Biochemical Pharmacology, Vol. 34, No. 22, pp. 4025–4031, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

COMPARISON OF THE MEMBRANE-RELATED EFFECTS OF CYTARABINE AND OTHER AGENTS ON MODEL MEMBRANES*

KARL A. KOEHLER,†‡§ JOHN HINES,‖ EDWARD G. MANSOUR,‡ YOUSEF M. RUSTUM,¶ DATTATREYA V. JAHAGIRDAR** and MAHENDRA K. JAIN**

Departments of † Biochemistry, ‡ Surgery, and ‖ Medicine, Case Western Reserve University, School of Medicine, Cleveland Metropolitan General Hospital, Cleveland, OH 44109; ¶ Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, NY 14263; and ** Chemistry Department, University of Delaware, Newark, DE 19716, U.S.A.

(*Received* 21 *February* 1984; *accepted* 4 *April* 1985)

Abstract—The membrane-associated effects of a series of chemotherapeutic and other drugs were examined via differential scanning calorimetry and by their modulation of the action of porcine phospholipase A_2 (PLA_2) on bilayer substrates. The drugs examined included: cytarabine, aminoglycoside antibiotics, adriamycin, dibucaine, butacaine, and VP-16. The bilayers employed were phase-separated ternary lipid mixtures containing dimyristoylphosphatidylcholine: palmitoyllysolecithin: and either hexadecanoic acid (fatty acid ternary mixture) or hexadecanol (alcohol ternary mixture). Effects of the more hydrophilic drugs (cytarabine and aminoglycoside antibiotics) on the calorimetric profiles of the negatively charged (fatty acid-containing) and the neutral (hexadecanol-containing) ternary lipid mixtures indicate that the interaction of these drugs with biomembranes is likely to be dominated by electrostatic interactions. All of the drugs investigated, including the more hydrophobic adriamycin, dibucaine, butacaine, and VP-16, affected the phase equilibrium in the membrane and exhibited apparent noncompetitive inhibition of the action of PLA_2 on bilayers composed of ternary lipid substrates. In addition, cytarabine inhibited fusion of fatty acid-containing ternary mixtures. Conclusions: These drug : membrane interactions leading to a shift in the phase equilibria were apparently regiospecific. Hydrophilic drug : membrane interactions included an important electrostatic component. The effects of all of the drugs employed in this study on the action of PLA_2 on a bilayer substrate (fatty acid-containing ternary lipid mixture) are hypothesized to be a result of the drug-mediated shift in phase equilibria away from the optimally active phase distribution. As a result, PLA_2 binds with normal affinity to the membrane, but its membrane substrate is not catalytically turned over. It is evident that these drugs can directly affect cellular homeostasis in a manner that can show a dependence on the nature of the membrane surface.

1-β-D-Arabinosylcytosine (AraC,†† cytarabine) is a nucloside analog with demonstrated therapeutic efficacy in preclinical tumor model systems [1, 2] and in patients with acute myelocytic leukemia [3]. While clinical resistance to AraC is uncommon in previously untreated patients, resistance to this agent occurs in the majority of relapsed patients. It has been reported that the therapeutic effectiveness of this drug can be modified in mice [1, 3] and in humans [4–8]. Utilization of higher doses of AraC, in the range of 2–3 g/m^2, via intravenous push, yield post-infusion plasma levels of greater than 0.02 mg/ml [4, 8]. Although the primary effects of AraC at normal dose levels are considered to involve DNA synthesis and repair [2], the effectiveness of high-dose protocols suggests that drug effects at an additional cellular locus may be involved. In addition to AraC, other chemotherapeutic agents may also exhibit membrane-mediated effects. For example, adriamycin is an effective chemotherapeutic agent with documented effects at the DNA level [7, 8]. It has been reported that the interaction of this agent with membrane components might be of considerable interest from the therapeutic point of view [9]. The nephrotoxicity of aminoglycoside antibiotics appears to be due to their abilities to modify the action of lysosomal phospholipases [10]. The studies of Jain *et al.* [10, 11] and Ruppel *et al.* [12] have suggested that some proteins and many drugs may interact with phospholipid bilayers at phase boundaries or defect structures. Effects of drugs, either directly or indirectly, on such membrane structures would be predicted to affect a wide variety of membrane : protein interactions [13]. Such effects could readily be transmitted to other processes in the metabolic cascades of the cell. Modulation of the extent or accessi-

* This investigation was supported in part by NIH Grants GM-29703 and HL-32159, a Grant-in-Aid from the Northeastern Ohio Cancer Society, a Grant from the Cuyahoga County Hospital Foundation, Inc., and CA 21071 from the National Cancer Institute.

§ Address all correspondence to: Karl A. Koehler, Ph.D., Surgery Dept. (R-401), Cleveland Metropolitan General Hospital, 3395 Scranton Road, Cleveland, OH 44109.

†† Abbreviations: AraC, 1-β-D-arabinosylcytosine (cytarabine); LPC_{16}, lysophosphatidylcholine (palmitoyl); FA_{16}, hexadecanoic acid; PLA_2, pig pancreatic phospholipase A_2; PC, phosphatidylcholine (dipalmitoyl); DMPC, phosphatidylcholine (dimyristoyl); DSC, differential scanning calorimetry; VP-16, glycosidic derivative of podophyllotoxin; OL_{16}, hexadecanol; adriamycin, doxorubicin; fatty acid ternary mixture, 20% LPC + 10% hexadecanoic acid + PC_{14}; and alcohol ternary mixture, 20% LPC + 10% hexadecanol + PC_{14}.

bility of defect structures in membranes could therefore have profound effects on cellular homeostasis. Defect structures in membranes may arise not only at the T_m of single component bilayers, but also in phase-separated mixed lipid bilayers. For example, the presence of as little as 2% of lysophosphatidylcholine plus fatty acid in phosphatidylcholine membranes can result in fusion of vesicles [11] and activation of pig pancreatic phospholipase A_2 (PLA_2) by enhancing its binding to bilayers [14, 15].

A solute-induced shift in the phase equilibrium in ternary codispersions and the resulting changes in organizational features of bilayers have far-reaching consequences vis à vis conceptualization of membrane active drug behavior [11]. The experiments reported here were designed to examine the effects of a number of polar and hydrophobic drugs on the properties of ternary phospholipid codispersions, particularly those containing DMPC + LPC + fatty acid. We have postulated that the defect structures present at the boundaries of the laterally phase-separated bilayer domains in such ternary codispersions act as a common locus for the binding of phospholipase A_2 [11], for the fusion of vesicles [16], and for the action of certain solutes which inhibit both of these processes [11]. Thus, the methodologies chosen for the studies reported here indirectly probe the effect of drugs on the presence and extent of the defect structures in the ternary codispersions. An *in vitro* highly characterized membrane system was chosen for these studies to allow detailed interpretation of the results in terms of phase equilibria.

EXPERIMENTAL

Materials. The following lipids were employed: palmitic acid (FA_{16}) (Sigma), L-α-lecithin (β-γ-dimyristoyl) synthetic (DMPC) (Calbiochem), and L-α-lysolecithin (γ-palmitoyl) synthetic (LPC_{16}) (Calbiochem). Gentamycin sulfate, streptomycin, and tobramycin were obtained from the Sigma Chemical Co. (St. Louis, MO). Purified VP-16 was obtained from the Bristol Laboratories (Syracuse, NY). Adriamycin was obtained from the Adria Laboratories, Columbus, OH. Pig pancreatic phospholipase A_2 (PLA_2) was a gift from Professor G. H. De Haas.

Methods. Drugs were dissolved in deionized water immediately prior to use. A dry film of premixed phospholipids was dispersed in appropriate aqueous buffers and sonicated in a bath-type sonicator (Sonicor SC-50T). The vesicles were "annealed" by incubating them at 55° for more than 2 hr. Unless otherwise stated, all the studies reported here were conducted on ternary codispersions of dimyristoylphosphatidylcholine + 1-palmitoyllysophosphatidylcholine + palmitic acid (3:1:1 mole ratio).

Phospholipase A_2 assays were performed by pH-stat titration of the liberated fatty acid. As described elsewhere, the ternary codispersion is hydrolyzed by PLA_2 without any latency phase [15]. A typical run consisted of 5.0 ml solution containing 100 mM KCl, 10 mM $CaCl_2$, and 150 μM phospholipids at pH 8.0 and 30°. The reaction was initiated by adding 500 ng PLA_2 in 1 μl solution. Results are reported in chart units (1 chart unit = 14 nmoles of protons released). For inhibition studies, drugs were added before initiating the hydrolysis and allowed to incubate in the reaction vessel for at least 3 min.

Differential scanning calorimetry was carried out on a Mettler TA 2000B instrument. Samples were prepared by dispersing a solid film of 2.5 mg DMPC alone or in a ternary lipid mixture with buffer containing 50 mM Tris + 100 mM KCl with and without $CaCl_2$ with and without drug at pH 8.0. The lipid dispersions (35 μl) in sealed aluminum pans were scanned at 1°/min from 10 to 55°. Enthalpies and transition temperatures were calibrated with dimyristoylphosphatidylcholine dispersions and indium standards.

Membrane fusion/aggregation studies were carried out on a Beckmann Acta III spectrophotometer by monitoring the change in turbidity as described by Elamrani and Blume [17]. Fusion studies were carried out at 30° and were organized as follows: 2.5 ml of buffer (0.1 M KCl/0.1 M Tris, pH 8.4) were placed in a thermostatted, stirred cuvette. After thermal equilibration, an appropriate volume of stock drug solution was added followed by 100 μl of 40 mg/ml of the ternary phospholipid dispersion which had been stored at 60°. This protocol was necessary in order to avoid complications due to spontaneous fusion of the stock solution of the vesicles at their phase transition temperature [11]. The reader is cautioned against making direct comparisons between therapeutic plasma concentrations of drugs such as those discussed in the beginning of the paper and those concentrations employed in the model systems utilized here. Ideally, in exploring membrane effects of drugs, one should compare only the mole fractions of drugs in the membrane. The mole fraction of a solute in a bilayer depends upon the bilayer/water partition coefficient and the aqueous phase concentration, which depend not only upon the relative amounts of the bilayer and aqueous phases but also upon the relative distribution of the solute in the various compartments and subphases in the bilayer. Thus, for example, for DSC studies the total concentration of drug employed is high because the lipid concentration in the sample is high. It is also important to keep in mind the possibility that metabolic or transport processes down a cellular/metabolic cascade may require only a partial modulation of the primary membrane process. Finally, it is clear that congruence between model system results and *in vivo* results will only be approached as the models become more related to the cell surface. This is particularly important while evaluating the effect of drugs on the phase-separated bilayer systems employed here, where the phase equilibria may be altered selectively by the drug partitioned into the phase boundaries.

RESULTS

Scanning calorimetry. Calorimetric profiles provide an important measure of the cooperative phase behavior of the phospholipid dispersion. The effects of AraC, butacaine, gentamycin, streptomycin, and adriamycin on the thermotropic phase transition behavior of pure DMPC and the ternary codispersions were examined. Typical calorimetric pro-

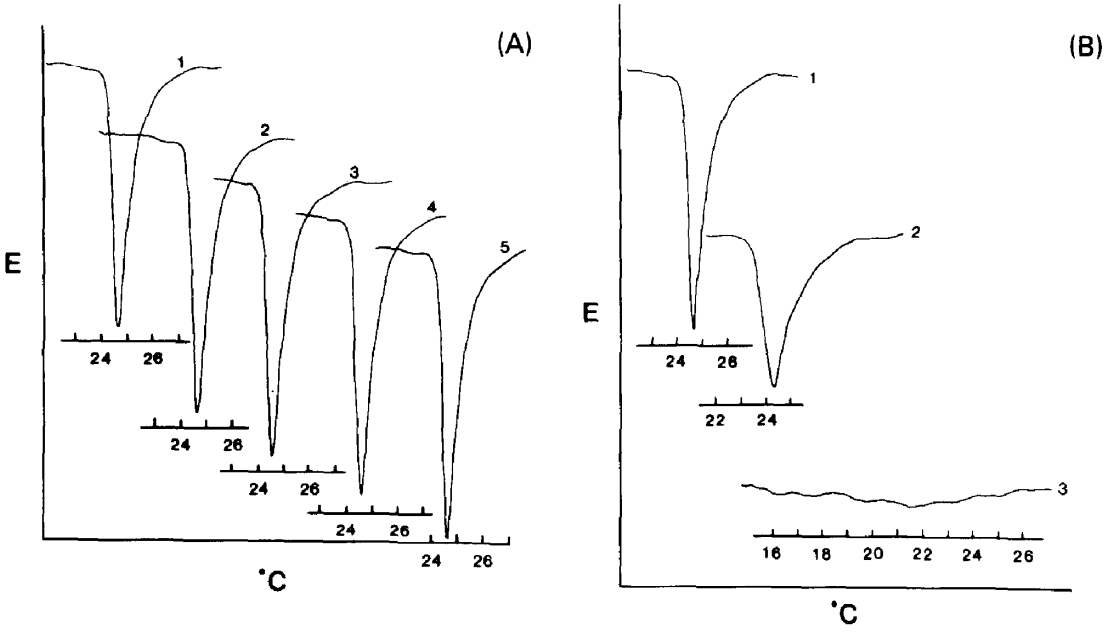

Fig. 1. (A) Effects of AraC, streptomycin, and gentamycin on the phase transition behavior of DMPC. Key: (1) DMPC alone; (2) DMPC + 2.9 mM AraC; (3) DMPC + 10.2 mM AraC; (4) DMPC + 12.2 mM streptomycin; and (5) DMPC + 13.1 mM gentamycin. (B) Effects of adriamycin and butacaine on the phase transition behavior of DMPC. Key: (1) DMPC alone; (2) DMPC + 9.2 mM adriamycin; and (3) DMPC + 23.3 mM butacaine. Run at 1°/min, 50 μV sensitivity, 50 mV chart span. Each pan contained 2.14 mg DMPC in Tris:KCl buffer. Sample volume = 35 μl. E = enthalpy.

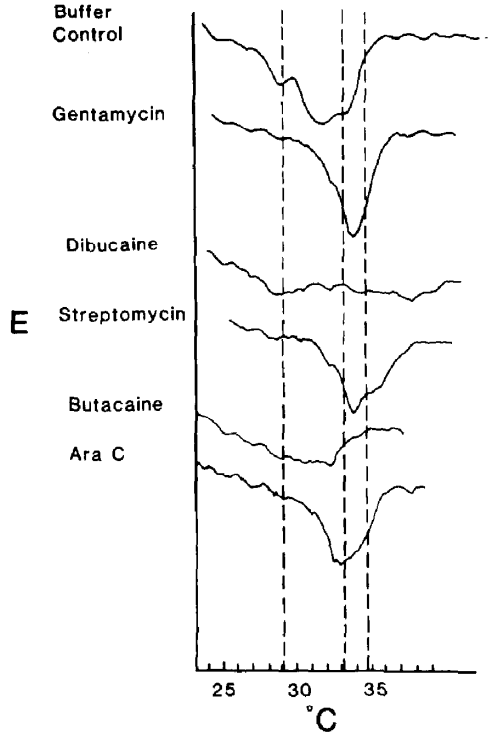

Fig. 2. Effects of AraC, butacaine, streptomycin, dibucaine, and gentamycin on the calorimetric profiles of the ternary fatty acid-containing lipid mixture. Lipid:Drug molar ratio = 40:1 (total lipid:total drug).

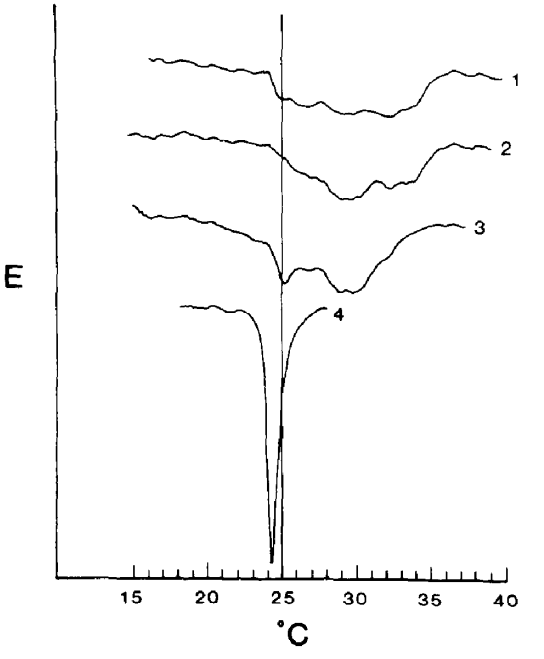

Fig. 3. Effect of adriamycin on the phase transition behavior of the fatty acid-containing ternary lipid mixture. Key: (1) ternary mixture + buffer; (2) ternary mixture + 13 mM adriamycin; (3) ternary mixture + 2.6 mM adriamycin; and (4) DMPC + 9.2 mM adriamycin.

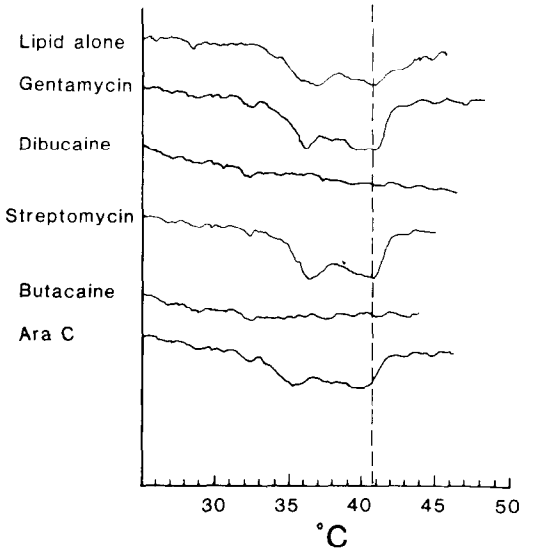

Fig. 4. Effects of several drugs on the calorimetric profiles of the alcohol-containing ternary lipid mixture. Total lipid:Total drug mole ratio = 40:1. The ordinate is enthalpy.

files are shown in Fig. 1, A and B. The sharp thermotropic transition of *pure DPMC* was unaffected by hydrophilic solutes such as AraC, streptomycin, and gentamycin. However, the thermotropic transition of pure DMPC was modified by the hydrophobic drug dibucaine and, to a lesser extent, by adriamycin. These latter drugs markedly broaden the transition; however, measurements of the effects of these drugs on the DPMC transition enthalpy indicate that dibucaine and adriamycin effects are mediated in quite different ways (see below). As shown in Fig. 2, in the absence of drug, the fatty acid-containing ternary lipid mixture exhibited a complex transition profile as expected from such a phase-separated system containing domains rich in DMPC (the lower melting region) and the domains rich in $LPC_{16} + FA_{16}$ [16]. At the lipid:drug mole ratios employed, gentamycin, streptomycin, and AraC eliminated or shifted the transition which was observed at the lower temperature end of the transition profile. This transition was presumably due to the DMPC-rich domain of the bilayer. However, since AraC does not influence pure DMPC bilayers, this suggests that AraC influences the phase transition behavior of the DMPC-rich regions of the ternary dispersions. At the mole ratios employed, the drugs dibucaine and butacaine had their most profound effects on the transitions located at the high temperature end of the transition profile (Fig. 2). Figure 3 illustrates the concentration dependence of adriamycin on the transition profiles of the fatty acid-containing ternary lipid mixture. Similar drug-dependent effects were observed in calorimetric studies of the alcohol-containing ternary mixtures (Fig. 4). Such dispersions are uncharged, but they possess essentially the same defect structures as the fatty acid-containing ternary mixtures [16]. Only dibucaine and butacaine had significant effects on the transition profiles of the ternary hexadecanol-

Table 1. Effects of drugs on the relative phase transition enthalpy of DMPC and the ternary lipid mixtures*

Lipid system	Drug	Enthalpy† (% of lipid alone)
DMPC		100
DMPC	Butacaine	0
DMPC	Streptomycin	100
DMPC	Gentamycin	100
DMPC	Arac	100
DMPC	Adriamycin	101
Ternary		100
Ternary	Adriamycin	138
Ternary	Adriamycin	95
Ternary	Arac	62
Ternary	Gentamycin	61
Ternary	Streptomycin	47
Ternary	Dibucaine	0
Ternary	Butacaine	38
Ternary-OL		100
Ternary-OL	Butacaine	0
Ternary-OL	Dibucaine	0
Ternary-OL	Gentamycin	121
Ternary-OL	Streptomycin	98
Ternary-OL	Arac	97

* Details of system composition can be found in the legends to Figs. 1–4.

† Enthalpy of DMPC dispersions was 6.4 kcal/mole, and those of the ternary codispersions containing DMPC + 1-Palmitoyllysolecithin + palmitic acid (3:1:1) was 8.8 kcal/mole of DMPC.

containing system (Fig. 4). These hydrophobic solutes essentially eliminated the transitions. Gentamycin, streptomycin, and AraC had minimal effects on the transition profiles. Comparison of these two sets of data involving the fatty acid-containing and the hexadecanol-containing ternary lipid mixtures suggests that electrostatic interactions between the positively charged drugs and the negatively charged head groups of the acid-containing bilayers play an important role in the observed drug–membrane interaction.

Table 1 summarizes the effects of various drugs on the relative enthalpy of the phase transition of DMPC and ternary lipid mixtures. In the pure DMPC system, which is not expected to contain defect structures of the sort described for the ternary mixtures [16], butacaine and adriamycin had profound effects on the transition half-width (Fig. 1B). Butacaine markedly reduced the transition enthalpy. The ability of adriamycin to broaden the DMPC phase transition without affecting the transition enthalpy suggests that this drug affected the cooperativity of the transition. Butacaine clearly interacted with the DMPC phase. Streptomycin, gentamycin, and AraC interacted minimally with pure DMPC under these conditions. Results involving the charged fatty acid-containing ternary lipid mixture suggest a qualitative picture of adriamycin-induced phase separation. At low adriamycin concentration, where phase separation is suggested by the observed transition profile (Fig. 3), the transition enthalpy increased to 138% relative to the ternary mixture alone. Increasing adriamycin concentration led to reduction of the transition enthalpy to 95%. AraC, gentamycin, streptomycin, and butacaine reduced

the transition enthalpy. Interactions of these drugs with uncharged ternary mixtures containing hexadecanol rather than palmitic acid yielded even more clear-cut results. Butacaine and dibucaine eliminated the transition whereas streptomycin and AraC had little effect on the transition enthalpy (Fig. 4). Gentamycin increased the enthalpy to 121% relative to the ternary mixture alone, suggesting that this antibiotic may enhance phase separations in this system. In summary, the enthalpy results reported here constitute additional evidence for the suggested electrostatic interactions between these drugs and the negatively charged bilayers. Thus, hydrophobic drugs such as butacaine have effects on all phases while the aminoglycoside antibiotics optimally interact with the ternary mixture containing negatively charged fatty acid. Two drugs, adriamycin and gentamycin, exhibited evidence of enhancing phase separation in ternary systems, at least at low drug concentrations.

Effects of drugs on the action of phospholipase A_2 on ternary lipid dispersions. These interactions were explored by observing the effects of drugs upon the PLA_2-catalyzed hydrolysis of the ternary phospholipid substrate. The equilibrium concentration-dependent inhibition of the effects of AraC, adriamycin, tobramycin, and streptomycin on the phospholipase A_2-catalyzed hydrolysis of the ternary lipid mixture is sigmoidal. The detailed inhibitor concentration-dependence of the rate of hydrolysis on AraC concentration, for example, is rather complex (Fig. 5C). At low inhibitor concentration there was little or no inhibition. Only beyond a certain inhibitor concentration did the extent of inhibition increase abruptly. This type of data suggests that the action of these solutes was not directly on the enzyme (see also Ref. 11). The abrupt increase in inhibition could be due to phase changes induced by the partitioning of the solute. The kinetics of inhibition of PLA_2 action by a variety of drugs were also evaluated via Dixon and double-reciprocal plots [18] (Fig. 5, A and B). All of the drugs examined showed noncompetitive inhibition. The initial rate of PLA_2-catalyzed hydrolysis of the ternary lipid dispersions decreased upon increasing the drug concentration, as did the ultimate extent of substrate hydrolyzed. The K_i values for several drugs are summarized in Table 2. Consistent with the previous results, the more hydrophobic drugs, such as butacaine and eto-

Table 2. Apparent K_i values characterizing the noncompetitive inhibition of the action of phospholipase A_2 on the ternary fatty acid-containing lipid mixture* by AraC and other drugs

Drug	K_i (mM)
AraC	1.24
Streptomycin	1.65
Gentamycin	1.28
Tobramycin	0.34
Adriamycin	0.16
Butacaine	0.08
VP-16†	0.06

* $LPC_{16} + FA_{16} + PC_{14}$; 18 mole% LPC + FA. See experimental section for details of experimental organization.

† Carrier free.

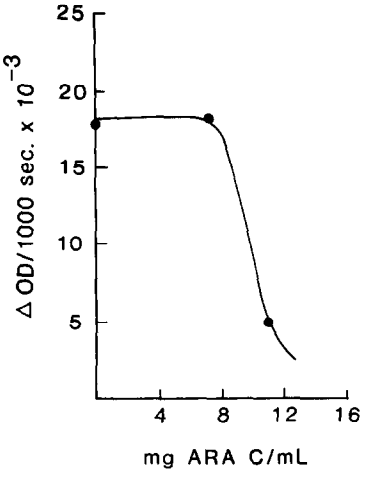

Fig. 6. Effect of AraC on the fusion rate of vesicles prepared by sonication of the fatty acid-containing ternary lipid mixture. See text for details.

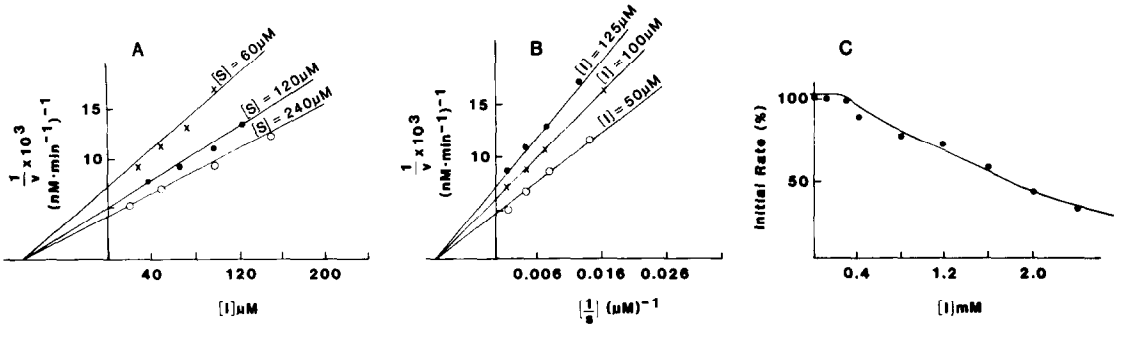

Fig. 5. Effects of butacaine and AraC on the PLA_2-catalyzed hydrolysis of the ternary lipid mixture. See the Experimental section for details of experimental organization. (A) Dixon plots for inhibition of hydrolysis of dimyristoylphosphatidylcholine in the fatty acid-containing ternary lipid mixtures by butacaine. (B) The $1/v$ versus $1/s$ plot in the presence of different fixed concentrations of the noncompetitive inhibitor butacaine. The hydrolysis was initiated by the addition of 0.4 μg of PLA_2 to the fatty acid-containing ternary lipid mixture in 100 mM KCl + 10 mM $CaCl_2$ at pH 8.0 and maintained at 30°. (C) Effect of AraC on the initial rate of hydrolysis of the fatty acid-containing ternary lipid dispersion. Assays were conducted at 30°, pH 8.0, in 100 mM KCl + 10 mM $CaCl_2$; 0.4 μg of PLA_2 was added per assay.

poside, had the lowest K_i values. Qualitatively, however, all of the drugs listed in Table 2 had similar effects on the PLA_2-catalyzed hydrolysis of the ternary lipid dispersion.

Effect of AraC on the rate of membrane aggregation/fusion. As shown in Fig. 6, the rate of fusion of ternary lipid vesicles containing fatty acid was determined as a function of the concentration of AraC. AraC inhibited the observed turbidity change with an approximate half-maximal effect at 4 mM. Such an effect would be anticipated if AraC modulated the boundaries of the phase-separated domains in the ternary dispersions [11], or affected the dehydration of the bilayer at membrane:membrane junctions.

DISCUSSION

The drugs utilized in this study included: AraC (cytarabine), which is a charged hydrophilic molecule; the aminoglycoside antibiotics gentamycin, streptomycin, and tobramycin; adriamycin (doxorubicin), a somewhat less hydrophilic drug; the anesthetic agents dibucaine and butacaine, both of which contain hydrophobic regions; and a glycosidic derivative of podophyllotoxin, VP-16, which has a low water solubility. This selection of drugs covers a broad range of structural types that have proven effective in discriminating various aspects of membrane structure and function.

It is clear from the results that the drugs examined here had profound effects on phase equilibria within the bilayer membrane. These effects were the result of selective interactions of these solutes with regions of the membrane. Perturbations of membrane phase equilibria are reflected in effects on the action of an extrinsic membrane protein (PLA_2) on the substrate bilayer and in inhibition of membrane fusion. Both of these functional consequences of drug:membrane partitioning are hypothesized to involve a common locus, that is, defect structures located at boundaries of laterally separated phases in the plane of the membrane. The detailed nature of the perturbation involved in inhibition of PLA_2 action on the membrane may be related to the extent of defect structures or the time distribution of defects in the membrane, but ultimately this effect is due to reduced binding of PLA_2 to bilayers in the presence of inhibitory solutes [19].

Many of the results are interpreted in terms of a model involving selective drug interactions with different regions of the ternary fatty acid-containing lipid dispersions. The DSC studies of the interaction of drugs with pure DMPC, fatty acid-containing ternary lipid mixtures, and the long chain alcohol-containing ternary lipid mixtures indicate the existence of regioselective interactions of these drugs with the membrane. While the relatively hydrophilic drugs such as gentamycin, streptomycin, and AraC did not interact with pure DMPC (single component membranes) to cause changes in the observed calorimetric profile, they did appear to interact preferentially with DMPC-rich regions of charged fatty acid-containing ternary lipid mixtures. Charge effects appeared to play an important role in the interaction of these solutes with the negatively charged ternary membrane system, since interactions observed via calorimetry were much reduced in the neutral long chain alcohol-containing ternary mixture. The effects of gentamycin, streptomycin, and AraC on the phase transition profile of the ternary fatty acid-containing lipid mixture appeared to be localized to the lower temperature transitions, those presumably involving DMPC-rich phases. More hydrophobic solutes such as dibucaine and butacaine appeared to generate their most pronounced effects in the higher temperature region, presumably involving domains relatively rich in $LPC_{16} + FA_{16}$.

The effects of the drugs on the relative transition enthalpies support and extend these conclusions. AraC, streptomycin, and gentamycin had essentially no effect on the transition enthalpy of pure DMPC membranes. Adriamycin strongly affected only the transition half-width without affecting the enthalpy of the transition. This suggests that adriamycin affects the cooperativity of the transition. In contrast, butacaine essentially eliminated the transition; hence, the cooperative unit was eliminated. Consistent with our observations of drug effects on the transition profiles of the neutral and negatively charged ternary lipid mixtures, substantial differences existed between drug effects on relative transition enthalpies. AraC, gentamycin, streptomycin, dibucaine, and butacaine all reduced the relative transition enthalpy of the fatty acid-containing ternary mixture. At low concentrations, adriamycin appeared to increase the relative transition enthalpy, again suggesting that phase separation was increased by this drug in this lipid system. AraC and streptomycin had little effect on the uncharged ternary mixtures containing long-chain alcohol. Gentamycin may enhance phase separation to some extent in this neutral system. Both butacaine and dibucaine eliminated the transition enthalpy. It is interesting to note that these latter two drugs were indistinguishable in their effects on the relative transition enthalpy of the uncharged ternary mixture, but they appeared to be distinguishable in their effects on the negatively charged ternary system.

Thus, these drugs were selective in their interactions with different regions of the membrane in the ternary systems. Charge played a role in the interaction of the charged hydrophilic drugs. Over some concentration ranges, membrane phase separations were enhanced by partitioning of adriamycin (fatty acid-containing ternary system) and gentamycin (long chain alcohol-containing ternary system). AraC affected the rate of membrane aggregation/fusion (with half-maximal effect at ~4 mM under the noted conditions of drug concentration, lipid phase, and aqueous phase). Such an effect would be anticipated if AraC modulated the boundaries of the phase-separated domains in the ternary dispersions [11].

The drugs evaluated in this study apparently interact with different domains of these phase-separated lipid dispersions. Whatever the detailed nature of the drug:membrane interactions, all of the drugs examined exhibited noncompetitive inhibition in the PLA_2:membrane system. The observation of noncompetitive inhibition may tentatively be interpreted

in terms of PLA_2:substrate interface interactions. This requires that membrane defect structure be altered in a manner that allows binding by PLA_2, but which affects enzyme turnover. The inhibitors presumably lowered the concentration of defect structures by shifting the equilibrium of phase-separated domains towards a mixed phase. PLA_2 binds to the defect sites in the catalytically active state. The concentration of this state was lowered by the solute-induced shift in the phase equilibrium. Thus, V_{max} and the extent of hydrolysis were lowered because only the enzyme bound to the substrate interface was catalytically active. The K_m in this system is a measure of interfacial binding [14]. Since the apparent K_m does not change in the presence of an inhibitor, it implies that only the formation of the catalytically active complex was blocked.

The observed sigmoidal concentration-dependence of AraC inhibition of PLA_2 action on membrane bilayers and on membrane fusion suggests that an apparently cooperative reorganization of membrane structure was induced once a threshold membrane concentration of drug was achieved. The drug concentration range over which the membrane structural transition occurs may depend to a great extent on the composition of the exposed membrane and its constituent proteins. For example, hydrophilic drugs, such as AraC, had little impact on the phase behavior of ternary alcohol-containing lipid mixtures. Thus, selective membrane sensitivity to chemotherapeutic agents may reasonably be expected. Sigmoidal drug concentration dependence also suggests that only a relatively narrow range of drug concentrations will determine the difference between therapeutic and toxic drug effects.

Whatever the exact mechanism for the membrane effects of these positively charged drugs, as reflected in decreased rate of PLA_2-catalyzed hydrolysis, it is clear that inhibition of phospholipases A and C can have pronounced pathological consequences [20]. Tolleshang *et al.* [21] interpret observed effects of dibucaine and related compounds on the uptake of asialo-glycoproteins in isolated hepatocytes in terms of inhibitory effects of local anesthetics on endocytosis and catabolic processes in isolated hepatocytes. Such reports and our observation of inhibition of the action of phospholipase A_2 suggest that perhaps the observed central nervous system toxicity occasionally observed during high-dose AraC therapy [22] may result from inhibition of phospholipases, or inhibition of lysosome function, or any other function that requires phase-separated domains in bilayers. Of course, Ara-C-induced phase changes in the membrane may affect its own transport across the cell membrane.

The studies reported here have demonstrated a number of important perturbations of membrane structure and function induced by cytarabine and other agents. Such observations satisfy a necessary condition supporting the hypothesis that drug:cell membrane interactions, at least in part, are involved in high dose cytarabine cytotoxic effects. These results alone, however, are not sufficient to demonstrate that this hypothesis is correct.

REFERENCES

1. S. Schwartz, B. Morgenstern and R. L. Capizzi, *Cancer Res.* **42**, 2191 (1982).
2. B. A. Chabner, in *Pharmacologic Principles of Cancer Treatment* (Ed. B. A. Chabner), pp. 387–401. W. B. Saunders, Philadelphia (1982).
3. Y. M. Rustum, *Analyt. Biochem.* **90**, 289 (1978).
4. A. P. Early, H. D. Preisler, H. Slocum and Y. M. Rustum, *Cancer Res.* **42**, 1587 (1982).
5. S. Rudnick, E. C. Cadman and R. L. Capizzi, *Cancer, N.Y.* **44**, 1189 (1979).
6. S. Rudnick, E. C. Cadman and R. L. Capizzi, *Blood* **50**, (Suppl.), 229 (1977).
7. H. S. Schwartz, H. D. Preisler and P. M. Kanter, *Leuk. Res.* **5**, 366 (1981).
8. K. W. Kohn, R. A. Ewig, L. C. Erickson and L. K. Zwelling, in *DNA Repair* (Eds. E. C. Friedberg and H. P. Hanawa), Vol. 1, Part B, pp. 379–401. Marcell Dekker, New York (1981).
9. T. R. Tritton and G. Lee, *Science* **217**, 248 (1982).
10. M. K. Jain and N. M. Wu, *J. membr. Biol.* **34**, 157 (1977).
11. M. K. Jain, M. Streb, J. Rogers and G. H. De Haas, *Biochem. Pharmac.* **33**, 2541 (1984).
12. D. Ruppel, H-G. Kapitza, H. J. Galla, F. Sixl and E. Sackmann, *Biochim. biophys. Acta* **692**, 1 (1982).
13. B. Fourcans and M. K. Jain, *Adv. Lipid Res.* **12**, 147 (1974).
14. M. K. Jain, M. R. Egmond, H. M. Verheij, R. Apitz-Castro, R. Dijkman and G. H. De Haas, *Biochim. biophys. Acta* **688**, 341 (1982).
15. R. Apitz-Castro, M. K. Jain and G. H. De Haas, *Biochim. biophys. Acta* **688**, 349 (1982).
16. M. K. Jain and G. H. De Haas, *Biochim. biophys. Acta* **642**, 203 (1980).
17. K. Elamrani and A. Blume, *Biochemistry* **21**, 521 (1982).
18. I. H. Segel, *Enzyme Kinetics*, p. 111. John Wiley, New York (1975).
19. M. K. Jain and D. V. Jahagirdar, *Biochim. biophys. Acta* **814**, 319 (1985).
20. K. Y. Hostetler and L. B. Hall, *Proc. natn. Acad. Sci. U.S.A.* **79**, 1663 (1982).
21. H. Tolleshang, T. Berg and K. Holte, *Biochim. biophys. Acta* **714**, 114 (1982).
22. M. D. Winkellman and J. D. Hines, *Ann. Neurol.* **14**, 520 (1983).