Vol. 84, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1978 Pages 802-808

ADRIAMYCIN: A PROPOSAL ON THE SPECIFICITY OF DRUG ACTION

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Received August 14, 1978

SUMMARY: Adriamycin has been found to decrease the gel to liquid crystal transition temperature of liposomal membranes of varying compositions. However, when a low level of cardiolipin was inserted into a lecithin-containing membrane matrix, drug interaction caused the opposite effect on the thermal transition. It is suggested that this phenomenon may be indicative of specificity in the cytotoxic action of adriamycin on tumors, because evidence exists which indicates that certain neoplastic cells may contain cardiolipin in their plasma membrane and thus present a different surface to the drug than a non-malignant cell.

INTRODUCTION

Adriamycin is an important agent in the treatment of malignant diseases of man (1). The mechanism of action of this agent is generally considered to involve intercalation into double helical DNA and subsequent inhibition of DNA and/or RNA synthesis (2), although other sites of action have been proposed. One of these additional actions of adriamycin, shown recently by our laboratory (3), is an effect on the cell surface; action is expressed as an increase in the rate of agglutination of Sarcoma 180 cells by concanavalin A (Con A) after their exposure in culture to relatively low (i.e., 10^{-7} M) concentrations of adriamycin. Since neither the rate nor the extent of Con A binding to Sarcoma 180 cells are affected by the drug, a direct membrane alteration, possibly at the level of fluidity, is conceivable. Other investigators have also reported membrane effects of adriamycin on red blood cells (4) and Ehrlich ascites cells (5). In addition, an important series of observations which appear to diminish the importance of the actions of the anthracycline antibiotics at the level of the nucleic acids, are that a congener of adriamycin, N-trifluoroacetyl adriamycin-14-valerate (AD-32), is an effective antineoplastic agent, but apparently does not enter the

0006-291X/78/0843-0802$01.00/0

cell nucleus (6) or bind to isolated DNA (7). Thus, there is a persuasive body of experimental results which implicate cellular membrane alterations in the cytotoxic action of the anthracyclines. Even if the plasma membrane is not the primary target for the cytotoxic action of agents of this class, it is clear that interaction at this level is still required for entry into the cell. Consequently, it is reasonable to assume that the structure of the cell membrane may be an important determinant of the therapeutic efficacy of adriamycin, regardless of the ultimate cellular target of the drug.

Because the cell surface is such a complex structural entity, we are attempting to dissect the problem of adriamycin-membrane interactions into more manageable proportions. To this end, we have been investigating the effect of this antibiotic on the lipid component of membranes by employing sonicated liposomes as a model of the phospholipid bilayer. One of the widely discussed features of biological membranes is fluidity, a property readily measurable in the liposome system. Fluidity is a measure both of the degree of flexibility of the hydrocarbon chains of the lipid bilayer and of the ease of their lateral motion within the plane of the membrane. Pure phospholipid dispersions undergo a thermotropic phase change from the solid (gel) to fluid (liquid crystal) states at a characteristic temperature, the T_m. The physical properties of the two states differ, such that the transition can be followed using various physical techniques; in the present study, we have used the easily measured property of light scattering in the forward direction (i.e., turbidity).

MATERIALS AND METHODS

Adriamycin (NSC 123127) was obtained from the Division of Cancer Treatment of the National Cancer Institute. Stearylamine was obtained from K & K Laboratories, cardiolipin from Miles Laboratories, and the other lipids from Calbiochem or Sigma Chemical Company. Methods are described in the legend to Figure 1.

RESULTS AND DISCUSSION

The effect of adriamycin on the phospholipid phase transition of various kinds of liposomes is shown in Figure 1 and Table 1. In each experiment,

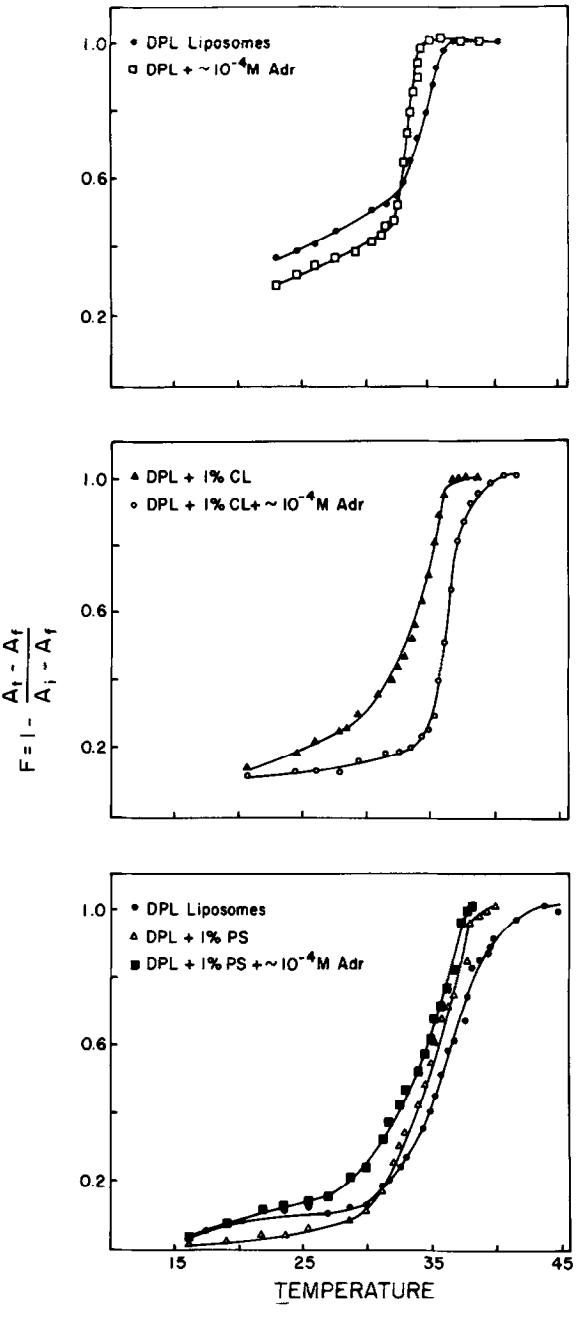

FIGURE 1: Transition curves for dipalmitoyl lecithin (DPL) liposomes with small amounts of other lipids incorporated into the bilayer in a Ca^{2+}-Mg^{2+}-free phosphate buffered 0.9% NaCl solution (pH 7.4). The liposomes were sonicated for 2 to 3 hours above the thermal phase transition temperature. Subsequently, the drug was added and allowed to equilibrate for at least 4 hours. The thermal transitions were determined by measuring the turbidity

TABLE 1

Transition temperatures for dipalmitoyl lecithin (DPL) liposomes containing small amounts of other lipids incorporated into the bilayer in the absence and presence of 10^{-4} M adriamycin.

Liposome composition	T_m Control	T_m Adriamycin
DPL	34.6	33.3
DPL + 1% cardiolipin, bovine	33.3	35.4
DPL + 1% phosphatidyl serine, bovine	33.2	31.7
DPL + 1% dipalmitoyl phosphatidyl glycerol	33.6	33.0
DPL + 1% sphingomyelin, bovine	33.7	33.3
DPL + 1% stearyl amine	35.4	35.0

Data were obtained as described in the legend to Figure 1.

a synthetic lecithin (dipalmitoyl phosphatidyl choline) provided a matrix in which other lipids were dispersed. These pure lecithin liposomes showed a cooperative phase transition centered at 34.6°, which is in reasonable agreement with the value of 37° obtained by Suurkuusk et al. (8) using calorimetric and fluorescence probe techniques. Addition of 10^{-4} M adriamycin to lecithin liposomes consistently lowered the transition temperature by about a degree, an effect indicative of a fluidizing action of the drug. Although 10^{-4} M adriamycin is higher than concentrations used in biological studies, it is not an unreasonable concentration for the type of physical observations described in this report (9). Adriamycin also lowered the T_m of various other membranes of mixed lipid composition, including those with a net negative surface charge (1% phosphatidyl serine inserted), and a net positive surface charge (1% stearyl amine inserted) and with spingomyelin. Conversely, however, liposomes

of the liposomes as a function of increasing temperature in a thermostatically controlled Gilford 2400-S spectrophotometer. In each case, the fraction (F) of the total transition is plotted versus temperature. The T_m is defined as the temperature at which the dispersion has progressed half-way between its initial (gel) and final (liquid crystal) states. The T_m values were generally reproducible to within $\pm$ 0.5°C and the trends of the data for the various combinations of lipids were consistently repeatable over several experimental determinations. Recycling the samples through a second heating yielded comparable results indicating that aggregation (8) or hysterisis, if they occurred, did not modify the T_m or the effects of the drug. Abbreviations used are: DPL, dipalmitoyl phosphatidyl choline; CL, cardiolipin; PS, phosphatidyl serine; Adr, adriamycin.

containing 1% cardiolipin (i.e., diphosphatidyl glycerol) exhibited a higher T_m in the presence of adriamycin, indicating that the antibiotic makes such bilayers less fluid. This difference is not simply a nonspecific effect of an acidic phospholipid because phosphatidyl serine-containing liposomes are made more fluid by the drug, in a manner analogous to pure lecithin. Likewise, phosphatidyl glycerol, which is one-half of a cardiolipin molecule, behaves like all of the other lipids studied, in that adriamycin fluidized lecithin membranes containing this lipid. Thus, adriamycin has a differential or unique effect on the fluidity of the liposomal membrane which contained cardiolipin.

A possible explanation for the different effects of adriamycin on cardiolipin-containing liposomes is that the drug has a different affinity for such membranes. Using a fluorescence titration technique (10), we have measured the equilibrium binding properties of adriamycin with both dimyristoyl lecithin liposomes and with these membranes containing 1% cardiolipin. Scatchard plots (not shown) of the data are nearly coincident for both types of liposomes. The plots are curved indicating the lack of a single type of binding site and yield a limiting affinity constant of about 6×10^3 M^{-1}. Thus, these data provide no suggestion that the presence of cardiolipin creates a unique binding site or altered affinity of the liposomes for adriamycin; for this reason, we have concluded that the differential effect of adriamycin on cardiolipin-containing membranes is due to altered membrane organization which occurs when this lipid is present. Since the differential change in membrane fluidity is manifested when only 1 molecule of cardiolipin is present with 99 other lipid molecules, it appears that cardiolipin may provoke a long range effect on membrane structure. That is, the influence of cardiolipin must extend over a range greater than simply its nearest neighbors. This action may be important in cellular membranes which exist as a fluid mosaic of organized neighborhoods, because long range ordering by specific components allows for communication between membrane domains.

It is also conceivable that this phenomenon may be involved in the sensitivity of neoplastic cells to the anthracycline antibiotics. Available data

on the phospholipid composition of normal and malignant fibroblast and liver cells show a striking anomaly with respect to cardiolipin (11,12). This lipid, which is normally restricted to mitochondrial membranes, may be found in all cellular membranes upon malignant transformation. Thus, there is a dedifferentiation of membranes with respect to the disposition of cardiolipin. This trend has been shown to be less pronounced in minimal deviation hepatomas and absent in regenerating rat liver (13) and, therefore, is a characteristic of certain cancers, and not simply related to the growth rate. Because of the potential long-range structural effects of cardiolipin discussed above, a tumor cell containing cardiolipin in its plasma membrane might be expected to present a different surface to an incoming adriamycin molecule than a normal cell (or a cancerous cell) which lacks cardiolipin in the surface membrane. A differential behavior of adriamycin on the fluidity of membranes containing cardiolipin could provide an explanation for some of the specificity of its cytotoxic action on normal and neoplastic cells. It is important to stress that this hypothesis does not require the ultimate cellular target for adriamycin to reside at the level of the surface membrane, only that the drug must interact with this membrane at some time during its encounter with a cell. Moreover, the concept may explain, at least in part, the observed cardiac toxicity of this agent (1), in that heart muscle, which is rich in respiring mitochondria, might be a sensitive target because membranes of mitochondria are the major repository for cardiolipin in normal cells. Thus, we suggest that the phospholipid composition and distribution in cellular membranes may play an important role in determining the susceptibility of neoplastic cells to the anthracycline antibiotics, as well as the toxic side effects of these agents to heart tissue.

ACKNOWLEDGEMENTS

This work was supported by U.S. Public Health Service Grants CA-10748, CA-02817, and CA-16359 and a Grant from the Swebilius Foundation.

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