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Comparison of the acute cardiotoxicity of the antimalarial drug halofantrine *in vitro* and *in vivo* in anaesthetized guinea-pigs

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1 Several unrelated drugs have pro-arrhythmic activity associated with an ability to prolong the QT interval of the ECG. The aim of this work was to examine the effects of the antimalarial drug halofantrine *in vivo* and *in vitro*.

2 In anaesthetized guinea-pigs consecutive bolus doses of halofantrine (0.3, 1, 3, 10 and 30 mg kg^{-1}, i.v.) at 25 min intervals caused dose-dependent prolongation of the rate corrected QTc interval and bradycardia. The change in heart rate became significant after administration of 10 mg kg^{-1} halofantrine (-23 ± 9 beats min^{-1}) whereas the increase in QTc was significant with only 1 mg kg^{-1} halofantrine (22 ± 10 ms). It was only with the highest dose of halofantrine that the PR interval was increased (from 52 ± 3 to 67 ± 4 ms) and second degree atrioventricular (AV) block (type 1 Mobitz) occurred in all animals. No changes were observed in any parameters in a separate group of guinea-pigs which received vehicle (dimethylacetamide 60% propylene glycol 40%) at equivalent time points.

3 The blood concentrations of halofantrine ranged from 0.26 ± 0.17 μM after administration of 0.3 mg kg^{-1} to 2.79 ± 0.87 μM after 30 mg kg^{-1}, i.v. There was a significant correlation between the blood concentrations of halofantrine and the changes in QTc interval.

4 In guinea-pig left papillary muscles the effective refractory period was increased significantly 60 min after addition of halofantrine; from 161 ± 4 to 173 ± 6 ms with 10 μM, 156 ± 8 to 174 ± 6 ms with 30 μM and 165 ± 6 to 179 ± 5 ms with 100 μM halofantrine. However, the vehicle (0.1% Tween 80 in DMSO; final concentration of vehicle in Krebs, 1%) also increased the effective refractory period from 164 ± 5 to 173 ± 6 ms. Similar results were obtained in right ventricular strips but left atrial effective refractory periods were not altered by either the vehicle or halofantrine.

5 The results of these experiments suggest that any direct effects that halofantrine may have had on the effective refractory period of cardiac muscle cannot be separated from those of the vehicle. The prolongation of QTc and consistent observation of AV block with halofantrine in anaesthetized guinea-pigs suggest that *in vivo* models may be more useful for further studies investigating the mechanisms underlying the cardiotoxicity of halofantrine.

Keywords: Halofantrine; QT interval; effective refractory period; cardiotoxicity; antimalarial; AV block; arrhythmias; heart rate

Introduction

Long QT syndrome, where the rate-corrected QT interval (QTc) of the ECG is abnormally long, may be either congenital in origin, or 'acquired', due to the effects of certain drugs. A diverse range of drugs has been associated with the acquired long QT syndrome and some cause serious cardiac arrhythmias such as torsade de pointes (Napolitano *et al*., 1994). Recently, concern has been expressed about the antimalarial drug, halofantrine, following reports of QTc prolongation, episodes of torsade de pointes or sudden cardiac death (Nosten *et al*., 1993; Castot *et al*., 1993; Monlun *et al*., 1993; 1995; Karbwang *et al*., 1993; Toivonen *et al*., 1994). Although some of these adverse events occurred in patients with congenital long QT syndrome (Toivonen *et al*., 1994), halofantrine-induced QT prolongation also occurred in individuals with normal QT intervals (Monlun *et al*., 1995). In addition, adverse effects of halofantrine have been observed in patients receiving standard doses as well as those receiving higher doses (Karbwang *et al*., 1993), suggesting that this problem may not just be confined to situations of overdose.

At present it is not known how halofantrine could cause such effects. A number of other drugs that prolong the QT interval may do so as a consequence of direct actions on cardiac ion channels (Tan *et al*., 1995). Alternatively, it has been suggested that the precipitation of arrhythmias such as torsade de pointes may result from an abnormal response to alterations in autonomic control of the heart. Increased adrenergic activity has been implicated in some types of long QT syndrome, whereas some ventricular tachycardias, such as torsade de pointes can be a consequence of preceding bradycardia. Marked hypokalaemia can also cause QT prolongation, trigger torsade de pointes and exacerbate the pro-arrhythmic effects of certain drugs, such as class Ia antiarrhythmics (Ben-David & Zipes, 1993; Tan *et al*., 1995). Thus, if halofantrine *per se* does cause QT prolongation a number of different mechanisms may contribute to the development of associated severe arrhythmias like torsade de pointes. In addition, the precipitation of serious arrhythmias may be more likely in the presence of some other confounding factor such as hypokalaemia or bradycardia. The aim of the present studies was to compare the actions of halofantrine *in vitro* and *in vivo*, by examining its effects on the effective refractory period of isolated cardiac muscle and on the ECG of anaesthetized animals. Some of this work has been presented to the British Pharmacological Society (Batey *et al*., 1996; Lightbown *et al*., 1996).

Methods

In vivo *studies*

Anaesthesia was induced in male Dunkin-Hartley guinea-pigs (380 to 530 g), with 30 mg kg^{-1} sodium pentobarbitone, i.p. The trachea was cannulated to allow ventilation (Bioscience pump) with room air at a rate of 54 strokes min^{-1}, and a stroke volume of 15 ml kg^{-1} body weight. The blood gases were monitored with a Corning 158 pH/blood gas analyser. A

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carotid artery and a jugular vein were cannulated to allow recording of arterial blood pressure and for i.v. administration of drugs, respectively. The cannulae were filled with heparin-treated saline (0.9% w/v NaCl containing 10 u ml^{-1} heparin). The arterial line was connected to a pressure transducer (PDCR 75, Druck Ltd.), linked to a Lectromed 5240 pre-amplifier and MT6 recorder. Anaesthesia was maintained as necessary, by administration of further doses of sodium pentobarbitone (1.5 to 3 mg kg^{-1}, i.v.). ECG signals were monitored by use of limb leads linked to a Lectromed 5340 ECG pre-amplifier. Either lead I or lead II was recorded, depending on which gave the better separation of T waves from the P wave of the next complex. PR, QRS and QT intervals were measured, and the values for QT interval were also corrected for heart rate to give QTc by use of Bazett's formula expressed in ms as recommended recently by Molnar *et al.* (1995). The PR interval is measured from the onset of the P wave to the onset of the R wave (i.e. Q), the QRS interval is from Q to S, and the QT interval is from Q to the end of the T wave.

After all cannulations were complete, and blood gases were satisfactory ($P_{O_2} > 70$ mmHg, P_{CO_2} 25–40 mmHg), the animal was allowed ten minutes to stabilize. Anaesthetic was flushed through the cannula with heparin-treated saline. To prevent the halofantrine from precipitating out in the cannula, heparin-treated glucose solution (5% w/v D-glucose containing 10 u ml^{-1} heparin), was used to flush the drug through into the circulation. Five blood samples were taken during the course of the experiment. For each sample, approximately 0.20 ml was withdrawn via the arterial cannula and 0.18 ml transferred to an Eppendorff tube containing 0.02 ml of heparin-treated saline. The samples were frozen and stored at $-20°C$, for later analysis of halofantrine concentrations.

Vehicle control experiments were carried out in a further six male Dunkin-Hartley guinea-pigs. In these experiments blood pressure was measured with a Bell and Howell type 4-422 transducer connected to a Grass 7P1/7DA amplifier and ECG monitored with a Grass 7P4 amplifier. The amplifiers were connected to a Po-Ne-Mah computerized data acquisition system (Linton, Diss, Norfolk) and recorded at a sampling rate of 1000 Hz. The vehicle for halofantrine, dimethylacetamide (40%)/propylene glycol (60%) v/v, was diluted with 5% w/v glucose solution, to provide vehicle concentrations similar to the drug group. The vehicle was flushed through the cannula with glucose solution as described above.

Experimental protocol After stabilization, initial readings of blood pressure, heart rate and ECG were obtained. These were taken as the basal values. The lowest dose of halofantrine (0.3 mg kg^{-1}) was then administered intravenously. Halofantrine was given in a volume of 1 ml kg^{-1}. Increasing doses of halofantrine (1, 3, 10 and 30 mg kg^{-1}) were administered at 25 min intervals and 20 min after each dose an arterial blood sample (0.2 ml) was taken and stored for later analysis of halofantrine concentrations. The protocol for the control group was identical to that for the halofantrine group, except that the appropriate volume and dilution of vehicle was administered instead of halofantrine. Data were recorded continuously and subsequently retrieved from the computer at the relevant time points.

Halofantrine assay The concentration of halofantrine in whole blood was measured by reversed-phase high performance liquid chromatography according to the procedure described by Mberu *et al.*, (1992) for liquid samples. Inter- and intra-assay coefficients of variation ranged from 6.2 to 10.4%. Calibration graphs were linear to 3.7 μM. The lower limit of quantification was 0.018 μM.

In vitro *studies*

Experiments were performed with tissues from male Dunkin-Hartley guinea-pigs (285 to 665 g) obtained from Halls, Burton-on-Trent. Guinea-pigs were killed by a blow to the head followed by exsanguination. The thorax was opened and the heart removed rapidly and placed in a Krebs solution of the following composition (mM): NaCl 119, KCl 3.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, D-glucose 10 and $CaCl_2$ 1.9. The left atrium and at least one right ventricular strip and one left papillary muscle were dissected out rapidly and a thread attached to one end of the tissue. The opposite end of each preparation was impaled on one pole of a bipolar platinum electrode, which was then placed in Krebs solution in a 30 ml organ bath maintained at 37°C and gassed with 95% O_2/5% CO_2. Preparations were suspended under a resting tension of 10 mN and paced at 1 Hz with square wave pulses of 5 ms duration at twice threshold voltage, using Grass S48 or S88 stimulators. Developed tension was measured using Lectromed UF1 isometric force transducers (sensitivity range, up to 570 mN) connected to Lectromed 5240 pre-amplifiers and a Lectromed MT6 recorder. Preparations were allowed to equilibrate for 1 hour. During this time the preparations were washed 3 to 4 times and the resting tension reset if necessary.

The effective refractory period was measured by a modification of the extra stimuli method of Scholtysik (1980). At 30 s intervals a series of five extra stimuli were applied. These extra stimuli were delivered at the same pulse width, voltage and frequency as the 1 Hz pacing stimulus, but at a known delay after each normal pacing stimulus. The delay between normal and extra stimuli was increased gradually until an increase in developed tension was noted in response to the extra stimuli. The lowest delay at which this occurred was assumed to be the effective refractory period. Care was taken to check the threshold voltage at regular intervals, and to adjust the stimulation voltage if necessary, to ensure that all measurements of effective refractory period were made at twice threshold stimulation voltage.

Experimental protocol Halofantrine was dissolved and diluted in dimethylsulphoxide (DMSO) containing 0.1% Tween 80 and added to the organ bath in 300 μl aliquots so that the final concentration of this vehicle in the organ bath was always 1%. Three measurements for the control value of effective refractory period were made, after which each preparation was washed. Ten minutes later the effective refractory period was checked again and then either vehicle, or a single concentration of halofantrine (10, 30 or 100 μM) was added to the organ bath. The effective refractory period was measured at 5, 10, 20, 30, 40, 50 and 60 min after addition of vehicle or halofantrine. Only one concentration of drug (or vehicle) was added to each tissue.

Drugs/reagents

All salts for Krebs solutions were of AnalaR grade or higher and obtained from BDH, Poole or Fisons, Loughborough. DMSO was purchased from Fisons, Loughborough and Tween 80, N,N-dimethylacetamide and propylene glycol were obtained from Sigma, Poole. Heparin sodium (mucous) injection, was obtained from CP Pharmaceuticals, Wrexham. Solid (±)-halofantrine HCl (batch no.3) for *in vitro* studies and an intravenous formulation of (±)-halofantrine HCl for the *in vivo* study (formula 9, batch no. 3D01HP; a 50 mg ml^{-1} solution), were supplied by SmithKline Beecham Pharmaceuticals Ltd.

Statistics

Values are expressed as the mean ± s.e.mean or mean ± s.d. of n experiments. Shapiro-Wilk tests revealed that some data may not be distributed normally. Within group comparisons between two values were therefore made by means of two-tailed Wilcoxon's signed ranks tests whereas Friedman tests were used for within group comparisons of three or more values. Between group comparisons of two values were made with Mann-Whitney U tests and comparisons between three or

more groups were made by Kruskal Wallis tests. A probability of $P<0.05$ was considered to be significant.

Results

In vivo *studies*

Haemodynamics and ECG intervals Administration of increasing bolus doses of halofantrine caused a progressive bradycardia but did not cause any significant alterations in systolic or diastolic arterial blood pressure. The QT, QTc and PR intervals of the ECG were prolonged, but the QRS duration was not altered by halofantrine. Table 1 details the values measured 10 min after administration of each dose of halofantrine. No changes in any of these parameters occurred in a separate group of anaesthetized guinea-pigs which received equivalent volumes of vehicle at the same time points (Table 1). The greatest effects of halofantrine seemed to be on the QT interval and heart rate. Since the baseline values for QTc in the vehicle and halofantrine groups were significantly different, no comparisons between the groups were made with the raw data.

The changes in heart rate and QTc from baseline within each group were calculated and these values are illustrated in Figures 1 and 2, respectively. Statistical comparison of the values 5 min after administration of each dose of halofantrine or vehicle revealed that the change in heart rate induced by halofantrine became significantly different from that in the vehicle group after administration of 10 mg kg^{-1} halofantrine (Figure 1). However, the change in the QTc interval became significantly different from that in the vehicle group after administration of only 1 mg kg^{-1} halofantrine (Figure 2). It can also be seen that the bradycardia (Figure 1) progressed with time after administration of each of the three highest doses of halofantrine, whereas the prolongation of the QTc interval was maximal within 5 min of giving halofantrine, except after the highest dose of halofantrine (Figure 2). The concentrations of halofantrine measured in blood samples taken during the progress of these experiments are detailed in Table 2. There was a significant correlation between the change in QTc and the blood concentration of halofantrine (Figure 3).

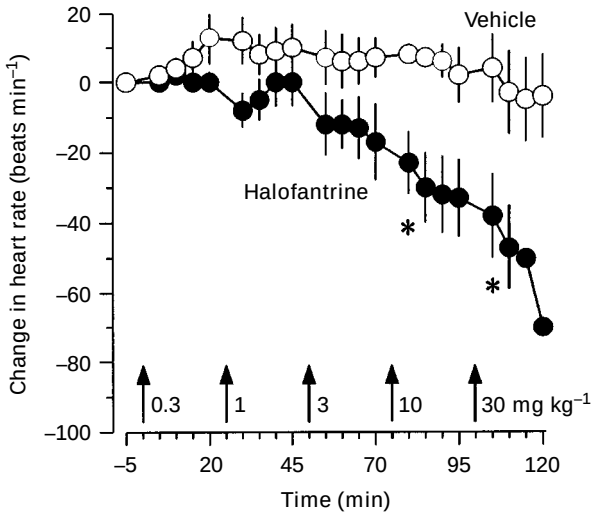

Figure 1 The change in heart rate in anaesthetized guinea-pigs which received vehicle or increasing doses of halofantrine at 25 min intervals. Each value is the mean and vertical lines show s.e.mean, $n=6$ per group. $^*P<0.05$ compared to vehicle, Kruskal-Wallis test comparing only the values measured 5 min after each dose.

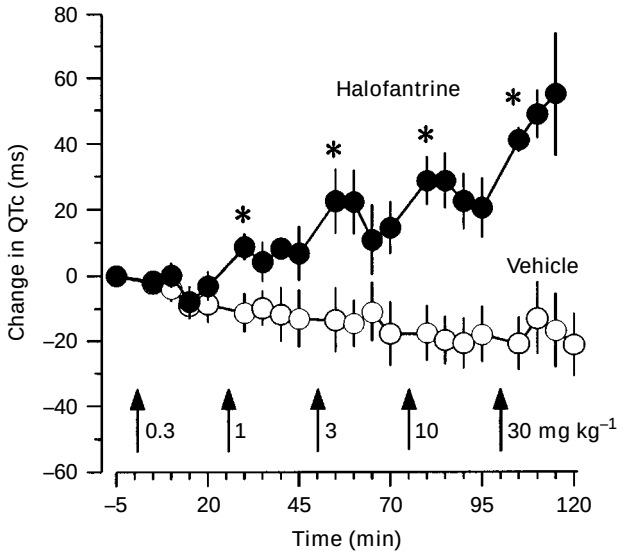

Figure 2 The change in rate corrected QT interval (QTc) in anaesthetized guinea-pigs which received vehicle or increasing doses of halofantrine at 25 min intervals. Each value is the mean and vertical lines show s.e.mean, $n = 6$ per group. $^*P<0.05$ compared to vehicle, Kruskal-Wallis test comparing only the values measured 5 min after each dose.

Table 1 Heart rate, systolic and diastolic blood pressure (BP) and ECG intervals (PR, QRS, QT and QTc) measured before and 10 min after each dose of halofantrine or vehicle in anaesthetized guinea-pigs

Time (min)	*Dose*	*Heart rate* (beats min^{-1})	*Systolic BP* (mmHg)	*Diastolic BP* (mmHg)	*PR* (ms)	*QRS* (ms)	*QT* (ms)	*QTc* (ms)
Vehicle								
0	0	277±12	59±5	42±5	60±3	34±3	152±8	324±11†
10	1 ml kg^{-1}	281±12	65±7	46±6	59±3	34±3	149±7	320±10
35	1 ml kg^{-1}	284±9	63±6	43±5	58±4	32±2	145±5	314±7
60	1 ml kg^{-1}	283±12	64±6	43±5	61±6	33±2	143±5	312±6
85	1 ml kg^{-1}	284±10	63±6	41±4	61±6	32±3	140±5	305±7
100	1 ml kg^{-1}	274±10	62±6	40±4	63±7	33±2	143±3	311±7
Halofantrine								
0	0	282±9	49±4	28±4	52±3	32±2	168±5	364±7
10	0.3 mg kg^{-1}	283±9	52±4	29±4	54±2	31±1	168±5	364±6
35	1 mg kg^{-1}	277±8	56±4	31±4	51±2	31±1	172±4	368±8
60	3 mg kg^{-1}	270±8	58±4	31±4	51±1	30±1	184±10*	387±16*
85	10 mg kg^{-1}	252±11**	59±6	31±5	54±2	30±1	194±9**	394±12**
100	30 mg kg^{-1}	235±11***	58±8	30±5	67±4**	32±2	210±12***	414±15***

Each value is the mean±s.e.mean, $n=6$ per group. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared to value at 0 min, Friedman test, †$P<0.05$ compared to halofantrine group, Mann-Whitney U test.

ECG morphology Changes in the QRS complex were evident at 10 mg kg^{-1} in most animals, and in all after 30 mg kg^{-1} halofantrine. In some animals the lengthening of the QT interval resulted in merging of the T wave with the subsequent P wave, which made it difficult to measure PR interval accurately at some time points. All animals had some form of atrioventricular (AV) block after administration of the highest dose of halofantrine. Sample traces from one guinea-pig illustrating changes in ECG morphology and the development of second degree type I Mobitz AV block are shown in Figure 4. The major point to note in Figure 4d is the lengthening PR interval, followed by a missed beat, after this point the PR value drops again, and the cycle begins anew. None of the animals exhibited 'torsade de pointes' or any other form of ventricular tachycardia. It should be noted that for both haemodynamic and ECG data, the effects of halofantrine at 30 mg kg^{-1} were such that values could not be obtained accurately for all animals at the later time points. No changes in ECG morphology were observed in the vehicle group and none of these animals had AV block.

In vitro *studies*

In isolated left atria, vehicle or halofantrine had no significant effect on effective refractory period measured at intervals throughout the 60 min contact period (Figure 5a). In isolated right ventricular strips and left papillary muscles, there appeared to be a time-dependent increase in effective refractory period in both vehicle and halofantrine-treated groups (Figure 5b,c). When statistical comparisons were made within groups, between the effective refractory period values measured 60 min after addition of drug or vehicle and those obtained before treatment, the two higher concentrations of halofantrine (30 and 100 μM) caused significant prolongation of the effective refractory period in right ventricular strips. However, the ve-

Table 2 Concentrations of halofantrine measured in whole blood sampled 20 min after administration of each dose in anaesthetized guinea-pigs

Dose (mg kg^{-1})	*Halofantrine* (μM)
0.3	0.26±0.17
1	0.28±0.18
3	0.47±0.35
10	0.79±0.27
30	2.57±0.87

Each value is the mean±s.d. of $n=5$ or 6.

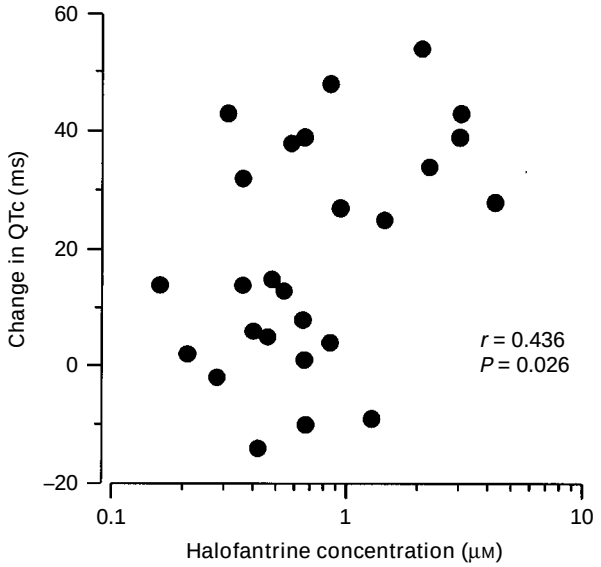

Figure 3 The correlation between the change in the QTc interval measured 5 min after administration of each dose of halofantrine (0.3, 1, 3, 10 and 30 mg kg^{-1}) and the concentrations of halofantrine measured in blood sampled 20 min after each dose in anaesthetized guinea-pigs.

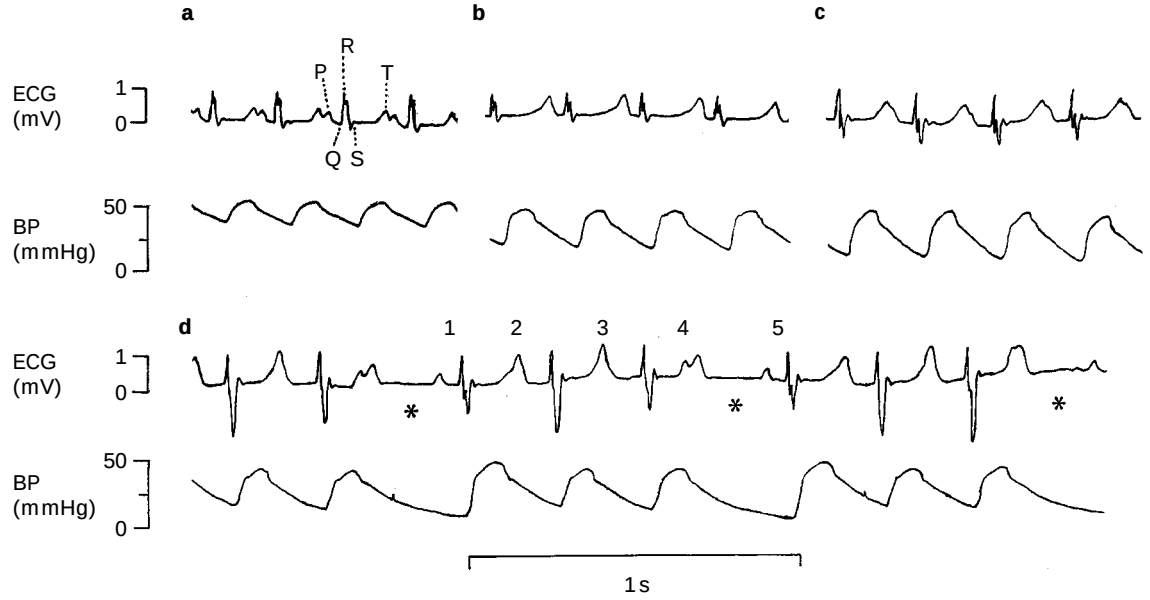

Figure 4 Sample traces illustrating the effects of halofantrine on the ECG and arterial blood pressure (BP). (a) Pre-drug, (b) 10 min after 10 mg kg^{-1}, (c) 10 min after 30 mg kg^{-1} and (d) 18 min after 30 mg kg^{-1}. The P wave, QRS complex and T wave are indicated on one cardiac cycle in (a). In (b) and (c), the P waves occur within the T wave of the previous complex and are masked by them. As the experiment progressed the QRS complex developed a larger downward component. In (d) there is second degree Mobitz type I AV block which is characterized by progressive lengthening of the PR interval until there is a missed beat (QRS complex), after which the PR interval returned to its shortest value and the cycle repeats. An asterisk indicates a missing QRS complex. Immediately after the first missed beat the PR interval was shortest (1). The next two P waves (2) and (3) were masked by the T waves of the preceding beats, but the interval from the end of the T wave to Q increased, indicating a lengthening of the PR interval. The next P wave (4) occurred just before the T wave of the previous complex leading to the next missed beat. The PR interval (5) following this missed beat was short.

hicle also prolonged the effective refractory period in these preparations (Figure 5b). Similar results were obtained in left papillary muscles, where all three concentrations of halofantrine and the vehicle significantly prolonged the effective refractory period after 60 min contact (Figure 5c). When comparisons were made between groups (i.e. between vehicle, 10, 30 or 100 μM halofantrine groups) at either 0 min or at 60 min there were no significant differences in left atria, right ventricular strips or left papillary muscles. Thus, when compared with the relevant time-dependent vehicle control, none of the concentrations of halofantrine altered the effective refractory period significantly.

Discussion

The results of these studies demonstrate that halofantrine does prolong the QT interval in anaesthetized guinea-pigs but that any effects that halofantrine may have had on the effective refractory period of isolated cardiac muscle *in vitro* could not be separated from those of the vehicle. One of the main problems encountered in studying the effects of halofantrine is its insolubility in water. The compound is extremely lipophilic (Milton *et al*., 1989; Humberstone *et al*., 1995) and although it will dissolve in several organic solvents, including those used in the formulation for parenteral use, it will not remain in solution when these are diluted with physiological salt solutions such as Krebs solution (AJ Batey & DA Hughes, unpublished observations). To overcome this problem a modified vehicle (0.1% Tween 80 in DMSO) for stock solutions of halofantrine was used for the *in vitro* studies. However, the apparent effects of halofantrine were not concentration-dependent and were shared by the vehicle. Thus no firm conclusions can be drawn about whether or not halofantrine increased the effective refractory period in isolated ventricular preparations. However, the data do suggest that halofantrine does not have an effect greater than that of the vehicle.

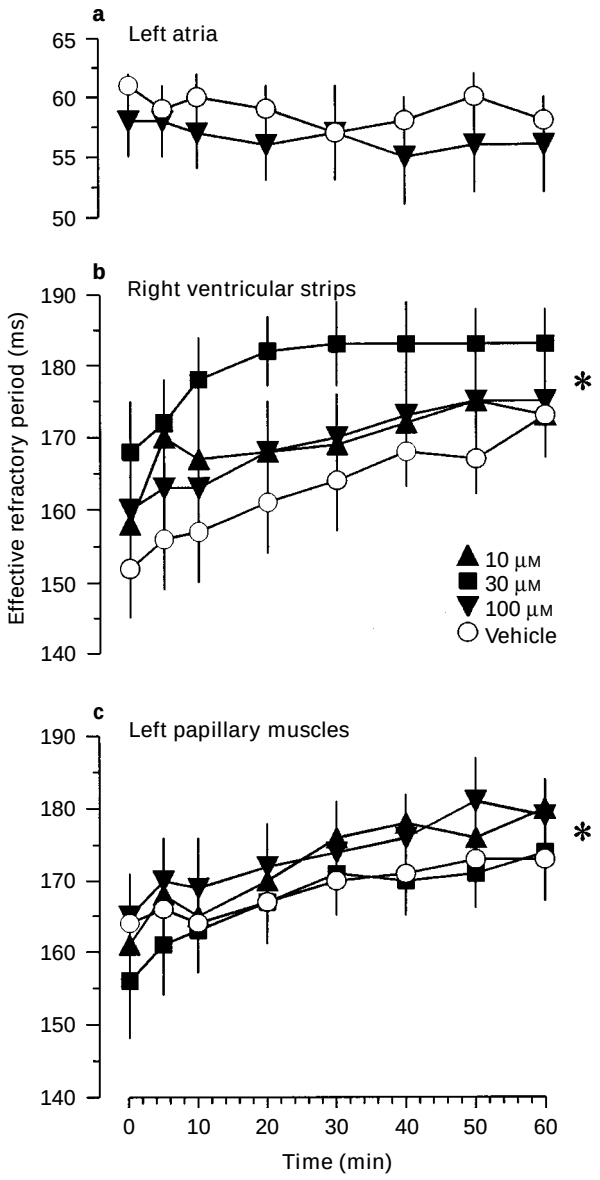

Figure 5 The effects of vehicle and halofantrine, 10 μM, 30 μM and 100 μM on effective refractory period (ms) measured in separate groups of guinea-pig isolated (a) left atria, (b) right ventricular strips and (c) left papillary muscles. Each value is the mean and vertical lines show s.e.mean, $n=6-10$ per group. *$P<0.05$ for 60 min values compared with 0 min values for all groups, except 10 μM halofantrine in the right ventricular strips, Wilcoxon test. There were no significant differences between groups at either time point, Kruskal Wallis test.

The effective refractory period only increased with time and/or vehicle in the ventricular preparations, not the atrial preparations. We have also found that varying the stimulation frequency altered the effective refractory period in ventricular preparations but not in left atria (AJ Batey & SJ Coker, unpublished observations). The effective refractory period of cardiac muscle is inherently reverse use-dependent (Baskin & Lynch, 1994), i.e. reducing the pacing frequency prolongs the effective refractory period and this is a result, at least in part, of decreased outward K^+ currents (Sheu *et al*., 1980). Drugs that reduce the delayed rectifier outward potassium current (I_k), such as Class III antiarrhythmics also display reverse-use dependence (Tande *et al*., 1990; Baskin & Lynch, 1994). The lack of reverse use-dependence in atrial preparations may be a consequence of differences in the importance of K^+ currents in atrial repolarization. Taken together, these observations suggest that the ability of time and/or the vehicle to prolong effective refractory period in the ventricular preparations may be due to blockade of an outward K^+ current such as I_k. Indeed, a paper published since we commenced our studies, shows clearly that DMSO can markedly depress I_k and that at the concentration used in our studies (1%), DMSO reduced I_k tail currents by more than 20% in guinea-pig isolated ventricular myocytes (Ogura *et al*., 1995). Thus it would seem likely that the observed increases in effective refractory period in ventricular but not atrial preparations could be due to DMSO.

In view of the difficulties in separating any potential effects of halofantrine *in vitro* from those of the vehicle, further studies were performed *in vivo* in anaesthetized guinea-pigs. These demonstrated clearly that halofantrine prolongs the rate-corrected QT interval in a dose-dependent manner. It could be argued that applying Bazett's correction for changes in heart rate is inappropriate, since this was originally devised for use in man assuming a normal rate of 60 beats min^{-1}. However, Hayes *et al*. (1994) have shown that Bazett's formula is of value in correcting QT intervals for changes in heart rate in anaesthetized guinea-pigs. Halofantrine did reduce heart rate in a dose-dependent manner, but it is clear that the QT interval was more sensitive to the effects of halofantrine than heart rate. Significant increases in QT were seen at a lower dose (3 mg kg^{-1}) than that at which heart rate was reduced significantly (10 mg kg^{-1}, see Table 1). In addition, QTc was significantly greater in the presence of 1 mg kg^{-1} halofantrine compared to the vehicle. The time courses of the changes in heart rate and QTc also differed, with the peak effect on QTc being seen within 5 min of administration, while bradycardia increased progressively (compare Figures 1 and 2). This suggests that changes in heart rate and QTc were independent events. Bradycardia has also been observed in malaria patients receiving parenteral halofantrine, although this was attributed to resolution of fever rather than a direct drug effect (Krishna *et al*., 1993).

The ability of halofantrine to prolong the QTc interval correlated with the blood concentrations, in agreement with

similar clinical observations (Nosten *et al*., 1993; Karbwang *et al*., 1993). The blood concentrations of halofantrine (0.26 to 2.79 μM, $\sim$140 to 1500 ng ml^{-1}) measured after i.v. injection of 0.3 to 30 mg kg^{-1} of drug were similar to the ranges observed after oral (Milton *et al*., 1989; Nosten *et al*., 1993; Karbwang *et al*., 1993) or i.v. (Krishna *et al*., 1993) administration of halofantrine in man. Measurement of the blood concentrations of halofantrine in the *in vivo* experiments also revealed that the concentrations examined *in vitro* reached values well in excess of those achieved *in vivo* or that could be expected in clinical use. This strengthens the above suggestion that halofantrine had little effect *in vitro*.

The AV block that occurred in all guinea-pigs after administration of the highest dose of halofantrine may be a consequence of the significant increase in PR interval at this time. Bradycardia developed progressively during the experiments, whereas the PR interval was unchanged with the lower doses of halofantrine. Prolongation of the PR interval without a concomitant increase in QRS duration, as was observed with the highest dose of halofantrine, may suggest reduced activity of the slow Ca^{2+} channels responsible for the upstroke of the action potential in nodal cells. However, this explanation is unlikely to have any bearing on the prolongation of the QTc interval which was seen with much lower doses of halofantrine. QTc prolongation and torsade de pointes are generally thought to be a consequence of enhancement of inward depolarizing Ca^{2+} or Na^+ currents or reductions of outward repolarizing K^+ currents (Tan *et al*., 1995). Again it is interesting to note the lack of change in the QRS duration with halofantrine. This suggests that the QTc prolongation was due solely to delayed repolarization and did not include a component relating to slowed conduction through the ventricles consequent to blockade of fast Na^+ channels.

Although QTc prolongation and AV block occurred in all animals, no serious arrhythmias such as torsade de pointes were observed, despite the administration of high doses of halofantrine. It is possible that if the animals had been followed for longer, the AV block may have degenerated into more serious arrhythmias. The lack of torsade de pointes in the present experiments may indicate that although QTc prolongation will occur with the drug, torsade de pointes will only be precipitated when some other aggravating factor is also present. Alternatively, as mentioned above, the increased PR interval that occurred only with the highest dose of halofantrine, suggestive of Ca^{2+} channel blockade, may indicate a self-limiting effect. Some drugs that prolong QTc, such as amiodarone, block more than one type of ion channel and rarely cause torsade de pointes (Lazzara, 1989). A novel compound, BRL 32872, which blocks both K^+ and Ca^{2+} channels has been shown recently to prolong the QTc interval but cause significantly less torsade de pointes than drugs which block K^+ channels selectively (Bril *et al*., 1996).

The ability of halofantrine to prolong QTc *in vivo* without any marked effect on effective refractory period *in vitro* suggests either, that the *in vivo* effects of halofantrine on cardiac repolarization are indirect, or that insufficient amounts of halofantrine get to the relevant site *in vitro*. Halofantrine is very lipophilic and its oral absorption is significantly enhanced by co-administration with fats (Milton *et al*., 1989). Recently, it has been shown that halofantrine binds extensively to plasma lipoproteins (Humberstone *et al*., 1995). It is possible that when added to tissues *in vitro* halofantrine does not gain access to intracellular sites because it is trapped in the lipid layers of cell membranes. However, *in vivo*, halofantrine may bind to lipoproteins and be transported into cells via their LDL receptors (Brown & Goldstein, 1986). Once inside the cells, breakdown of lipoproteins may release halofantrine to act intracellularly, e.g. by binding to the inner gates of ion channels. Thus it may be premature to conclude that the ability of halofantrine to cause QT prolongation is not due to a direct effect on cardiac muscle. Further studies are necessary to investigate these points.

In conclusion, the results detailed above indicate that the antimalarial drug halofantrine causes dose-dependent prolongation of the QTc interval in anaesthetized guinea-pigs, with a significant correlation between blood concentrations of halofantrine and the changes in QTc. Although AV block occurred with the highest dose, torsade de pointes was not seen. Due to effects of the vehicle *per se*, no firm conclusions could be drawn from *in vitro* experiments examining the possible effects of halofantrine on the effective refractory period. Further experiments are necessary to try to determine how halofantrine causes QTc prolongation and whether this presents a serious risk of precipitating torsade de pointes. In light of the difficulties encountered in finding a solvent suitable for use in *in vitro* studies, and the apparent lack of effect of halofantrine *in vitro*, it may be better to perform further studies in *in vivo* models such as the anaesthetized guinea-pig.

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