The Surface Charge of Membranes Modulates the Interaction with the Anthracycline Daunomycin[a]

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The cytotoxic effects observed for nonpenetrating, polymerimmobilized anthracycline[1] strongly suggest that the interaction of the drugs with the plasma membrane of tumor cells may be important in the molecular mechanisms of drug cytotoxicity. In the present study, we have used positively (PCL) and negatively (NCL) charged liposomes (Avanti Polar Lipids) as model systems to determine how the surface charge of the membrane influences its interaction with ionized drugs such as daunomycin (DNM), an anthracycline antibiotic that contains an ionizable amino group (pK $\simeq$ 7.6–8.2) at the daunosamine sugar moiety.

Equilibrium binding of DNM to liposomes was carried out by using ultracentrifugation and fluorescence anisotropy techniques to distinguish between free and liposome-bound drug (FIG. 1). pH values ranging from 6 to 8.3 were chosen for the studies to produce different ionization states of the drug without changing the net charge of the stearylamine and the dicetyl phosphate groups present in the PCL and NCL, respectively. Binding parameters were obtained from Klotz plots, which gave straight lines with a slope equal to $1/n$, where n is the maximum number of binding sites per phospholipid molecule, and a y-intercept of $1/(n \cdot K_{app})$, where K_{app} is the apparent binding constant and $(n \cdot K_{app})$ equals the overall binding constant, K_s.

FIGURE 1 shows direct binding data corresponding to the interaction between DNM and NCL (**A**) or PCL (**B**) at different pH. It should be noted that the abscissa in **B** needs to include much higher liposome concentrations (in terms of phospholipid contents) to begin to evidence saturation.

The results clearly suggest that the interaction between the drug and the vesicles is mainly governed by the presence of negative charges at the liposome surface, while the ionization state of the drug, as evidenced from the results obtained at different pH, is less important in altering drug binding. Nonetheless, drug-binding parameters, as determined from Klotz plots, were sufficiently sensitive to the pH as to discriminate between binding of neutral and cationic species of the drug (TABLE 1). For either NCL or PCL, high pH values favoring the presence of un-ionized drug species result in larger K_s values due to an increased binding stoichiometry. Furthermore, fluorescence resonance energy transfer studies similar to those reported previously[2] indicate that the

[a]This work is supported by Grants 950/84 from CAICYT and 87/1634 from FISS.

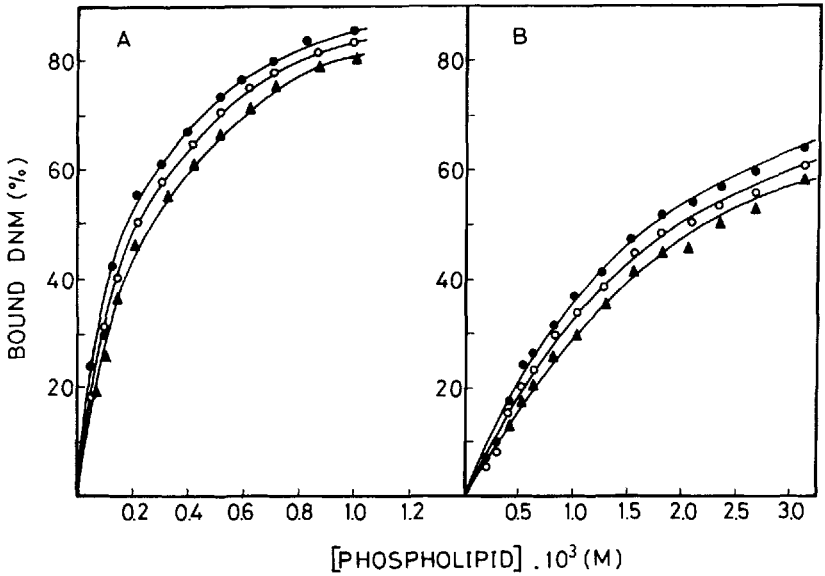

FIGURE 1. Binding of DNM to negatively (**A**) and positively (**B**) charged liposomes at 25°C. The steady-state fluorescence anisotropy of a 2.5-μM DNM solution was determined in the presence of increasing amounts of liposomes by using a Perkin-Elmer LS-5 spectrofluorimeter equipped with total emission and automatic polarization accessories. Excitation wavelength was 480 nm and a Corning 3-68 filter was used to eliminate scattered light. The concentrations of free and liposome-bound DNM were determined at each phospholipid concentration as described by Burke and Tritton.[5] Buffers used in the experiments contained 100 mM NaCl in addition to either (●) 10 mM EPPS, pH 8.3; (○) 10 mM HEPES, pH 7.6; 10 mM HEPES, pH 7.0 (not shown), or (▲) 10 mM PIPES, pH 6.1.

TABLE 1. Binding Constants and Stoichiometries for the Interaction between DNM and Positively (PCL) or Negatively (NCL) Charged Liposomes at Different pH[a]

	pH			
	6.1	7.0	7.6	8.3
NCL				
n^b	0.032	0.037	0.050	0.080
$K_s(M^{-1})$	4764	4975	5180	5515
$K_{app} \cdot 10^5\ (M^{-1})$	1.50	1.34	1.04	0.70
PCL				
n	0.0048	0.0055	0.0071	0.0089
$K_s\ (M^{-1})$	485	500	570	675
$K_{app} \cdot 10^5\ (M^{-1})$	1.0	0.91	0.78	0.74

[a]Binding parameters are averages from 2–3 measurements and were determined from Klotz plots, as indicated in the text. Linear correlation coefficients were better than 0.9 in all cases.
[b]n: moles of DNM/mol of phospholipid.

un-ionized form of the drug has access to hydrophobic domains located deep within the lipid bilayer, which are less accessible to ionized DNM (data not shown).

The observations reported here might have implications in cellular drug resistance. Since some drug-resistant cells have less negatively charged phospholipids than their parental drug-sensitive lines,[3] it is possible that a decreased drug binding to the resistant cell membrane could partly account for the observed decreased intracellular drug accumulation exhibited by most drug-resistant cells.[4]

REFERENCES

1. TRITTON, T. R. & G. YEE. 1982. The anticancer agent adriamycin can be actively cytotoxic without entering cells. Science **217:** 248–250.
2. FERRER-MONTIEL, A. V., J. M. GONZALEZ-ROS & J. A. FERRAGUT. 1988. Association of daunomycin to membrane domains studied by fluorescence resonance energy transfer. Biochim. Biophys. Acta **937:** 379–386.
3. FERRAGUT, J. A. Submitted for publication.
4. MYERS, C., K. COWAN, B. SINHA & B. CHABNER. 1987. The phenomenon of pleiotropic drug resistance. *In* Important Advances in Oncology. V. T. DeVita, S. Hellman & S. A. Rosenberg, Eds. Lippincott. Philadelphia, PA.
5. BURKE, T. G. & T. R. TRITTON. 1985. Structural basis of anthracycline selectivity for unilamellar phosphatidylcholine vesicles: An equilibrium binding study. Biochemistry **24:** 1768–1776.