with post-prandial lipoproteins is coincident with a decrease in clearance and volume of distribution in fed beagle dogs and rats (Humberstone et al 1998a; Brocks & Wasan 2002). In-vitro studies have also demonstrated that an increased association of halofantrine with post-prandial plasma lipoproteins leads to an increase in IC50 value (concentration producing 50% inhibition) when incubated with *P. falciparum* (Humberstone et al 1998b). These pharmacokinetic and pharmacodynamic changes have been attributed to the likely decrease in the free concentration of halofantrine in plasma in the presence of increased lipoprotein concentrations. Drug association with plasma lipoproteins and lipoprotein-receptor-mediated uptake has also been implicated in altering the pharmacokinetic and pharmacological/toxicological profile of other lipophilic drugs, including amphotericin B and ciclosporin (Wasan & Cassidy 1998; de Groen 1988; Wasan et al 1998).

The effect of food on halofantrine-induced prolongation of the QTc interval is difficult to interpret because post-prandial administration of halofantrine leads to significantly higher plasma concentrations (in comparison with fasted subjects (Milton et al 1989; Humberstone et al 1996)) as well as a change in the profile of halofantrine distribution across the plasma lipoprotein classes. The myocardium has receptors for the uptake of very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (Takahashi et al 1992), and when assessed in light of the significant and changing association of halofantrine with post-prandial lipoproteins, it raises the interesting possibility that such an uptake pathway may contribute to the observed post-prandial prolongation of the QTc interval. This concept has been proposed previously for probucol-induced QTc prolongation (Eder 1982). Hence, this study was undertaken to examine whether the altered association of halofantrine with plasma lipoproteins in post-prandial plasma is a likely contributory factor to QTc prolongation.

Materials and Methods

Animal studies

New Zealand White (NZW) rabbits have been used extensively for in-vitro and in-vivo electrophysiologic studies (Coker 1989), and preliminary investigations of the cardiac effects of halofantrine and other antimalarials have been conducted in rabbits (Lightbown et al 2001). NZW rabbits are categorised as LDL mammals (Chapman 1986), with the predominant lipoprotein fraction in plasma being LDL. Lipoprotein function, structure and lipid transfer protein-1 (LTP1) activity in rabbit plasma are also similar when compared with human plasma (Wasan et al 1998). The presence of cardiac VLDL and LDL receptors in rabbits is well established (Takahashi et al 1992).

Male NZW rabbits were obtained from Charles River (Margate, Kent, UK). Rabbits were individually housed under ambient conditions of a 12-h light–dark cycle at 18–23 °C with food (Labsure diet R14; SDS, Witham, Essex) and water freely available. The weight range for experimental use was 2.5–3.1 kg. The animal handling protocols were in accordance with the Guidance on the Operation on the Animals (Scientific Procedures) Act 1986, London, UK and also adhered to European Community guidelines for the use of experimental animals. Experiments were carried out under the authority of Project Licence no. 40/1702 and approved by the University of Liverpool Animal Welfare Committee.

Surgical preparation of the rabbits was as previously reported (McIntosh et al 2003). Hyperlipidaemia, to mimic post-prandial lipid changes, was induced with a 30-min infusion of Intralipid 10% (Baxter Healthcare), comprised of 10% fractionated soybean oil, 1.2% fractionated egg phospholipid and 2.25% glycerol, at a dose of 1.5 mL kg^{-1}, and induced changes in plasma lipoprotein composition broadly representative of the acute post-prandial changes observed in human plasma. The plasma triglyceride concentration 30 min after cessation of the Intralipid infusion typically increased by 219%, which compares favourably to changes in triglyceride concentrations observed in man 3 h following a high-fat meal (222%) (McIntosh et al 1998).

A schematic representation of an ECG for a rabbit is shown in Figure 1. Five successive ECG complexes were analysed and the intervals averaged for each recording time point. The PR interval was defined as the interval between the onset of the P wave and the onset of the QRS complex. The QRS complex was measured from the onset of the Q wave to the point at which the ST segment intersected the isoelectric line. The QT interval was measured from the onset of the Q wave to the end of the T wave. Bazett's formula was used to correct the QT measurement for heart rate effects, as the QT interval has been shown to vary in direct proportion to the square root of the RR interval.

Experimental protocol

Halofantrine hydrochloride (30 mg mL^{-1}) was formulated in dimethylacetamide (DMA) and propylene glycol (40:60 v/v) (Krishna et al 1993). The solution was freshly prepared on the day of the experiment and the concentration of halofantrine confirmed via HPLC.

A 15-min stabilization period after surgery was allowed before a 5-mL blood sample was taken for baseline lipid and lipoprotein levels, after which an infusion of either saline (fasted) or Intralipid (hyperlipidaemic) was administered via the femoral vein at a rate of 1.5 mL kg^{-1} over 30 min. After the saline or Intralipid infusion, the femoral vein cannula was flushed with 5% dextrose solution before administration of 10 mg kg^{-1} halofantrine in the DMA–propylene glycol vehicle over a 5-min period using a syringe infusion pump. A 5-mL blood sample was taken 15 and 45 min after the start of the halofantrine infusion for lipoprotein analysis, HPLC analysis of

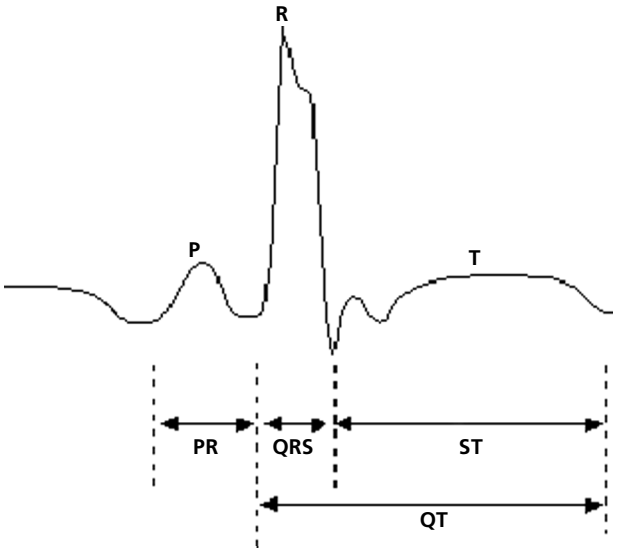

Figure 1 A schematic representation of a lead II ECG profile from a NZW rabbit. The profile and points of measurement of the individual intervals are indicated.

plasma halofantrine levels and blood gas determinations. The protocol for the control rabbits was identical to that for the fasted group except the DMA–propylene glycol vehicle alone was administered as a 5-min infusion. ECG data were continuously recorded and subsequently retrieved from the computer database at the relevant time points for analysis and interpretation.

Blood samples were anticoagulated with disodium EDTA (1.5 mg mL^{-1}). Following centrifugation, a portion (1 mL) of each plasma sample was stored at –20 °C for later analysis of halofantrine concentrations using a validated HPLC method (Humberstone et al 1995), and 1 mL of each plasma sample was immediately layered in a density gradient for ultracentrifugation and lipoprotein separation.

Minor adjustments to the lipoprotein separation methods previously described (McIntosh et al 1999) were required for the isolation of rabbit plasma lipoproteins due to species-related variation in lipoprotein concentration and density. The method used here was identical to that published previously except that the density gradient formed within the ultracentrifuge tube included an additional layer of 1.006 mg mL^{-1} sodium chloride (NaCl) solution, such that the complete density gradient was composed of 0.31 mL of a potassium bromide (KBr) solution with a density of 1.21 mg mL^{-1}, 1.25 mL of a KBr solution with a density of 1.15 mg mL^{-1}, a 1.0 mL plasma sample followed by 0.72 mL of a KBr solution with a density of 1.02 mg mL^{-1} and the final layer of 0.72 mL of the NaCl solution with a density of 1.006 mg mL^{-1}. Following ultracentrifugation, the density gradient was fractionated, using an automated fraction collector (Gilson FC 203; Gilson, WI), into 20 × 200-μL volumes. Each fraction was identified as containing triglyceride-rich lipoprotein (TRL; chylomicrons plus VLDL), LDL, high-density lipoprotein (HDL) or lipoprotein-deficient plasma (LPDP) based on UV absorbance at 280 nm and triglyceride and cholesterol concentrations in the fractions. In rabbit plasma TRL was contained in fractions 1–4, LDL was collected in fractions 5–10, HDL was within 11–16 and the remaining four fractions (17–20) were defined as LPDP.

Free fraction determination

Halofantrine free fraction data was generated in blood obtained from anaesthetized fasted or hyperlipidaemic rabbits via cardiac puncture. Fasted plasma samples (n 3 at each concentration) were spiked with halofantrine to concentrations of 1000, 1700, 5600 and 11 500 ng mL^{-1} and incubated in a water bath for 60 min at 37 °C. Hypertriglyceridaemic samples were similarly spiked at halofantrine concentrations of 1000, 6200, and 25 600 ng mL^{-1}. The halofantrine plasma concentrations employed were chosen to cover the range of concentrations measured 15 or 45 min after the 10 mg kg^{-1} intravenous dose of halofantrine to fasted or hyperlipidaemic rabbits in the QTc study.

Ultracentrifugation is frequently used for determination of the free fraction of highly lipophilic compounds in plasma and has previously been shown to compare well with results obtained using other techniques (Oellerich & Muller-Vahl 1984; Legg & Rowland 1987). The ultracentrifugation technique employed in these studies was a modified version of the methods employed by Legg & Rowland (1987). Spiked plasma (4 mL) was added to each of six ultracentrifuge tubes (Ultra-Clear; Beckman, CA) and the plasma was ultracentrifuged (Beckman Optima XL-100K; Beckman, CA; SW60 Ti rotor) at 37 °C for 30 h at 50 000 rev min^{-1} (average 250 000 *g*).

Plasma water samples were obtained by piercing the side of the ultracentrifuge tube with a 23-gauge needle attached to a 1-mL syringe (Terumo, Elkton, MD). A sample of approximately 100 μL of plasma water was aspirated for analysis of halofantrine, protein and lipid content. The absence of protein and lipoproteins in the sample of plasma water was confirmed by analysing the sample for triglyceride (TG), total cholesterol (CH) and total protein (TP) concentrations using commercial enzymatic colorimetric kits (Boehringher Mannheim, Germany) on a COBAS MIRA clinical analyser (Roche, Basle, Switzerland). All three assays were externally calibrated.

For a highly plasma bound compound such as halofantrine, the free concentration was expected to be extremely low, thereby requiring an analytical technique with a lower limit of quantitation than the 20 ng mL^{-1} available by UV detection. Hence, a liquid chromatography coupled with mass spectrometry (LC/MS) assay was developed, consisting of a Hewlett Packard Series 1100 system containing a binary pump, an ALS auto-injector (Hewlett Packard, CA) and a Wakosil II microbore C18 column (5 μm particle size, 150 mm × 1 mL; SGE instruments, TX). The MS detector was a Micromass Platform II (Micromass, Manchester, UK) operating under positive ion electrospray

conditions, with a source temperature of 80 °C. Data were analysed on a Digital Celebris GL 5120 computer using MassLynx software (Micromass, Manchester, UK). The mobile phase consisted of 66:34 acetonitrile–water with 5 mM NH_4OAc. The aqueous component of the mobile phase was adjusted to pH 2 by the addition of trifluoroacetic acid. The flow rate was 100 μL min^{-1} and all separations were performed at room temperature.

Plasma water samples (50 μL) were diluted 3-fold with acetonitrile before injection onto the LC/MS. Samples in which the concentration of halofantrine exceeded the concentration range of the standard curve (1–20 ng mL^{-1}) were further diluted in mobile phase and re-assayed against a new standard curve, prepared with dilutions matching those of the sample. The halofantrine LC/MS assay was validated for precision and accuracy using standard procedures (Snyder et al 1997).

Statistical analysis

The haemodynamic data were analysed as a change from the baseline value (rather than the absolute values) to take into account the baseline variability between the treatment groups. Data normality was confirmed using the Ryan-Joiner test and the homogeneity of variance was evaluated using Bartlett's test for equal variance. The within-treatment effect was determined using a *t*-test comparing baseline values to the maximum observed effect, whereas a one-way analysis of variance was employed to identify between-treatment effects. Multiple comparisons were performed, when required, using Tukey's pairwise comparisons. The compositional changes in lipoproteins were analysed using repeated measures analysis of variance. A standard statistical software package was employed for data analysis (Minitab, CA) with $\alpha = 0.05$.

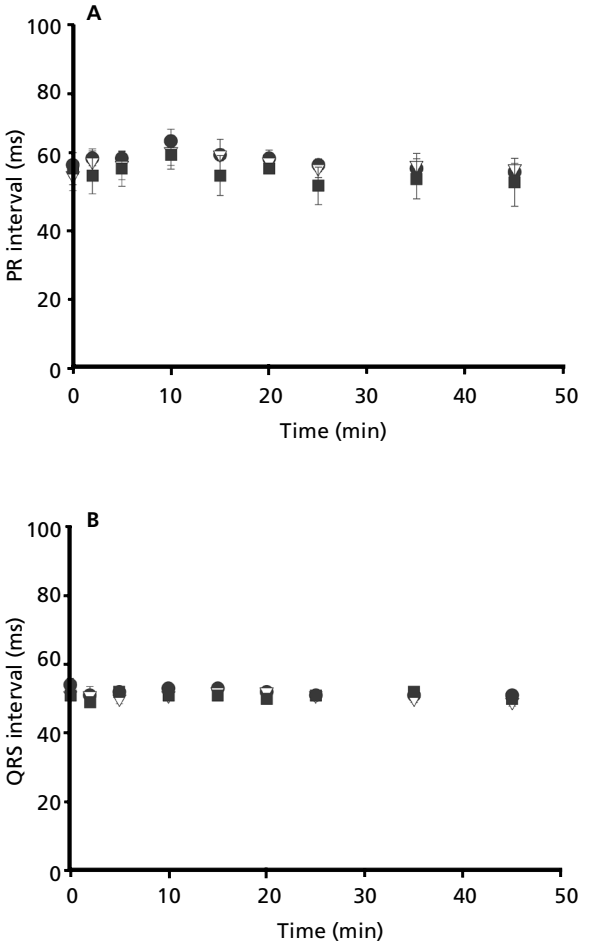

Figure 2 Measured PR intervals (A) and QRS intervals (B) in the vehicle control group (■, n = 3) and after administration of halofantrine to fasted (●, n = 5) and hyperlipidaemic (▽, n = 6) rabbits. Data are presented as mean ± s.e.m.

Results

QTc interval prolongation

ECG and haemodynamic changes were assessed in three intravenous treatment groups: vehicle control, fasted; 10 mg kg^{-1} halofantrine, fasted; and 10 mg kg^{-1} halofantrine, hyperlipidaemic. Figure 2 depicts the effect of these treatments on the PR (Figure 2A) and QRS intervals (Figure 2B) of the ECG. The administration of halofantrine caused a marginal increase in the PR interval 10 min after the start of the infusion, although the extent of prolongation was not statistically significant and the PR interval had returned to baseline within 20 min. This marginal effect was likely mediated by the DMA–propylene glycol vehicle as it was observed in both drug treatment groups and the vehicle control. The administration of halofantrine, or vehicle alone, induced no changes in the QRS interval duration.

In contrast to the lack of effect of halofantrine on the PR and QRS intervals, administration of halofantrine to the fasted treatment group resulted in a significant prolongation of the QT interval ($P = 0.011$) (data not shown). The standard form of assessing such data is as heart-rate-corrected QTc intervals, and these are presented in Figure 3A. Consistent with the uncorrected QT measurements, administration of halofantrine to the fasted treatment group resulted in prolongation of the QTc interval. However, administration of the same halofantrine dose to the hyperlipidaemic treatment group did not significantly lengthen the QT or QTc interval and the QT/QTc interval in the hyperlipidaemic group was not significantly different to the vehicle control treatment.

Figure 3B presents the change in the QTc interval (i.e. the measured value less baseline value measured immediately before halofantrine administration) for the various treatment groups. Correction for the variation in baseline QTc resulted in a more pronounced and more reproducible effect in the fasted treatment group (increase to 430 ms from a baseline of 361 ms) when compared to the hyperlipidaemic group (maximum measured QTc interval was 388 ms from a baseline value of 373 ms). Importantly,

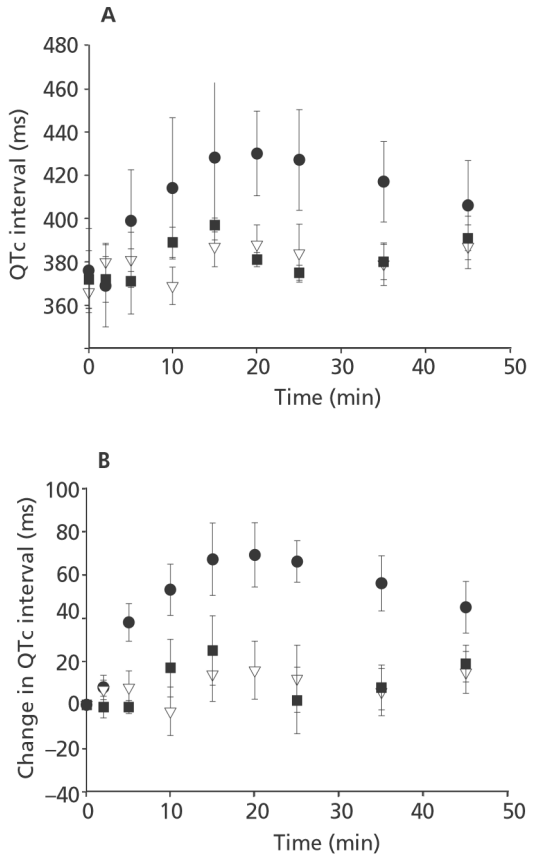

Figure 3 The QTc intervals (A) and the change in QTc interval, expressed as a deviation from the QTc value immediately before dosing (B) in the vehicle control group (■, n = 3) and after administration of halofantrine to fasted (●, n = 5) and hyperlipidaemic (▽, n = 6) rabbits. Data are presented as mean ± s.e.m.

the trends observed in the heart-rate-corrected data were consistent with the change in absolute QT intervals. These data demonstrate that administration of halofantrine to fasted rabbits induced a significant lengthening of the QTc interval ($P = 0.002$) and that this effect was significantly different to that observed for both the hyperlipidaemic and vehicle control groups ($P = 0.010$).

Relationship between halofantrine lipoprotein distribution and QTc interval

Each rabbit in the fasted and hyperlipidaemic treatment group was intravenously administered 10 mg kg^{-1} halofantrine over a 5-min infusion period. Plasma and lipoprotein fractions (TRL, LDL and HDL) were analysed for halofantrine concentration at 15 and 45 min after the commencement of the infusion. Table 1 presents the halofantrine plasma concentrations and the percent QTc prolongation relative to the baseline QTc interval at these time points in each treatment group. In fasted rabbits, where the QTc interval was prolonged to its most significant level (an increase of 18 ± 4%), the halofantrine plasma concentration was 5.6 ± 1.3 μg mL^{-1}. Over the subsequent 30 min, the plasma concentration decreased to 1.7 ± 0.4 μg mL^{-1} coincident with an alteration in the increase in QTc interval relative to baseline (12 ± 3% prolongation).

In hyperlipidaemic rabbits, halofantrine plasma concentrations were significantly higher than those observed at the equivalent time points in fasted rabbits after administration of the same dose. However, despite the high plasma halofantrine concentrations (25.7 ± 3.5 μg mL^{-1}) at 15 min post-dosing, the QTc interval was not significantly lengthened (4 ± 3%) compared with baseline. During the subsequent 30 min, the plasma halofantrine concentration declined to 6.2 ± 1.1 μg mL^{-1}, although no change in the QTc interval was observed during this period.

The profile of halofantrine distribution across the various lipoprotein fractions remained constant throughout the time course of the experiment and this was consistent with a relatively small change in the total triglyceride concentration in plasma over the same time period (Table 2). The fasted halofantrine lipoprotein distribution 15 min post-dosing was 36% in TRL, 13% in LDL, 28% into HDL and 22% in the LPDP, and this distribution did not change significantly over the subsequent 30-min period. The lipoprotein distribution of halofantrine in the hyperlipidaemic group was markedly different to that observed in the fasted group. More than double the proportion of halofantrine was contained within the TRL fraction (80%), with corresponding decreases in the proportion of halofantrine contained within LDL (5%), HDL (9%) and LPDP (6%), and these changes were apparent at both 15 and 45 min post-dosing.

Table 1 Plasma concentration of halofantrine and the percentage prolongation of the QTc interval at 15 and 45 min post-infusion of halofantrine in fasted and hyperlipidaemic rabbits.

Treatment	Time	QTc prolongation (%)	Plasma halofantrine concn (μg mL^{-1})
Fasted (n = 5)	15 min	18 ± 4	5.6 ± 1.3
	45 min	12 ± 3	1.7 ± 0.4
Hyperlipidaemic (n = 6)	15 min	4 ± 3	25.7 ± 3.5
	45 min	4 ± 3	6.2 ± 1.1

Data are presented as the mean ± s.e.m.

Table 2 Percentage distribution of halofantrine between plasma lipoprotein fractions at 15 and 45 min post-infusion of halofantrine in fasted and hyperlipidaemic rabbits.

Treatment	Time	Plasma triglyceride concn ($mg\,mL^{-1}$)	Plasma lipoprotein fraction			
			TRL	LDL	HDL	LPDP
Fasted (n = 5)	15 min	1.4 ± 0.5	36.3 ± 11.5	13.4 ± 6.0	28.3 ± 4.2	22.0 ± 11.7
	45 min	1.8 ± 0.6	41.4 ± 13.6	15.2 ± 10.9	25.9 ± 7.7	17.4 ± 12.4
Hyperlipidaemic (n = 6)	15 min	6.4 ± 2.4	80.9 ± 4.2	4.9 ± 1.4	8.3 ± 3.0	5.8 ± 2.5
	45 min	6.2 ± 2.1	80.2 ± 6.2	4.8 ± 1.3	10.0 ± 4.2	5.0 ± 2.2

Hyperlipidaemia induced an overall 20% increase in the extent of association of halofantrine with plasma lipoproteins compared with distribution in fasted rabbit plasma. Data are presented as mean ± s.d. TRL, triglyceride-rich lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPDP, lipoprotein-deficient plasma.

Determination of unbound fraction of halofantrine in plasma

The observation that the extent of QTc prolongation noted after intravenous administration of halofantrine to hyperlipidaemic rabbits was reduced when compared with halofantrine administration to rabbits in the fasted state suggested that the association of halofantrine with plasma lipoproteins did not enhance QTc prolongation through uptake of halofantrine–lipoprotein complexes via VLDL and LDL receptors, and also implied that a simple linear relationship did not exist between the pharmacological effect and the total halofantrine plasma concentration. However, for highly plasma bound drugs such as halofantrine, pharmacological effect is often better related to the free drug concentration rather than the total plasma concentration (Oellerich & Muller-Vahl 1984; Woosley et al, 1984; Legg & Rowland 1987). Ultracentrifugation was therefore used to determine the unbound halofantrine concentration in rabbit plasma such that the relationship between free fraction (f_u) and pharmacological effect might be examined. The free fraction of halofantrine was determined in blank plasma from fasted and hypertriglyceridaemic rabbits, which was then spiked with a range of halofantrine concentrations to reflect the concentration range observed in the QTc study (where, despite an increase in total halofantrine plasma concentration, the effect of halofantrine on QTc intervals was reduced). As there was no significant difference in f_u as a function of halofantrine concentration, mean values were calculated in both fasted and hypertriglyceridaemic plasma (the lack of concentration dependence is consistent with halofantrine solubilisation within plasma lipoproteins, rather than a specific binding phenomenon to, for example, plasma proteins (McIntosh et al 1999)). As expected, the f_u for halofantrine in fasted plasma was considerably higher (20.9 ± 3.0%, mean ± s.d., n = 18) than that observed in hypertriglyceridaemic plasma (10.2 ± 2.5%, mean ± s.d., n = 12). The f_u for halofantrine determined here in rabbit plasma (10–20%) was considerably higher than that observed in other species, including dogs (~1%, unpublished results) and rats (< 0.1%; Brocks 2002). These differences most likely reflect lower levels of α_1-acidic glycoprotein (AGP) in rabbit plasma when compared to other species. Similar trends (i.e. significantly higher unbound fractions measured in rabbit plasma versus other species) have previously been reported for other weak bases including oxprenolol and propranolol (Belpaire et al 1984).

Table 3 shows the percent QTc prolongation and total plasma halofantrine concentration at 15 and 45 min post-dosing in fasted and hyperlipidaemic rabbits. The predicted free concentrations of halofantrine are included in the final column in Table 3 and were calculated as the fraction of the actual concentration measured in the QTc study. Although the f_u of halofantrine was reduced in hyperlipidaemic plasma from approximately 20% to 10%, the calculated unbound plasma concentration (C_u) of halofantrine in the hyperlipidaemic rabbits in the QTc study was still higher than the C_u of halofantrine in the fasted rabbits, since the magnitude of the increase in total halofantrine plasma concentration was greater than the reduction in f_u.

Discussion

QTc prolongation after halofantrine administration in man has most commonly been reported after oral post-prandial halofantrine administration, an effect that occurs in conjunction with a significant increase in the plasma halofantrine concentration and an increase in halofantrine association with post-prandial plasma lipoproteins. These two observations stimulated the current examination of the possibility that an increase in post-prandial association of halofantrine with plasma lipoproteins may contribute to the development of QTc interval prolongation via receptor-mediated myocardial uptake of halofantrine–VLDL or halofantrine–LDL.

Consistent with previous observations (Monlun et al 1993; Nosten et al 1993; Batey et al 1997), the administration of halofantrine did not significantly alter atrioventricular conduction (PR interval) or ventricular depolarisation (QRS interval) as depicted in Figure 2,

Table 3 Percent QTc prolongation and total and free plasma halofantrine concentration at 15 and 45 min post-dosing in fasted and hyperlipidaemic rabbits.

Treatment	Time	QTc prolongation (%)	Measured plasma halofantrine concn ($\mu g\,mL^{-1}$)	Free fraction (%)	Predicted free plasma halofantrine concn ($\mu g\,mL^{-1}$)
Fasted	15 min	18 ± 4	5.77 ± 1.33	20.9 ± 3.1[a]	1.18 ± 0.62
	45 min	12 ± 3	1.74 ± 0.37	20.9 ± 3.1[a]	0.36 ± 0.17
Hyperlipidaemic	15 min	4 ± 3	25.65 ± 3.15	10.2 ± 2.5[b]	2.54 ± 1.00
	45 min	4 ± 3	6.16 ± 1.05	10.2 ± 2.5[b]	0.67 ± 0.32

The free plasma concentration of halofantrine was determined in-vitro, by spiking plasma to the equivalent total plasma concentration as measured in the in-vivo study. The measured QTc interval prolongation values induced by halofantrine administration 15 and 45 min post-dosing are included for comparison with the total and free halofantrine plasma concentration. The predicted free halofantrine concentration data are presented as the mean ± s.d (n = 3). [a]Mean value obtained from the overall mean of n = 18 samples, where at least n = 3 samples of fasted plasma were spiked with halofantrine at 1000, 1700, 5600 and 11 500 ng mL^{-1} and f_u determined. No significant differences were seen in data obtained at each concentration. [b]Mean value obtained from the overall mean of n = 18 samples, where at least n = 3 samples of hypertriglyceridaemic plasma were spiked with halofantrine at 1000, 6200, and 25 600 ng mL^{-1} and f_u determined. No significant differences were seen in data obtained at each concentration.

and the 18% prolongation of the QTc interval observed after a single 10 mg kg^{-1} halofantrine dose to fasted rabbits (Figure 3) was similar to a previous study in anaesthetized rabbits where a cumulative dose of 14 mg kg^{-1} of halofantrine induced a 32% prolongation of the QTc interval (Lightbown et al 2001). Considering only the fasted rabbit data, there was a broad relationship between the total halofantrine plasma concentration and the extent of QTc prolongation (Table 1), which is consistent with several other previously published studies (Karbwang et al 1993; Karbwang & Bangchang 1994; Giudicelli et al 1996).

In contrast, administration of halofantrine to the hyperlipidaemic treatment group resulted in a 4-fold decrease in the extent of QTc prolongation relative to the fasted group (Table 1). This reduction in effect of halofantrine on the QTc interval occurred in spite of a 5-fold increase in plasma halofantrine concentrations. The high plasma halofantrine concentration seen in hyperlipidaemic rabbits is consistent with both reduced distribution and decreased clearance of halofantrine from lipid-rich plasma when compared with fasted plasma, as described previously (Humberstone et al 1998a).

The lack of effect on QTc, despite the raised plasma concentrations in the hyperlipidaemic group, suggests that the relationship between total halofantrine plasma concentration and cardiac effect was not a simple linear pharmacodynamic relationship, at least in this rabbit model of hyperlipidaemia. These findings also suggested that enhanced cardiac delivery of halofantrine via lipoprotein receptors in the myocardium was not a major contributory factor to the onset of QTc prolongation after post-prandial halofantrine administration.

The absence of a direct relationship between pharmacological effect and total plasma drug concentration is not uncommon, and in these instances, an improved correlation between pharmacological effect and the free concentration of drug is often observed (Aarons & Rowland 1981; Oellerich & Muller-Vahl 1984; Woosley et al 1984). This appears to be particularly true for compounds that act on the cardiovascular system (Woosley et al 1984), where the unbound fraction is generally assumed to be the only species capable of interaction with receptors or other binding sites (Oellerich & Muller-Vahl 1984; Woosley et al 1984). For example, quinidine and disopyramide can prolong the QT interval and these effects have been best correlated with the free concentration of drug in plasma rather than the total plasma drug concentration (Karbwang & Bangchang 1994). This effect may also reflect the fact that many of the drugs that increase the QT interval are highly lipophilic and therefore highly protein bound.

Direct measurement of halofantrine f_u in the in-vivo samples generated during the rabbit QTc studies was not possible, as the large sample volume required (4 mL) would have reduced the circulating blood volume to a level that would most likely have had an adverse effect on the haemodynamic profile. Consequently, to gain a reasonable estimate of the likely C_u for halofantrine in plasma, blank fasted and blank hyperlipidaemic rabbit plasma was spiked with halofantrine at a range of concentration covering those observed in the in-vivo study and the unbound drug fraction was determined. Table 3 presents the total halofantrine plasma concentrations measured in fasted and hyperlipidaemic plasma 15 and 45 min after the intravenous administration of 10 mg kg^{-1} halofantrine and the predicted free concentration, based on the unbound fraction measured in spiked plasma. At 15 min post-infusion, the total plasma halofantrine concentration in hyperlipidaemic plasma (25.6 $\mu g\,mL^{-1}$) was five times that of the total plasma concentration in fasted plasma (5.7 $\mu g\,mL^{-1}$). Applying the measured f_u from the spiked in-vitro studies, the predicted C_u of halofantrine in hyperlipidaemic plasma was 2.5 $\mu g\,mL^{-1}$, compared with 1.2 $\mu g\,mL^{-1}$ in fasted plasma. Therefore, although the difference in free halofantrine concentration (2.5 fold) was smaller than the difference in total halofantrine concentration (5 fold) the concentration of the pharmacologically active (free) halofantrine was still greater in

hyperlipidaemic plasma, in contrast to the diminished effect on the QTc interval seen in hyperlipidaemic rabbits.

These data suggest that the reduced QTc prolongation observed in hyperlipidaemic rabbit plasma (when compared with normolipidaemic plasma) was not simply a function of a reduction in free halofantrine concentration resulting from altered plasma binding. This complexity may arise from the presence of individual dynamic binding equilibria between halofantrine and TRL, LDL, HDL and each plasma protein class (albumin, α-acidic glycoprotein, etc.), and the realisation that alterations in halofantrine distribution between the plasma components in the hyperlipidaemic state (in general a shift to greater TRL binding) will likely impact upon the rate at which the equilibrium between bound and free drug is established, producing a situation where the kinetics of free drug (and therefore pharmacodynamic activity) may be altered. Thus, the in-vitro studies described here examine the equilibrium of halofantrine in hyperlipidaemic plasma and normal plasma, when in-vivo, in addition to the position of the bound–free equilibrium, the rate of replenishment of the free fraction may also be critical. In support of this hypothesis, similar conclusions were drawn in a previous in-vitro investigation (using an isolated rabbit heart model) into the likely effects of altered plasma protein binding on myocardial uptake of propafenone, a highly protein bound drug that shows a dose-dependent widening of the QRS interval in the ECG profile (Gillis & Kates 1986). Under steady-state conditions, a linear relationship existed between C_u and QRS interval duration but under non-steady-state conditions the relationship between C_u and pharmacodynamic effect was not evident. Interestingly, at all times a linear relationship existed between the myocardial concentration of propafenone and the extent of QRS prolongation, suggesting that protein binding impacted upon the partitioning of propafenone into the myocardium, thereby modulating the cardiac effect.

The studies evaluating halofantrine administration and QTc prolongation conducted in this rabbit model were designed to explore one aspect of the clinical situation, which was the pharmacodynamic relationship between halofantrine, lipoproteins and QTc interval prolongation. To further examine the factors involved in the clinical situation, beyond halofantrine association with plasma lipoproteins, an animal model in which co-administration of halofantrine (orally) with food, where the entire range of food-initiated physiological events were stimulated, would be preferred. One of the primary benefits associated with such a model (aside from the obvious advantage of a conscious ambulatory animal in comparison to an anaesthetized animal) is that it would allow for both oral administration of halofantrine and stimulation of the post-prandial state, such that the absorption pathway of halofantrine into the systemic circulation would be similar to the situation after post-prandial oral administration in man. Khoo et al (2001) recently reported that post-prandial oral administration of halofantrine to dogs resulted in greater than 50% of the dose being transported into the systemic circulation via the lymphatics, compared with less than 5% after fasted administration. These notable changes in halofantrine absorption route in post-prandial animals, when compared with pre-prandial animals, may be significant in terms of the potential effect of halofantrine on the heart (and in particular on QTc intervals) and will be investigated in future studies.

Conclusions

This investigation was undertaken to test the hypothesis that increased association of halofantrine with post-prandial plasma lipoproteins would result in a more pronounced QTc interval prolongation relative to fasted administration, due to lipoprotein-receptor-mediated uptake of halofantrine–lipoprotein complexes into the myocardium. In contrast to the suggested hypothesis, however, the halofantrine-induced prolongation of the QTc interval was reduced (and not increased) in hyperlipidaemic rabbits relative to fasted rabbits. It is therefore unlikely that lipoprotein-based uptake of halofantrine into the myocardium is a major contributor to the previously observed increase in QTc prolongation after post-prandial administration of halofantrine. Although the free fraction of halofantrine in plasma was reduced under conditions where lipoprotein concentrations were elevated, it seems likely that the reduction in free concentration of halofantrine in plasma was not sufficient to account for the reduction in QTc interval prolongation. The reduced QTc effect more likely reflects altered myocardial uptake, possibly stemming from changes to plasma binding kinetics. Further studies designed to monitor the partitioning of halofantrine into the myocardium are planned.

References

Aarons, L. J., Rowland, M. (1981) Kinetics of drug displacement interactions. *J. Pharmacokinet. Biopharm.* **9**: 181–190

Batey, A. J., Lightbown, I. D., Lambert, J. P., Edwards, G., Coker, S. J. (1997) Comparison of the acute cardiotoxicity of the antimalarial drug halofantrine *in vitro* and *in vivo* in anaesthetized guinea pigs. *Br. J. Pharmacol.* **122**: 563–569

Belpaire, F. M., Braeckman, R. A., Bogaert, M. G. (1984) Binding of oxprenolol and propranolol to serum, albumin and alpha-1 acidic glycoprotein in man and other species. *Biochem. Pharmacol.* **33**: 2065–2069

Brocks, D. R. (2002) Stereoselective halofantrine and desbutyl halofantrine disposition in the rat: cardiac and plasma concentrations and plasma protein binding. *Biopharm. Drug Dispos.* **23**: 9–15

Brocks, D. R., Wasan, K. M. (2002) The influence of lipids on stereoselective pharmacokinetics of halofantrine: important implications in food-effect studies involving drugs that bind to lipoproteins. *J. Pharm. Sci.* **91**: 1817–1826

Cenni, B., Meyer, J., Brandt, R., Betschart, B. (1995) The antimalarial drug halofantrine is bound mainly to low and high density lipoproteins in human serum. *Br. J. Clin. Pharmacol.* **39**: 519–526

Chapman, M. J. (1986) Comparative analysis of mammalian plasma lipoproteins. In: Segrest, J. P., Albers, J. J. (eds) *Plasma lipoproteins. Part A. Preparation, structure and molecular biology*. 128th edn, Academic Press, Inc., New York, pp 70–143

Coker, S. J. (1989) Anesthetized rabbits as a model for ischemia- and reperfusion-induced arrhythmias: effects of quinidine and bretylium. *J. Pharmacol. Methods* **21**: 263–279

de Groen, P. C. (1988) Cyclosporine, low-density lipoprotein, and cholesterol. *Mayo Clin. Proc.* **63**: 1012–1021

Eder, H. A. (1982) The effect of diet on the transport of probucol in monkeys. *Artery* **10**: 105–107

Gillis, A. M., Kates, R. E. (1986) Influence of protein binding on the myocardial uptake and pharmacodynamics of propafenone. *J. Cardiovasc. Pharmacol.* **8**: 1163–1167

Giudicelli, C. P., Touze, J. E., Bernard, J. (1996) Electrocardiographic changes in antimalarial treatment with halofantrine, pharmacokinetic study, therapeutic deductions. *Bull. Acad. Natl Med.* **180**: 71–82

Humberstone, A. J., Currie, G. J., Porter, C. J. H., Scanlon, M. J., Charman, W. N. (1995) A simplified liquid chromatography assay for the quantitation of halofantrine and desbutylhalofantrine in plasma and identification of a degradation product of desbutylhalofantrine formed under alkaline conditions. *J. Pharm. Biomed. Anal.* **13**: 265–272

Humberstone, A. J., Porter, C. J. H., Charman, W. N. (1996) A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine. *J. Pharm. Sci.* **85**: 525–529

Humberstone, A. J., Porter, C. J. H., Edwards, G. A., Charman, W. N. (1998a) Association of halofantrine with postprandially derived plasma lipoproteins decreases its clearance relative to administration in the fasted state. *J. Pharm. Sci.* **87**: 936–942

Humberstone, A. J., Cowman, A. F., Horton, R. J., Charman, W. N. (1998b) Effect of altered serum lipid concentrations on the IC_{50} of halofantrine against *Plasmodium falciparum*. *J. Pharm. Sci.* **87**: 256–258

Karbwang, J., Bangchang, K. N. (1994) Clinical pharmacokinetics of halofantrine. *Clin. Pharmacokinet.* **27**: 104–119

Karbwang, J., Bangchang, K. N., Bunnag, D., Harinasuta, T., Laothavorn, P. (1993) Cardiac effect of halofantrine. *Lancet* **342**: 501

Khoo, S. M, Edwards, G. A., Porter, C. J. H., Charman, W. N. (2001) A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine. *J. Pharm. Sci.* **90**: 1599–1607

Krishna, S., ter Kuile, F. O., Supanaranond, W., Pukrittayakamee, S., Teja-Isavatharm, P., Kyle, D. E., White, N. J. (1993) Pharmacokinetics, efficacy and toxicity of parenteral halofantrine in uncomplicated malaria. *Br. J. Clin. Pharmacol.* **36**: 585–591

Legg, B., Rowland, M. (1987) Cyclosporin: measurement of fraction unbound in plasma. *J. Pharm. Pharmacol.* **39**: 599–603

Lightbown, I. D., Lambert, J. P., Edwards, G., Coker, S. J. (2001) Potentiation of halofantrine-induced QTc prolongation by mefloquine: correlation with blood concentrations of halofantrine. *Br. J. Pharmacol.* **132**: 197–204

McIntosh, M. P., Porter, C. J. H., Charman, W. N. (1998) The development of an acute post-prandial hyperlipidemia rabbit model. *Pharm. Sci.* **1**: S91

McIntosh, M. P., Porter, C. J. H., Wasan, K. M., Ramaswamy, M., Charman, W. N. (1999) Differences in the lipoprotein binding profile of halofantrine in fed and fasted human or beagle plasma are dictated by the respective masses of core apolar lipoprotein lipid. *J. Pharm. Sci.* **88**: 378–384

McIntosh, M. P., Batey, A. J., Porter, C. J. H., Charman, W. N., Coker, S. J. (2003) Desbutylhalofantrine: evaluation of QT prolongation and other cardiovascular effects after intravenous administration in vivo. *J. Cardiovasc. Pharmacol.* **41**: 406–413

Milton, K. A., Edwards, G., Ward, S. A., Orme, M. L. E., Breckenridge, A. M. (1989) Pharmacokinetics of halofantrine in man: effects of food and dose size. *Br. J. Clin. Pharmacol.* **28**: 71–77

Monlun, E., Pillet, O., Cochard, J. F., Fararel Garrigues, J. C., Le Bras, M. (1993) Prolonged QT interval with halofantrine. *Lancet*. **341**: 1541–1542

Monlun, E., Le Metayer, P., Szwandt, S., Neau, D., Longy Boursier, M., Horton, R. J., Le Bras, M. (1995) Cardiac complications of halofantrine: a prospective study of 20 patients. *Trans. R. Soc. Trop. Med. Hyg.* **89**: 430–433

Nosten, F., ter Kuile, F. O., Luxemburger, C., Woodrow, C., Kyle, D. E., Chongsuphajaisiddhi, T., White, N. J. (1993) Cardiac effects of antimalarial treatment with halofantrine. *Lancet* **341**: 1054–1056

Oellerich, M., Muller-Vahl, H. (1984) The EMIT FreeLevel ultrafiltration technique compared with equilibrium dialysis and ultracentrifugation to determine protein binding of phenytoin. *Clin. Pharmacokinet.* **9** (Suppl. 1): 61–70

Snyder, L. R., Kirkland, J. J., Glajch, J. L. (1997) Completing the method: validation and transfer. In: Snyder, L. R., Kirkland, J. J., Glajch, J. L. (eds) *Practical HPLC method development* 2nd edn, John Wiley and Sons, Inc., New York, pp 687–695

Takahashi, S., Kawarabayasi, Y., Nakai, T., Sakai, J., Yamamoto, T. (1992) Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl Acad. Sci. USA* **89**: 9252–9256

Touze, J. E., Bernard, J., Keundjian, A., Imbert, P., Viguier, A., Chaudet, H., Doury, J. C. (1996) Electrocardiographic changes and halofantrine plasma levels during acute falciparum malaria. *Am. J. Trop. Med. Hyg.* **54**: 225–228

Wasan, K. M., Cassidy, S. M. (1998) Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. *J. Pharm. Sci.* **87**: 411–424

Wasan, K. M., Kennedy, A. L., Cassidy, S. M., Ramaswamy, M., Holtorf, L., Chou, J. W., Pritchard, P. H. (1998) Pharmacokinetics, distribution in serum lipoproteins and tissues, and renal toxicities of amphotericin B and amphotericin B lipid complex in a hypercholesterolemic rabbit model: single-dose studies. *Antimicrob. Agents Chemother.* **42**: 3146–3152

Woosley, R. L., Siddoway, L. A., Thompson, K., Cerskus, I., Roden, D. M. (1984) Potential applications of free drug level monitoring in cardiovascular therapy. *Clin. Pharmacokinet.* **9** (Suppl. 1): 79–83