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INTERACTION OF ANTITUMOR DRUGS WITH HUMAN ERYTHROCYTE GHOST MEMBRANES AND MASTOCYTOMA P815 : A SPIN LABEL STUDY

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SUMMARY

The cytotoxic and mutagenic properties of antitumor drugs such as adriamycin, acridines, diacridine, actinomycin D and Pt compounds are related to their interaction with nucleic acids and inhibition of protein synthesis. We have examined their interaction with human erythrocyte ghost membranes and murine mastocytoma cells using spin labeling techniques. These drugs induce changes in electron spin resonance of the spin labeled ghost membranes and in the mastocytoma cells. These alterations suggest that these drugs induce changes in protein conformation of the membranes. The membrane binding properties of these drugs may be important in their mechanism of action.

The antitumor activities of anthracycline antibiotics (adriamycin, daunomycin), acridines (diacridine, AMSA), actinomycin D and Pt-compounds are believed to result from their interactions with DNA and consequent inhibition of DNA and RNA synthesis (1-8). While the intercalative binding of these drugs to DNA is important for their cytotoxic and mutagenic actions, the molecular mechanism(s) of action remains poorly understood. Recent studies of Murphee et al. (9) have shown that anthracyclines such as adriamycin, a strong intercalator to DNA, bind to the membrane of Sarcoma 180 ascites cells

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Abbreviations: MSL, N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinly)-maleimide. 5-doxylstearate, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazdidinyloxyl methyl ester. AMSA, 4'-(9-Acridinylamino)-methanesulfon-m-anisidide.

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in a concanavalin A-mediated agglutination assay. Diacridines, prepared (3,4) as antitumor agents due to their high affinity towards nucleic acids, have been shown to bind with membranes (10). Fico et al. (10) have also shown that diacridines show a significant inverse correlation between in vivo percent increase in life span (ILS%) and their ability to enhance the rate of agglutination of S-180 cells. Furthermore, ILS% of diacridines did not correlate with inhibition of nucleic acids synthesis. These studies suggest that antitumor effectiveness may be associated with some mechanism of action other than inhibition of nucleic acid synthesis.

The spin labeling technique has been well established as a valuable tool for studying drug-macromolecule interactions (11-15). The most useful spin labels are those containing a nitroxide group, since this free radical is stable in aqueous solution over a wide range of temperatures and pH values. Holmes and Piette (16), in a pioneer investigation, have used spin labeled membranes to study the effect of phenothiazine derivatives on erythrocyte ghost membranes. Schneider et al., (17) and Butterfield et al., (18) have shown that the nitroxide spin labels, at low concentrations, do not perturb the morphology of the erythrocyte membranes and hence, are useful tools for studying membrane properties. In this paper, we describe the effects of three classes of known antitumor drugs on human erythrocyte ghost membranes and murine mastocytoma P815 cells using the spin labeling technique.

Materials: The spin labels N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) maleimide (MSL) and 5-doxylstearate were purchased from Synvar Associates, Palo Alto, California. Adriamycin (NSC-123,127), daunomycin (NSC-82,151), actinomycin D (NSC-3503) and cis-dichlorodiammine platinum II (NSC-119,875) were gifts of Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. AMSA (NSC-141,549) was a gift of Dr. C.L. Cysyk of the Laboratory of Pharmacology, National Cancer Institute. N,N-Di(9-acridyl)-1, 8-diaminooctane dihydrochloride was prepared according to the published method (3).

Methods: Human erythrocyte ghost membranes were prepared from fresh human red blood cells according to the method of Dodge et al. (19) by gentle hemolysis in 5 mM phosphate buffer pH 7.4. Murine mastocytoma P 815 cells (20) were grown and isolated from CDF-1 mice. These cells were washed three times with Lockes solution pH 7.4. Spin labeling of mastocytoma cells and ghosts with maleimide spin label were achieved by the method of Holmes and Piette (16). Spin labeling of the ghosts with 5-doxylstearate was achieved

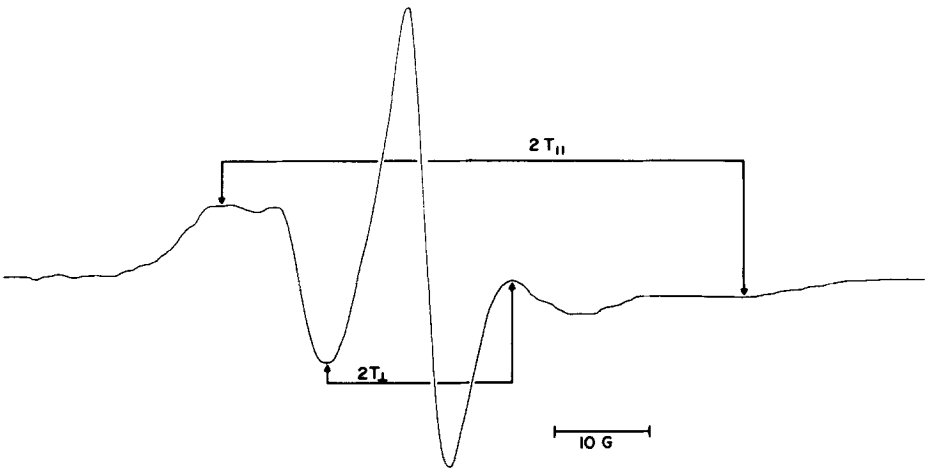

Figure 1: Electron Spin resonance spectrum of labeled ghost membranes (5-6mg protein/ml) with 5-doxylstearate (5 x 10^{-6}M).

according to the methods of Butterfield et al. (18). Samples for ESR studies were prepared by the addition of a methanolic solution (1x10^{-2}M) of drugs (except the Pt-compound which was dissolved in water) to the spin labeled ghosts in 5 mM phosphate (pH 7.4) and to the spin labeled cells in Lockes solution (pH 7.4). The controls contained equivalent amounts of methanol. The drugs were incubated with spin labeled ghosts for 18 hrs at 4°C and warmed to room temperature before ESR spectra were recorded. The drugs were incubated with P 815 cells for 30 min at room temperature. The ESR spectra were recorded with a Varian E-109 spectrometer operating at 100 kHz. The samples were introduced into an E-238 TM_{110} cavity in quartz flat cells.

Results and Discussion: Butterfield et al. (21) and Kury et al. (22) have used 5-doxylstearate spin label successfully to detect small changes in the fluidity of erythrocyte membranes. The ESR spectrum of the erthrocyte membrane labeled with 5-doxylstearate is shown in Figure 1 and is similar to that obtained by others (18). The interpretation of such a spectrum is based on the S-formalism (changes in T_{11} and $T_{\perp}$) of Hubbel et al. (23). The addition of antitumor drugs (5x10^{-5}M) produced no detectable changes in T_{11} or $T_{\perp}$ of the ESR spectrum of the spin labeled erythrocyte membranes. This suggested that these drugs did not induce fluidity changes in membrane phospholipids.

The reaction of MSL with sulfhydryl groups of proteins and erythrocyte membranes is well established (16). The ESR spectrum of MSL labeled ghost

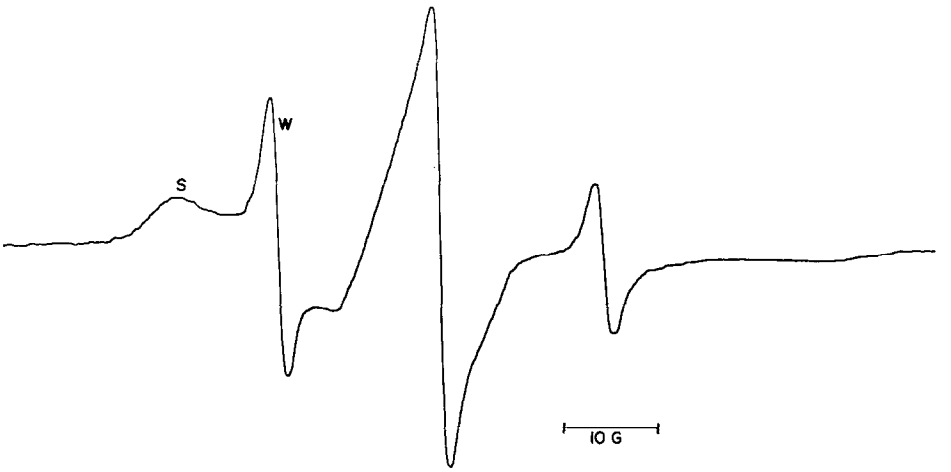

Figure 2: Electron spin resonance spectrum of labeled ghost membranes with MSL. S and W represent strongly immobilized and weakly immobilized nitroxide radicals respectively.

membranes (shown Figure 2) is similar to that obtained by others (16,17). Such a spectrum is a composite of two spectra; one due to a strongly-immbolized radicals(S) and the other due to a weakly-immobilized(W) radicals. The ratio of the amplitudes of the two peaks (W/S) was taken as a relative amount of two conformations of the proteins present in the membranes. The interaction of the antitumor drugs with the ghost membranes was followed by observing the ESR spectrum of the nitroxide radical and the data is presented in Table 1. The results clearly indicate that a small (5-15%) but significant change in protein conformation occurred due to an interaction with the drugs. Sandberg, et al. (24) have suggested that the strongly immobilized spectrum results from the labeling of proteins located within the lipoprotein matrix of the membrane. The weakly-immobilized spectrum, by contrast, is probably associated with labels that are in a polar environment on the membrane surface. The data in Table 1 show that all the antitumor drugs (except diacridine at higher concentration) cause a conversion of the strongly immobilized labels to weakly immobilized labels. This suggests that these drugs cause a change in the conformation of labels

TABLE-1: INTERACTION OF ANTITUMOR DRUGS WITH MSL LABELED ERYTHROCYTE MEMBRANES[a]

Drug	W/S ratio[b] $1x10^{-5}M$	$5x10^{-5}M$
Control	5.85	
Daunomycin	6.135 (4.8)	6.1 (4.8)
Adriamycin	6.37 (8.8)	6.54 (11.8)
Actinomycin D	6.45 (10.2)	6.58 (12.5)
AMSA	6.58 (12.5)	6.71 (14.7)
Diacridine (C_8)	6.17 (5.5)	5.21 (11)
cis-Pt Complex	6.58 (12.5)	6.58 (12.5)

[a]Contained 2.5 - 3.0 mg protein/ml.

[b]The values represent the ratio of weakly to strongly immobilized labels. The % increase or decrease is given in parenthesis.

that are buried within the lipoprotein matrix. The diacridine also causes a conformational change which appears to be associated with protein that are located at the surface.

The ESR spectrum of MSL-labeled mastocytoma cells is similar to that observed with labeled ghost membranes. The interaction of the antitumor drugs with these cells was similarly followed by observing the ESR spectrum and the changes in W/S ratio are presented in Table 2. The results suggest that the antitumor drugs interact with membrane proteins in a similar fashion to that seen with the ghost membrane. In both systems, the cis-Pt compound and AMSA bring about the largest changes (15%) suggesting a stronger interaction with membrane proteins. It seems possible that the antitumor drugs may have a similar site on both human ghost membranes and the mastocytoma cell membranes. It is of interest that the interaction of these drugs is different from that of the phenothiazines which cause a conversion of weakly immobilized label to strongly immobilized labels (16). Recently, Jones and Woodbury (25) have shown that MSL reacts extensively with the membrane sulfhydryl groups and

TABLE 2: INTERACTION OF ANTITUMOR DRUGS WITH MSL LABELED MURINE MASTOCYTOMA P 815 CELLS[a]

Drug	W/S ratio[b] 1×10^{-5} M	5×10^{-5} M
Control	7.22	
Daunomycin	7.81 (8.2)	7.97 (10.4)
Adriamycin	7.71 (6.8)	7.63 (5.7)
Actinomycin-D	7.680 (6.4)	7.684 (6.4)
AMSA	7.94 (10)	8.366 (15.8)
Diacridine (C_8)	8.132 (12.6)	7.786 (7.8)
cis-Pt complex	8.333 (15.4)	8.476 (17.4)

[a]contained 1.1×10^8 cells/ml.

[b]The values represent the ratio of weakly to strongly immobilized labels. The % increase or decrease is given in parenthesis.

penetrates deeply into the hydrophobic regions of the membrane due to its high lipid solubility. It is tempting to suggest that these antitumor drugs, due to high lipid solubility, may be interacting in the hydrophobic regions of the membranes. Conformational changes in the membrane proteins induced by drugs are known to inhibit physiologically significant functions of erythrocyte membranes, such as glucose and ion transport (26-28). Thus, it appears possible that antitumor drugs may exert their biological effects not only by interaction with DNA but also by modifying cell membrane function.

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