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The modulation of thermal properties of vinblastine by cholesterol in membrane bilayers

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Abstract

It has been shown that the partitioning of vinblastine in 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) single and multiple bilayer dispersions induces partial interdigitation of the lipid alkyl chains. Similar behavior has been observed for abietic and ursodeoxycholic acids and may well be generalized for the partitioning of bulky amphoteric molecules, which tend to localize in the vicinity of the polar heads. For the present study, differential scanning calorimetry (DSC) has been employed to investigate the role of lipid molecular characteristics such as the alkyl chain length and the polarity of the head-group, as well as the impact of cholesterol upon vinblastine-induced interdigitation. It is found that vinblastine does not induce interdigitation in lipids with either shorter or longer alkyl chains than DPPC, or having head-groups of different polarity. In addition, it is shown that the presence of cholesterol in the lipid bilayer tends to modulate the phase behavior of the lipid/vinblastine bilayer system. Preliminary studies show that such properties directly affect the encapsulation efficiency and the pharmacokinetics of liposomes.

Keywords: DPPC; Interdigitation; Lipid/vinblastine bilayer system

1. Introduction

It is suggested through several biophysical studies that interdigitation of the alkyl chains in lipid bilayers may be related to important biological phenomena [1–13]. Etherlipids, dipalmitoylphosphatidylglycerol (DPPG) and saturated mixed-chain phosphatidylcholine bilayer dispersions all have been shown to form interdigitated bilayers [14,15]. Short-chain alcohols, glycerol, chlorpromazine, KSCN, bulky amphoteric molecules such as adiabetic and ursodeoxycholic acids and bromocylated taxanes are all known to induce the formation of interdigitated bilayer membranes when incorporated in phosphatidylcholines [1,4,9,12]. In a previous publication [13], we have studied the thermal effects of the vinca alkaloid vinblastine upon the phase behavior of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) bilayers and compared them with analogous effects exhibited by interdigitated membranes. Vinblastine (Fig. 1) is a mitotic inhibitor isolated from the plant *Catharanthus roseus G. Don.* (Apocynaceae) which is widely used in the treatment of leukemia and Hodgkin's disease, breast carcinoma, Wilms tumor, Ewing's sarcoma and small cell lung cancer either alone or in combination with other chemotherapeutic agents [16]. We have suggested that the presence of vinblastine in DPPC bilayers caused partial interdigitation of the lipid alkyl chains as evidenced by an increase of the total enthalpy change of the main lipid phase transition (ΔH) as well as of the transition temperature for single- and multi-bilayer vesicles and also by molecular modeling studies. Apart from the increase in ΔH, X-ray diffraction and fluorescence spectroscopy provide diagnostic techniques for interdigitated membranes [2,3,5,6].

Cholesterol has been reported to obstruct interdigitation of the alkyl chains. The interdigitated phase of DHPC is

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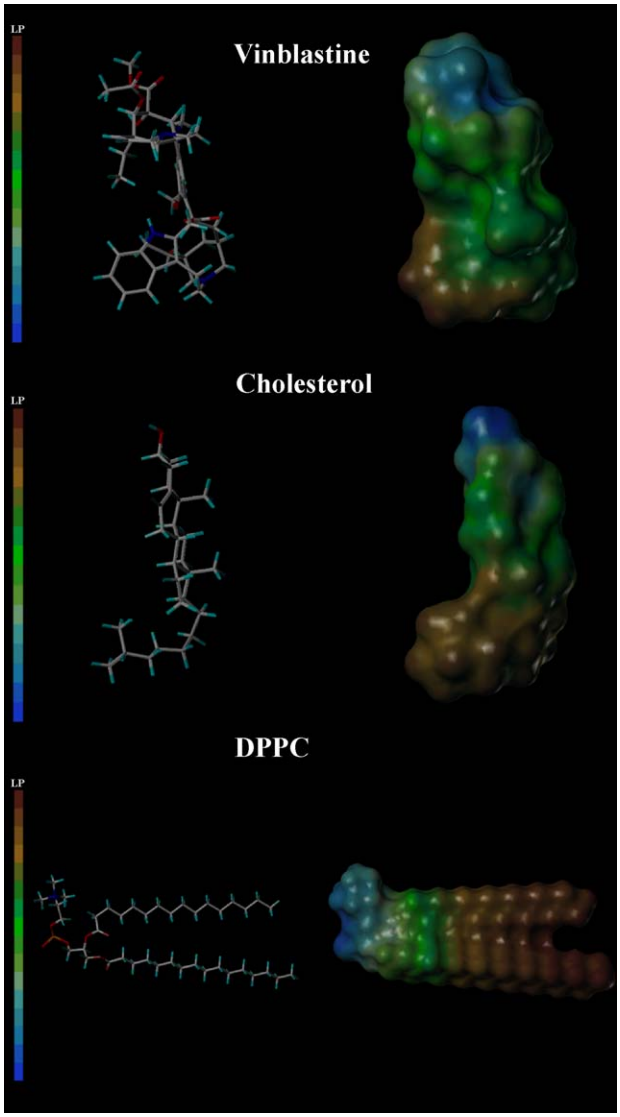


Fig. 1. Molecular structures of cholesterol, vinblastine and DPPC and their lipophilic profiles. Blue colors represent the polar moiety and brown the hydrophobic segments.

abolished in the DHPC/cholesterol membranes [2]. Membrane-partitioned short-chain alcohols are countered by cholesterol leading to the loss of interdigitization potential of the membranes [10,11]. The presence of cholesterol primarily affects the fluidity of lipid membranes [17]. X-ray diffraction studies have shown that it positions itself parallel to the lipid bilayers with its hydrophobic steroidal and alkyl chain parts extending in the lipophilic part of the membrane while the polar part localizes in the vicinity of the esterified carbonyls. The hydroxyl group probably forms a hydrogen bond with the oxygen of the esterified carbonyl groups [18]. The thermal effects of cholesterol upon the phase transitions of mesomorphic states of phospholipid bilayers have been extensively studied by differential scanning calorimetry (DSC). At low concentrations cholesterol induces localized disorder in the gel phase. At concentrations ~30% cholesterol gives rise to the formation of the liquid-ordered phase characterized by the decoupling of the positional and conformational ordering. Although the phase is liquid, there exists a high degree of conformational ordering related to the lipid alkyl chains [19–22]. NMR experiments have demonstrated that cholesterol causes a conformational ordering effect above the phase transition temperature of phosphatidylcholine bilayers and a positional disordering effect below the phase transition. This is termed as the "buffer effect" [23–28]. The unique properties of cholesterol in lipid bilayers are all attributed to its molecular structure. More explicitly, it contains: (a) a β-OH group at position 3, which gives its amphipathic character, (b) a small relatively flexible tail, and (c) a flat fused ring system.

In the present study, we have examined the role of cholesterol (Fig. 1) in the lipid/vinblastine bilayer system using DSC. In addition, we have examined the role of lipid molecular characteristics in the vinblastine-induced interdigitation of the bilayer. Such study may shed light upon the molecular requirements for bilayer interdigitation induced by bulky amphoteric molecules. Moreover, the properties of cholesterol in phospholipid bilayers can be used to improve the drug-encapsulation capacity of liposomal carriers. This work is currently in progress in our laboratory.

2. Materials and methods

All the lipids used in the study were obtained from Avanti Polar Lipids Inc. Vinblastine sulfate was obtained from Eli Lilly. The organic solvents, i.e. chloroform ($CHCl_3$) and methanol (CH_3OH) used were of spectroscopic grade. Liposomes with or without cholesterol were prepared by dissolving the lipid mixture in chloroform. The solvent was subsequently slowly evaporated in a rotary evaporator. Multilamelar large vesicles (MLVs) were formed by adding ammonium sulfate 150 mM (pH = 5.3, 300 mOs). The preparation was then treated by freeze–thaw for 15 cycles. The so-produced vesicles were subsequently extruded 10 times through two polycarbonate membranes of 200 nm pore size diameter, using an extruder by Lipex Biomembranes Inc., heated at 50 °C. The final lipid concentration of the formulations was 10 mg/ml. The size distribution for the DPPC liposomes was determined using the Mastersizer by Malvern Instruments Ltd. Repetitive extrusion of the MLVs through two-stacked polycarbonate filters with 100 nm pore size after 10 freeze–thaw cycles resulted in large unilamelar vesicles (LUVs) with a size distribution of 180 nm in diameter. Subsequently, the LUVs were passed through Sephadex G-75, pre-equilibrated with 100 mM TES + 100 mM NaCl (pH = 7.5, 300 mOs) to create a transmembrane pH gradient. LUVs were incubated with the vinblastine sulfate at 60 °C for 5 min. The mixed DPPC/vinblastine vesicles with encapsulated vinblastine were separated from un-entrapped vinblastine by filtration through a Sephadex

G-75 column. Vinblastine was assayed by UV spectroscopy at 262 nm and the drug/phospholipid molar ratio was determined by phosphate assay from 0.13 to 0.18. The rate of vinblastine uptake was fast and efficient to more than 98% within 5 min.

2.1. Differential scanning calorimetry

High-precision calorimetry using the VP-DSC microcalorimeter by Microcal Inc., Northampton, USA, was employed for the study of LUVs as well as mixtures of LUVs and MLVs prepared by the method described above. Heating and cooling scans were carried out at 15 K/h in order to study thermal history and hysteresis effects. A conventional DSC technique was applied for the study of MLV samples of DMPC 1,2-dimyristoyl-*sn*-glycero3phosphatidylcholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1,2-distearoyl-*sn*-glycero3phosphatidylcholine (DSPC) and 1,2-dipalmitoyl-*sn*-3-phosphatidylglycerol (DPPG) using a Perkin Elmer DSC-7 calorimeter. DPPC with or without cholesterol and vinblastine were dissolved in chloroform. The solvent was then evaporated by rotavapore under vacuum (0.1 mm Hg) at temperature above the transition temperature of the phospholipid. For measurements, this dry residue was dispersed in appropriate amounts of bi-distilled water by vortexing. An aliquot (ca. 5 mg) was sealed into stainless steel capsules obtained from Perkin Elmer. Thermograms were obtained on a Perkin Elmer DSC-7 calorimeter. Prior to scanning, the samples were held above their phase transition temperature for 1–2 min to ensure equilibration. All samples were scanned at least twice until identical thermograms were obtained using a scanning rate of 2.5 °C/min.

3. Results

We have applied high-precision and conventional DSC to study the thermal effects of cholesterol in uni- and multilamellar DPPC vesicles. The DSC thermograms are displayed in Fig. 2 while the corresponding calorimetric results are shown in Table 1. Details of the calorimetric results for DPPC and DPPC/vinblastine (83:17 phospholipid/molar ratio or $\chi = 0.17$) membranes can be found in our previous publication [13]. Briefly, for unilamellar preparations the partitioning of vinblastine at $\chi = 0.17$ causes abolishment of the weak $L_{\beta}' - P_{\beta}'$ heat capacity C_p peak found in the pure DPPC bilayers and a sharpening of the C_p anomaly for the main gel/liquid-crystalline transition at 41.76 °C (Fig. 2a and b). The molar enthalpy change increases to $\Delta H = 7.8$ kcal/mol from $\Delta C_{\varsigma} = 5.3$ kcal/mol observed for pure DPPC bilayers. The calorimetric results revealed a thermotropic phase behavior, which provides strong evidence for close packing within the bilayers, compatible to vinblastine-induced interdigitization of the alkyl chains.

The addition of cholesterol at $\chi = 0.17$ enhances the positional heterogeneity of DPPC bilayers in the gel phase rendering them more fluid-like (see Table 1). The C_p peak for the main lipid phase transition of the LUVs broadens, the pretransitional peak disappears and ΔH decreases (Fig.

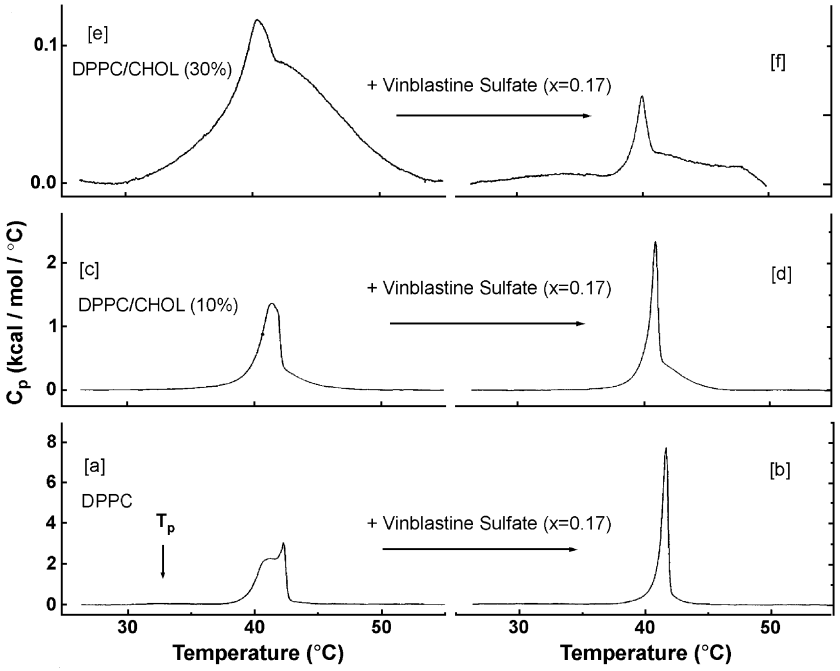


Fig. 2. High-sensitivity DSC scans for LUVs of DPPC containing vinblastine ($\chi = 0.17$) and various concentrations of cholesterol $\chi = 0$, 0.10 and 0.30.

Table 1
Quantitative thermal data for DPPC bilayers with or without vinblastine and with or without cholesterol

	LUV			MLV	
	T_m (°C)	ΔH (kcal/mol)	C.U. (no. of DPPC molecules)	T_m (°C)	ΔH (kcal/mol)
DPPC	41.20	5.32	76	42.3	7.51
DPPC/vinblastine (100:17)	41.76	7.77	180	40.5	10.44
DPPC/CHOL (10:1)	41.45	3.62	82	40.6	4.25
DPPC/CHOL/vinblastine (100:10:17)	40.97	3.82	300	40.0	5.81
DPPC/CHOL (10:3)	40.43	0.52	430	42.8	1.95
DPPC/CHOL/vinblastine (100:30:17)	40.19	0.44	850	43.0	1.49

2c). The T_m values and the size of the cooperative unit (C.U.), on the other hand, show negligible effects (see Table 1). When vinblastine at ($\chi = 0.17$) is incorporated in the DPPC/($\chi = 0.10$) cholesterol bilayers the C_p peak sharpens while ΔH and C.U. increase and T_m decreases (Fig. 2d). The T_m value of these preparations is 41 ± 0.3 °C. Analogous phase behavior characterized by an increase in ΔH is revealed when conventional DSC is used to study the effects of incorporating vinblastine ($\chi = 0.17$) in DPPC/cholesterol ($\chi = 0.10$) MLVs (Fig. 3). The quality of these thermal scans may also be interpreted as a reversible transition from a vesicular suspension to an extended peak bilayer network. In particular, Schneider et al. [29] published an article in which they combine calorimetry, viscosity and electron microscopy methods to explain this thermal profile. They state that these structural transitions arise from two effects: (i) the enhanced membrane elasticity accompanying the lipid state fluctuations on chain melting, and (ii) solvent-associated interactions (including electrostatics) that favor a change in membrane curvature. Other authors explain similar thermal behavior of bioactive molecules as the "inherent inhomogeneity" of the membrane bilayer. Thus, this preparation may contain domains consisting mainly of pure DPPC bilayers and others rich in drug.

We have also studied vinblastine incorporation in LUVs and MLVs prepared from DPPC/cholesterol ($\chi = 0.30$). In the case of LUVs the addition of cholesterol at this relatively high concentration triggers phase separation effects that are readily detectable in the C_p profiles (Fig. 2e). At higher temperature, the endothermic C_p peak is broad. It has been shown to describe the free lipid domains within the bilayers. The sharp peak at lower temperature on the other hand, is characterized by a small value of ΔH. It corresponds to DPPC/cholesterol domains with a very high degree of alkyl-chain conformational order above and below T_m (liquid-ordered phase) [20]. The incorporation of vinblastine ($\chi = 0.17$) in these DPPC/cholesterol bilayers readily induces a sharp increase in the size of C.U. Moreover, the broad free-lipid associated anomaly is suppressed. However, vinblastine can no longer cause an increase in ΔH but instead a marginal decrease. The T_m values for the DPPC/cholesterol ($\chi = 0.30$) and DPPC/cholesterol ($\chi = 0.30$)/vinblastine ($\chi = 0.17$) preparations remain lower by 1–1.5 °C than the corresponding values for pure DPPC bilayers. The C_p peaks for MLV preparations containing $\chi = 0.30$ cholesterol are significantly broad. Once again as for LUVs, ΔH for the MLV preparations contain-

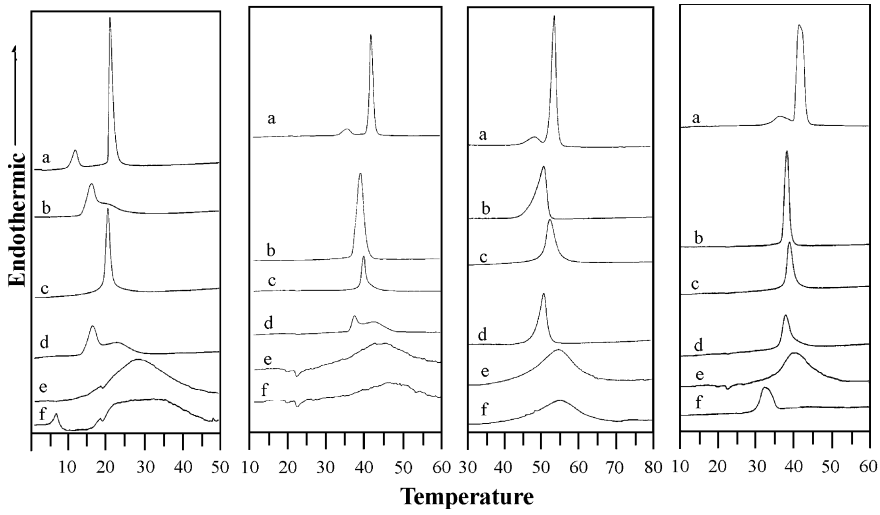


Fig. 3. DSC scans for lipid bilayers containing DMPC (left), DPPC (second from the left), DSPC (second from the right) and DPPG (right). (a) Phospholipid bilayers alone; (b) phospholipid bilayer containing $\chi = 0.17$ cholesterol; (c) phospholipid bilayers with incorporated $\chi = 0.10$ cholesterol; (d) addition of $\chi = 0.17$ vinblastine in phospholipid bilayers with incorporated $\chi = 0.10$ cholesterol; (e) phospholipid bilayers with incorporated $\chi = 0.30$ cholesterol; (f) addition of $\chi = 0.17$ vinblastine in phospholipid bilayers with incorporated $\chi = 0.30$ cholesterol.

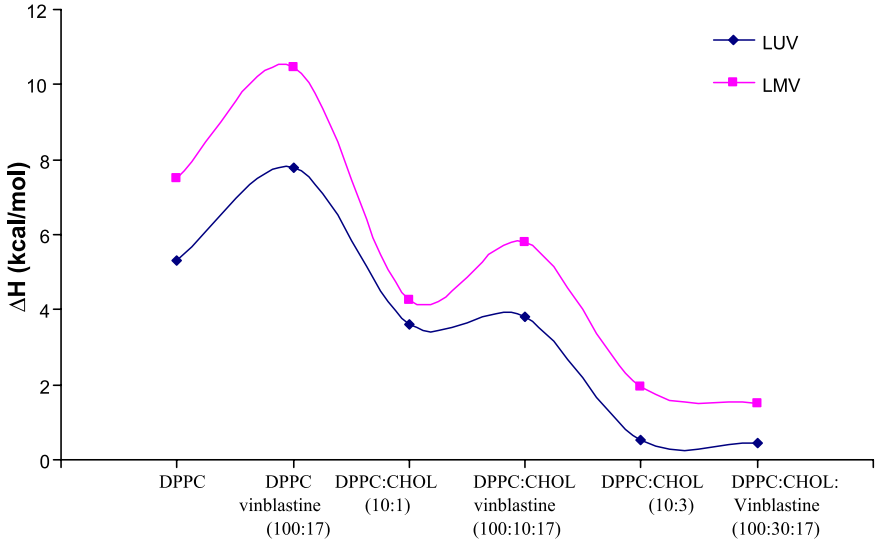
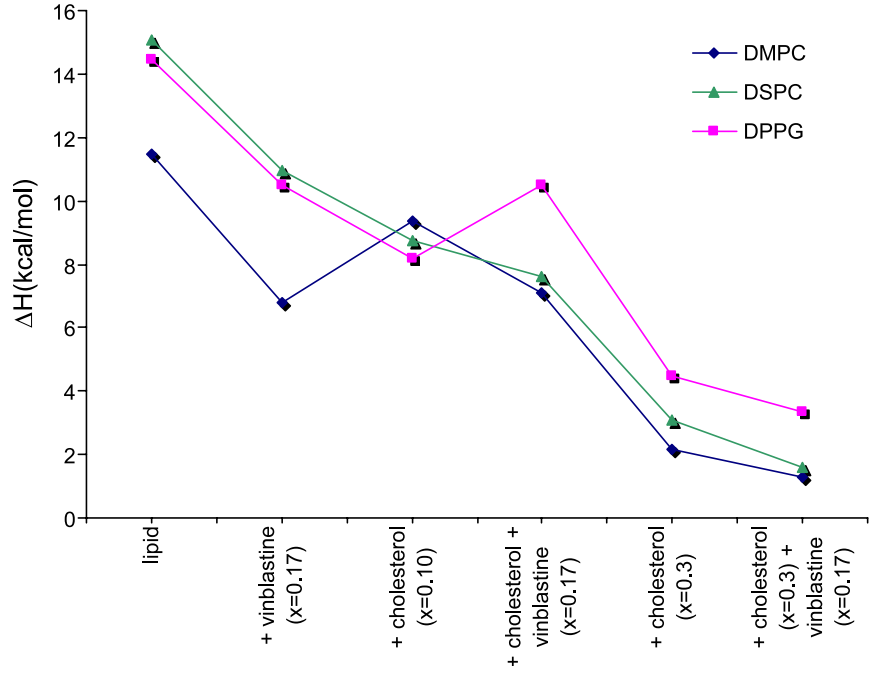


Fig. 4. Δ*H* changes versus cholesterol concentration for LUV and MLV preparations.

ing DPPC/cholesterol ($\chi = 0.30$)/vinblastine ($\chi = 0.17$) is lower than that of the corresponding preparation without vinblastine. All the calorimetric results obtained in the present study provide strong evidence that vinblastine-induced interdigitation is obstructed by the presence of cholesterol in the DPPC bilayers. For high enough cholesterol concentration, interdigitation is impossible for either LUVs or MLVs. Fig. 4 shows Δ*H* changes versus cholesterol concentration for LUV and MLV preparations.

Vinblastine-induced interdigitation of the membrane bilayers was studied separately in membranes containing phospholipids with shorter (DMPC) or longer alkyl chains (DSPC) or bearing a different head-group (DPPG). For DMPC, DSPC and DPPG, the modulation of the thermal properties induced by the addition of cholesterol was also studied. The DSC results for all the membrane bilayers used are also illustrated in Fig. 3. They show that in all cases vinblastine fails to induce interdigitation. This is readily indicated by the ΔH trends recorded, which always show a decrease of Δ*H* in the presence of vinblastine in the bilayer. The lowering of Δ*H* signifies that the interaction of bulky vinblastine with the various phospholipids induces disorder



(Fig. 5). As expected, the obtained results also indicate that cholesterol augments vinblastine-induced disordering. This is an interesting observation and shows that partial interdigitation induced by vinblastine is only specific to DPPC bilayers and thus depends on the size of its alkyl chain and the kind of its head-group.

The perturbing effect of vinblastine in these lipids is also specific as it can be observed from the DSC scans of Fig. 3 (Table 2). Hydrated DMPC lipids exist in the gel phase for temperatures lower than 15 °C and in the liquid crystalline phase for temperatures higher than 24 °C [29]. The presence of $\chi = 0.17$ vinblastine causes significant broadening of the phase transition temperature range and thus the formation of lipid domains containing different vinblastine concentrations as well as lowering of ΔH. The wide component at higher temperature may consist of pure DMPC bilayers while the narrow component of DMPC bilayers may contain the drug molecules intercalating into membrane bilayers. The presence of $\chi = 0.10$ cholesterol in the DMPC bilayers results in the abolishing of the pretransition, marginal lowering of the phase transition temperature and decrease of ΔH. When a concentration of $\chi = 0.17$ of the drug is added, the profile resembles that of DPPC/$\chi = 0.17$ vinblastine. This indicates that the predominant concentration of drug over cholesterol governs the thermal effects of DPPC/cholesterol bilayers. The presence of $\chi = 0.30$ cholesterol causes more significant broadening of the phase transition and further lowering of ΔH. At this concentration of cholesterol the bilayers undergo a solid-disordered to a liquid-ordered phase transition. As in the figure, the addition of $\chi = 0.17$ vinblastine enhances the creation of more disorder effects almost entirely abolishing the main lipid phase transition. The results for DSPC are analogous, which has longer alkyl chains than DPPC (C-18 versus C-16). DSPC bilayers exhibit the main lipid phase transition around 54 °C. The intercalation of $\chi = 0.17$ vinblastine into DSPC bilayers causes pronounced broadening of the temperature width of the phase transition, lowering of the phase transition temperature and decrease of ΔH. The presence of $\chi = 0.10$ cholesterol in DSPC bilayers results in the abolishment of the pretransition, marginal lowering of the phase transition temperature and decrease of ΔH. When $\chi = 0.17$ of vinblastine is added, a small additional decrease of the phase transition temperature as well as of ΔH is observed. DSPC/cholesterol ($\chi = 0.30$) preparation shows a DSC thermogram exhibiting a broad peak that has phase transition about at the same temperature as DSPC bilayers. It is possible that phase separation effects are dominant and responsible for this C_p profile. When $\chi = 0.17$ vinblastine is added in DPPC/cholesterol ($\chi = 0.30$) preparation, no significant difference from that of DPPC/cholesterol ($\chi = 0.30$) is observed suggesting again as in DPPC bilayers that cholesterol determines the shape of the DSC profiles. DPPG bilayers, show the pretransition and the main phase transitions at 37 and 42 °C, respectively. The addition of $\chi = 0.17$ vinblastine resulted in the lowering of the phase transition and abolishment of pretransition as well as in the decrease of ΔH. Similar effects were produced by $\chi = 0.10$ cholesterol. When $\chi = 0.17$ vinblastine is added in the DPPG/$\chi = 0.10$ cholesterol bilayer, an additional decrease of the main lipid phase transition temperature was observed. The DPPG/$\chi = 0.30$ cholesterol preparation exhibits a very broad peak. The addition of $\chi = 0.17$ vinblastine results in a peak ranged between 30 and 35 °C and a barely observable, very broad shoulder extended up to 55 °C.

Table 2
Values of the half-width temperature ($T_{m1/2}$), peak temperature (T_m) and enthalpy change ($\Delta H>$) of phospholipid bilayers without and with vinblastine and phospholipid/cholesterol without or with vinblastine

Samples	T_m (°C)	$T_{m1/2}$ (°C)	ΔH (kcal/mol)
DMPC	24.1	0.91	2.04, 11.49
DMPC + vinblastine ($x = 0.17$)	19.5	2.72	6.77
DMPC + cholesterol ($x = 0.10$)	23.4	1.27	9.38
DMPC + cholesterol + vinblastine ($x = 0.17$)	19.7	2.72	7.09
DMPC + cholesterol $x = 0.3$)	–	–	2.15
DMPC + cholesterol ($x = 0.3$) + vinblastine ($x = 0.17$)	–	–	1.30
DSPC	54.4	1.5	1.51, 15.06
DSPC + vinblastine ($x = 0.17$)	49.5	3.5	10.95
DSPC + cholesterol ($x = 0.10$)	52.4	3.0	8.77
DSPC + cholesterol + vinblastine ($x = 0.17$)	51.6	2.5	7.59
DSPC + cholesterol ($x = 0.3$)	–	–	3.09
DSPC + cholesterol ($x = 0.3$) + vinblastine ($x = 0.17$)	–	–	1.60
DPPG	41.8	2.25	1.54, 14.44
DPPG + vinblastine ($x = 0.17$)	38.0	1.5	10.52
DPPG + cholesterol ($x = 0.10$)	39.1	1.9	8.18
DPPG + cholesterol + vinblastine ($x = 0.17$)	37.8	2.5	10.49
DPPG + cholesterol ($x = 0.30$)	–	–	4.46
DPPG + cholesterol ($x = 0.3$) + vinblastine ($x = 0.17$)	–	–	3.34

4. Discussion

In a previous study we examined the effects of the incorporation of vinblastine in DPPC bilayers upon their thermotropic phase behavior. The stereoelectronic similarities of vinblastine with other bulky amphoteric molecules exerting analogous thermal effects upon lipid bilayers led to the hypothesis that vinblastine induces interdigitation of the DPPC alkyl chains. Molecular modeling techniques were used to establish that this hypothesis was indeed possible. The topography of the vinblastine molecules illustrates that it is localized in the area starting in the vicinity of the polar head-groups and ending at the upper lipophilic part of the lipid chains.

In the present study we have examined the thermal effects of vinblastine upon other lipids. We have found that interdigitation cannot be induced in lipids with shorter (DMPC) or longer alkyl chains (DSPC). This is an important observation since it shows that interdigitation is "lipid-specific" and can therefore be induced when strict molecular requirements are met. This argument is reconfirmed by analogous studies using a lipid with different head-group (DPPG).

The effects of cholesterol partitioning within the lipid bilayers, for all the lipids used in the present study, are equivalent to each other and to literature reports. Even at the lowest concentration used ($\chi = 0.10$), the presence of cholesterol induced the abolishment of the pretransition, caused significant broadening of the main lipid phase transition peak, and led to the decrease of ΔH. At $\chi = 0.30$, a liquid-disordered phase is induced for almost all the lipids, leading to the extreme broadening of the main lipid phase transition peak and the steep decrease of ΔH. On the other hand, when vinblastine is partitioned within the bilayers, the effects for DPPC are different than for any of the other lipids. The increase in ΔH observed for DPPC in the presence of $\chi = 0.17$ vinblastine has not been observed in of the other lipids. This increase has been associated to an interdigitization of the lipid alkyl chains and indicates that such interdigitization is only possible for DPPC. For most of the other lipids the presence of vinblastine caused a significant broadening of the main lipid phase transition peak (DMPC and DSPC) and a decrease of T_m, in DMPC bilayers by 4.6 °C, in DSPC, by 4.9 °C and in DPPG by 4.6 °C.

In an attempt to elucidate the role of cholesterol in the DPPC/vinblastine bilayers, a structure comparison between the two molecules is made showing that: (a) cholesterol has only one polar site localized in the 3β-OH group, while vinblastine has several polar molecular segments; (b) cholesterol has a flat steroidal part that accommodates between adjacent phospholipids. This is not possible for vinblastine, which although containing several flat aromatic rings, are not properly connected to form a flat conjugation; (c) cholesterol contains a small alkyl that extends its lipophilic steroidal part. Such structural moiety is lacking from vinblastine. These structural features may account for the thermal data. Cholesterol, having different stereoelectronic properties from vinblastine exerts different perturbation effects upon the DPPC bilayers. Cholesterol is a bulky molecule but its major structural entity is steroidal and flat so that it can easily fit between adjacent alkyl chains. This structural feature is lacking from vinblastine. Cholesterol, at low concentrations causes positional disordering due to mismatch of the steroidal part with the alkyl chains of phospholipids. In contrast, vinblastine in analogously low concentrations may enhance positional ordering since, due to its bulkiness, packing voids between adjacent phospholipid alkyl chains can be formed and the bilayer may eventually lead to interdigitation. Indeed, at cholesterol concentration $\chi = 0.10$ and vinblastine concentration $\chi = 0.17$, the calorimetric data show that vinblastine-induced interdigitation of the alkyl chains to a lesser extend than in the DPPC/vinblastine bilayers. Thus, the presence of lower concentration of cholesterol in the membrane is not strong enough to prevent it.

At higher cholesterol concentrations though, as $\chi = 0.30$, the liquid-ordered phase formed is characterized by a high degree of conformational order and positional disorder. Interdigitated packing of the alkyl-chains does not take place. The addition of vinblastine further disrupts the positional ordering of the lipid molecules. This explains the observed lowering of ΔH in the DSC data.

Vinblastine did not induce interdigitation when bilayers contained lipids other than DPPC. The magnitude of the alkyl chain length appears to be an important molecular characteristic. It would seem that for a vinblastine-induced interdigitated phase to stabilize, the degree of partial overlap between the alkyl chains and also the bilayer thickness are equally important parameters. Both a higher degree of alkyl chain overlap and a smaller bilayer thickness would appear to favor interdigitization. So, while longer alkyl chains could signify a higher degree of overlap and promote interdigitization in the DPPC versus the DMPC bilayers, the larger bilayer thickness would obstruct it, favoring interdigitation in the DPPC versus the DSPC bilayers. Another molecular characteristic of the lipid important for vinblastine-induced interdigitization appears to be the polarity of the head-group. This is indicated by the DPPG bilayers, which although having the same length as DPPC failed to form a vinblastine-induced interdigitated phase. This may be attributed to the lack of electrostatic interactions possible for choline head-group and sulfate salt of vinblastine. For the DPPG bilayers, vinblastine was a disorder-enhancing agent.

In conclusion, vinblastine-induced interdigitization of lipid bilayers depends strongly upon the degree of overlap of the alkyl chains, the bilayer thickness and the polarity of the lipid head-groups. Among DMPC, DPPC, DSPC and DPPG lipids, only the DPPC/vinblastine system leads to interdigitation. Cholesterol appears to play an important role in the modulation of thermal properties of lipid bilayers containing vinblastine and obstructs the enhancement of interdigitation. Thus, cholesterol appears not only to be a buffer of fluidity of membrane bilayers, but also a biomolecule that affects thermal properties of pharmaceutical molecules in membrane bilayers. Extensive similar studies with other pharmaceutical molecules will certainly elucidate further the role of cholesterol in the modulation of thermal changes that cause drug molecules.

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