Interaction of the Antimalarial Agents Halofantrine and Lumefantrine with Lipid Bilayers

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The effects of antimalarial drugs halofantrine and lumefantrine on the fluoresence anisotropy of diphenylhexatriene (DPH)-containing phospholipid vesicles have been examined. Lumefantrine increases DPH anisotropy, indicating a condensing effect on bilayers of dipalmitoylphosphatidyl choline (DPPC), dioleoylphosphatidylcholine (DOPC), egg lecithin and mouse erythrocyte membranes (including membranes isolated from plasmodial-infected mice). Its condensing effect is more pronounced in bilayers of lower microviscosity. In contrast, increases or decreases in DPH anisotropy are observed with halofantrine, depending on the nature of the lipid. Decreases in anisotropy, which reflect a perturbing effect, are observed in bilayers of high microviscosity (for example, gel state of DPPC bilayers). Increases in anisotropy are observed in bilayers of low microviscosity (such as DOPC and egg lecithin bilayers). The perturbing effect of halofantrine is further confirmed by the increases in permeability of calcein-containing DPPC vesicles in the presence of the drug. However the perturbative effects of halofantrine are observed to the same magnitude in uninfected and plasmodial-infected erythrocyte membranes, and may not be relevant to the antimalarial action of the drug. In contrast, the condensing effect of lumefantrine is significantly greater in infected erythrocyte membranes and may contribute to its antimalarial action.

Key words antimalarial agent; fluorescence anisotropy; lipid bilayer organisation; halofantrine; lumefantrine; diphenylhexatriene

Halofantrine and lumefantrine are widely used arylmethanol antimalarial agents characterized by erratic oral absorption patterns, considerable binding to lipoproteins and poor aqueous solubility.[1—4] Both drugs are amphipathic molecules with tertiary amino functions that are protonated at physiological pH (Fig. 1).

Lipid membranes are ubiquitous in their distribution and involvement in biological phenomena. A study of drug-membrane interactions can give a better understanding of many aspects of drug action, including mode of action, distribution and accumulation in tissue compartments, toxicity and selectivity profile.[5] The antimalarial action of halofantrine and lumefantrine is targeted against the plasmodial life stages in the erythrocytes. Some arylmethanol antimalarial agents (quinine, mefloquine) have been observed to disrupt membrane trafficking events that are involved in the uptake of nutrients into the parasitized erythrocytes,[6] but it is not known if halofantrine and lumefantrine have similar actions. Quinine and mefloquine have been reported to exhibit strong interactions with dipalmitoylphosphatidyl choline (DPPC) bilayers[7,8] and it is tempting to hypothesize that their disruptive effects on phospholipid organisation may contribute to changes in the permeability and transport capability of infected erythrocytes.

Previous reports have shown that halofantrine penetrates and disrupts the organisation of phosphatidylcholine bilayers.[8—10] In one study, it was found to have a greater perturbative effect on DPPC bilayers than quinine and mefloquine.[8] Less is known of the bilayer effects of lumefantrine which shares many of the characteristics of halofantrine (lipophilicity, poor aqueous solubility, amphipathic character, erratic absorption). The present study examines the effects of halofantrine and lumefantrine on the fluidity of various phospholipid vesicles using diphenylhexatriene (DPH) as a fluorescence probe. The aim of the study is to assess the scope of their effects on lipid bilayers and to determine how these membrane effects may be related to pharmacological activity (pharmacokinetics, toxic/side effects, mode of action as antimalarial agents).

The investigations involve monitoring drug-induced changes in the fluorescence anisotropy of DPH in vesicles prepared from synthetic (DPPC, dioleoylphosphatidylcholine (DOPC)) and naturally occurring (egg lecithin, erythrocyte membrane) phospholipids. DPPC is an example of a C_{16} saturated phosphatidyl choline which is widely distributed in biological membranes. DOPC is an example of a C_{18} unsaturated phosphatidyl choline. The naturally occurring egg lecithin is composed of about equal amounts of C_{16} and C_{18}

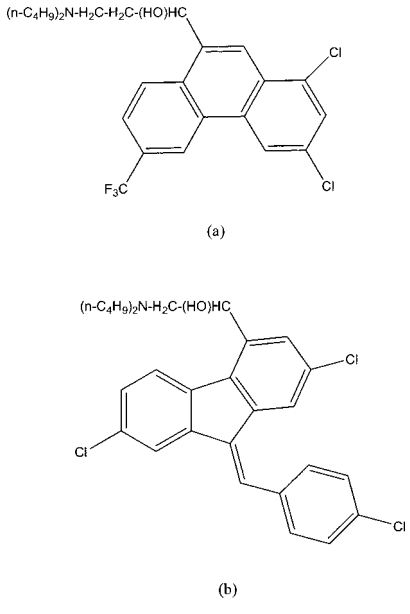

Fig. 1. Structures of (a) Halofantrine and (b) Lumefantrine

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fatty acids. There is also a higher proportion of unsaturated phospholipids in egg lecithin. Erythrocyte membranes are widely studied as models for drug-membrane interaction[11,12] because they are readily isolated and are simple compared to other cell types due to the absence of a nuclear and cytoplasmic membranes. In addition, since halofantrine and lumefantrine act primarily against the plasmodial life stages in the erythrocytes, it would be interesting to evaluate their effects on erythrocyte membranes from malarial infected mice. Uninfected erythrocyte membranes were also investigated for comparison.

The effects of lumefantrine and halofantrine on membrane permeability were also monitored by investigations on drug-induced release of calcein from DPPC vesicles.

Experimental

Materials Lumefantrine and halofantrine hydrochloride were gifts from Novartis (Basel, Switzerland) and SmithKline Beecham Pharmaceuticals (Hertfortshire, U.K.) respectively. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1,2-di[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine (DOPC) and cholesterol were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Egg phosphatidylcholine (egg lecithin) and 1,2-diphenyl-1,3,5-hexatriene (DPH) were purchased from Avanti Polar Lipid, Inc (Alabester, AL, U.S.A.) and Molecular Probes (Eugene, OR, U.S.A.) respectively. Calcein was obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Preparation of Phospholipid Vesicles Unilamellar DPPC, DOPC or egg lecithin vesicles were prepared in the following manner. An aliquot (0.5 ml) of the phospholipid in chloroform (0.5 mM) was transferred to a test tube and evaporated under a steady stream of nitrogen gas. The lipid, deposited as a thin film on the inner wall of the test tube, was protected from light and dried overnight *in vacuo*, after which phosphate buffer (5 ml, 0.1 mM, pH 7.0) was added to hydrate the lipid layer over 2 h at 25 °C. The mixture was placed in a beaker of ice and sonicated for 10 min using a probe sonicator (Heat Systems Sonicator XL, with settings of 3—4 and a cycle of "30 s-on- and 30 s-off") to produce unilamellar vesicles.

DPPC-cholesterol vesicles were similarly prepared, with aliquots of DPPC (0.5 ml of 0.5 mM stock solution) and cholesterol (25 μl of 1 mM stock solution) added together and dried down as described above. The ratio of DPPC and cholesterol was maintained at 10 : 1.

For the preparation of drug-containing phospholipid vesicles, an aliquot of the drug in chloroform (1 mM) was added to the phospholipid (or phospholipid-cholesterol) solution at the stage of drying down with nitrogen gas. Hydration and sonication were carried out as described earlier. The mole ratio of drug to phospholipid concentration varied from 0.6 : 1 to 2 : 1.

Preparation of Vesicles from Mice Erythrocytes Vesicles were prepared from membranes isolated from mice erythrocytes. The mice were either normal Swiss Albino mice (male, 30±2 g) or mice infected with *Plasmodium berghei ANKA*. Erythrocyte membranes were isolated following the method of Rock *et al.*,[13] with some modifications. Briefly, the method involved removing platelets from whole blood using adenosine diphosphate and filtration through a column of glass beads. Leucocytes were removed by filtering through a column of cellulose fibre powder (Whatman CF11). The effluent blood was washed with an equal volume of calcium–magnesium saline (15.4 mM NaCl, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, pH 7.0) by centrifugation (400 g, 10 min, 4 °C) in a Beckman Avanti J-25 centrifuge using a JA 25.50 rotor. The packed cells resuspended to the original volume with the same saline solution, were lysed by incubating (37 °C, 30 min) with an equal volume of saponin (0.2% w/v) in 15.4 mM NaCl, 5 mM $MgCl_2$. The supernatant from the lysed solution was centrifuged (20000 ***g***, 20 min, 4 °C) to obtain the erythrocyte membranes. The membranes were washed three times with calcium–magnesium saline by centrifugation (20000 ***g***, 20 min, 4 °C), and finally resuspended in a solution of 0.25 M sucrose and 5 mM $MgCl_2$ (pH 7.0) to give an approximate 5% w/v solution, and stored at 4 °C (up to 10 d) until use. Erythrocyte membranes from infected mice were similarly prepared. Blood was collected from animals that have developed 40—60% parasitemias as determined from microscopic examination of blood smears. The Stewart assay for organic phosphate[14] was used to estimate the yield of the membrane phospholipid. The phosphate content was found to be 16.8(±7) μmol per ml of the final suspension. There was no significant difference in phosphate content of membranes obtained from normal and infected blood.

For the preparation of vesicles from erythrocyte membranes, 5 ml of the suspension in sucrose/$MgCl_2$ solution, containing 84 μmol organic phosphate, was sonicated under conditions described earlier. Drug containing vesicles were prepared by drying down an aliquot of the drug in chloroform (1 mM) using nitrogen gas. To the dried film was added 5 ml of the stock solution containing the erythrocyte membrane, allowed to stand (2 h) for hydration and sonicated under conditions described previously.

Fluorescence Polarisation Measurements 1,6-Diphenylhexatriene (DPH) was used as a probe to investigate membrane fluidity of the phospholipid vesicles. A stock solution of DPH in tetrahydrofuran (1 mM) was prepared and an aliquot (10 μl) was added to 5 ml of phosphate buffer solution (0.1 mM, pH 7.0), and stirred vigorously for 15 min. Freshly sonicated phospholipid vesicles (or drug-containing phospholipid vesicles) in the same buffer solution (5 ml) was then added and the solution was left to stand in the dark for 2 h (25 °C), after which anisotropy measurements were immediately taken on a Perkin Elmer LS-50B luminescence spectrophotometer. Excitation and emission wavelengths were set at 360 and 425 nm respectively, at slit widths of 10 nm. Measurements were carried out at 25 °C and in the case of DPPC and DPPC-cholesterol vesicles, at 47 °C as well, with temperature maintained in the cuvette holder by a thermostated water bath (Gallenkamp). The final concentration of DPH is 1 μM and the ratio of DPH to phospholipid was kept at 1 : 25 for DPPC, DOPC and egg lecithin. The final concentrations of halofantrine/lumefantrine are 15, 25 and 50 μM, corresponding to mole ratios (drug : phospholipid) of 0.6, 1 and 2 respectively. At least 3 separate determinations were carried out for each concentration of drug.

Permeability Experiments DPPC vesicles containing calcein were prepared in the following way: An aliquot of DPPC in chloroform (0.3 ml of 10 mM stock solution) was dried down under nitrogen gas as described earlier. A solution of calcein in water (87.5 mM, pH 7.0) was prepared and its osmolality measured using a vapour pressure osmometer (VaproTM, Wescor). The osmolality of the calcein solution normally ranged from 292 mOsm to 319 mOsm. 3 ml of the calcein solution was added to the lipid sample, allowed to stand (2 h) and sonicated (20 min) as described earlier. Calcein-encapsulated DPPC vesicles were separated from the free (unencapsulated) calcein by gel-filtration on a Sephadex G-50 column using phosphate buffer (1 mM, pH 7.0) which had the same osmolality as the calcein solution. Upon collection of the fraction containing calcein-encapsulated DPPC vesicles from the column, experiments to monitor the release of calcein from these vesicles were carried out immediately. An aliquot (400 μl) of the calcein-containing liposomal suspension was added to the stirred contents of a cuvette containing 3 ml of the same phosphate buffer (1 mM, pH 7.0) used earlier. Fluorescence was monitored at excitation and emission wavelengths of 492 nm and 512 nm respectively, at slit widths of 5 nm. The fluorescence reading was monitored at room temperature (25 °C) over 10 min or until a steady baseline was obtained. An aliquot (200 μl) of the drug in methanol was added and the flourescence monitored for the next 50 min. No methanol-induced release of calcein was noted. However, the addition of the halofantrine (1.0, 2.0 mM) and lumefantrine (0.5 mM) to non-calcein containing DPPC vesicles gave rise to fairly consistent increases in fluorescence intensity and these were subtracted from the increases caused by drug-induced calcein release. Another control experiment that was simultaneously carried out involved the addition of an aliquot (120 μl) of Triton X-100 (25% v/v) solution to a sample of the same liposomal suspension. An increase in fluorescence was immediately observed upon addition, and this was taken to represent the maximum increase in fluorescence that could be attained from that sample. The drug-induced increase in fluorescence (due to release of calcein) was expressed as a percentage of the increase observed in the presence of Triton X, and is given by the following expression:

$$\text{drug-induced release of calcein (\%)} = [(F_t - F_o)/(F_{max} - F_o)] \times 100$$

where F_o = initial fluorescence reading, before addition of drug, F_t = fluorescence reading obtained at time t in the presence of drug, and F_{max} = fluorescence reading obtained at time t in the presence of Triton X.

The drugs halofantrine and lumefantrine were investigated over the concentration range of 0.1 to 2 mM (0.5 mM in the case of lumefantrine). Higher concentrations were not investigated as they caused turbidity on addition to the phosphate buffer solution. At least two separate determinations were carried out for each concentration of drug.

Results

The fluorescent probe DPH is widely used to monitor the

microviscosity of membrane bilayers. It is generally thought to penetrate the hydrophobic core of the bilayer. When DPH is immobilized in a viscous medium, like the highly ordered gel phase of a phospholipid bilayer, a strong polarized fluorescence signal (anisotropy) is detected. A decrease in anisotropy occurs when DPH is incorporated into the less viscous liquid crystalline state of the lipid bilayer. This can be seen from the anisotropies of DPH in DPPC vesicles at 25 °C (gel phase) and 47 °C (liquid crystalline phase) (Table 1).

Halofantrine (HF) and lumefantrine (LF) have contrasting effects on the anisotropy of DPH in DPPC vesicles. Measurements carried out at 25 °C (gel phase of DPPC) show that HF caused DPH anisotropy to decrease significantly, with a maximum decrease of 81% at 50 μM HF. LF had an opposite effect of increasing DPH anisotropy. LF-induced increases in anisotropy are not correlated to concentration.

When measurements were carried out at 47 °C (liquid crystalline phase of DPPC), only the highest concentration of HF (50 μM) caused a fall in DPH anisotropy. No significant change in anisotropy was detected at lower concentrations of the drug (15, 25 μM). In the case of LF, significantly larger increases in anisotropy were noted at 47 °C. For example, in the presence of 25 μM LF, DPH anisotropy increased by 84% at 47 °C, compared to only 13% at 25 °C.

DPH anisotropy was also measured in DPPC-cholesterol vesicles at 25 and 47 °C. As seen from Table 1, the DPH anisotropies recorded at both temperatures were not significantly different from those obtained from cholesterol-free DPPC vesicles, possibly due to the low concentration of cholesterol used in this study (2.5 μM; DPPC : cholesterol = 10 : 1) From DSC experiments (not shown here), it was noted that the transition temperature of these vesicles (38.2 °C, ±0.09, n=3) did not differ significantly from that of DPPC vesicles (39.06 °C, ±0.19, n=3). This might explain the small change in DPH anisotropy observed in this instance.

The results show that the presence of cholesterol in the DPPC vesicles caused HF (15 μM) to have an even greater perturbing effect on the mixed bilayer at both 25 °C and 47 °C. At 25 °C, a decrease of 69% is observed, compared to 29% decrease in cholesterol-free DPPC vesicles. At 47 °C, HF exerted a strong perturbing effect, unlike the insignificant change observed in cholesterol-free DPPC vesicles. LF caused increases in the DPH anisotropies of DPPC-cholesterol vesicles. A smaller increase is observed at 47 °C when compared to that observed for cholesterol-free DPPC vesicles.

DOPC has a transition temperature of −20 °C and exists in its liquid crystalline phase at 25 °C. Under these conditions, both HF and LF caused DPH anisotropy to increase, with LF causing a larger increase (91%) than HF (46%) at the same concentration (15 μM).

Egg lecithin is made up of about equal amounts of C_{16} and C_{18} fatty acids, and has a higher proportion of unsaturated fatty acids. Egg lecithin exists in the liquid crystalline state at 25 °C. Both HF and LF increased anisotropy of DPH-containing egg lecithin vesicles. The increase is again more marked for LF but are poorly correlation to concentration. A smaller increase in DPH anisotropy is observed for HF.

Erythrocyte membranes were prepared from mice blood after separation of platelets and leucocytes, and hemolysis of the erythrocytes with saponin. Membranes were collected from both uninfected mice and plasmodial-infected mice. Investigations have shown that the fluidity of erythrocyte membranes increases on plasmodial infection.[15—17] This was also observed in the present study where anisotropies recorded from vesicles prepared from infected mice were lower than that obtained from uninfected mice. LF cause an increase in DPH anisotropy when incorporated into vesicles prepared from both infected and uninfected erythrocyte membranes. These increases were concentration dependent and a larger increase (43%, 50 μM LF) was observed in vesicles prepared from infected erythrocyte membranes than from normal membranes (18%, 50 μM LF). In contrast, HF caused decreases in DPH anisotropy when incorporated into vesicles prepared from both infected and uninfected erythrocyte membranes. These decreases are similar for both types of membranes.

The effects of HF and LF on membrane permeability were investigated using calcein-containing DPPC vesicles. When encapsulated into DPPC vesicles, calcein exhibits self

Table 1. Effect of HF and LF on the DPH Anisotropy of Phospholipid Vesicles

Lipid vesicles	DPH anisotropy at 25 °C[a)]	DPH anisotropy at 47 °C[a)]
a) DPPC		
Control	0.31 (0.01)	0.12 (0.02)
+15 μM HF	0.24 (0.03)[b)] −29%	0.13 (0.01) +8%
+25 μM HF	0.19 (0.04)[b)] −39%	0.10 (0.03) −17%
+50 μM LF	0.06 (0.04)[b)] −81%	0.03 (0.01)[b)] −75%
+15 μM LF	0.35 (0.02)[b)] +13%	0.18 (0.05)[b)] +50%
+25 μM LF	0.35 (0.01)[b)] +13%	0.22 (0.04)[b)] +84%
+50 μM LF	0.36 (0.02)[b)] +16%	0.21 (0.08)[b)] +75%
b) DPPC–cholesterol[c)]		
Control	0.29 (0.02)[d)]	0.16 (0.02)[d)]
+15 μM HF	0.09 (0.02)[b)] −69%	0.04 (0.02)[b)] −75%
+15 μM LF	0.35 (0.01)[b)] +21%	0.18 (0.02)[b)] +13%
c) DOPC		
Control	0.11 (0.02)	
+15 μM HF	0.16 (0.01)[b)] +46%	
+15 μM LF	0.21 (0.04)[b)] +91%	
d) Egg lecithin		
Control	0.14 (0.02)	
+15 μM HF	0.17 (0.01)[b)] +21%	
+25 μM HF	0.19 (0.01)[b)] +36%	
+50 μM HF	0.20 (0.01)[b)] +43%	
+15 μM LF	0.25 (0.02)[b)] +79%	
+25 μM LF	0.24 (0.03)[b)] +71%	
+50 μM LF	0.24 (0.03)[b)] +71%	
e) Erythrocyte membranes		
Control	0.32 (0.01)	
+50 μM HF	0.27 (0.02)[b)] −14%	
+100 μM HF	0.25 (0.02)[b)] −21%	
+25 μM LF	0.34 (0.01)[b)] +8%	
+50 μM LF	0.37 (0.01)[b)] +18%	
f) Erythrocyte membranes (infected mice)		
Control	0.28[e)]	
+50 μM HF	0.25 (0.01)[b)] −11%	
+100 μM HF	0.22 (0.01)[b)] −21%	
+25 μM LF	0.32 (0.01)[b)] +14%	
+50 μM LF	0.40 (0.02)[b)] +43%	

a) Anisotropy readings are given as a mean of 3 readings with SD in brackets. +/− refer to the % increase or decrease in anisotropy readings compared to control. *b*) Statistically significant from control anisotropy readings ($p<0.05$), using paired *t*-test, SPSS Ver. 10.0. *c*) Ratio of DPPC to cholesterol = 10 : 1. *d*) Statistically not significant from DPPC vesicles ($p>0.05$), using independent *t*-test, SPSS Ver 10.0. *e*) Statistically significant from anisotropy readings obtained from uninfected erythrocyte membranes ($p<0.05$), using independent *t*-test, SPSS Ver 10.0.

Table 2. Halofantrine and Lumefantrine-Induced Calcein Release from DPPC Vesicles at 25 °C

Compound	% Release of calcein[a)]
Halofantrine	
0.1 mM	5.0 (2.3)
0.5 mM	11.6 (2.5)
1.0 mM	19.8 (0.5)
2.0 mM	20.5 (1.1)
Lumefantrine	
0.1 mM	—[b)]
0.25 mM	6.1 (1.1)
0.5 mM	8.0 (2.7)[c)]

a) Release of calcein (%)=$[(F_t-F_o)/(F_{max}-F_o)]\times100$ where F_o=initial fluorescence reading, before addition of drug, F_t=fluorescence reading obtained at time t in the presence of drug, and F_{max}=fluorescence reading obtained at time t in the presence of Triton X. Readings represent the mean of 2 different experiments. *b*) Baseline fluorescence decreased on addition of drug. *c*) Statistically not significant from release observed at 0.5 mM HF ($p>0.05$), independent t-test, SPSS Ver. 10.0.

quenching of its fluorescence. However, when released from the vesicles, its fluorescence increases. Table 2 tabulates the effect of HF and LF on the release of calcein from DPPC vesicles, expressed as a percentage of the maximum amount released when a detergent Triton X is added to the vesicles. HF enhances the release of calcein (up to 20%, Fig. 2a) over the concentration range of 0.1 mM to 1.0 mM. A higher concentration (2 mM) of HF did not result in greater calcein release. In the case of LF, only a small proportion of calcein (8%) was released at the highest concentration investigated (0.5 mM, Fig. 2b). Concentrations greater than 0.5 mM LF caused turbidity and were not be investigated.

Discussion

The effects of HF and LF on DPH anisotropy vary according to the nature of the phospholipid vesicles investigated. In the case of DPPC vesicles, the decrease in DPH anisotropy observed in the presence of HF suggests that the drug disrupts the arrangement of the phospholipid bilayer. This disruption is greater when the phospholipid is in the organised gel phase (at 25 °C) than in the randomly arranged liquid crystalline phase (at 47 °C). In contrast, LF increases DPH anisotropy at both temperatures, suggesting that the presence of the drug caused the lipid bilayers to become more compact and condensed. Larger increases were observed at 47 °C, when the lipid is in its liquid crystalline phase. This suggests that the condensing effect of LF is more pronounced when it is in an environment of lower microviscosity.

The results from the calcein permeability experiments lend further support to the perturbing effects of HF. HF enhanced the permeability of DPPC vesicles and a maximum of 20% of the encapsulated calcein is released by 1.0 mM HF. It should be noted that the concentrations of HF (0.1—1.0 mM) required for calcein release are at least 10 fold higher than the concentrations of HF (15—50 μM) required for decreasing DPH anisotropy. The effect of LF on the permeability of calcein-containing DPPC vesicles stands in contrast to that of HF. No calcein release was detected at low concentrations (0.1 mM) and only a small increase (8%) was observed with 0.5 mM LF, the highest concentration used due to the low solubility of LF.

The anisotropy of DPH was also measured in vesicles containing DPPC and cholesterol (10 : 1). Cholesterol is a major component of most lipid membranes. In human erythrocytes, the proportion of cholesterol is estimated to be as high as 20% of total lipids.[18] The effect of cholesterol on bilayers depends on whether it is incorporated into the gel state or liquid crystalline state of the lipid. In the liquid crystalline state of the phospholipid bilayer, cholesterol condenses the bilayer and decreases fluidity/permeability. The opposite effect is noted when cholesterol is incorporated into the gel state of a phospholipid. Thus, the incorporation of cholesterol into DPPC vesicles would modify the bilayer arrangement so that it is less ordered at 25 °C and more ordered at 47 °C. This is observed to be so, even at the low concentration of cholesterol used in this investigation. If the condensing effect of LF is more pronounced in an environment of lower microviscosity as proposed earlier, one would expect its condensing effect to be greater at 25 °C in cholesterol-containing DPPC vesicles compared to cholesterol-free DPPC vesicles. This is because at 25 °C, DPPC exists in its gel state and cholesterol would increase the fluidity of the organised gel state. This is indeed observed. At 25 °C, LF (15 μM) increased DPH anisotropy by 21% in DPPC-cholesterol vesicles and by 13% in DPPC vesicles. The corresponding increases at 47 °C (when the lipid exists in the liquid crystalline state) are 13% in DPPC-cholesterol vesicles and 50% in DPPC vesicles.

In the case of HF, its perturbing effects are generally greater in the more organised gel phase of DPPC, although at the highest concentration of HF investigated (50 μM), comparable perturbing effects are noted at 25 °C and 47 °C. The inclusion of cholesterol in DPPC vesicles should lessen the perturbing effects of HF at 25 °C and enhanced its perturbing effects at 47 °C. However, greater perturbing effects are observed at both temperatures.

Unlike DPPC, DOPC is an unsaturated phospholipid with *cis* (Δ9) double bonds on both C_{18} alkyl chains. This causes DOPC molecules to be less cohesive and DOPC exists in its liquid crystalline phase at room temperature conditions. Both LF and HF increase the anisotropy of DOPC vesicles containing DPH. The LF-induced increase is to be expected, based on its effects on DPPC vesicles in its liquid crystalline state. However, the HF-induced increase in DPH anisotropy of DOPC vesicles has not been observed before. HF may have dual effects of perturbing and condensing bilayers depending on the nature (saturated *versus* unsaturated) of the phospholipid.

Egg lecithin contains both unsaturated and saturated phospholipids, with a greater proportion of the former (4 : 1). An increase in DPH anisotropy is again observed in the presence of HF and LF. The apparent condensing effects of HF and LF observed in this instance mirror the observations made earlier with vesicles prepared from the unsaturated phospholipid DOPC.

Erythrocyte membranes are widely used in studies of drug–membrane interaction.[19,20] Since both halofantrine and lumefantrine act as antimalarial agents by targeting the life-cycle of the parasites in the erythrocytes, it is particularly relevant to examine how these drugs influence the fluidity of erythrocyte membranes. Erythrocyte membranes contain phosphatidylcholine, phosphatidylethanolamine and cholesterol as the major lipid components on a weight basis.[18] Plasmodial infection is known to cause greater fluidity and diminished microviscosity of the erythrocyte membranes due

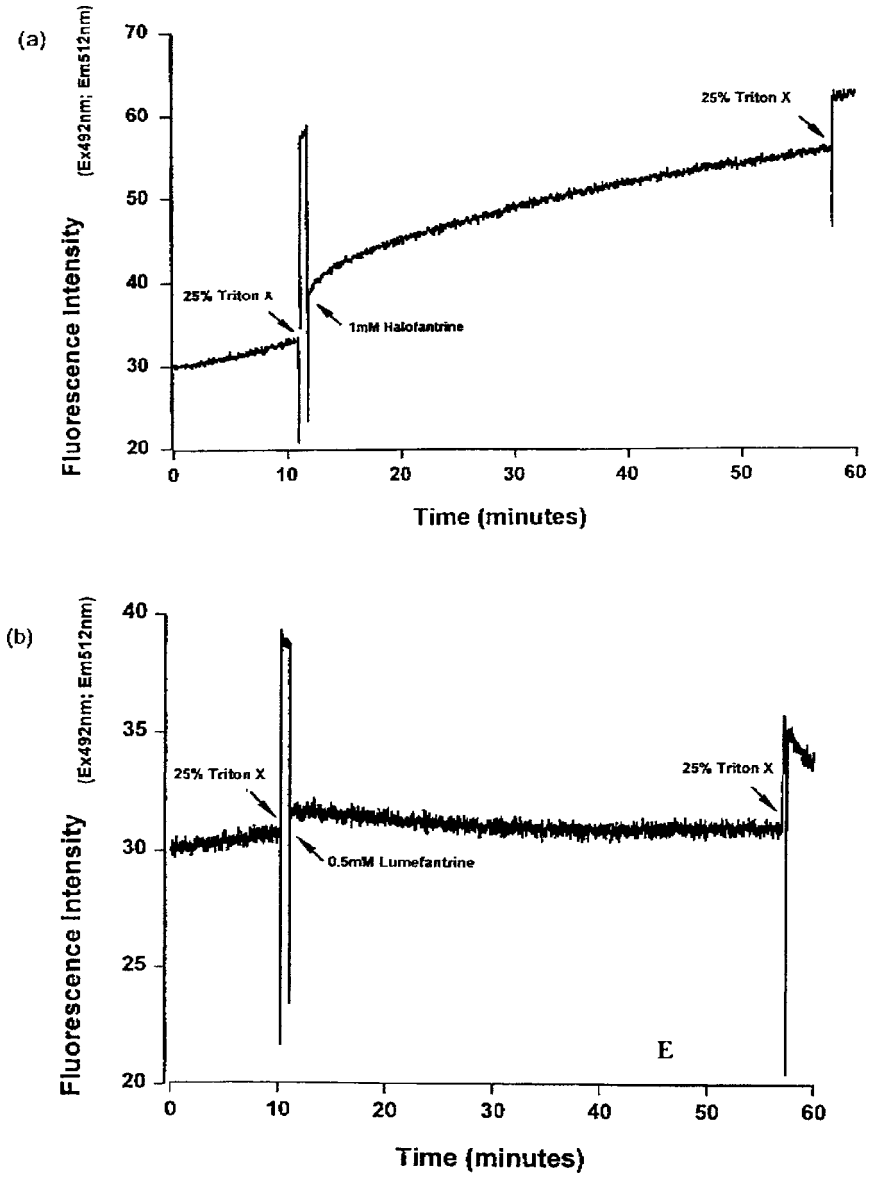

Fig. 2. (a) Increase in Fluorescence Intensity Due to Release of Calcein on Addition of 1 mM Halofantrine to the Stirred Contents of Calcein-Containing DPPC Vesicles in Phosphate Buffer, pH 7.0, 25 °C

In this experiment, 2 cuvettes of similar contents are taken and to one is added Triton X, and to the other 1 mM halofantrine. The baseline fluorescence of both cuvettes are similar as they came from the same preparation of vesicles. The strong increase in fluorescence on addition of Triton X is shown here as an overlapping plot of the slower increase caused by halofantrine addition. Near the end of the experiment, Triton X is added to the cuvette to which halofantrine was added. The increase in fluorescence signal is due to the release of the remaining calcein.

(b) Increase in Fluorescence Intensity Due to the Release of Calcein on Addition of 0.5 mM Lumefantrine to the Stirred Contents of Calcein-Containing DPPC Vesicles in Phosphate Buffer, pH 7.0, 25 °C

The description given in (a) also applies.

to changes in the membrane composition and/or organization.[15—17] There are suggestions that the greater fluidity may be due to the depletion of cholesterol from the membrane of infected erythrocytes.[16]

Vesicles prepared from egg lecithin and erythrocytes are often used to represent biological membranes. The presence of cholesterol in erythrocyte membranes would mean that there is more rigidity in these bilayers compared to egg lecithin bilayers. Under these circumstances, one would expect LF to have a greater stabilizing effect on the membrane with lesser microviscosity (*i.e.* egg lecithin). This is found to be so, although other factors (like phospholipid composition, presence of proteins) may account for this observation.

The perturbing effect of HF is generally more pronounced in an environment of greater microviscosity, as exemplified by gel phase DPPC. In environments of lower microviscosity, such as DOPC and egg lecithin vesicles, the perturbing effect of HF is not observed and a condensing effect becomes evident.

In view of reports indicating that erythrocyte membranes become more fluid on infection, the expected responses of HF and LF on such vesicles should be a lesser perturbing effect for HF and a greater condensing effect for LF. This was not observed for HF, which gave comparable perturbing ef-

fects on both types of membranes. However, LF did have a greater condensing effect on the infected membranes.

Conclusion

In conclusion, this investigation has shown that LF and HF have contrasting effects on lipid bilayers. These differences may arise from the steric characteristics of HF and LF, which would influence their insertion into the lipid bilayers. LF has a fluorene ring linked *via* a double bond to a phenyl moiety. This would make the LF more bulky than HF which has a compact phenanthrene ring.

LF has a predominant condensing effect that is more pronounced in bilayers of low microviscosity. In contrast, HF has a predominant perturbing effect on bilayers and its action is generally more pronounced in bilayers of higher microviscosity. LF has a significantly greater condensing effect on membrane vesicles prepared from infected erythrocytes. In contrast, the perturbing effects of HF are largely the same when investigated on infected and uninfected erythrocytes. This would suggest that the perturbing effects of HF are not selective for infected erythrocytes but the condensing effect of LF may be relevant in altering erythrocyte permeability pathways. The significance of these lipid interactions on the antimalarial activity of HF and LF remains unclear. However, the occurrence of these interactions should be considered when evaluating the pharmacokinetic and toxicological effects of these drugs.

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