Biochemical Pharmacology, Vol. 30, pp. 411–415.

0006-2951/81/050411-05 $02.00/0

VINBLASTINE AND VINCRISTINE ACTION ON GEL–FLUID TRANSITION OF HYDRATED DPPC

L. TER-MINASSIAN-SARAGA*, G. MADELMONT*, C. HORT-LEGRAND† and S. METRAL†

* Laboratorie de Physico-Chimie des Surfaces et des Membranes, Equipe de Recherche du CNRS associée à l'Université Paris V, and † Laboratorie de Biophysique Neuromusculaire, UER Biomédicale 81, 45 rue des Saints-Pères, 75270 Paris cedex 06, France

(*Received* 29 *February* 1980; *accepted* 12 *September* 1980)

Abstract—The effect of two anti-cancer agents, vinblastine sulphate (VLBS) and vincristine sulphate (VCRS), on the gel–liquid crystal transition of fully hydrated dipalmitoylphosphatidylcholine (DPPC) has been studied by differential scanning calorimetry (DSC). DSC diagrams were established for various mixtures of DPPC + agent and a fixed (50%) amount of water. It is concluded that VLBS perturbs the hydrated DPPC structure more strongly than VCRS. This conclusion confirms the idea proposed by Hort-Legrand and Métral [5] that these anti-mitotic drugs may also affect the functioning of cell membranes.

Pharmacological studies with human leukocytes have shown that the anti-mitotic and anti-inflammatory effects of vinblastine (VLBS) and vincristine (VCRS) at very low concentrations and at pH 7.4, are related to their binding to units of microtubules [1, 2]. The behaviour of vinblastine sulphate (VLBS) aqueous solutions was studied by Nimni [2] and it was concluded that the anti-mitotic character of VLBS might be related to its lipophilic character and to its hydrophobic interaction with proteins being polymerized endothermically. However it has been shown also that VLBS may increase the aggregation of membrane components (possibly proteins) either by decreasing their motion (lipid fluidity) or by binding to the membrane proteins itself [3].

The hydrophobic character of these anti-mitotic drugs is relevant also to the problem of drug encapsulation in phospholipid liposomes [4]. Finally, it is well known that in addition to the carcinostatic action of the drugs, based on their interaction with microtubular proteins, they involve neuro-muscular effects.

A recent study of VCRS effects on frog muscular synapses has been performed [5]. To explain the results obtained it has been assumed that the drugs may modify the functioning of biological membranes specifically. This hypothesis has been tested here using fully hydrated (50%) dipalmitoylphosphatidylcholine (DPPC) as a model for the lipophilic regions of membranes. The effects of VCRS and VLBS on the thermotropic behaviour of this model system have been compared.

MATERIALS AND METHODS

Reagents. VLBS and VCRS were a gift from Elly Lily (USA). They were used as received. DPPC was purchased from Fluka, its purity being established by thin-layer chromatography.

Preparation of DSC samples. Mixed (DPPC + VLBS) and (DPPC + VCRS) samples for DSC were prepared as follows. Aliquots of DPPC solutions in methanol/chloroform $CH_3OH/CHCl_3$ (1/9) were deposited in the cups and the solvent evaporated under vacuum. Pure drug (VLBS or VCRS) was added to these cups and its weight determined using a microbalance. Chloroform was then added to dissolve both the drug and the DPPC (to give a final concentration of 1 mg VLBS/50 μl or complete dissolution of the VCRS). The solvent was evaporated to constant weight (10^{-6} g accuracy), the dry drug mixture being of the order of 0.5 mg. Water was then added, to a final 50% by weight, and the cup sealed. The cups were incubated at 65° for 5 hr to allow the mixing of the drug with the liquid crystal phase of DPPC. The reference cup contained an equal amount of water. Two samples were prepared for each of the following compositions: $x = 0.25, 0.5, 0.7, 0.8, 0.9$ where x is phospholipid mole fraction.

Thermograms. The DSC scanner was a Du Pont Thermoanalyser 990–910. The scans were started at 1–2° and stopped at 60°. For each sample four scans were performed.

The heating rate was 2° min^{-1}. The areas below the peaks were evaluated using a planimeter and the heat of transition, ΔH_t, was deduced. This heat and the characteristic temperatures of the peaks: T_m, the main transition temperature, read at the maximum deflection, and T_o, obtained by extrapolation of the low temperature (right hand) side of the peak (as shown in Fig. 1), are plotted as a function of the molar fraction x of DPPC in the dry mixtures. With the drugs, the bars on the figures represent the dispersion of ΔH_t values (corresponding to four or eight) thermograms.

RESULTS

Figure 1(a) represents the heating scans for the DPPC + VLBS mixtures. Figure 1(b) shows the scans for the DPPC + VCRS mixtures. The pre-

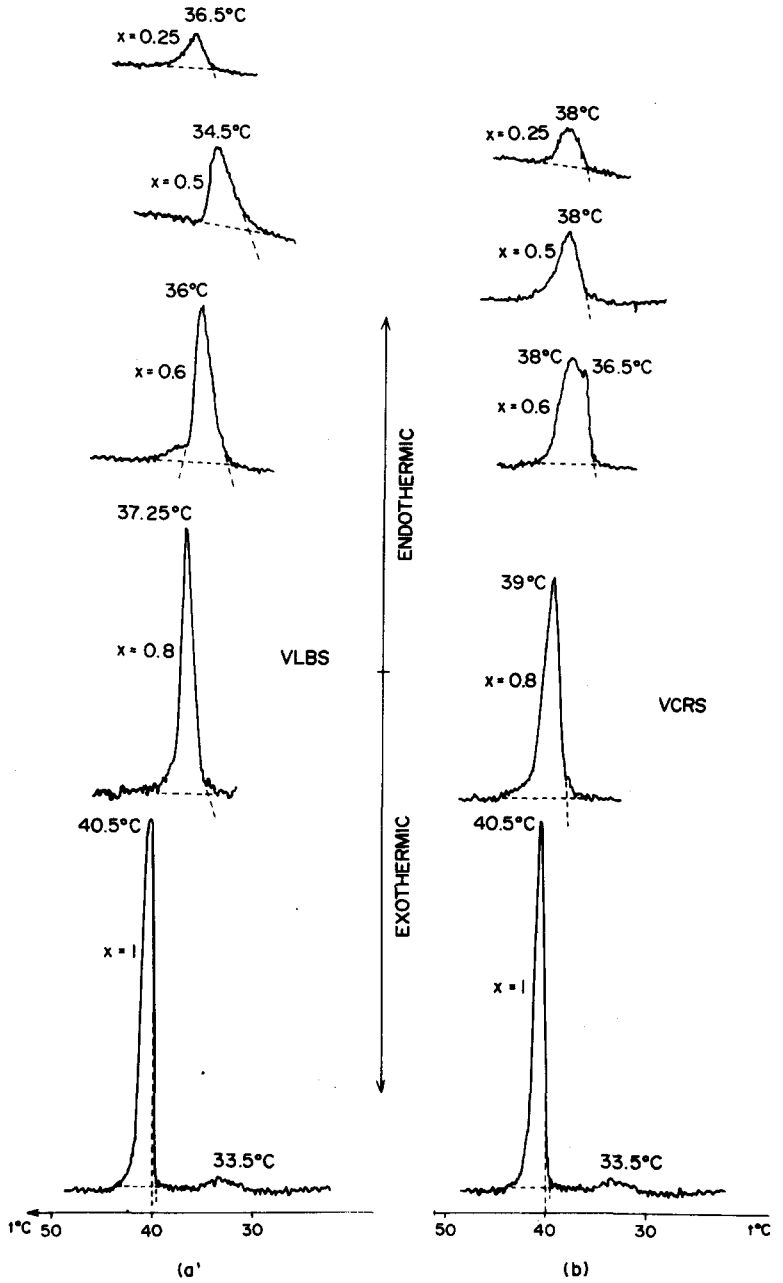

Fig. 1. DSC scans for mixtures of VLBS and of VCRS with DPPC. Heating rate: 2° min^{-1}. Sensitivity: 0.2 mV/cm. x = mole fraction of DPPC in the dry mixture. Only temperatures T_m at maxima of peaks are given. The extrapolations shown provide T_o, the temperature of onset of the transition. (a) VLBS. Weight of DPPC in the dry mixtures for the various values of x: 1 (0.5 mg); 0.8 (0.392 mg); 0.6 (0.288 mg); 0.5 (0.203 mg, 0.265 mg); 0.25 (0.116 mg). (b) VCRS. Weight of DPPC (as under (a)) corresponding to the various values of x: 1 (0.5 mg); 0.8 (0.392 mg); 0.6 (0.288 mg); 0.5 (0.237 mg); 0.25 (0.116 mg).

transition peak of DPPC, at ~33°, vanished on the scans corresponding to $x < 0.9$. The characteristic temperatures T_o, T_m of the peaks are represented as a function of the mixtures compositions in Fig. 2(a) and in Fig. 2(b). The enthalpy of the transition per mole of DPPC, ΔH_t, both in the presence of VLBS and of VCRS is shown in Figure 3.

For pure DPPC ($x = 1$), the gel–liquid crystal main transition temperature, corresponding to the melting of the palmitoyl chains, is located at 40.5° and the heat of melting is $\Delta H_t = 8.1 \pm 0.4$ cal mol^{-1}. This is close to the value given by Phillips *et al.* [6]. The pretransition occurring in the gel phase occurs at 33.5° (peak maximum).

When the drugs were present ($x < 1$) the pretransition peak was not observed on the scans and the main peak was broadened and displaced to lower temperatures.

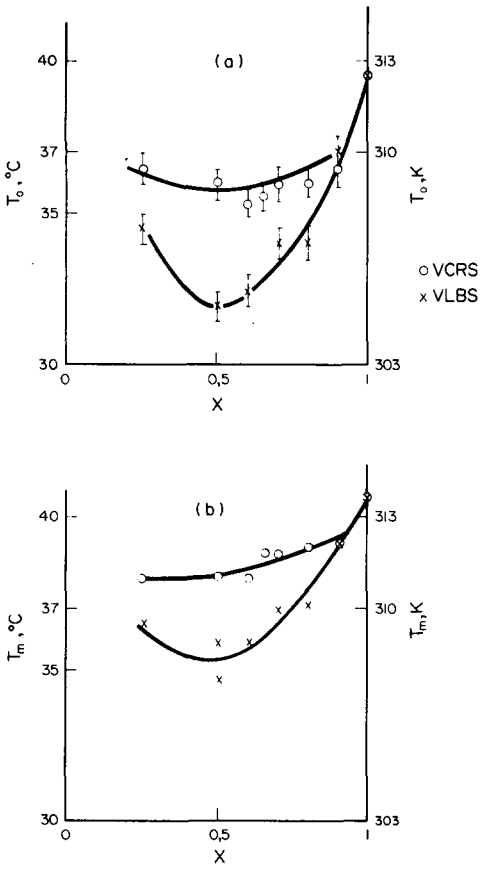

Fig. 2. The effect of the anti-mitotic drugs on the transition temperature of fully hydrated (50%, w/w) DPPC. Heating thermograms. (a) Temperature at the onset of the transition T_o. The bars are equal to the errors involved by the extrapolations shown in Fig. 1. (b) Main transition temperature (peak maxima, Fig. 1) $T_m \cdot x$ = mole fraction of DPPC in the dry mixture.

Effect of the drugs on DPPC characteristic temperatures

Both VLBS and VCRS decrease the characteristic temperatures T_o and T_m, of DPPC, the effect of VLBS being larger (Fig. 2(a) and (b)).

At $x = 0.5$ in the mixtures with VLBS, the melting of the chains started at 32° and the peak maximum occurred between 35 and 36° (Fig. 2(b)). The melting of the chains in the mixtures with VCRS started at 36° and the peak maximum occurred at 38°. Pure VLBS did not display any peak in the range 2–80°.

Below we consider separately the mixtures in which either DPPC or the drug are the major constituent (in excess).

When DPPC was the major molar constituent ($1 > x > 0.5$) in the mixtures, both T_o and T_m decreased while the main peak broadened (Fig. 1). For $x \geqslant 0.9$, both drugs showed the same results whereas significant differences between them are observed for the range $0.5 \leqslant x \leqslant 0.8$. A discontinuous broadening of the DPPC main peak occurred in the presence of VCRS at $x = 0.8$ followed by a 'saturation' effect (almost constant T_o and T_m) for $x < 0.8$ (see Fig. 2(a) and (b)). In this range of composition for the system VLBS + DPPC the transition peak broadened and the transition temperature decreased continuously.

When the drug was the major constituent ($0 < x < 0.5$), for VLBS both the onset of chain melting T_o and the peak maximum temperature T_m increased above the value at $x = 0.5$ whereas for VCRS the peak maximum temperature T_m was equal to 38° for the values of x equal to 0.25, 0.5 and 0.6.

Effect of drugs on molar enthalpy ΔH_t of DPPC chain melting

The effect of the drugs on the molar melting enthalpy of the chains, as for the temperatures, T_o and T_m, was different according to whether DPPC or the drug was the major constituent in the mixtures.

When DPPC was a major constituent of the mixtures ($0.6 < x < 1$), both VLBS and VCRS *increased* ΔH_t, while the characteristic temperatures T_o and T_m decreased. Such an increase has previously been found with gramicidin S [7]: a mole fraction of 0.15 of gramicidin S increased ΔH_t of DPPC by 30 per cent. For our systems, this increase was about 20 per cent at, respectively, $x = 0.6$ for DPPC + VLBS and $x \simeq 0.8$ for DPPC + VCRS.

When the drug VLBS was the major constituent in the mixtures with DPPC ($0 < x < 0.5$), ΔH_t decreased continuously when $x \to 0$. The results for VCRS are less clear than for VLBS. The heat of chain melting was the same and equal to $\Delta H_t =$ 7 kcal/mole DPPC at $x = 0.25$ and at $x = 0.5$. The result for $x = 0.25$ was dislocated from the curve drawn in Fig. 3. Although the corresponding peak was small (Fig. 1(b)) it was sharp enough to allow the measurement of its area on the scans and to deduce the molar enthalpy of transition of DPPC.

DISCUSSION

The interpretation of the diagrams in Fig. 2 is based on the conclusions of Nimni [2] and Wunderlich *et al.* [3] concerning the thermal behaviour of VLBS aqueous solutions. This interpretation is qualitative. For systems simpler than the one studied

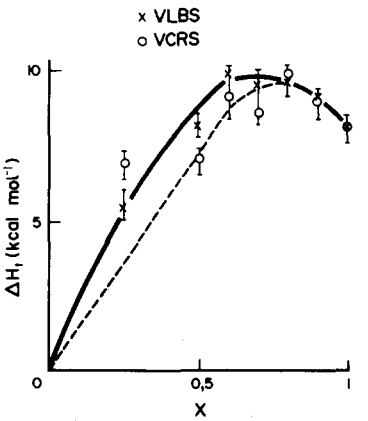

Fig. 3. The average molar enthalpy of transition ΔH_t for DPPC.

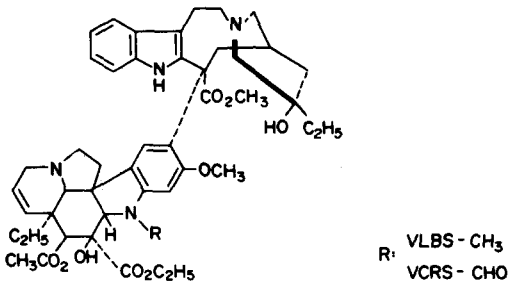

Fig. 4. The chemical structure of VLBS and VCRS.

here; no clear interpretation is yet available [8, 9, 12, 14].

Figure 4 shows the chemical formulae of VLBS and VCRS. These molecules are formed by two rigid parts, one of which bears the cationic $> NH_2^+$ group. VLBS is lipophilic [3] and aggregates in aqueous solution when heated and the lipophilic solvent, octanol, extracts 99.8 per cent of the drug from 10^{-3} M VLBS solutions in water. Nimni [2] has related this behaviour to the lipophilic character of VLBS. Similar experiments for VCRS have not yet been performed; however, the isotropic solvent octanol differs from the anisotropic gel and lamellar phases of hydrated DPPC [10]. This is why, for the last 15 years, hydrated DPPC has been considered a suitable model for the study [8–16] of properties such as fluidity and permeability of the phospholipids constituting the biological membranes.

It has been shown that when mixed with unlike molecules [8, 11, 12] the enthalpy of DPPC chain melting and the corresponding transition temperature may be lowered. From these studies two models have emerged for the structure of the mixed (with DPPC) systems. In the 'solution' model DPPC forms the continuous solvent phase containing separated solute molecules [13]. In the 'alloy' model the segregation of like molecules produces domains (Fig. 5) of 'pure' constituents. The state of DPPC located at the boundary between domains is strongly perturbed [8, 9, 11, 14]. Therefore the smaller the size of the domains the larger the quantity of DPPC molecules in the perturbed state [8, 9] and the smaller the average molar enthalpy of transition.

The 'solution' model predicts the depressing effect of 'solute' on the transition temperature of the 'solvent' and a constant heat of transition ΔH_t[13]. The domain model predicts no effect of the composition on the main transition temperature but a variation of ΔH_t with the composition [9, 12].

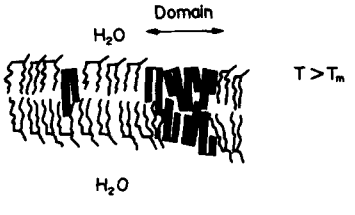

Fig. 5. A model for liquid–crystalline bilayer in fully hydrated DPPC + drug systems; mole fraction of DPPC in the dry mixture: $1 > x > 0.5$.

Neither of the two models predicts the *increase* of the enthalpy of DPPC transition observed with the drugs VLBS and VCRS by us and with gramicidin S (G-S) [7].

The thermograms (Fig. 1) correspond to a thermal behaviour of drug contaminated DPPC domains as shown in Fig. 5. The diagrams T_o–x and T_m–x for VLBS and VCRS (Fig. 2(a) and (b)) imply that in that 'dilute' drug mixtures, $x \geq 0.9$, both drugs mix well with DPPC and affect it in the same way.

For $0.5 < x \leq 0.8$ VCRS has only a small additional effect on the 'main' transition temperature T_m (upper curve in Fig. 2(b)) in contrast to VLBS (lower curve in same figure). Thus at $x = 0.5$ the lowering of the temperatures T_o and T_m of DPPC by VLBS is 3–4° larger than the lowering by VCRS. The difference between the effects of the two drugs in this range of x is assigned to different miscibilities of the drugs with the hydrated DPPC: VCRS would be less miscible than VLBS due to the replacement of the $> N\text{–}CH_3$ group of VLBS by the $> N\text{–}CHO$ group of VCRS. We assume that increasing miscibility with the lipophilic areas of a membrane implies increase of the perturbation of membrane structure.

Biological implications. The effect of VLBS and VCRS on biological membranes may be inferred using the 'lipid annulus' model [11, 15–17]. Lee [15, 16] has used this model to explain the non-specific effect of lipophilic local anesthetics. The solubilisation of local anesthetics in the lipid annulus around the conducting pores perturbs the structure of the lipid chains and the functioning of pores.

Therefore, our results on model phospholipids (Fig. 2 and 3) would predict that under comparable conditions VLBS might be a more efficient perturbing agent than VCRS. On the other hand, Hort-Legrand and Métral [5] have observed that VCRS increases the frequency of spontaneous miniature and plate potential by about 200 per cent, while VLBS increase this frequency by 300–500 per cent. This difference between the effects of the two drugs on this synaptic membrane might be related to the stronger perturbation of DPPC lamellar structure by VLBS as inferred from the results of the present DSC study.

In conclusion, the different effects of VLBS and VCRS on hydrated DPPC (50%) thermal behaviour in the range of temperature 2°–80° points to the very important role of the lipophilic or lyophilic character of just one internal group of these molecules. This difference in character may affect the 'miscibility' of the drugs with the organized phases of hydrated DPPC (50%) and the melting temperature and enthalpy of its chains.

If this perturbing effect of the drugs on DPPC lamellar phase was relevant to the functioning of biological membranes then VLBS would be a more powerful agent than VCRS.

REFERENCES

1. W. A. Creasey, K. G. Beusch and S. E. Malawista, *Biochem. Pharmac.* **20**, 1579 (1971).
2. M. E. Nimni, *Biochem. Pharmac.* **21**, 485 (1972).
3. F. Wunderlich, R. Muller and V. Speth, *Science* 182, 1136 (1973).

4. S. B. Kaye and V. J. Richardson, *Cancer Chemother. Pharmac.* **3**, 81 (1979).
5. C. Hort-Legrand and S. Metral, *C. R. Acad. Sci. Paris* **282**, Série D, 933 (1976).
6. M. C. Phillips, R. M. Williams and D. Chapman, *Chem. Phys. Lipids* **3**, 234 (1969).
7. En-Shinn Wu, K. Jacobson, F. Szoka and A. Portis, *Biochemistry* **17**, 5543 (1978).
8. E. Sackmann and B. Bunsenger, *Phys. Chem.* **82**, 891 (1978).
9. T. N. Estep, D. B. Mountcasle, R. L. Biltonen and T. E. Thomson, *Biochemistry* **17**, 1984 (1978).
10. D. Chapman, R. M. Williams and B. D. Ladbrooke, *Chem. Phys. Lipids* **1**, 445 (1967).
11. P. Jost, O. H. Griffith, R. Capaldi and G. Van Der Kooi, *Biochim. biophys. Acta* **311**, 141 (1973).
12. D. Bach and I. R. Miller, *Biochim. biophys. Acta* **433**, 13 (1976).
13. A. G. Lee, *Biochim. biophys. Acta* **742**, 285 (1977).
14. D. A. Pink and D. Chapman, *Proc. natn. Acad. Sci.* U.S.A. **76**, 1542 (1970).
15. A. G. Lee, *Molec. Pharmac.* **13**, 474 (1977).
16. A. G. Lee, *Nature* **262**, 545 (1976).
17. W. Schwarz, P. T. Palade and B. Hille, *Biophys. J.* **20**, 343 (1977).