ELSEVIER

International Journal of Pharmaceutics 135 (1996) 167–176

international journal of pharmaceutics

Interaction of anthracyclines with human erythrocytes at hyperthermic temperature

Agnieszka Szwarocka[a], Tadeusz Robak[b], Euzebiusz Krykowski[b], Zofia Jóźwiak[a,*]

[a]*Department of Thermobiology, University of Łódź, Banacha 12/16 Str., 90-237 Łódź, Poland*
[b]*Department of Internal Medicine, Medical Academy Łódz, Poland*

Received 12 December 1995; accepted 30 January 1996

Abstract

The effects of anthracyclines on the structure of human erythrocytes were examined by flow cytometry and electron spin resonance spectroscopy. Erythrocytes were exposed to adriamycin, aclarubicin and daunorubicin at 37°C and at higher temperatures. We have shown that drugs at low concentrations (1–5 μg/ml) did not exhibit significant changes in the structure of erythrocyte membranes. Higher doses of drugs induced changes in the membrane fluidity at hydrophobic parts of the lipid bilayer and conformation of the membrane proteins both at 37°C and elevated temperature. These results suggest that simultaneous exposure of cells to drugs and hyperthermia did not markedly enhance the effects of all drugs on the organization of erythrocyte membranes.

Keywords: Erythrocytes; Plasma membranes; Anthracyclines; Hyperthermia; EPR; Flow cytometry

1. Introduction

The anthracyclines form a large family of drugs widely used in the treatment of several human cancers. Drugs bind to various targets in the cell; DNA, DNA-associated enzymes and cellular membranes (Capranico et al., 1990; Tritton, 1991; Gieseler et al., 1994). Drug membrane interactions result in perturbation of cellular functions such as signal transduction (Posada et al., 1989), translocation of protein and lipids (Eilers et al., 1989; Voelker, 1991) or electron transfer (Sun et al., 1992). The standard anthracycline, adriamycin (ADR) binds preferentially to lipid domains and forms an electrostatic complex with negatively charged phospholipids (Duarte-Karim et al., 1976). It has been demonstrated that adriamycin induces an increase in the plasma membrane fluidity in Ehrlich ascites tumor cells (Sugiyama et al., 1986) and in cultured lymphoma cells (Oth et al., 1987; Lameh et al., 1989).

Studies on the interaction of anthracyclines with human red blood cells have shown that drugs bind rapidly to plasma proteins and red cells in vivo as well as in vitro (Garnier-Suillerot and Gattegno, 1988; Bandak and Czejka, 1994). Adriamycin may penetrate into the lipid bilayer and is also able to link to membrane spectrin (Mikkelsen et al., 1977). Arancia et al. (1988),

* Corresponding author: Fax: +042 354473.

PII S0378-5173(96)04482-1

Arancia and Donelli (1991) using freeze-fracture and electron microscopy have indicated that ADR induces morphological and ultrastructural changes of human red blood cells.

The cytotoxicity of anthracyclines to tumor cells is enhanced at elevated temperatures. Hyperthermia at 40–43°C increases the doxorubicin uptake and may also increase the delivery of drugs to target cells (Hahn, 1982; Engelhardt, 1987). Regarding the thermal enhancement of the toxicity of adriamycin or other anticancer antibiotics, experimental data are still controversial and uncompleted. Particularly, little is know about the interaction of anticancer drugs with erythrocytes at hyperthermic temperature. Due to the fact that erythrocytes play an active role in anthracyclines distribution and metabolism, it was necessary to carry out this study to estimate whether cells are affected or damaged by the simultaneous exposure to drugs and hyperthermia. Thus, in this paper, we have compared the effects of drugs at 37°C and combination of drugs and hyperthermia on the structure of plasma membranes. The interactions of adriamycin, aclarubicin and daunorubicin with human erythrocytes were monitored using flow cytometry and electron spin resonance spectroscopy (EPR).

2. Materials and methods

2.1. Chemicals

Doxorubicin (adriamycin-ADR) was obtained from Farmitalia Carlo Erba., Milan, Italy. Daunorubicin (DNR) was purchased from Laboratoire Roger Bellon and Aclarubicin (ACL) from Behringwerke AG, Marburg. All drugs were stored protected from light at 4°C.

Spin labels, 5-doxylstearic acid (5-DSA), 16-doxylstearic acid (16-DSA) and 4-maleimido-tempo were purchased from the Sigma Chemical Company. Spin labels were dissolved in absolute ethanol and stored at 4°C. All other chemicals were of the best quality commercially available.

2.2. Preparation of erythrocyte suspensions

Human peripheral blood from healthy donors in ACD solution was centrifuged at 600 g for 10 min. After removal of the plasma and buffy coat, the erythrocytes were washed three times in phosphate buffered saline, PBS, (5 mM sodium phosphate buffer, containing 0.15 M NaCl, pH 7.4) and suspended at the same medium.

2.3. Membrane preparation

Erythrocyte membranes were obtained by hypotonic lysis according to the procedure of Dodge et al. (1963) at 4°C and then were suspended in PBS, pH 7.4. Protein concentration was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

2.4. Treatment of erythrocyte and membrane samples with drugs

Three percent erythrocyte suspensions in PBS, pH 7.4, and the membrane samples (3 mg protein/ml) were incubated in the absence or with various amounts of adriamycin, daunorubicin or aclarubicin in the dark for 30 min at 37°C, 42°C and 50°C. Heating of cells was performed in precision controlled (± 0.1°C) waterbaths. Drugs were added to the samples at a final concentration of 1–20 μg/ml. Fresh dilutions of drugs were prepared in PBS just before each experiment. After incubation, the drug-treated samples were washed three times with PBS and labeled with spin labels or investigated by flow cytometry analysis.

2.5. Preparation of spin-labeled erythrocytes and erythrocyte membranes

Erythrocytes suspended in PBS to the hematocrit of 50% were labeled with 5- and 16-doxylstearic acids for 1 h at 37°C in the dark. The lipid spin labels were added to the erythrocyte samples in the ratio 1:100.

Erythrocyte membranes were labeled with maleimide spin label for 12 h at 4°C also in the dark, in a ratio of 1 mg of label per 25 mg of membrane protein. The spin labeled samples were

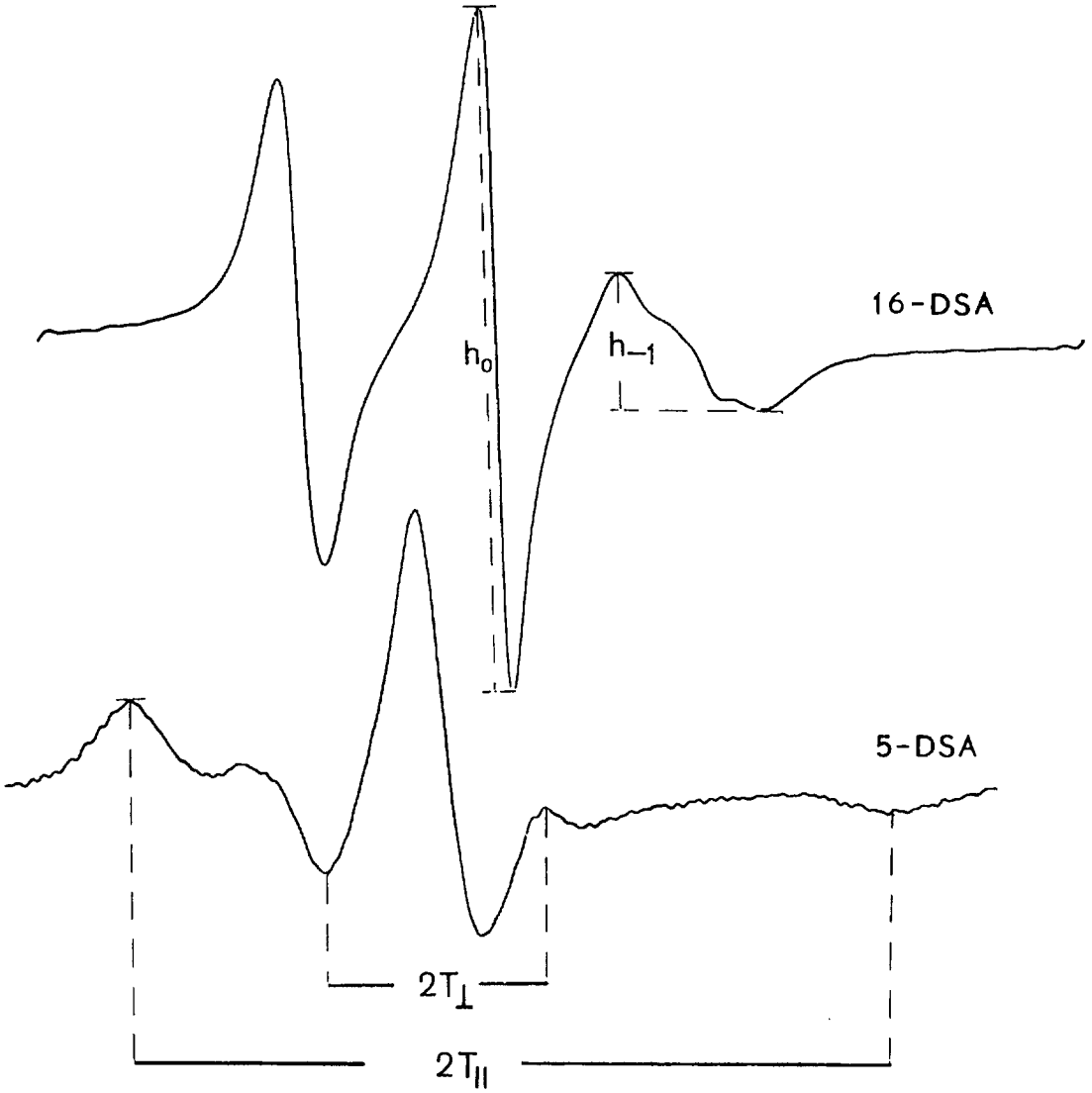

Fig. 1. EPR spectra of 5-DSA and 16-DSA in control erythrocytes. Measurements of the outer hyperfine ($2T_{\|}$) and inner hyperfine ($2T_{\perp}$) splittings and midline height (h_0) and high-field height (h_{-1}) are indicated.

washed several times with PBS, pH 7.4, to remove unbound spin labels.

2.6. EPR measurements

EPR spectra were measured at room temperature using a Brücker ESP 300E spectrometer with computerized data acquisition and analysis capabilities.

Typical instrumental parameters during these measurements were as follows: modulation frequency 100 kHz, modulation amplitude 2.02 G, time constant 20.48 ms, scan time 400 s, field set 3240 G and scan range 100 G.

From the spectrum of 5-DSA (Fig. 1) an order parameter (S) was derived by measuring the outer and inner hyperfine splittings $2T_{\|}$ and $2T_{\perp}$ (Gordon et al., 1989) using the formula:

$$S = (T_{\parallel} - T_{\perp})(a_N)/(T_{zz} - T_{xx})(a'_N)$$

where

$$a_N = 1/3(T_{zz} + 2T_{xx})$$

$$a'_N = 1/3(T_{\parallel} + 2T_{\perp})$$

T_{zz} (32.4G) and T_{xx} (6.1G) are the principal hyperfine splittings that correspond to the main axis of the spin probe (Sauerheber et al., 1980). For 16-DSA spectra, the amplitudes of the two lines h_0 and h_{-1} were measured (Fig. 1). The ratio of h_0/h_{-1} is related to the fluidity of the local environment of the label (Laurent et al., 1980).

Fig. 2 gives an example of an EPR spectrum of maleimide spin label. From these spectra, the ratio of W/S was determined. Maleimide spin label binds covalently to membrane proteins giving rise to EPR absorptions which represent, respectively, weakly (W) and strongly (S) immobilized state of the labeled sites in the membrane proteins. In these studies, the W/S ratio has been used as monitor of changes in protein conformation and environment within the membrane (Berliner, 1983; Wyse and Butterfield, 1988).

2.7. Flow cytometric analysis

Control cells and erythrocytes preincubated with anthracyclines were analysed with a flow cytometer ARGUS (Norway) supplied with a 100 W-HBO mercury arc lamp. Cell size and shape were appreciated with simultaneous separate detection of low angle (LS-1) and right angle (LS-2)

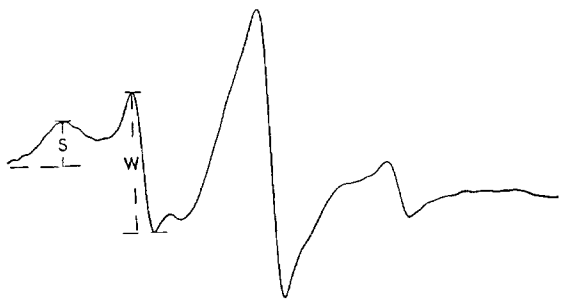

Fig. 2. A tipical EPR spectrum of the maleimide labeled erythrocyte membranes. The spectral amplitudes of the spin label covalently bound to strongly and weakly immobilized sites are indicated by S and W.

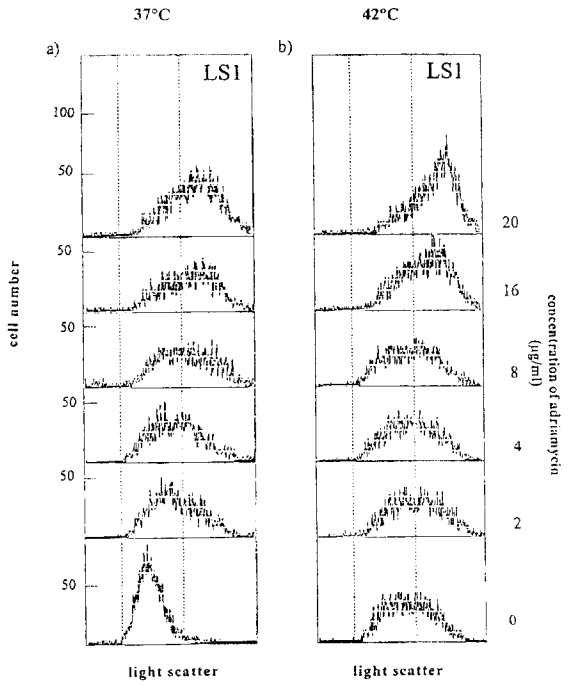

Fig. 3. Flow cytometric analysis of adriamycin-treated erythrocytes at 37°C and 42°C for 30 min.

light scattering. The data obtained were displayed in the form of a diagram of cell number versus light scatter and were analyzed by standard computer programs of ARGUS Company.

2.8. Statistical analysis

Data were analyzed by two-tailed Student's t-test of paired data. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. The effect of anthracyclines and temperature on shape of erythrocytes

Using flow cytometry technique, we estimated prehemolytic changes in size and shape of erythrocytes in the absence and the presence of anthracyclines. A representative diagram of erythrocytes incubated with adriamycin for 30 min at 37°C and 42°C is shown in Fig. 3a and b,

respectively. The effect of drugs concentrations and temperature on the erythrocyte shape is also demonstrated in Fig. 4a–c. Population of erythrocytes incubated without drugs at 37°C contained only negligible amount of cells with unnormal shape. Exposure of cells to hyperthermia (42°C, 30 min) increased the fraction of erythrocytes with the altered shape up to 30%. The addition of adriamycin to cells at concentration of 1–20 μg/ml caused dose-dependent increase of cell population with the modified shape both at physiological temperature and at 42°C (Fig. 4a). However, at 42°C only the higher concentrations of adriamycin (8–20 μg/ml) significantly potentiated the effect of hyperthermia alone.

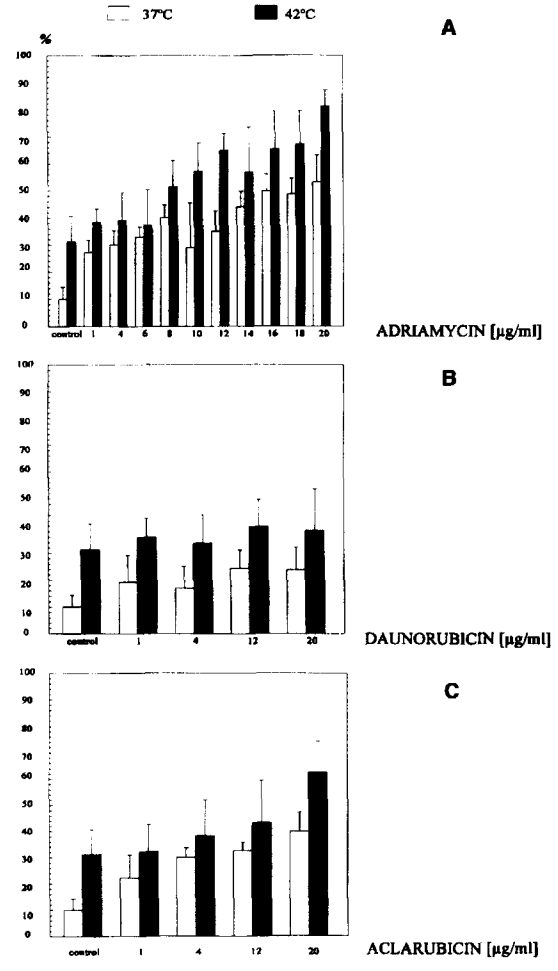

Fig. 4. Effect of drugs concentrations on prehemolytic changes in shape of human erythrocytes incubated for 30 min at 37°C and 42°C. Mean ± S.D. of four independent experiments. (A) adriamycin-treated cells; (B) daunorubicin-treated cells; (C) aclarubicin-treated cells.

Changes of erythrocyte shape in the presence of aclarubicin were also dose dependent at 37°C and 42°C but they were smaller about 10–20% than in the adriamycin treated cells (Fig. 4c). In contrast to the adriamycin and aclarubicin, the daunorubicin caused the dose independent changes in the shape of erythrocytes at 42°C. At 37°C, daunorubicin also exhibited no significant difference in the number of modified cells with increasing concentration of drug (Fig. 4b). These results indicate that the effect of daunorubicin on the shape of erythrocytes is different from those of adriamycin and aclarubicin and that ADR and ACL greatly affect erythrocyte morphology.

3.2. The effect of drugs and hyperthermia on the membrane lipids

Pretreated erythrocytes with anthracyclines at 37°C or at 42° and 50°C were labeled with two lipid spin labels: 5 and 16 doxylstearic acids. 5-DSA was used to determine the membrane fluidity at the surface of the lipid bilayer by calculation of the order parameter, S. 16-DSA is a diffusible lipid spin probe to explore the hydrophobic membrane core. Spectra of 16-DSA were analyzed by using the parameter h_0/h_{-1} (Fig. 1). The effect of drugs and temperature on the order parameter of 5-DSA in human erythrocyte membranes is presented in Tables 1–3. Data demonstrate that hyperthermia (42°C and 50°C, 30 min) alone significantly reduced the order parameter. When erythrocytes were exposed to adriamycin, daunorubicin or aclarubicin and hyperthermia simultaneously or kept at 37°C in the presence of drugs, no alterations in the order parameter were observed.

The values of the h_0/h_{-1} for 16-DSA are reported in Figs. 5–7. All tested drugs induced considerably higher changes in the lipid organization of erythrocyte membranes at hydrophobic regions of lipid bilayers than in polar parts of membranes. Under indicated conditions, the elevated temperatures in the absence of drugs caused

Table 1
Effect of adriamycin on the order parameter of 5-DSA in erythrocyte membranes

T	Control	0.1(μg/ml)	0.5(μg/ml)	1(μg/ml)	2(μg/ml)	20(μg/ml)
37°C	0.747 ± 0.007	0.741 ± 0.009	0.742 ± 0.012	0.740 ± 0.008	0.736 ± 0.006	0.731 ± 0.011
42°C	0.731* ± 0.004	0.733 ± 0.005	0.727 ± 0.007	0.733 ± 0.002	0.729 ± 0.004	0.723 ± 0.005
50°C	0.724** ± 0.006	0.730 ± 0.009	0.728 ± 0.005	0.731 ± 0.001	0.729 ± 0.007	0.723 ± 0.002

Cells were incubated with drug at 37°C, 42°C and 50°C for 30 min in phosphate-buffered saline (pH 7.4). EPR spectra were measured at room temperature. Mean ± S.D. of four experiments. Significantly different, $*P < 0.05$; $**P < 0.01$.

Table 2
Effect of daunorubicin on the order parameter of 5-DSA in erythrocyte membranes

T	Control	0.1(μg/ml)	0.5(μg/ml)	1(μg/ml)	2(μg/ml)	20(μg/ml)
37°C	0.745 ± 0.006	0.742 ± 0.007	0.740 ± 0.005	0.742 ± 0.008	0.740 ± 0.003	0.738 ± 0.011
42°C	0.728* ± 0.009	0.733 ± 0.005	0.733 ± 0.006	0.726 ± 0.005	0.729 ± 0.005	0.727 ± 0.008
50°C	0.722** ± 0.003	0.725 ± 0.007	0.726 ± 0.005	0.720 ± 0.003	0.722 ± 0.007	0.726 ± 0.009

Cells were incubated with drug at 37°C, 42°C and 50°C for 30 min in phosphate-buffered saline (pH 7.4). EPR spectra were measured at room temperature. Mean ± S.D. of four experiments. Significantly different, $*P < 0.05$; $**P < 0.01$.

Table 3
Effect of aclarubicin on the order parameter of 5-DSA in erythrocyte membranes

T	Control	0.1(μg/ml)	0.5(μg/ml)	1(μg/ml)	2(μg/ml)	20(μg/ml)
37°C	0.745 ± 0.005	0.744 ± 0.004	0.738 ± 0.007	0.739 ± 0.008	0.739 ± 0.004	0.741 ± 0.007
42°C	0.731* ± 0.008	0.728 ± 0.011	0.730 ± 0.005	0.726 ± 0.008	0.729 ± 0.003	0.727 ± 0.005
50°C	0.722** ± 0.007	0.720 ± 0.005	0.723 ± 0.004	0.726 ± 0.005	0.727 ± 0.003	0.719 ± 0.005

Cells were incubated with drug at 37°C, 42°C and 50°C for 30 min in phosphate-buffered saline (pH 7.4). EPR spectra were measured at room temperature. Mean ± S.D. of four experiments. Significantly different, $*P < 0.05$; $**P < 0.01$.

the statistically significant decrease of the h_0/h_{-1} ratio with respect to the control samples incubated at 37°C. It was found that effect of drugs was dose and temperature dependent. At 37°C, adriamycin, as well as aclarubicin and daunorubicin, at low concentrations of 0.1–2 μg/ml, did not change the h_0/h_{-1} ratio for 16 doxylstearic acid. At higher drugs concentrations (10–20 μg/ml), the h_0/h_{-1} ratio significantly increased with increasing doses. Incubation of cells at 42°C for 30 min with low doses of adriamycin (0.1–2 μg/ml) caused a gradual increase of the h_0/h_{-1} to the values noted at 37°C (Fig. 5). However, adriamycin at concentration of 20 μg/ml led to an enhancement of the h_0/h_{-1} from 4.19 ± 0.12 to 5.11 ± 0.18 in comparison with the control values at 42°C. At the same temperature of 42°C, only high doses of aclarubicin produced significant increase of the h_0/h_{-1} ratio (Fig. 6). In contrast to the adriamycin and aclarubicin, the daunorubicin both at low and high doses did not induce alterations in the h_0/h_{-1} at 42°C (Fig. 7).

Elevation of the temperature to 50°C resulted in progressively enhancement of the h_0/h_{-1} parameter in the presence of increasing concentrations of adriamycin, aclarubicin and daunorubicin. From these findings, it can be said that hyperthermia alone caused an increase of the lipid fluidity both at the surface and in the hydrophobic parts of the erythrocyte membranes. The combination of hyperthermia and anthracyclines exhibited different effect on the lipid fluidity in polar and hydrophobic areas of membranes. Exposure of erythrocytes to drugs at low and high

doses and hyperthermia (42°C and 50°C) altered no the organization of lipids in polar parts of membranes. However, the high concentrations of ADR and ACL (10—20 μg/ml) at physiological and elevated temperatures and DNR at high doses at 37°C and 50°C markedly decreased the lipid fluidity at deeper regions of membranes.

The comparison of the drugs effects on the physical state of erythrocyte membrane lipids suggests that daunorubicin produced much less changes in the lipid fluidity than adriamycin and aclarubicin.

3.3. Effect of anthracyclines and elevated temperature on the conformational changes of membrane proteins

In order to demonstrate that anthracycline drugs also interact with the erythrocyte proteins, the membranes were labeled with maleimide spin label and analysed by EPR. The most common spectral parameter for protein-bound label is

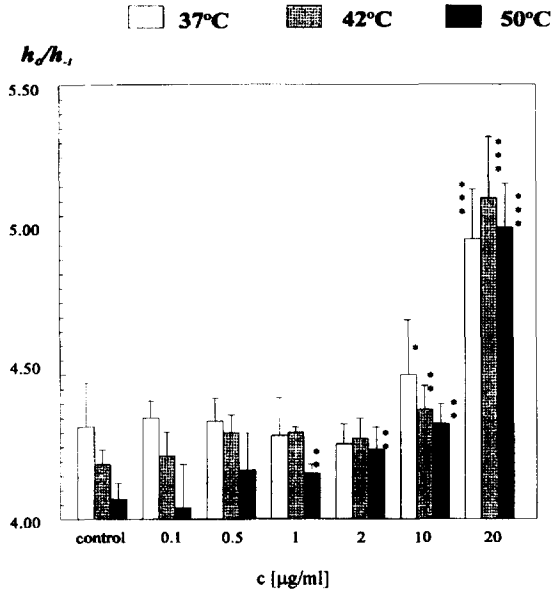

Fig. 5. Effect of adriamycin on the h_0/h_{-1} parameter of membrane bound 16-DSA. Cells were incubated with adriamycin for 30 min at indicated temperature in PBS. Each point represents the average ± S.D. of four experiments. Significant differences as compared with control at tested temperature: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

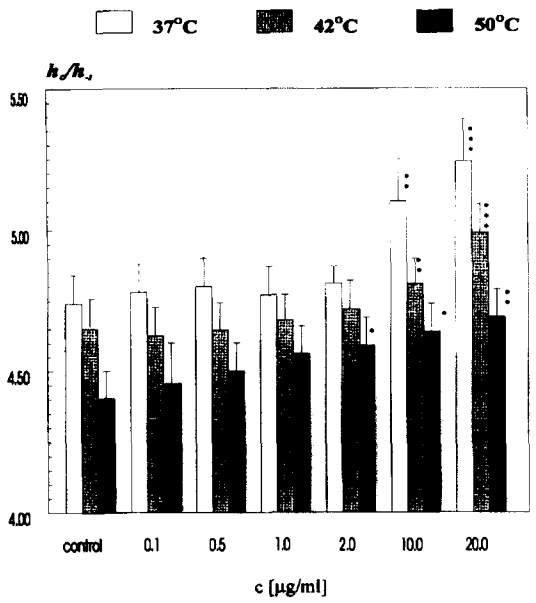

Fig. 6. Effect of aclarubicin on the h_0/h_{-1} parameter of membrane bound 16-DSA. Cells were incubated with aclarubicin for 30 min at indicated temperature in PBS. Each point represents the average ± S.D. of four experiments. Significant differences as compared with control at tested temperature: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

defined as the W/S ratio. This parameter is particularly sensitive to changes in the membrane structure. An increase in the W/S ratio indicate conformational changes of the spin labeled proteins and imply a decrease in protein-protein interactions. The effect of temperature and drugs at concentrations of 5 μg/mg protein and 50 μg/mg protein on the W/S ratio are summarized in Table 4. Heat treatment alone (42°C and 50°C, 30 min) caused considerably increase of the W/S ratio with increasing temperature. The values of W/S altered from 3.15 ± 0.41 at 37°C to 5.41 ± 0.54 at 50°C. Simultaneous exposure of the erythrocyte membranes to drugs and hyperthermia diminished the W/S ratio in a concentration and temperature dependent manner. As shown in Table 4, the adriamycin, aclarubicin and daunorubicin produced similar decrease in the W/S ratio with increasing concentration of drugs. The results indicate that adriamycin, aclarubicin and daunorubicin produced significant reduction in the segmental motion of spin-labeled binding sites

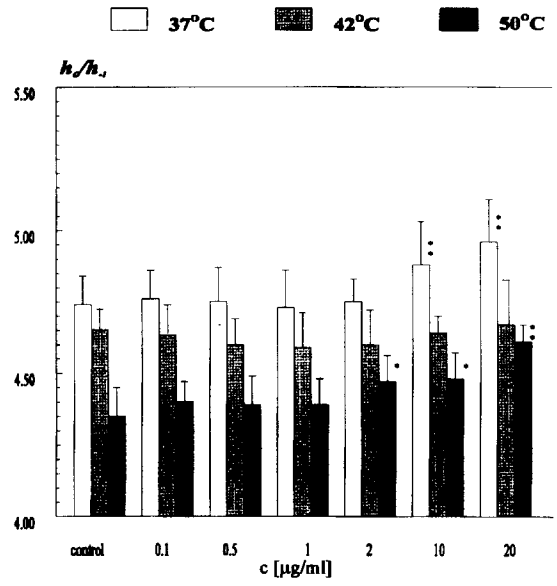

Fig. 7. Effect of daunorubicin on the h_0/h_{-1} parameter of membrane bound 16-DSA. Cells were incubated with daunorubicin for 30 min at indicated temperature in PBS. Each point represents the average ± S.D. of four experiments. Significant differences as compared with control at tested temperature: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

leading to an increase in protein-protein interactions in human erythrocyte membranes.

4. Discussion

These results show that adriamycin, aclarubicin and daunorubicin induced several kinds of damage in human erythrocytes, including shape alterations, changes in the membrane fluidity and conformation of proteins. An extent of cell injury is related to type of drug, its concentration and temperature. At 37°C, only adriamycin and aclarubicin significantly affected the morphology of erythrocytes and the lipid fluidity in hydrophobic parts of membranes. High concentrations of anthracyclines both at 37°C and elevated temperatures increased damage to cells leading to rigidification of membrane lipids in hydrophobic core and changes in conformation of membrane proteins. Daunorubicin caused a smaller, concentration-dependent alterations in the membrane fluidity and shape erythrocytes.

The mechanism of toxicity of anthracyclines at hyperthermic temperature has not yet been clearly elucidated. Although much information has been gained by the use of model synthetic membranes and the culture cell lines, there are no reports which have examined the interaction of aclarubicin and daunorubicin with human erythrocytes at elevated temperature.

Anthracyclines interactions with human erythrocytes were investigated mainly by freeze-fracture, scanning electron microscopy methods or circular dichroic spectroscopy (Garnier-Suillerot and Gattegno, 1988; Arancia et al., 1988). Particular attention was focused on the effect of adriamycin on the distribution of intramembrane particles and on the erythrocyte shape (Arancia et al., 1988; Arancia and Donelli, 1991). Studies performed by other investigators have indicated that the interactions of adriamycin with human erythrocyte membranes occurs mostly at the level of negatively-charged phosphate groups (Garnier-Suillerot and Gattegno, 1988). It has been pro-

Table 4
Changes in the W/S ratio for a maleimide spin label bound to erythrocyte membranes pretreated with drugs at indicated temperature

	Control	5 μg of the drug/mg protein			50 μg of the drug/mg protein		
		ADR	ACL	DNR	ADR	ACL	DNR
37°C	3.15 ± 0.41	2.88 ± 0.90	2.46 ± 0.22*	2.55 ± 0.26*	2.41 ± 0.24**	2.15 ± 0.27**	2.27 ± 0.18**
42°C	3.90 ± 0.90	3.31 ± 0.84*	2.98 ± 0.15*	3.24 ± 0.73*	2.99 ± 0.41**	2.50 ± 0.21**	2.59 ± 0.16**
50°C	5.41 ± 0.54	4.10 ± 0.40**	4.75 ± 0.63**	4.08 ± 0.80**	3.73 ± 0.50***	3.83 ± 0.14***	3.76 ± 0.47***

Mean ± S.D. of eight experiments. Statistically significant differences in comparison to values at indicated temperature: $^*P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0005$

posed that also spectrin may be the target for ADR, since this protein is known to be involved in maintaining erythrocyte morphology (Mikkelsen et al., 1977). However, the structural and functional consequences of such interaction are not well defined. It is well known that adriamycin and epirubicin bind rapidly to human erythrocytes both in vivo and in vitro (Garnier-Suillerot and Gattegno, 1988; Bandak and Czejka, 1994) and that about 30–50% of drugs circulating in the blood is associated with erythrocytes following intravenous administration (Brogini et al., 1980). Taking into account the above data, the binding of drugs to erythrocytes may affect the structure of erythrocyte membranes and limit their function.

In the work reported here, we have demonstrated that adriamycin, aclarubicin and daunorubicin at doses above 5 μg/ml caused morphological and structural changes of human erythrocytes not only at elevated temperature but also at 37°C. Using nitroxide spin labels, we found that anthracyclines were able to interact both with the membrane lipids and proteins. An apparent differences in the lipid fluidity between the surface and deeper regions of erythrocyte membranes induced by drugs suggest that anthracyclines are predominantly intercalated into the hydrophobic areas of the lipid bilayer. In model membrane systems, ADR is also intercalated into the hydrocarbon part of the bilayer with deeper penetration into fluid phase than into solid phase vesicles (Constantinides et al., 1990). Changes in the W/S ratio observed in drug treated erythrocyte membranes have shown that interaction of adriamycin, aclarubicin and daunorubicin occur at the level of cytoskeletal proteins. This indicates also that binding of drugs to cytoskeletal proteins is responsible for increased protein-protein interactions at hyperthermic temperature. The preferentially binding of maleimide spin label to SH groups of cytoskeletal proteins; spectrin, actin, bands 2.1, 4.1 and to the cytoplasmic region of band 3 is known from papers of Butterfield (1982), Wyse and Butterfield (1988) and Palmieri and Butterfield (1990).

The comparison of the alterations induced by anthracyclines in human erythrocytes suggest that aclarubicin produced similar damage to cells as adriamycin at 37°C as well as at temperature above 40°C. However, under the same conditions, daunorubicin induced smaller perturbations in the structure of erythrocytes than ADR and ACL. Differences in toxicity may be based on the differences in chemical structure of drugs. Adriamycin differs from daunorubicin by one hydroxygroup, while aclarubicin differs from adriamycin and daunorubicin by two extra sugar moieties (Speth et al., 1989; Booser and Hortobagyi, 1994).

The data reported in this paper confirmed that adriamycin, daunorubicin and aclarubicin due to their amphipatic nature were easily incorporated within the erythrocyte membranes. Furthermore, EPR studies have demonstrated the existence of two different kinds of binding sites of anthracyclines to erythrocytes, membrane lipids and proteins. Addition of drugs at low doses to erythrocytes produced only not significant morphological and structural changes of cells. Of course, the incorporation of drugs at higher doses into cells caused similar rigidification of membrane lipids at hydrophobic areas and conformational alterations of membrane cytoskeletal proteins at physiological and at higher temperatures. This indicates, also, that simultaneous exposure of human erythrocytes to drugs and hyperthermia did not significantly enhance the extent of perturbations in the organization of membrane lipids and proteins. Thus, these results suggest that erythrocytes may play an active role in distribution low doses of daunorubicin, aclarubicin and adriamycin at physiological temperature and moderate hyperthermia.

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