Involvement of Plasma Membrane Lipid Structural Order in Adriamycin Resistance in Chinese Hamster Lung Cells

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ABSTRACT

Plasma membrane preparations from Chinese hamster lung cells, which are resistant to the antitumor agent Adriamycin, were analyzed using fluorescence polarization of the membrane lipid probe trans-parinaric acid. The results of these studies reveal that membranes from several drug-resistant isolates have a substantial decrease in lipid structural order relative to membranes from drug-sensitive cells. Additional studies have shown that certain isolates are unstable and undergo a sequential phenotypic reversion after continuous passage in culture. Thus, we have identified cells which have reverted for membrane lipid physical changes but which still remain highly resistant to Adriamycin. At later passages, these cells are found to revert to drug sensitivity. These results indicate that an alteration of plasma membrane lipid structural order is not an essential component of the Adriamycin-resistant phenotype. However, in certain isolates, drug resistance and changes in membrane physical properties are both associated with an unstable genetic element.

INTRODUCTION

Previous studies have shown that cells resistant to the antitumor agent Adriamycin are highly defective in the cellular accumulation of drug (2, 3, 5, 15, 16). This defect appears to be primarily due to impaired drug transport into the cell (15, 16), and/or a major enhancement of a drug efflux mechanism (4, 5, 16). The results of these studies suggest that plasma membrane alterations may make a significant contribution to the drug-resistant phenotype. Consistent with this is the finding that plasma membranes of drug-resistant cells contain a phosphorylated glycoprotein (P-180) which is not detected in cells sensitive to Adriamycin (1, 2). Recent studies have provided evidence that there is a strong correlation between the presence of this protein and drug resistance (1). Additional studies also indicate that phosphorylation plays an important role in regulating the biological activity of P-180 (1, 2).

Recently, several laboratories have reported that cells resistant to Adriamycin have an alteration in the lipid "fluidity" of the cell surface (9, 13, 18). It was thus speculated that drug resistance may be related to this cellular change. In the present study, we use steady-state fluorescence polarization to determine that membrane physical properties of resistant cells are significantly altered relative to membranes from cells sensitive to drug. However, by analyzing cells which have reverted to drug sensitivity, we show that the alterations in these membrane physical properties are not required in order for a cell to exhibit drug resistance.

MATERIALS AND METHODS

Cells. Chinese hamster lung cells resistant to Adriamycin were isolated as described previously (3). Both sensitive and resistant cells were cultured in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal calf serum.

Plasma Membranes. Plasma membranes from sensitive and resistant cells were isolated as previously described (10). The crude cell membrane preparation was applied to a discontinuous sucrose gradient (15 to 60% sucrose) and centrifuged for 2 hr at 35,000 rpm in the Spinco SW50.1 rotor. The plasma membranes and endoplasmic reticulum fractions were collected, diluted in 0.01 M Tris-HCl (pH 7.6), and thereafter pelleted by centrifugation for 1 hr at 35,000 rpm in the Spinco SW50.1 rotor. The isolated membrane preparations were suspended in 0.01 M Tris-HCl (pH 7.6). The purity of the plasma membrane fraction was determined as described previously (2). As determined by electron microscopy and marker enzyme analysis (12), there is no detectable difference in the plasma membranes from drug-sensitive and -resistant cells.

Fluorescence Polarization. Fluorescence polarization analysis, using TPNA (10, 11) (obtained from Dr. R. D. Simoni, Biological Sciences, Stanford University, Stanford, CA) was performed as previously described (11). Corrections for scattering depolarization were made, when necessary, by the method of Lentz et al. (7). Computer analysis and curve-smoothing methods have also been previously reported (11).

RESULTS

The results of a typical fluorescence polarization experiment, using TPNA, are shown in Chart 1. Chart 1A shows the temperature-dependent fluorescence polarization of the probe in plasma membrane preparations from sensitive and resistant cells. Standard deviations of the values shown are not presented in this figure, since in all cases the deviations are less than 0.03 unit. It is apparent that the polarization ratio, which is inversely correlated with probe rotational mobility (14), was lower in the resistant cell membranes. This difference was maintained throughout the temperature range of 10–45°. Equivalent preparations from a separate resistant isolate also exhibit a decreased polarization ratio throughout this temperature range (Chart 1). As shown in Table 1, the plasma membranes from 3 independent isolates of Adriamycin-resistant cells exhibit similar changes in fluorescence polarization using TPNA as a probe of lipid motion. These results suggest that plasma membranes from resistant cells exhibit less structural order than do those isolated from cells sensitive to

1This investigation was supported in part by Research Grant CA-28120 from the National Cancer Institute, Department of Health and Human Services, and by American Cancer Society Grant IN-115, through the Mid American Cancer Center.

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3In the absence of independent measurements of fluorescence lifetimes, decreases in steady state fluorescence depolarization cannot be unambiguously attributed to increases in "fluidity." Thus the expression fluidity, or decrease in lipid structural order, is used here in its broadest sense, denoting changes in the rate of rotation and/or the distribution of the fluorophore in the anisotropic lipid bilayer of plasma membranes.

Received December 6, 1983; accepted July 30, 1984.

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This abbreviation is used here: TPNA, trans-parinaric acid (all-trans-9,11,13,15-octadecatetraenoic acid); DMS, double minute chromosomal spheres.
Membrane Changes in Adriamycin-resistant Chinese Hamster Lung Cells

Chart 1. Fluorescence polarization of TPNA in plasma membranes and endoplasmic reticulum was not altered in resistant cells (Chart 1B), thus suggesting that this change in lipid physical properties is specific for the plasma membrane.

In order to examine the involvement of membrane structural order in Adriamycin resistance, selected revertants were isolated and characterized. Isolation of these revertants is based on the finding that isolate R3 becomes sensitive to Adriamycin after several passages in culture. We have found that by following phenotypic reversion after several passages we can demonstrate that distinct genetic loci are involved in membrane changes and drug resistance. In these studies, we analyzed plasma membrane lipid physical properties and drug resistance in the R3 isolate after passages 10, 40, 70, and 110. As shown in Chart 2, the TPNA fluorescence polarization in plasma membranes from R3P40 is considerably lower than TPNA fluorescence polarization in isolated plasma membranes from sensitive cells. This isolate is also highly resistant to Adriamycin (Table 1). The early passage R3 isolate, R3P10, also exhibits membrane physical properties which are indentical to those of R3P40 (Table 1). This isolate is also highly resistant to Adriamycin (Table 1). However, an analysis of R3P70 showed that the plasma membranes from these cells were no longer less ordered than those from drug-sensitive cells (Chart 2). These cells are however still highly resistant to Adriamycin (Table 1). If R3P70 is grown until passage 110, we now find that these cells have reverted to drug sensitivity (Table 1), and have the same membrane physical properties as R3P70 (Chart 2).

DISCUSSION

In the present study fluorescence polarization has been used to analyze the lipid fluidity of plasma membranes from cells resistant to Adriamycin. Isolated plasma membranes were examined, since studies with whole cells have been shown to be unreliable, due to probe accumulation in intracellular lipid droplets which are very fluid (8, 17). The results of the present study demonstrate that membranes from several independent drug-resistant isolates are considerably less ordered than are membranes from sensitive cells. This alteration in membrane physical properties appears to be confined to the plasma membrane, since similar changes are not observed for isolated endoplasmic reticulum. Recently, several laboratories have also reported plasma membrane fluidity changes in cells resistant to Adriamycin. Siegfried et al. (13) have utilized electron spin resonance spectroscopy to analyze fluidity changes in drug-resistant Sarcoma 180 cells. The results of these studies demonstrated that several different drug-resistant isolates had an increase in membrane fluidity. Wheeler et al. (18) have also found that the murine tumor line, MDAY-K2, selected for Adriamycin resistance, exhibits an increase in membrane fluidity, as determined by fluorescence polarization analysis in the presence of diphenylhexatriene. In contrast to these results and those which we have reported, Ramu et al. (9) have found that Adriamycin-resistant P388 murine leukemia cells exhibit a decrease in lipid fluidity, as compared to drug-sensitive cells. These results were obtained by analyzing fluorescence polarization of diphenylhexatriene incubated with cells sensitive and resistant to drug. Since all of these studies were carried out without an analysis of cell revertants, it becomes of considerable interest to determine if an alteration in membrane lipid physical properties is actually required for a cell to exhibit drug resistance. The results of the present study indicate that this is not the case.
selected revertants reveals that cells can exhibit membrane physical properties closely similar to the parent cell, but still be resistant to Adriamycin. It should be pointed out that the changes observed in fluorescence polarization values for the membranes used in this study, and for the whole cells used in the previous studies (9, 18), are not necessarily due solely to changes in membrane lipid rotational motion. Changes in the fluorescence lifetime of the probes used (7, 8, 14), either due to association with other lipids or with membrane proteins, could conceivably be responsible for the differences in fluorescence polarization ratios between sensitive and resistant cells. In the absence of direct measurements of fluorescence lifetime, therefore, our results and previous results must be interpreted with caution. However, the electron spin resonance measurements of Siegfried et al. (13) also suggest that membrane lipid motion is increased in resistant cells; these results do not suffer from this limitation. We feel that it is safe to assert that alterations in membrane lipid physical properties, loosely referred to as fluidity, are associated with resistance to Adriamycin. The results presented in this report also allow us to say that these alterations, regardless of the exact nature of the motional change, are not a direct cause of Adriamycin resistance. It is interesting, however, that of several drug-resistant isolates tested, all exhibit an alteration in plasma membrane lipid structural order at early passages. This may suggest that these changes have an indirect role in drug resistance. One possible explanation is that the development of resistance is a multistage process. Membrane lipid changes may play a role in the early development stages and would thus not be required once resistance is established.

Another possible explanation for the present findings is related to the study of Kaufman et al. (6), who have provided evidence that amplified gene-containing DMS are responsible for an unstable phase of methotrexate resistance. It thus seems possible that DMS or some other unidentified unstable genetic element are formed in response to cell treatment with Adriamycin. These chromosomal elements would contain the genetic loci for the development of both drug resistance and altered membrane physical properties. These 2 phenotypes would be closely linked genetically, and would appear in a high proportion, or perhaps all, cells resistant to Adriamycin. In certain instances, such as the R6 isolate, the DMS may be incorporated into the genome, and thus generate a stable phenotype. In other cases such as the R3 isolate this may not be the case, and the cells would be unstable and undergo a reversion for membrane physical properties and resistance.

ACKNOWLEDGMENTS

Expert technical assistance was provided by Debra Garman and Leisa Albers.

REFERENCES

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Cancer Res 1984;44:4978-4980.

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