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Sensitization of multidrug-resistant colon cancer cells to doxorubicin encapsulated in liposomes*

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Summary. The effectiveness of liposome-encapsulated doxorubicin in overcoming multidrug resistance was studied in various human colon cancer cells. Colon-cancer cell lines SW403, HT29, SW620, and SW620/R overexpressed P-glycoprotein as determined by immunoflow cytometry, thereby confirming the presence of the multidrug-resistant phenotype. Important differences were observed in the cytotoxicity of free doxorubicin as represented by IC_{50} values of 0.168, 0.058, 0.023, and 9.83 μM for SW403, HT29, SW620, and SW620/R, respectively. Liposomally encapsulated doxorubicin provided an IC_{50} that was 1.4 times lower than that of the free drug in the doxorubicin-resistant SW 620/R cell line, whereas no difference was evident in the sensitive parental SW620 cells. In addition, liposome-encapsulated doxorubicin exhibited 1.31- and 2.33-fold cytotoxicity to HT-29 and SW403 cells, respectively. The intracellular drug accumulation in SW620/R cells was enhanced by liposomally encapsulated doxorubicin, whereas it was reduced in all other cell lines as compared with that of free drug. The colon-cancer cell lines demonstrated different degrees of doxorubicin-induced DNA strand breakage that correlated with their sensitivities to drug-induced cytotoxicity. However, no difference was observed between DNA breakage caused by the free drug and that induced by liposome-encapsulated doxorubicin in any of the cell lines. The results suggest that the enhanced cytotoxicity of liposomal doxorubicin to colon cancer cells was due to some secondary non-DNA target. However, liposomally encapsulated doxorubicin appears to be effective in diminishing the multidrug-resistant phenotype and may have clinical applications.

Introduction

Colorectal cancer is one of the major causes of cancer death in the world. In the United States, cancer of the colon is the second leading cause of neoplastic death [11]. Despite extensive studies on chemotherapeutic approaches to the treatment of this human cancer, the survival statistics remain unchanged. One of the reasons for failures of chemotherapy of colon cancer relates to expression of the multidrug resistance (MDR) phenotype [11].

The development of resistance to multiple chemotherapeutic agents by cultured cells has been used as a model system for the clinical problem of intrinsic and acquired MDR. Resistance of cultured cells to a variety of drugs, including vinca alkaloids, anthracyclines, dactinomycin, and epipodophyllotoxins, is frequently due to the expression of a membrane P-glycoprotein (molecular weight 170 kDa) encoded by a family of MDR genes [15]. Expression of the human MDR I gene is sufficient to confer the MDR phenotype on sensitive cells [12]. Although elucidation of the role of MDR I and P-glycoprotein in mediating MDR in a clinical setting awaits prospective studies, Goldstein et al. [11] have demonstrated that P-glycoprotein may play a significant role in conferring resistance on tumors originating from the gastrointestinal tract, the kidney, the liver, the pancreas, and the adrenal glands.

Since intracellular drug accumulation has been found to be decreased in drug-resistant cells, P-glycoprotein has been called an energy-dependent drug-efflux pump [23]. In attempts to inhibit this transport system, a number of agents have been shown to modulate the activity of P-glycoprotein and, thus, to diminish drug resistance. Most prominent among these agents are calcium channel blockers such as verapamil [34], which has been shown to reverse the MDR phenotype. However, the use of these compounds is significantly compromised by their toxic side effects at optimal clinical doses [29].

Recently, liposomes have been extensively used as drug carriers in cancer chemotherapy, especially for anthracyclines [3, 25]. We have demonstrated that liposomally encapsulated doxorubicin enhances the cytotoxicity as-

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sociated with the free drug [24] and have recently shown that the use of liposomal doxorubicin increases the therapeutic efficacy of the drug in the treatment of human breast cancers [33]. In our laboratory, we have shown for the first time that liposome-encapsulated doxorubicin may be useful in circumventing the MDR phenotype in MDR cells [30]. In Chinese hamster LZ cells, whose resistance to doxorubicin is 3,000-fold that of the parental V-79 cells, we have demonstrated that liposomal doxorubicin produces sensitivity 7 times higher than that achieved using the free drug and doubles the intracellular concentration of drug [30].

To investigate further the correlation between the expression of P-glycoprotein and its possible modulation by liposome-encapsulated doxorubicin, we carried out the present study in a number of colon-cancer cell lines. The studies reported herein demonstrate that liposomal doxorubicin enhances the sensitivity of colon cancer cells as compared with free drug. We correlated the cytotoxicity with DNA damage, drug uptake, and expression of P-glycoprotein in these colon cancer cells. These experiments indicate that liposomal doxorubicin effectively modulates the MDR phenotype.

Materials and methods

Cells and cell culture. The SW403, HT-29, and SW620 (sensitive) human colon-carcinoma cell lines were obtained from the ATCC (American Type Culture Collection, Rockville, Md.). The doxorubicin-resistant subline SW620/R was kindly provided by Dr. A. Fojo of the National Cancer Institute (Bethesda, Md.). This subline was established by the continuous exposure of cells to gradually increasing concentrations of doxorubicin and was maintained in medium supplemented with 1 μg doxorubicin/ml. Cultures were grown as monolayers in RPMI-1640 medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine, and penicillin/streptomycin. Cell lines were cultured at 37° C under 95% relative humidity in an atmosphere containing 5% CO_2. The doxorubicin-resistant subline was maintained in drug-free medium for at least 1 week prior to study.

Preparation of liposomes. Doxorubicin (DOX) was obtained from Adria Laboratories (Columbus, Ohio), and cardiolipin, phosphatidylcholine, and cholesterol were supplied by Sigma Chemical Co. (St. Louis, Mo.). Liposome-encapsulated doxorubicin (DOX-lip) was prepared as described elsewhere [25]. Briefly, DOX was encapsulated in liposomes that were composed of cardiolipin, phosphatidylcholine, and cholesterol (molar ratio, 2 : 10 : 6.8) at approximately 12% by weight. DOX-lip was diluted with normal sterile saline (0.9% NaCl). The concentration of DOX entrapped in liposomes was determined by fluorescence [25]; in all experiments, equivalent doses of DOX-lip were used as compared with free drug. Blank liposomes (blank lip) were also prepared using the same composition and ratio of lipids in the absence of DOX; they were diluted in sterile saline to yield lipid doses equivalent to those of DOX-lip.

Cytotoxicity assays. The growth-inhibition method was used to determine the cytotoxicity of free drug and DOX-lip in colon cancer cells. In all, 5×10^5 SW403, HT-29, SW620, and SW620/R cells were plated into 25-cm^2 flasks. The cells were cultured, and those in the exponential growth phase were exposed to varying concentrations of drugs (free DOX, DOX-lip, or blank lip) for 48 h at 37°. The cells were then rinsed twice, trypsinized, and harvested to obtain single-cell suspensions. The cells were then counted by a hemacytometer and viability was determined by the trypan-blue exclusion method. The data were expressed in terms of IC_{50} which corresponds to a 50% reduction in the cell number as compared with controls.

P-glycoprotein determination by immunoflow cytometry. P-glycoprotein was detected by flow cytometry as previously described by Kartner et al. [16]. The murine monoclonal antibody to P-glycoprotein, C219 (Centocor, Malvern, Pa.), was used as the primary antibody, and goat antimouse IgG fluorescein conjugate (Boehringer Mannheim Biochemical, Indianapolis, Ind.) was used as the second antibody. Cells were analyzed using a Becton Dickinson FACStar Plus. Fluorochromes were excited with the 488-nm line of a coherent Innovo 90-C argon laser (200 mW) and fluorescence emission was measured using a 530/30-nm band-pass filter. In all, 5,000 cells were acquired for each 1,024-channel histogram. The relative expression of P-glycoprotein among the cell lines tested was proportional to the fluorescence ratio of cells preincubated with vs without primary antibody.

Drug accumulation study. The intracellular accumulation of DOX was quantified by measuring the fluorescence intensity using a spectrofluorometer following incubation of the drug at 37° C with 1×10^6 cells grown as monolayers. The cells were treated for specific periods of 1–8 h with either free DOX or DOX-lip, were rinsed twice with phosphate-buffered saline (PBS), and were then trypsinized and harvested [17]. The harvested cells were suspended in 25 ml ice-cold PBS, counted, and centrifuged. For fluorometric analysis, the cells were lysed in 20 μl 4% sodium dodecyl sulfate (SDS) and sonicated for 5 min; thereafter, the DOX was solubilized by the addition of 4 ml butanol and recentrifuged at 3,000 rpm for 20 min. The samples were read using a spectrofluorometer at wavelengths of 470 (excitation) and 580 nm (emission). For all cell lines, the relative fluorescence intensity of untreated cells was subtracted from that of treated cells. Relative fluorescence units were converted to absolute amounts of DOX in 10^7 cells using a standard calibration curve for DOX-spiked cells and were then treated similarly.

Glutathione assay. The total levels of reduced glutathione (GSH) in the four colon-cancer cell lines were measured according to Ellman's method [9]. Briefly, the colon cancer cells were trypsinized and washed twice in nitrogen-bubbled PBS. Cells were resuspended at 10^7 cells/ml in 0.1 M nitrogen-bubbled sodium phosphate buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA). Cells were lysed by 1 : 1 dilution with 4% sulfosalicylic acid under rapid mixing, and the samples were centrifuged at 10,000 *g* for 10 min at 4° C. Aliquots of 1 ml supernatant were added to 2 ml Ellman's reagent [8 mg/100 ml dinitro-5-thiobenzoic acid in 0.1 M sodium phosphate buffer (pH 8)], and the absorbance was recorded at 412 nm after 5 min. GSH content was expressed in nanomoles per milligram of protein. Protein determinations were done using the method of Lowry et al. [20].

DNA unwinding assay. The alkali unwinding assay described by Ahnstrom and Erixon [1] was used to measure DNA strand breaks. First, 1×10^6 cells were added to dishes containing 10 ml medium. [^{3}H]-Thymidine ([^{3}H]-Tdr; sp. act., 25 Ci/mmol) was added to the medium to a final concentration of 0.10 μCi/ml. Plates were then incubated for one cell-doubling period. Next, the medium was aspirated from each of the plates and replaced with 10 ml fresh medium that had not been supplemented with radiolabelled thymidine. The plates were returned to the incubator for 2 h. After this chase period, the cells were treated with drugs for 2 h at 37° C, rinsed twice with PBS, trypsinized, and harvested. The cells were centrifuged at 500 *g* for 5 min. The pelleted cells were resuspended in medium at 2×10^6 cells/ml and then dispensed into sample tubes on ice in 25 μl aliquots; 0.5 ml freshly prepared ice-cold unwinding solution (0.02 M NaOH/1 M NaCl; pH 12.1) was added to each tube. After the samples had unwound on ice for 1 h, 0.5 ml neutralizing solution (0.02 M NaH_2PO_4; pH 4.7) was added to stop the reaction and the samples were immediately sonicated for 15 s to break the DNA into small single- and double-strand fragments. Next, 40 μl 10% SDS was added to each tube.

Single- and double-strands of DNA in each tube were separated by hydroxylapatite chromatography. Columns were prepared by the addition of 150 mg hydroxylapatite crystals (Bio-Gel HTP, Biorad Laboratories) to a small polyethylene column followed by one wash with 3 ml 0.01 M potassium phosphate buffer (pH 6.7). To each of the prewashed columns, the contents of one sample tube were added and allowed to run

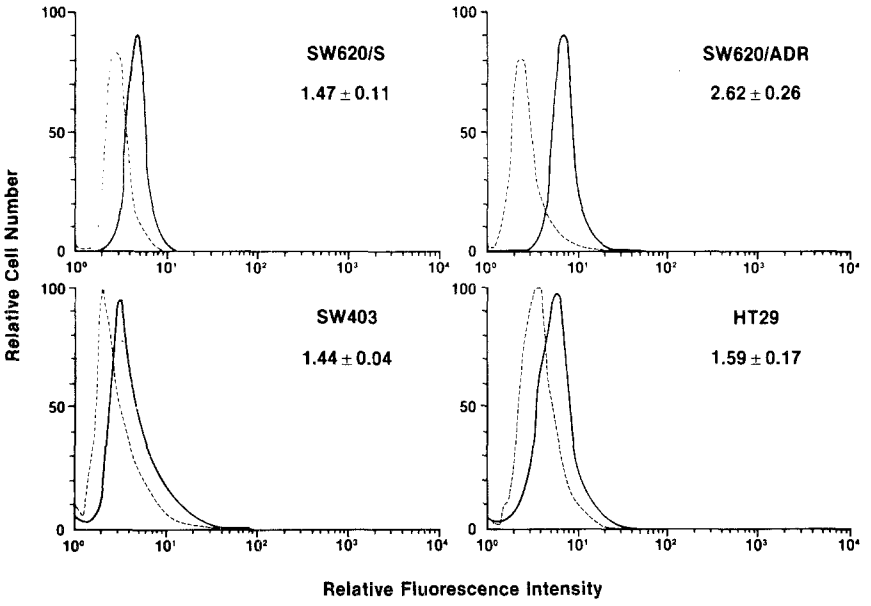

Fig. 1. Detection of P-glycoprotein by immunoflow cytometry in SW620, SW620/R, HT-29, and SW403 colon cancer cells. Peaks indicated by *solid lines* represent the histogram distribution of sorted cells incubated with C219 antibody and the antimouse fluorescein second antibody, and those indicated by *broken lines* represent the histogram distribution of sorted cells incubated with the second antibody only. Cells were analyzed using a Becton Dickinson FACStar Plus as described in Materials and methods

through; then, each column was again washed with 3 ml 0.01 M phosphate buffer. Single-strand DNA was eluted with 3 ml 0.10 M phosphate buffer and collected in a scintillation vial. Double-strand DNA was eluted with 1.5 ml 0.20 M phosphate buffer and collected. (All elutions were performed at 62° C to melt small, imperfectly base-paired regions of single-strand DNA.) To each vial of double-strand DNA, 1.5 ml distilled water was added to bring the volume to 3 ml. All vials then received 3 ml scintillation cocktail (Scintiverse E, Fisher Scientific) and were counted for 5 min.

Disintegrations per minute (DPM) in the single- and double-strand fractions were calculated using efficiency determinations based on standards counted under the same conditions. The fraction of DNA in the double-strand form (F) decreases logarithmically with increasing strand breakage [27] and was calculated as follows:

$$F = \frac{\text{DPM double-strand DNA}}{\text{DPM double-strand DNA+DPM single-strand DNA}}$$

Results

Cytotoxicity studies in colon cancer cells

The IC_{50} data from survival curves for the four cell lines treated with either free DOX or DOX-lip are presented in Table 1. The three nonselected colon-cancer cell lines SW620, HT-29, and SW403 demonstrated different sensitivities to free DOX. The HT-29 and SW403 colon cancer cells, respectively were 2.5 and 7.3 times more resistant to DOX than was the SW620 cell line. Based on the IC_{50} values, at least a 425-fold difference in resistance was established between the DOX-resistant cell line SW620/R and the sensitive cell line SW620: the latter exhibited an IC_{50} value of 0.023 μM for free DOX as compared with the 9.83 μM displayed by the former. When DOX-lip was used, the IC_{50} values shown by the sensitive lines were similar to those obtained following incubation with the free drug; however, the resistant cell line SW620/R demonstrated a value of 6.96 μM, which was 1.4 times lower than that obtained using free DOX. In addition, DOX-lip produced cytotoxicity to HT-29 and SW403 cells, respectively, amounting to 1.31- and 2.33-fold that induced by the free drug.

Table 1. Growth inhibition study

Drug	IC_{50} (μM)			
	SW620	SW620/R	HT-29	SW403
DOX	0.023	9.83	0.058	0.168
DOX-lip	0.023	6.96	0.044	0.072

Colon cancer cells (5×10^5) were plated into 25-cm^3 flasks. Cells in the exponential growth phase were exposed to varying concentrations of drugs (DOX, DOX-lip) and cytotoxicity was determined as described in Materials and methods

It is apparent that DOX-lip was quite effective in enhancing the cytotoxicity of DOX to colon cancer cells, although this enhancement was not directly correlated with the level of resistance observed in the cells. Investigations of the cytotoxicity of blank liposomes in the absence of drug resulted in a maximal cell kill of 5%–10% at lipid concentrations equivalent to those of DOX-lip over the same period of exposure in all cell lines (data not shown).

P-glycoprotein determination

The DOX-resistant cell line SW620/R was selected for its resistance to anthracyclines and the cells were propagated in 1 μg DOX/ml medium. The levels of expression of P-glycoprotein in SW620/S, SW403, HT-29, and SW620/R colon cancer cells were determined by flow cytometric analysis after immunochemical staining using a murine monoclonal antibody against P-glycoprotein C219 [15]. Figure 1 shows the histogram distribution of fluorescence intensity related to P-glycoprotein in each cell line. P-glycoprotein was detected in all cell lines, especially in the resistant cell line SW620/R. The relative levels of expression of P-glycoprotein were 1.5 ± 0.11, 1.4 ± 0.04, 1.6 ± 0.17, and 2.62 ± 0.26 in SW620, SW403, HT-29, and SW620/R colon cancer cells, respectively.

This study shows that colon-cancer cell lines express P-glycoprotein at varying degrees. Thus, SW620/R ex-

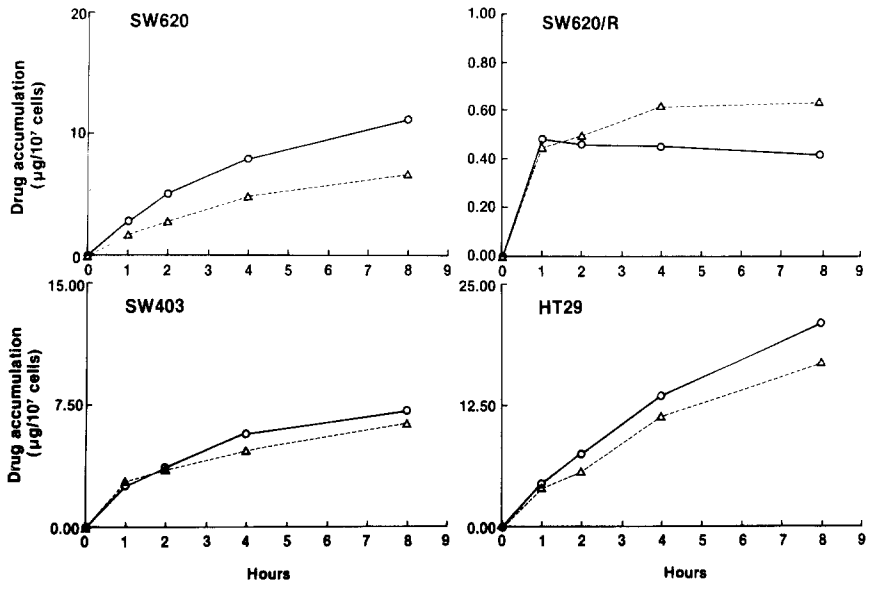

Fig. 2. Cellular drug accumulation following exposure of SW620, SW620/R, HT-29, and SW403 colon cancer cells to 10 μg free DOX/ml (○) or 10 μg DOX-lip/ml (△). Intracellular drug content was determined by fluorometry after extraction with butanol-1 as described in Materials and methods. The results represent the mean of at least three separate experiments

hibited fluorescence intensity that was approximately 2-fold that displayed by the parent cell line and the SW403 and HT-29 cells. Using immunoblotting, Mickey and co-workers [21] have shown that cell line SW620 expresses the P-glycoprotein, which supports our observation. Under similar experimental conditions, we observed an elevated level of expression of P-glycoprotein in SW403 and HT-29 cells, although we found no direct correlation with the absolute level of expression of this protein and relative drug resistance. In Fig. 1, the peaks to the left represent the histogram distribution of cells incubated with the second antibody only; this distribution was used to determine background cellular fluorescence and to calculate the fluorescence ratios reported herein.

Drug accumulation study

Figure 2 illustrates the accumulation of DOX in the four colon-cancer cell lines following treatment with 8.3 μM free drug or DOX-lip for a period of 1–8 h. After 8 h incubation with free drug, the parent SW620 cell line exhibited cellular accumulation of 11.2 ± 0.4 μg DOX/10^7 cells as compared with 0.42 ± 0.01 μg/10^7 cells for the resistant SW620/R cell line. Hence, as compared with the sensitive cell lines, the resistant line demonstrated a 26-fold decrease in total drug uptake. Among the cell lines that were not selected for resistance to DOX, significant differences in the cellular uptake of free drug were observed. The SW403 cells demonstrated drug accumulation of 7.2 ± 0.3 μg/10^7 cells at 8 h whereas HT-29 cells showed accumulation of 21 ± 0.7 μg DOX/10^7 cells over the same period of observation. Hence, SW620 and HT-29 cells accumulated 1.5 and 2.9 times more drug, respectively, than did SW403. In addition, there was no direct relationship between cytotoxicity and drug accumulation in the various cell lines.

The SW620/R cells selected for resistance to DOX demonstrated enhanced cellular uptake following incubation with DOX-lip. The total cellular accumulation was 0.64 ± 0.04 μg DOX/10^7 cells, which was 1.7-fold that observed using free DOX in these cells. Moreover, following incubation of SW620/R cells with free drug, the cellular accumulation of DOX reached a plateau at 1 h, whereas after treatment with DOX-lip, drug accumulation continued for 8 h. In contrast to the resistant SW620 cells, all non-selected cell lines demonstrated a decrease in cellular drug uptake when exposed to DOX-lip (Fig. 2).

Table 2. GSH levels in colon cancer cells

GSH (nmol/10^6 cells)			
SW620	SW620/R	HT-29	SW403
12 ± 5.6	15 ± 4.2	15.5 ± 3.55	13 ± 1.4

The total levels of reduced GSH in colon cancer cells were measured according to Ellman's method [9] (for details, see Materials and methods)

GSH assay

The GSH assay in the four colon-cancer cell lines did not reveal any significant difference between the cell lines. The values obtained for SW620, SW620/R, SW403, and HT-29 were 12 ± 5.6, 15 ± 4.2, 13 ± 1.4, and 15.5 ± 3.5 nmol/10^6 cells, respectively. The closeness of GSH values shows that the expression of resistance in these cell lines is not related to their GSH content (Table 2).

Alkali unwinding assay

The alkali unwinding assay [1, 27] was used to measure the DNA strand breakage produced by doxorubicin treatment in the different cell lines. In this assay, cellular DNA is exposed to mild denaturation conditions. Denaturation (unwinding) begins at sites of strand breakage and is allowed to progress for a limited period (1 h). After this interval, the

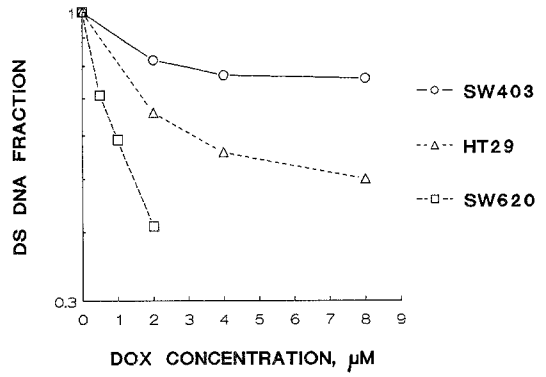

Fig. 3. DNA strand breaks were measured by alkali unwinding assays [1] in SW620, HT-29, and SW403 cells. The cells were radiolabeled with [^{3}H]-thymidine (0.10 μCi/ml; sp. act., 25 Ci/mmol) and were incubated for one doubling period. They were then washed with fresh medium and exposed to varying concentrations of free DOX or DOX-lip for 2 h. The values are presented as the double-strand DNA fraction and represent the mean of duplicate experiments (for details, see Materials and methods)

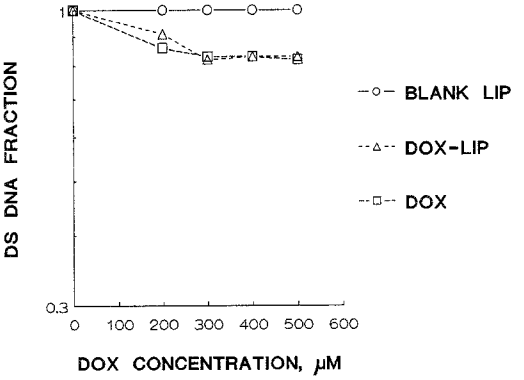

Fig. 4. DNA strand breaks were measured in SW620/R cells by alkali unwinding assays [1] following exposure of the cells to free DOX or DOX-lip for 2 h. The assays were carried out as shown in the legend to Fig. 3

unwinding is stopped by neutralization and the DNA is fragmented by sonication. Single- and double-strand DNA fragments are separated by hydroxylapatite chromatography. The double-strand DNA fraction decreases logarithmically with increasing strand breakage.

The different cell lines were treated with varying concentrations of free DOX at 37° C for a 2-h period and were then immediately assayed for DNA strand breakage. The results showed that the cell lines exhibited different degrees of DOX-induced DNA strand breakage (Fig. 3), which correlated with their sensitivities to drug-induced cytotoxicity. Concentrations required to produce a double-strand DNA fraction of 0.8 for each cell line were 0.3, 1, 2.8, and >300 μM for SW620, HT-29, SW403, and SW620/R, respectively (Figs. 3, 4). Control experiments with ^{137}Cs irradiation showed that as expected, all cell lines exhibited the same sensitivity to radiation-induced strand breakage (data not shown). Encapsulation of DOX in liposomes did not enhance the induction of DNA strand breakage by the drug in the resistant SW620/R cells (Fig. 4) or in any of the other cell lines (data not shown).

Discussion

Gastrointestinal cancers are relatively resistant to systemic chemotherapy. One reason for this resistance may be elevated levels of the MDR I gene, which has been shown to be present in 80% of untreated colon cancers by Goldstein et al. [11]. The protein product of this gene, P-glycoprotein, is thought to be related to decreased drug accumulation secondary to enhanced drug efflux. The present report describes the behavior of different human colon-cancer cell lines, three of which were nonselected (SW620, SW403, HT-29) and one of which was selected for resistance to DOX (SW620/R). All of the non-selected cell lines showed different degrees of resistance to DOX. The level of drug resistance exhibited by these cell lines was not directly correlated with the cellular drug accumulation (Table 1).

In general, all of these colon cancer lines expressed significant amounts of P-glycoprotein, especially SW620/R (Fig. 1). Mickey and co-workers [21] have demonstrated the absence of P-glycoprotein expression in SW620 using immunoelectrophoresis under normal conditions but found detectable levels following longer periods of exposure. This difference could be attributable to the high sensitivity of flow cytometric analysis and the high specificity of the monoclonal antibody used in the present study. According to Kartner et al. [16], expression of the MDR I gene roughly correlates with the degree of resistance and is accompanied by a decrease in drug accumulation. Our study shows that the accumulation of DOX correlated well with the degree of P-glycoprotein expression. We observed a low accumulation of DOX in SW620/R cells, a higher level in the SW403 line, and an intermediate content in HT-29 cells. In contrast, the expression of P-glycoprotein by SW620 cells was slightly lower than that by other cell lines; however, contrary to expectations, its drug accumulation was not the highest. This discrepancy could be related to other mechanisms of resistance in SW620 cells.

Our data demonstrated a decrease in the resistance of SW620/R cells to DOX when the drug was encapsulated in liposomes. The drug uptake in SW620/R cells treated with DOX-lip was 1.7-fold that obtained following incubation with free DOX (Fig. 2). This enhanced accumulation of DOX may be the result of a direct interaction of liposomes with P-glycoprotein. Recently, Thierry and co-workers [31] have demonstrated a direct interaction of empty liposomes with P-glycoprotein-enriched membrane vesicles isolated from LZ Chinese hamster cells. In addition, the resistant cells exhibit membrane fluidity different from that of the sensitive cells [6] and it is possible that liposomes interact differently with the resistant cell membranes to affect higher cellular transport of drug. It is also probable that liposomes modify the plasma membrane environment, affecting its structure.

In addition to the resistant SW620/R cell line, the non-selected cell lines HT-29 and SW403 were 1.3 and 2.4

times more sensitive to DOX-lip than to free DOX. It appears that the liposomes used in this study have the capacity to reduce the resistance of these cells. This view is supported by our recent observations [30] that LZ cells, which are 3,000 times more resistant to DOX than are the parent V-79 cells, were sensitized 7-fold by DOX-lip treatment. This partial reversal was accompanied by a 2-fold increase in drug accumulation by LZ cells exposed to DOX-lip as compared with free drug. In contrast, no enhancement of drug accumulation or cytotoxicity was observed following exposure of the V-79 parental cell line to DOX-lip [30]. The present study also demonstrates that treatment of sensitive SW620 cells with DOX-lip does not increase the cytotoxicity or accumulation of drug as compared with free DOX.

A number of biochemistry and pharmacology studies performed by various investigators have demonstrated that different mechanisms are involved in drug resistance [4, 5, 13, 22]. Deffie and co-workers [7] suggested that decreased drug uptake, decreased DNA damage, increased GSH activity, enhanced DNA repair, and elevated P-glycoprotein levels are characteristic of multifactorial drug resistance. In some MDR cell lines, overexpression of GSH has been demonstrated, and the use of buthionine sulfoximine has been shown to enhance the cytotoxicity of DOX to human MDR breast-cancer cells [8]. However, the present data indicate that GSH levels are equivalent in colon-cancer cell lines SW620, SW620/R, SW403, and HT-29 (Table 2). Hence, it can be concluded that the MDR reversal observed following treatment with DOX-lip is not affected by alterations in GSH functions.

DNA strand breakage induced by free DOX in the various cell lines correlated well with both cellular toxicity ($r = 0.99$) and drug accumulation ($r = 0.99$; Table 1, Fig. 2). This finding is in agreement with the premise that DNA is the main cellular target of lethality for DOX. Liposomal encapsulation of the drug had only a slight effect on drug uptake and no effect at all on DNA strand breakage in any of the cell lines tested. The resistant SW620/R cells, however, appeared to be specifically sensitized for cytotoxicity by liposome encapsulation. In the absence of enhanced DNA damage, the increased toxicity suggests the involvement of a secondary target for lethality (e.g., the cell membrane) when liposome encapsulation is used. In a recent study, Fan et al. [10] have shown enhanced cytotoxicity to UV-2237M/ADR cells using DOX encapsulated in multilamellar liposomes as compared with free drug. However, this enhanced sensitivity was not related to enhanced DNA strand breakage, in agreement with the present study.

Regardless of the mechanisms involved, DOX-lip was more active than free DOX in human colon cancer cells. Liposomes as carriers of DOX have been shown to attenuate the dose-dependent cardiotoxicity of the drug [14, 24, 25, 35]. Liposomes are biodegradable, nonimmunogenic, and relatively nontoxic and can be safely delivered to most of the major organs in animals and humans [2, 19, 28]. Recently, DOX-lip and liposomally encapsulated immunomodulators have been shown to minimize clinical complications [18, 26, 32] and, in some cases, to display enhanced therapeutic efficacy. Hence, in addition to its MDR-reversing capacity, DOX-lip is a potential chemotherapeutic modality for the treatment of cancer.

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