Halofantrine–Phospholipid Interactions: Monolayer Studies

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The antimalarial agent halofantrine penetrates dipalmitolylphosphatidylcholine (DPPC) monolayers resulting in an increase in surface pressure and an expansion in area occupied by the lipid components of the monolayer. This phenomenon is observed at concentrations (0.05—0.2 μM) of halofantrine that have no surface activity. Penetration increases with drug concentration and is greatest at low initial surface pressures of the monolayer. A critical surface pressure of the DPPC monolayer has been determined from constant area and constant pressure conditions. The magnitude of these values support the hypothesis that halofantrine readily penetrates the DPPC monolayers. The presence of cholesterol in the DPPC monolayer hampers penetration and a lower critical surface pressure is obtained under such conditions. Even then, a slower rate of penetration is observed only in monolayers maintained at high initial surface pressures (10, 15 mN/m), corresponding to the liquid condensed phase of the monolayer, and not at low surface pressures (2.5, 5.0 mN/m). These results help to give a better understanding of the dynamics of the halofantrine–phospholipid interaction as well as the pharmacodynamic character of the drug.

Key words halofantrine; dipalmitolylphosphatidylcholine monolayer; critical surface pressure

The antimalarial agent halofantrine (HF) (Fig. 1) is highly effective against multidrug resistant strains of the malarial parasite.[1] A serious shortcoming of the drug is its poor and variable absorption after oral administration which compromises the clinical efficacy of the drug and predispose it to the emergence of resistant strains of the parasite.[2] More recently, lipid based solid dispersions and lipidic self-emulsifying formulations of HF have been investigated to solve these problems.[3,4] Because of its lipophilic character (calculated log $p>8.0$), HF has a strong affinity for lipids and is often cited as a representative lipophilic drug that binds significantly to plasma lipoproteins.[5] As lipoprotein receptors are widely distributed throughout the body, drugs bound to lipoproteins may be more accessible to cellular transportation and metabolic pathways than the unbound drug. This may be the reason why HF is more readily taken up into *Plasmodium falciparum* infected erythrocytes as compared to uninfected erythrocytes.[6] High density lipoproteins (HDL) are taken up by the parasites as an essential source of nutrition. Not unexpectedly, HF associated with HDL particles is advantageously concentrated in infected erythrocytes as well.

The molecular basis of the interaction of HF with lipids is still unknown. Calorimetric investigations have revealed that the drug caused significant perturbation of dipalmitolylphosphatidylcholine (DPPC) bilayers. This is seen from the disappearance of the transition endotherm of DPPC in the prescence of HF,[7] and has been further confirmed by fluorescence polarisation experiments using pyrene and diphenylhexatriene as probe molecules.[8] The current understanding is that HF inserts itself into the hydrocarbon interior of the phospholipid bilayer and causes considerable disorganisation of the bilayer arrangement.

In order to gain more insight into the physicochemical nature of the interaction, we have carried out investigations on the interaction of HF with DPPC monolayers spread out on a Langmuir–Blodgett trough. Monolayers are a convenient experimental model system for the study of drug–lipid interactions under controlled conditions. The phospholipid monolayer may be considered as constituting one-half of the bilayer and is therefore a good mimic of the cell membrane model.[9,10] Liposomal bilayers are also important carriers for drug delivery systems, and an understanding of how a drug interacts with a monolayer may be usefully extrapolated to predict its penetration and stability within the liposomal bilayer.

DPPC monolayers are used in the present investigation as phosphatidylcholines are one of the major lipids found in mammalian plasma membranes. In the erythrocyte membrane, phosphatidylcholines are found in the outer layers of the membrane while phosphatidylethanolamines, another important phospholipid component, are concentrated in the inner layers. The distribution of these two neutral phospholipids is important for maintaining the integrity of the red cell membrane.[11] In addition, our earlier investigations on HF have been carried out using DPPC bilayers, and continuing the investigations with DPPC monolayers would facilitate the interpretation of the results.[7,8]

Experimental

Materials 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) and cholesterol were purchased from Sigma (St. Louis, MO, U.S.A.). HF hydrochloride was a gift from SmithKline Beecham (Hertfordshire, U.K.). Analytical grade chloroform and methanol (BDH), free from surface active impurities, were used without purification. MOPS buffer (pH 7.4) was prepared from 3-(*N*-morpholino)propanesulfonic acid (SigmaUltra) and double distilled water.

Monolayer Experiments Studies on monolayers were investigated on a computer-controlled Langmuir film balance (NIMA Langmuir–Blodgett

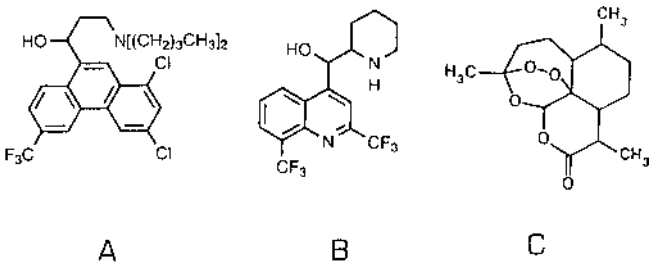

Fig. 1. Structural Formulae of Halofantrine (A), Mefloquine (B) and Artemisinin (C)

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trough, Model 601M). The trough (15×7×0.5 cm) is mounted on an aluminium base plate with built-in water channels for temperature control *via* an external circulator. It is placed on a vibration free table and enclosed in an environmental chamber. The trough is thoroughly washed with chloroform and rinsed with distilled water before the start of the experiment. It is then filled with the aqueous phase (MOPS buffer, 10 mM, pH 7.4). The contents of the trough is gently agitated using a magnetic stirrer bar placed in a cavity in the base of the trough. The cleanliness of the trough and its contents was ensured before each run by cycling the full range of trough area and aspirating the air–water surface while at the minimal surface area. The air–water interface was repeatedly compressed and expanded at a desired rate by controlling the movement of the 2 mechanical barriers. When the surface pressure fluctuation was less than ±0.2 mN/m during the compression of the entire surface pressure range, an aliquot of the sample (chloroform or methanolic solution of DPPC or HF at 0.2 mM) was spread on the surface of the aqueous phase with a Hamilton microsyringe and after a period of 15 min, the experiment was started. The compression rate was kept at 1 Å^2 molecule^{-1} min^{-1}. In this way, measurements of the surface pressure (π)–area isotherms for the spread films of DPPC or HF were made.

In another set of experiments to investigate the effect of time on the surface pressure of various concentrations of HF (0.1—1.0 μM), the adsorption kinetics of HF to the air–water interface was investigated by injecting an aliquot of a methanolic solution of HF (0.1—1.0 μM) into the buffered aqueous phase and following the increase in surface pressure over 30 min.

The adsorption kinetics of HF was also investigated in the presence of a DPPC monolayer in the following way: An aliquot of a solution of DPPC in chloroform (0.2 mM) was gently deposited as a monolayer at the air–water interface as described earlier. The surface pressure was adjusted to the desired value (ranging from 2.5 to 15 mN/m) and kept constant for 15 min before addition of HF (0.05—1.0 μM) into the aqueous phase. The adsorption kinetics of HF in the presence of a DPPC monolayer was monitored over a period of 30 min either as an increase in surface pressure (with the mechanical barriers held in fixed positions so that the area of the film is maintained constant) or as an increase in surface area (with the mechanical barriers allowed to move while maintaining a constant surface pressure). Similar experiments were carried out using a monolayer consisting of equimolar concentrations of DPPC and cholesterol (each at 0.1 mM). At least 2 determinations were made for each reading. The temperature of the experiments was kept constant at 28 °C (room temperature).

Measurement of Surface Tension of HF The surface tension of a saturated solution of HF in MOPS buffer (10 mM, pH 7.4) was measured in the following way: MOPS buffer (5 ml) was added to a known weight of HF hydrochloride (2 mg) in a test tube and shaken on a Heidolph Vibramax 110 Shaker overnight at room temperature. The undissolved material was removed by filtration and the surface tension of the saturated solution measured using a torsion balance, with correction made for the surface tension of the medium.

Results and Discussion

The surface pressures of HF and DPPC were monitored as a function of the area occupied by one molecule of the film-forming component (HF or DPPC) (Fig. 2). The π–area isotherm of DPPC is characterized by the horizontal "gas" phase, the slowly rising "liquid" phase (surface pressures between 0 to 30 mN/m), and the steeply rising "solid" phase (surface pressure >30 mN/m), a pattern which is in good agreement with reported literature.[12] In comparison, the π–area isotherm of HF lies below that of DPPC and has a gentler gradient compared to DPPC. Compression of the HF monolayer (down to 25 Å^2) resulted in only a small increase in surface pressure, implying that the monolayer is readily compressible and flexible. Based on the definition of surface pressure as the difference in surface tensions of the clean air–water interface and a surface covered with the monolayer (Eq. 1),[13—15] it can be deduced that the presence of a HF monolayer has caused only a small reduction in the surface tension, and hence a small increase in surface pressure. This would be the case if HF molecules were poorly aligned at the air–water interface, thus expanding the interface to a limited

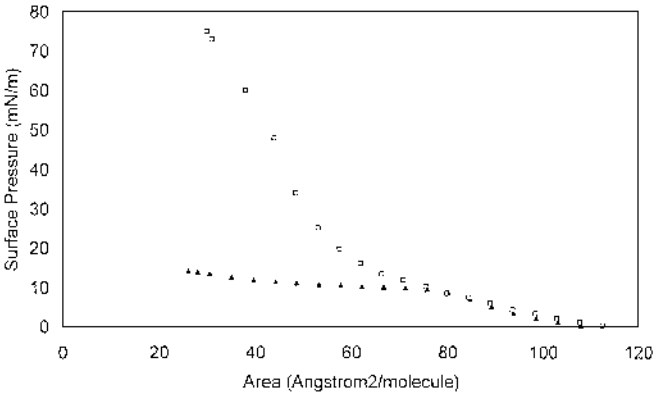

Fig. 2. Surface Pressure–Area Isotherms of DPPC (□) and HF (▲)

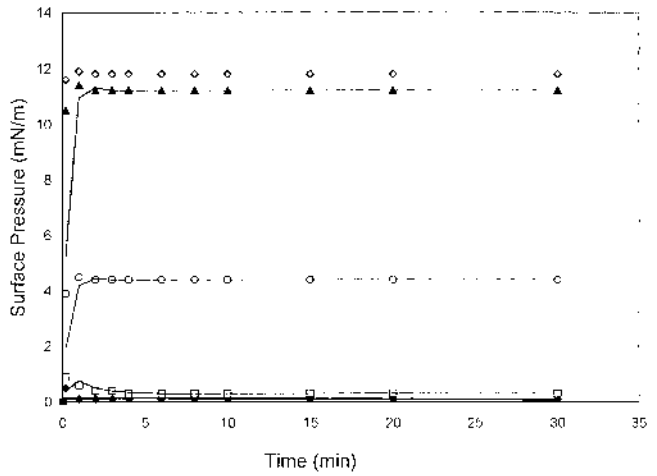

Fig. 3. Change in Surface Pressure of HF with Time
Concentrations of HF: 0.1 μM (◆), 0.2 μM (□), 0.25 μM (○), 0.3 μM (▲), 1.0 μM (◇).

degree.

$$\pi = \sigma_o - \sigma \quad (1)$$

where π is the surface pressure, σ_o is the surface tension of the clean air–water interface (72 mN/m) and σ is the surface tension of the monolayer.

Figure 3 shows the surface pressures of various concentrations of HF (0.1—1 μM) over time. The results show that the adsorption of HF at the air–water interface was only significant at higher concentrations (≥0.3 μM) of the drug and an equilibrium level of surface activity was attained quickly (within 5 min).

An important factor to consider in these experiments is the solubility of HF in the MOPS buffer (pH 7.4). This was not determined in the present investigation but in an earlier study, the solubility of HF in phosphate buffer (pH 7.4) was found to be low (0.102 μM, 28 °C).[16] It was also found that the solubility of HF increases as the ionic strength of the buffer was lowered. Since the MOPS buffer employed in this study has a lower ionic strength than the phosphate buffer used in the solubility determination (0.01 *vs.* 0.08), HF may have a greater solubility (>0.1 μM) in MOPS buffer. This was indirectly confirmed when a separate experiment was conducted to determine the surface tension of a saturated solution of HF in MOPS buffer (pH 7.4, 10 mM). After correc-

Table 1. Increase in Surface Pressure ($\Delta\pi$) of Monolayers (DPPC or DPPC–Cholesterol) Maintained at Various Initial Surface Pressures in the Presence of HF

HF (μM)	Initial surface pressure of monolayer (mN/m)[a]										
	2.5		5.0		10.0		15.0		20.0	25.0	30.0
	DPPC	DPPC +CHE	DPPC	DPPC +CHE	DPPC	DPPC +CHE	DPPC	DPPC +CHE	DPPC	DPPC	DPPC
0.05	4.1 (0.3)	11.0 (0.4)	5.3 (0.4)	9.3 (0.5)	7.6 (0.5)	6.8 (0.3)	11.6 (0.3)	4.2 (0.3)	10.8 (0.2)	9.5 (0.2)	6.2 (0.3)
0.10	10.7 (0.6)	13.9 (0.7)	12.0 (0.5)	11.7 (0.7)	11.7 (0.5)	8.1 (0.8)	11.2 (0.7)	4.3 (0.3)	10.8 (0.8)	9.6 (0.3)	6.5 (0.3)
0.15	11.6 (0.4)	13.5 (0.3)	12.5 (0.3)	12.9 (0.1)	16.6 (0.4)	9.8 (0.6)	14.2 (0.1)	8.2 (0.5)	11.3 (0.2)	9.5 (0.3)	6.2 (0.5)
0.20	13.9 (0.4)	14.8 (1.3)	12.9 (0.6)	11.0 (0.8)	11.1 (0.4)	8.0 (0.1)	8.9 (0.5)	3.0 (0.3)			
1.0	18.43 (0.1)		16.6 (0.3)		14.53 (1.1)		11.9 (2.0)				

a) Values in parentheses represent SD for 3 determinations. CHE; cholesterol.

tion for the MOPS buffer, a value of 13.6 mN/m was obtained, which is close to the surface pressures recorded for HF concentrations of 0.3 μM (11.2 mN/m) and 1.0 μM (11.8 mN/m) on the Langmuir–Blodgett trough (Fig. 2). Thus although no accurate determination of the solubility of HF in MOPS buffer was made, it is estimated that its solubility falls in the range of 0.3 to 1 μM.

HF has a tertiary amino function in its side chain, the pK_a of which is estimated to be 10.8, after related compounds listed in the literature.[17] Therefore, at the pH of the monolayer experiments (pH 7.4), the protonated form of HF would predominate (*ca.* 99%). The combined presence of a hydrophobic phenanthrene ring and a protonated side chain in HF would classify it as an amphiphilic molecule, which is normally associated with good surface activity. Contrary to such expectations, HF is seen to have a weak tendency to penetrate or adsorb on to the air–water interface. Stereochemical factors may account for this: HF has a bulky hydrophobic phenanthrene ring and a flexible protonated side chain. Orientation of these molecules at the interface to give a compressible monolayer may be difficult to achieve.

The surface pressure of a DPPC monolayer is increased upon addition of HF. This can be seen from Table 1 which tabulates the increases in surface pressure ($\Delta\pi$) of DPPC monolayers maintained at initial pressures of 2.5—30 mN/m in the presence of different concentrations of HF. Two trends can be seen from this data: at low concentrations of HF (0.05, 0.1, 0.15 μM), a biphasic response was observed, *viz.* $\Delta\pi$ increased from 2.5 mN/m to about 10—15 mN/m, followed subsequently by a decline at higher surface pressures (>15 mN/m). At higher concentrations of HF (0.2, 1.0 μM), $\Delta\pi$ decreased linearly from 2.5 to 15 mN/m.

One factor that needs to be considered when interpreting the results is the solubility of HF in the aqueous phase. As stated earlier, HF is poorly soluble in the aqueous subphase, with an estimated solubility range of 0.3—1 μM. When HF is added to the aqueous phase, it will partition between the aqueous subphase and the phospholipid monolayer, so that the concentration of HF attained in the monolayer, after the equilibration period of 30 min, may be less than what was injected into the aqueous phase. The underlying assumption that is made here is that the concentration of HF in the aqueous phase is constant during the course of the experiment. This is reasonable because HF has a poor solubility in the aqueous phase (estimated at 0.3—1 μM) and several concentrations of HF (0.05—0.2 μM) used in the experiments are below the HF solubility limit. In addition, measurements were made after a reasonable period (30 min), thus permitting equilibrium between the aqueous phase and monolayer to be established.

Considering that low concentrations of HF (0.05—0.2 μM) are not surface active (Fig. 3), the increases in the surface pressures of the DPPC monolayer on addition of HF must be due to the penetration of HF into the monolayer. This would lead to a fall in the surface tension at the lipid/air interface, leading to an increase in surface pressure. In the case of 1.0 μM HF which has intrinsic surface activity, an overall increase in surface pressure is still observed after correction for the initial surface activity of HF. Another noteworthy feature is that DPPC monolayers maintained at high surface pressures are generally less readily penetrated by HF (small $\Delta\pi$) compared to monolayers maintained at low surface pressures (large $\Delta\pi$). At high surface pressures, the monolayer is closely packed and condensed and entry by external agents would be more difficult. Part of Table 1 is depicted in Fig. 4 (*viz.* HF concentrations of 0.05 and 1.0 μM).

Similar experiments were also carried out with other antimalarial agents in order to establish if this effect of HF is unique to itself or a general characteristic of antimalarial agents. Two representative antimalarial agents, artemisinin and mefloquine (Fig. 1) were investigated for their surface activity in the presence/absence of a DPPC monolayer. Both compounds are amphiphilic molecules. The quinolinemethanol, mefloquine is structurally related to HF and has a hydrophobic ring (trifluoromethyl-substituted quinoline) and a polar piperidine ring. Artemisinin is a sesquiterpene lactone and the five polar oxygen atoms are localised on the same edge of the lipophilic hydrocarbon framework.[18] Both mefloquine and artemisinin have negligible surface activities *per se* (Fig. 5), but unlike HF, they did not cause appreciable changes to the surface pressure of the DPPC monolayer. As seen from Fig. 5, artemisinin (1 μM) and mefloquine

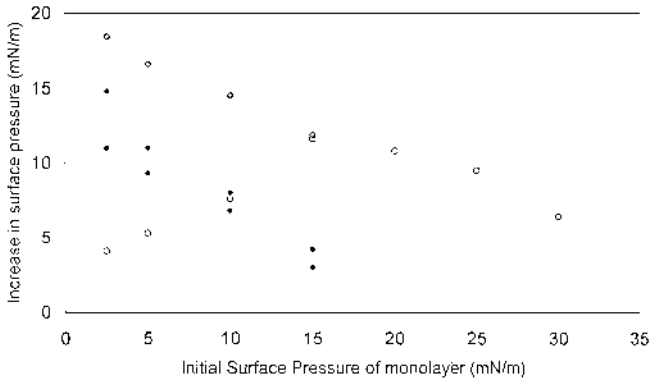

Fig. 4. Change in Surface Pressure ($\Delta\pi$) with Initial Pressure of Monolayer on Addition of the Following Concentrations of HF

DPPC monolayer: 0.05 μM (○), 1.0 μM (◇). DPPC/cholesterol monolayer: 0.05 μM (●), 0.2 μM (◆).

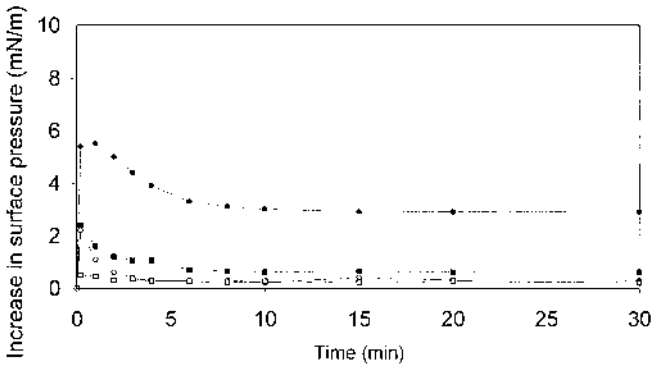

Fig. 5. Change in Surface Pressure ($\Delta\pi$) of DPPC Monolayer (5 mN/m) as a Function of Time in the Presence of Mefloquine 0.5 μM (○) and Artemisinin 1 μM (□)

Surface pressures of mefloquine 0.5 μM and artemisinin 1 μM are given by (●) and (■) respectively (on the same scale).

(0.5 μM) caused an initial small rise in surface pressure ($\Delta\pi$=2—5 mN/m) which rapidly decreased and reached an equilibrium level after 15 min. In these experiments, the DPPC monolayer was maintained at a low surface pressure (5 mN/m) that is more conducive to penetration by external agents, and both mefloquine and artemisinin were investigated at higher concentrations than HF. Yet only a small increase in surface pressure was recorded for these antimalarials. HF is therefore quite unusual in its ability to penetrate and increase the surface pressure of the phospholipid monolayer.

Another way of interpreting the results in Table 1 is to extrapolate the descending linear portions of the plots so that they intersect the *x* (surface pressure)-axis. This will give the critical surface pressure (π_c) at which the DPPC film must be maintained in order to prevent the penetration of HF (*i.e.* where there is no increase in surface pressure, $\Delta\pi$=0). This value varies according to the concentration of HF at which the measurement was made and was found to be 44.68 (±7.34) mN/m for the DPPC monolayer over a HF concentration range of 0.05 to 1.0 μM (Table 2). This means that the HF readily penetrates the DPPC monolayer and entry into monolayer is halted only when a very rigid monolayer maintained at a surface pressure of about 45 mN/m is present. Such a high surface pressure is equivalent to that of an unnatural "solid" phase.

Table 2. Critical Surface Pressures of the DPPC and DPPC/Cholesterol Monolayers as Determined from Constant Area and Constant Pressure Methods

Mode of determination	Critical surface pressure π_c (mN/m)	
	DPPC monolayer	DPPC/Cholesterol monolayer
From constant area conditions[a)]	44.68 (7.34)	23.67 (6.33)
From constant pressure conditions[b)]	21.92	15.59

a) An increase in surface pressure was recorded under these conditions. π_c values at different concentrations of HF were determined (Fig. 3) and averaged. π_c values for the DPPC and DPPC/cholesterol monolayers are significantly different at $p<0.005$. *b*) The area occupied by per mole of DPPC or DPPC/cholesterol was recorded under these conditions. π_c was determined from Fig. 7 (plot of area expanding effects of HF *vs.* π) by extrapolating the straight lines obtained for DPPC and DPPC–cholesterol monolayers to the *x* axis.

Cholesterol is a normal lipid component of most natural membranes. When incorporated among phospholipid molecules, cholesterol aligns itself with its hydroxyl group alongside the carboxyl group of the phospholipid and the rigid, planar steroidal ring among the first 10 or so carbon atoms of the acyl chain. At temperatures below the phospholipid transition temperature, cholesterol interferes with the tight packing of the phospholipid molecules in the gel phase, giving rise to a fluidizing effect. Packing in the monolayer will be looser with an increase in area and there will be greater permeability and fluidity.

The effect of cholesterol on the penetration of HF into DPPC monolayers is shown in Table 1 and partly depicted in Fig. 4. For monolayers maintained at low surface pressures (2.5, 5 mN/m), HF penetrates the DPPC monolayer more readily in the presence of cholesterol. As the surface pressure of the monolayer increases to 10 and 15 mN/m, penetration is slowed down by the presence of cholesterol, as seen from the smaller increases in surface pressure. As stated earlier, cholesterol has a fluidizing effect on the DPPC monolayer at temperatures below its phase transition temperature (41 °C) and this should faciliate the entry of amphiphilic molecules like HF into the monolayer. However, the fluidizing influence of cholesterol may be limited or even offset when the surface pressure of the monolayer is increased, as the latter has the effect of condensing the monolayer. This would explain the overall trend of declining $\Delta\pi$ as the surface pressure of the DPPC–cholesterol monolayer is increased. As in the case of the DPPC monolayer, the critical surface pressure π_c of the DPPC–cholesterol monolayer for each concentration of HF could be obtained by extrapolating the descending linear part of the plot to the *x*-axis. An average π_c of 23.67 mN/m (±6.33) is estimated for the mixed DPPC–cholesterol monolayer, which is the surface pressure at which the monolayer must be maintained to prevent penetration by HF (0.05 to 0.2 μM). This value is about half the π_c value of the DPPC monolayer, indicating that HF penetrates the DPPC monolayer less readily in the presence of cholesterol and that the penetration can be halted by maintaining the monolayer at a surface pressure (24 mN/m) equivalent to the liquid condensed phase.

The experiments described in preceding paragraphs were carried out under "constant area" conditions, *viz*., the mechanical barriers of the trough were held at fixed positions and the area of the monolayer was kept constant during the investigation. Penetration of HF into the monolayer would cause an expansion of area, but since this has been prevented, an increase in surface pressure would be recorded. Another approach to investigate the penetration of HF into the DPPC monolayer is to perform the experiments under conditions of constant surface pressure. In these experiments, the initial surface pressure of the monolayer is kept constant and penetration of the monolayer would lead to an increase in area.

Figure 6 shows the equilibrium area occupied by 1 molecule of DPPC in the monolayer in the presence of different HF concentrations. The surface pressure of the monolayer is also varied from 2.5 to 15 mN/m. The area occupied by 1 molecule of DPPC increases with increasing HF concentration. The presence of HF expands the area occupied by the lipid, *i.e*. it has an "area expanding" effect. This effect can be quantified by measuring the gradients of the straight lines seen in Fig. 6. By plotting the gradients of the lines (=change in area of monolayer/change in HF concentration) against the initial surface pressure of the monolayer (Fig. 7), it can be seen that the expanding effect of HF is greatest at low surface pressures of the monolayer. As the expanding effect of HF can only occur when the drug has penetrated the monolayer, it follows that monolayers maintained at low surface pressures are more susceptible to penetration by HF. This was also observed from the constant area experiments but only at higher concentrations of HF (Table 1, Fig. 4).

Figure 7 also shows the area expanding effects of HF in a monolayer containing equal proportions of cholesterol and DPPC. A similar trend is observed, namely that the area expanding effect of HF declines as the surface pressure of the DPPC/cholesterol monolayer increases. When the rates of expansion are compared for the DPPC and DPPC/cholesterol monolayers, it is seen that penetration is significantly slower for the cholesterol containing monolayers maintained at high surface pressures (10, 15 mN/m). This is similar to what was observed earlier from the constant area experiments and can be attributed to the "loss" of the fluidizing effects of cholesterol when the monolayer is maintained at high surface pressures. In contrast, for monolayers maintained at low surface pressures of 2.5 and 5 mN/m, cholesterol did not significantly affect the penetration of HF into the monolayer.

Extrapolating the straight lines in Fig. 7 to the *x*-axis gives the critical surface pressure of the film at which the area expanding effects of HF will not be evident. This is found to be 21.92 mN/m for a DPPC monolayer and 15.59 mN/m for a DPPC/cholesterol monolayer (Table 2). Theoretically, this should correspond numerically to the critical surface pressure π_c determined from the constant area experiments but this was not observed. The deviation may be attributed to the method of measurement employed (*i.e.* constant area *versus* constant pressure approaches). Other authors have employed a constant area approach to determine π_c[19] but in this investigation, the constant pressure approach is preferred mainly because a linear trend is observed in the area-expanding effects of HF over a range of HF concentrations (0.05—0.2 μM) and constant pressure conditions. (Figs. 6, 7). In contrast, the constant area approach gave a biphasic response for some concentrations of HF and this has cast doubt on the legitimacy of the π_c determination. However, qualitatively the same trend is observed, namely that a DPPC monolayer must be maintained at a higher surface pressure than a DPPC/cholesterol monolayer to prevent penetration by HF.

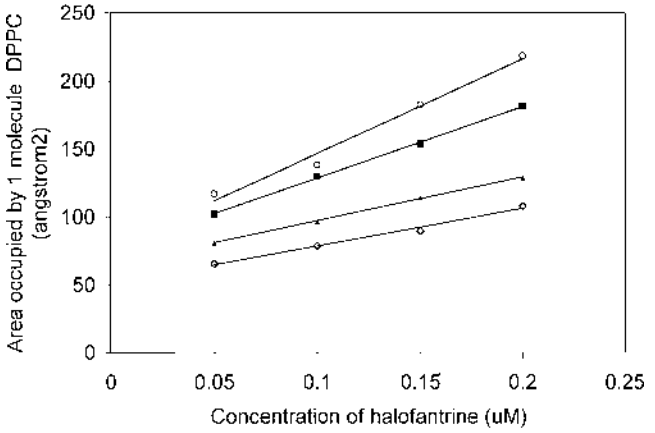

Fig. 6. Area (Å^2) Occupied by 1 Molecule of DPPC at Surface Pressures of 2.5 mN/m [○], 5 mN/m [■], 10 mN/m [▲] and 15 mN/m [◇] as a Function of HF Concentration

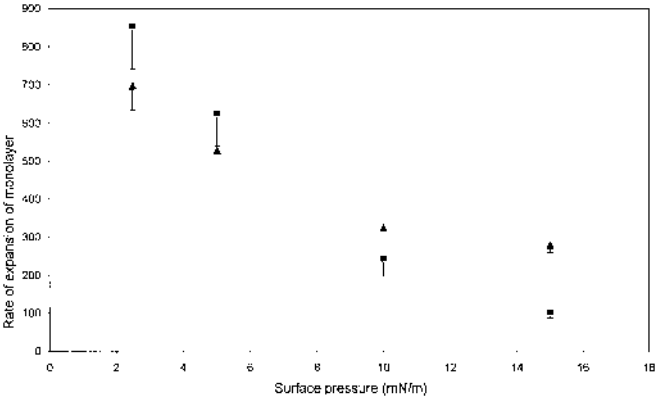

Fig. 7. Rate of Expansion of a DPPC (▲) Monolayer or a DPPC/Cholesterol (■) Monolayer as a Function of the Initial Surface Pressure of the Monolayer

Rate of expansion is significantly different for determinations carried out at surface pressures of 10 and 15 mN/m ($p<0.01$). Linear regression for DPPC monolayer: $r^2=0.899$; DPPC/cholesterol monolayer: $r^2=0.952$.

Conclusion

HF increases the surface pressure of DPPC monolayers due to its ability to penetrate the monolayer. Greater penetration is observed at higher concentrations of HF and lower surface pressures of the monolayer. The presence of cholesterol in the monolayer hampers penetration of HF, as evident from the lower critical surface pressures (π_c) obtained for a DPPC/cholesterol monolayer as compared to a DPPC monolayer.

Two approaches were used to determine π_c of the monolayer. Constant area experiments (*i.e.* monitoring the change in surface pressure) gave higher estimates of π_c for both DPPC and DPPC/cholesterol monolayers compared to values obtained from constant pressure experiments (*i.e.* monitoring the change in area occupied). The latter method of measurement is preferred in this study. Regardless of the method em-

ployed, it is seen that HF penetrates the DPPC monolayer more readily than the DPPC/cholesterol monolayer. These results are useful as they cast insight into the interaction of HF with phospholipids on a molecular level, which may be applied for the development of lipid drug delivery systems of HF as well as understanding the pharmacodynamics of this important antimalarial agent.

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