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Prolonged Circulation Time and Enhanced Accumulation in Malignant Exudates of Doxorubicin Encapsulated in Polyethylene-glycol Coated Liposomes[1]

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ABSTRACT

In preclinical studies, a doxorubicin liposome formulation containing polyethylene-glycol (Doxil) shows a long circulation time in plasma, enhanced accumulation in murine tumors, and a superior therapeutic activity over free (unencapsulated) doxorubicin (DOX). The purpose of this study was to characterize the pharmacokinetics of Doxil in cancer patients in comparison with free DOX and examine its accumulation in malignant effusions. The pharmacokinetics of doxorubicin and/or liposome-associated doxorubicin were analyzed in seven patients after injections of equivalent doses of free DOX and Doxil and in an additional group of nine patients after injection of Doxil only. Two dose levels were examined, 25 and 50 mg/m^2. When possible, drug levels were also measured in malignant effusions. The plasma elimination of Doxil followed a biexponential curve with half-lives of 2 and 45 h (median values), most of the dose being cleared from plasma under the longer half-life. Nearly 100% of the drug detected in plasma after Doxil injection was in liposome-encapsulated form. A slow plasma clearance (0.1 liter/h for Doxil *versus* 45 liters/h for free DOX) and a small volume of distribution (4 liters for Doxil *versus* 254 liters for free DOX) are characteristic of Doxil. Doxorubicin metabolites were detected in the urine of Doxil-treated patients with a pattern similar to that reported for free DOX, although the overall urinary excretion of drug and metabolites was significantly reduced. Doxil treatment resulted in a 4- to 16-fold enhancement of drug levels in malignant effusions, peaking between 3 to 7 days after injection. Stomatitis related to Doxil occurred in 5 of 15 evaluable patients and appears to be the most significant side effect in heavily pretreated patients. The results of this study are consistent with preclinical findings indicating that the pharmacokinetics of doxorubicin are drastically altered using Doxil and follow a pattern dictated by the liposome carrier. The enhanced drug accumulation in malignant effusions is apparently related to liposome longevity in circulation. Further clinical investigation is needed to establish the relevance of these findings with regard to the ability of liposomes to modify the delivery of doxorubicin to solid tumors and its pattern of antitumor activity.

INTRODUCTION

The administration of doxorubicin in liposome-associated form has been advocated as a means to reduce the cardiotoxicity of the drug. This is based on the reduced cardiac uptake of liposome-encapsulated doxorubicin and on pathological observations in preclinical animal models using a variety of liposome formulations (reviewed in 1). In recent years, the development of new formulations of long-circulating liposomes (also referred to as Stealth[3] or sterically stabilized liposomes) with reduced uptake by the RES[4] and enhanced accumulation in tumors has broadened the potential applications of these carriers in cancer drug delivery (reviewed in 2 and 3). In studies in rodents and dogs with some of these new formulations, liposome-associated doxorubicin has been shown to circulate with very long half-lives in the range of 15 to 30 h (4, 5). An increased accumulation of drug in murine transplantable tumors and in ascitic tumor exudates has been reported using long-circulating liposomes as doxorubicin carriers (6, 7). Doxorubicin encapsulated in long-circulating liposomes also shows a superior therapeutic antitumor activity and decreased toxicity when compared to free DOX[5] in a variety of mouse models (6, 7). Thus, long-circulating liposomes appear to offer a double advantage as an anticancer drug delivery system: toxicity buffering as with other previous liposome formulations and selective tumor accumulation leading to an enhanced antitumor activity. We have proposed (8) that the latter phenomenon is the result of liposome longevity in circulation on the one hand and increased microvascular permeability of tumors (9) on the other.

As a preamble to Phase I/II studies, it was important to determine whether the pharmacokinetic observations with doxorubicin liposomes in animals are extrapolable to humans. We thus undertook a pilot clinical study whose aims were to examine the pharmacokinetics of doxorubicin administered in free and liposome-encapsulated form at two dose levels (25 and 50 mg/m^2). The drug levels were also determined in malignant effusions in an attempt to estimate the putative drug levels in the tumor interstitial fluid. The liposome formulation used in this study, referred to hereafter as Doxil, contains a PEG derivatized phospholipid which has been shown to confer optimal prolongation of vesicle circulation time in animal models (10, 11).

PATIENTS AND METHODS

Patients. Sixteen cancer patients (male/female, 6/10) with a median Zubrod performance status of 2 (range, 1–3) and a median age of 59.5 years (range, 38–73) were entered into this study. The distribution by tumor type was: breast cancer (6 patients), non-small cell lung cancer (three patients), ovarian cancer (three patients), mesothelioma (two patients), soft tissue sarcoma (one patient), and pancreatic cancer (one patient). The criteria of eligibility included: bilirubin <35 μM; creatinine <150 μM; WBC $\geq$ 4,000/μl; neutrophils $\geq$ 1,500/μl; platelets $\geq$ 100,000/μl; normal prothrombin time; and left ventricle ejection fraction $\geq$ 55%. The study was carried out under the approval of the Institutional Review Board of the Hadassah University Hospital and Israel Ministry of Health, and written informed consent was obtained from all patients entered. Twelve of the 16 patients entered into the study had failed at least one line of chemotherapy. Four patients had not received prior chemotherapy. In 12 patients, malignant effusions (ascites and pleural effusion) were present. When technically feasible and clinically indicated, these effusions were tapped, and samples were processed to obtain information on drug levels.

Study Design. To rule out the possibility that interpatient variability may affect the interpretation of the results, a group of seven patients received the drug in free form in the first course of treatment and in encapsulated form (Doxil) in a second course of treatment. In this group, free DOX and Doxil were given at the same dose: 25 mg/m^2 in three patients and 50 mg/m^2 in four patients. To further characterize the pharmacokinetics of Doxil, a second group

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[3] Stealth Liposomes and Doxil are registered trademark names of Liposome Technology, Inc.

[4] The abbreviations used are: RES, reticuloendothelial system; DOX, doxorubicin; PEG, polyethylene-glycol; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography; AUC, area under the concentration $\times$ time curve.

[5] The abbreviation of DOX or free DOX is used to designate doxorubicin as the product used for injection only.

of nine patients received Doxil upfront without prior treatment with free DOX: six of them received 25 mg/m^2 in the first course, followed by a second course at 50 mg/m^2; and the remaining three received two successive courses of 50 mg/m^2. The top dose chosen (50 mg/m^2) is close to the recommended dose for free DOX as a single agent (12). The choice of the low initial dose (25 mg/m^2) derives from the need of a 2-fold difference in dose to obtain a clear picture of the dose dependency of the pharmacokinetic parameters.

All patients were scheduled for pharmacokinetic analysis in the two first courses. However, in three patients, only the first pharmacokinetic study was done due to patient deaths (2 cases) and patient refusal (1 case). Altogether, pharmacokinetic data were obtained after 7 courses of free DOX (3 at 25 mg/m^2 and 4 at 25 mg/m^2) and 22 courses of Doxil (8 at 25 mg/m^2 and 14 at 50 mg/m^2).

If there was evidence of disease stabilization or response, treatment with Doxil was continued at a dose of 50 mg/m^2 (every 21–28 days) with reevaluation after each 2 or 3 additional courses, up to a maximum of 10 courses. The safety of Doxil was monitored by periodic clinical and laboratory evaluations including weekly complete blood counts and three-weekly blood biochemistry profiles. MUGA scan (radionuclide ventriculography by multiple gated acquisition) to determine the left ventricle ejection fraction, electrocardiogram, and chest X-ray were done before the start of Doxil and after each five courses. Toxicity and antitumor response were graded using the WHO scale. In case of grade 3–4 toxicity, the dose of subsequent courses was reduced by 20%.

Drugs. Doxil is a liposome preparation containing doxorubicin provided by Liposome Technology, Inc. (Menlo Park, CA) and having the following lipid composition expressed as % mole ratio: hydrogenated soybean phosphatidylcholine (56.2), cholesterol (38.3), polyethylene-glycol (M_r 1, 900) derivatized distearoyl-phosphatidylethanolamine (5.3), and α-tocopherol (0.2). Doxorubicin is encapsulated in the liposome internal aqueous space at a drug-to-phospholipid ratio of approximately 150 μg/μmol in the presence of 155 mM ammonium sulfate and 200 μM deferoxamine mesylate. More than 98% of the drug is in the encapsulated form. The liposomes are suspended in 10% sucrose. The Gaussian mean vesicle size as measured by dynamic laser light scattering is in the range of 80 to 120 nm. Doxil was stored in frozen form (−10 to −20°C) at a concentration of 2 mg doxorubicin/ml. Under this storage condition, it is stable for more than 1 year with respect to drug potency, particle size, drug encapsulation, and phospholipid degradation. Doxil was administered without further dilution. The dose of Doxil is measured and expressed on the basis of its doxorubicin content.

Free DOX (Adriamycin-RDF) was obtained from Farmitalia-Carlo Erba (Milan, Italy) and diluted to 2 mg/ml in physiological saline before injection. Both Doxil and free DOX were given by slow bolus injection (5 to 10 min) through a peripheral arm vein.

Pharmacokinetic Studies. Blood was sampled from an arm vein contralateral to the site of injection into K_3-EDTA Vacutainer tubes and kept at 5°C. Four-ml blood samples were obtained before drug injection at 5, 15, and 30 min, and 1, 2, 4, 10, 24, 48, 72, and 168 h following Doxil; and at 5, 10, 15, and 30 min, and 1, 2, 4, 8, 12, and 24 h after free DOX injection. Plasma was separated by centrifugation within less than 24 h after blood collection. In Doxil-injected patients, a fraction of 0.5 ml plasma was passed through a Dowex resin column to separate the liposome-associated fraction from free and protein-bound drug which are retained by the resin as previously reported (13). The original plasma samples and the Dowex-treated plasma fractions were stored at −20°C.

In patients with malignant effusions (protein-rich and cytology-positive pleural effusion or ascites), the fluid was tapped one or several times. Usually, a large amount of fluid (>500 ml for pleural effusions; >1,000 ml for ascites) was removed to achieve a palliative effect. Fifty ml of effusion fluid was centrifuged in a preweighed tube. The supernatant was removed, and the tube was reweighed to estimate the weight of the cell pellet. Supernatants and cell pellets were stored at −20°C. Urine was collected for 24 h after injection of free DOX and for 72 h, in daily fractions, after injection of Doxil. Urine samples were centrifuged, filtered, and stored at −20°C.

To determine doxorubicin and doxorubicin-equivalents by a fluorescence assay, the samples were treated with 0.075 N HCl in 90% isopropanol (1/9, v/v or w/v) as previously described (7). Measurement was made by determining the intensity of fluorescence emission at 590 nm with an excitation wavelength of 470 nm using either the LS-5B (Perkin-Elmer, Buckinghamshire, England) or the SFM-25 (Kontron, Zurich, Switzerland) spectrofluorometers. The fluorimetric reading was converted to μg/ml by interpolation with the readings of a standard curve of DOX in the linear range.

HPLC analysis was used to detect metabolites in plasma, urine, and effusions. The extraction procedure for plasma samples and the HPLC system for detection and quantification of doxorubicin and its metabolites have been described elsewhere (5). Effusion supernatants, cell pellets, and urine samples were treated in the same way as plasma samples for the sake of drug extraction and HPLC analysis. To validate the HPLC procedure and, especially, to identify the polar metabolites, some of the urine samples were also analyzed by TLC on silicic acid (TLC aluminium sheets of silica gel 60 from Merck, Darmstadt, Germany) using two solvent systems as described by Takanashi and Bachur (14). System A is choloroform:methanol:glacial acetic acid:water (80:20:14:6). System B is acetone:butanol:water (50:40:10). The R_f values obtained in our analysis for various markers (doxorubicin, doxorubicinol, and aglycones) were similar to those reported (13).

Pharmacokinetic analysis was done by nonlinear least-squares analysis using Rstrip software (MicroMath, Inc., Salt Lake City, UT). The plasma concentration-time data were fitted to a biexponential equation as

$$C_{(t)} = A_1 * e^{-k_1 * t} + A_2 * e^{-k_2 * t}$$

where $C_{(t)}$ is the drug concentration (Y-axis) at time t (X-axis), A_1 and A_2 are the Y-intercepts, and k_1 and k_2 are the slopes or apparent first-order elimination rate constants. The area under the concentration*time curve (AUC) was calculated from the sums of the ratios A_1/k_1 and A_2/k_2. There were no significant differences between AUC values based on compartmental parameters and those based on the trapezoidal rule. In Doxil-injected patients, the AUC extrapolation from the last time point to infinity, was always less than 20% of the total AUC.

Clearance (CL) was calculated by dividing dose over AUC. Volume of distribution at steady state (V_{ss}) and mean residence time (MRT) were calculated using Equations A and B, respectively (15, 16), as

$$V_{ss} = \text{Dose} * \text{AUMC}/(\text{AUC}^2) \quad \text{(A)}$$

$$\text{MRT} = \text{AUMC}/\text{AUC} \quad \text{(B)}$$

where AUMC is the area under the product of $C*t$ plotted against t from time 0 to infinity.

RESULTS

Clinical Observations. A total of 53 courses (average per patient, 3; range, 1–9) of Doxil were given. Treatment was well tolerated. In four instances, there was an immediate reaction characterized by facial flushing and shortness of breath which resolved within min by discontinuing or reducing the rate of injection. These reactions did not occur when the rate of injection was maintained below 5 ml/min. Nausea occurred frequently, generally in a delayed (*i.e.*, 24 h post-treatment) and mild fashion (WHO grades 1 and 2), extending for several days and responding well to 5-HT_3 antagonists. Vomiting was reported only in sporadic cases. The most severe side effect was stomatitis, occurring 7 to 14 days after treatment in 5 of 15 evaluable patients dosed with 50 mg/m^2 Doxil. In two instances of patients heavily pretreated with chemotherapy, grade 3–4 stomatitis requiring 20% dose reduction was observed. No significant stomatitis was reported in seven patients receiving the single course of free DOX. Myelosuppression in the form of leukopenia/neutropenia was generally mild (grade 1 or 2) and always afebrile. In two instances coinciding with stomatitis, grade 3 granulocytopenia was detected. Nadir WBC counts below 4000/μl were observed on day 14 postinjection in only 6 of 15 patients dosed with 50 mg/m^2 Doxil. Grade 1 to 3 leukopenia occurred in 3 of 4 patients dosed with 50 mg/m^2 free DOX. No significant thrombocytopenia or anemia related to Doxil or free DOX were observed at any dose level. Following the 25 mg/m^2 dose of free DOX or Doxil, there was neither myelosuppression nor stomatitis.

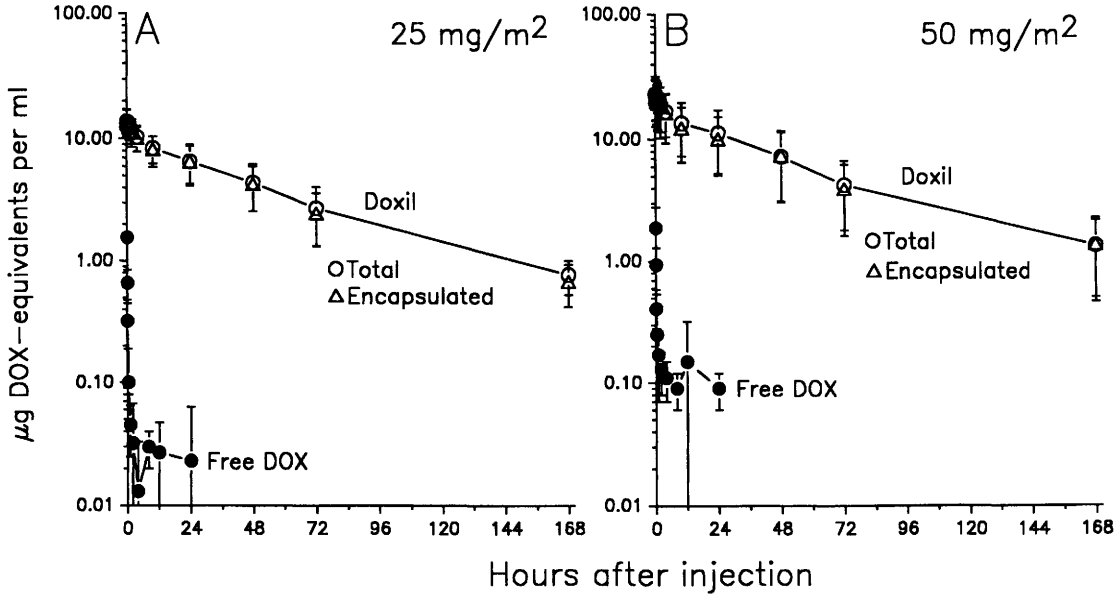

Fig. 1. Plasma levels of doxorubicin (total or liposome-encapsulated) in Doxil- and free DOX-treated patients. *A*, 25 mg/m^2 (n = 8 for Doxil; n = 3 for free DOX). *B*, 50 mg/m^2 (n = 14 for Doxil; n = 4 for free DOX).

In two patients, a desquamating dermatitis (grades 2 and 3) in both hands, resembling the hand-foot syndrome (17), was noticed after three courses of Doxil. This reaction was fully reversible after a 2-week rest period and may be related to a cumulative toxic effect of Doxil on the skin. The hand-foot syndrome is a known side effect of various chemotherapy regimes and has also been reported with continuous infusion of doxorubicin (18). There was no decrease of the left ventricle ejection fraction in four patients receiving five or more courses of Doxil, including a patient (#2) who received a cumulative dose of 235 mg/m^2 Doxil after a prior dose of 540 mg/m^2 free DOX.

Two antitumor responses were documented. In patient 2 with peritoneal mesothelioma, a decrease of the peritoneal thickening and of ascites was found by computed tomography scan. In patient 8 with ovarian cancer, who failed to respond to cisplatin, carboplatin, and etoposide, a sustained reduction of CA-125 blood levels was detected.

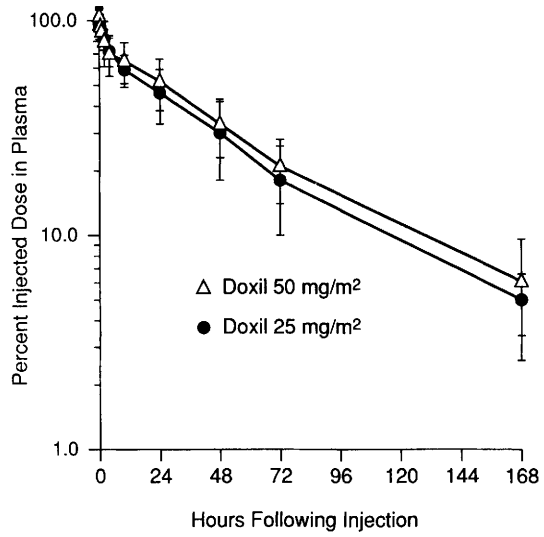

Fig. 2. Relative plasma clearance of Doxil (% injected dose) in relation to dose. Patients (n = 6) received consecutive doses of Doxil (25 and 50 mg/m^2) with a 3-week interval. To determine the percentage of injected dose in plasma, the patient's blood volume was calculated as 7.5% of the body weight, and the % hematocrit was sustracted to obtain the estimated plasma volume. The plasma drug concentration was then multiplied by the plasma volume and divided by the injected dose to obtain the % injected dose in plasma.

The time to failure for these responses was 6 and 7 months, respectively. Since both of these patients received a first course of free DOX, the respective contribution of free DOX and Doxil to the antitumor response is unknown.

Description of Doxil Pharmacokinetics. The pharmacokinetics of plasma doxorubicin after injection of Doxil was best described by a biexponential clearance curve. In many instances, very close fittings could also be obtained with a single exponential, but, except for one case, the goodness of fit, as based on the coefficient of determination and the Akaike Coefficient Criterion (19), was superior for the biexponential fit. Fig. 1 shows the plasma clearance curves of doxorubicin in patients receiving free DOX and Doxil at 25 mg/m^2 (Fig. 1*A*) or 50 mg/m^2 (Fig. 1*B*). Since no significant differences were found in the pharmacokinetics of doxorubicin, with or without previous treatment with free DOX, the results of both groups of Doxil patients were pooled and averaged to obtain a larger and more representative sample. At both dose levels, there is a striking difference between plasma doxorubicin clearance patterns when the drug is administered in free or liposome-encapsulated form. As noticed also in Fig. 1, the levels of total doxorubicin (○) and liposome-encapsulated doxorubicin (△) in Doxil-treated patients are almost superimposable, stressing the fact that most, if not all, of the drug in plasma is circulating in liposome-associated form.

In Fig. 2, the plasma clearance of doxorubicin in six patients receiving Doxil at 25 and 50 mg/m^2 has been plotted as a percentage of the injected dose to illustrate the effect of dose on clearance. Note that the two curves are almost identical, indicating that clearance is independent of dose within the range tested. This is in agreement with animal observations showing that the clearance of some types of long-circulating liposomes is independent of dose (20).

The pharmacokinetic parameters of Doxil and free DOX are summarized in Table 1. With Doxil, approximately 1/3 of the injected dose was cleared from plasma with an initial distribution half-life of 1–3 h. The rest of Doxil was cleared very slowly (second $t_{1/2}$, 42–46 h). This second phase accounted for more than 95% of the total AUC. We did not detect a terminal elimination phase of drug released from liposomes after Doxil administration, possibly because the high concentrations of liposome-associated drug may have masked a low concentration of free drug in the process of terminal clearance. This is in contrast to a previous study with a conventional liposome formulation

Table 1 *Pharmacokinetic parameters: median values (ranges)*

	Total doxorubicin equivalents in plasma		Liposome-associated doxorubicin in plasma		Total doxorubicin equivalents in plasma	
	Doxil 25 mg/m² (n = 8)	Doxil, 50 mg/m² (n = 14)	Doxil, 25 mg/m² (n = 8)	Doxil, 50 mg/m² (n = 14)	Doxorubicin, 25 mg/m² (n = 3)	Doxorubicin, 50 mg/m² (n = 4)
A_1 (mg/liter)	4.7 (2.8–9.1)	6.9 (4.5–13.6)	4.5 (1.7–7.7)	6.9 (3.0–19.2)	3.3 (2.8–5.2)	5.7 (1.6–10.5)
A_2 (mg/liter)	8.2 (6.7–12.6)	13.2 (7.8–34.5)	8.3 (6.5–12.5)	12.2 (6.8–32.6)	0.06 (0.03–0.08)	0.22 (0.11–0.34)
C_0 (mg/liter)	12.6 (11.9–20.4)	21.2 (12.7–43.4)	12.9 (11.5–19.7)	20.6 (9.8–42.6)	3.3 (2.9–5.3)	5.9 (1.7–10.8)
1st $t_{1/2}$ (h)[a]	3.2 (0.2–5.4)	1.4 (0.2–7.3)	1.8 (0.5–7.7)	2.3 (0.2–4.5)	0.07 (0.05–0.09)	0.06 (0.06–0.08)
2nd $t_{1/2}$ (h)[b]	45.2 (20.8–59.1)	45.9 (29.3–74.0)	41.7 (21.1–60.5)	46.2 (29.7–82.1)	8.7 (3.6–13.3)	10.4 (5.4–26.8)
AUC_0^∞ (mg·h/liter)	609 (227–887)	902 (335–2,497)	597 (208–903)	893 (304–2,457)	1.0 (0.7–1.3)	3.5 (2.6–6.0)
CL (liters/h)	0.08 (0.05–0.21)	0.09 (0.03–0.24)	0.06 (0.05–0.23)	0.09 (0.03–0.26)	45.3 (39.7–48.6)	25.3 (13.3–35.2)
V_{SS} (liters)	4.1 (3.0–6.5)	5.9 (2.3–10.1)	3.9 (3.0–6.7)	6.4 (2.4–11.3)	254 (126–393)	365 (131–501)
MRT (h)	62.7 (28.6–81.3)	65.0 (41.8–100.3)	58.2 (29.0–79.6)	65.6 (42.5–110.5)	5.2 (28–9.9)	11.8 (6.2–16.8)

[a] 1st $t_{1/2}$, $\ln 2/k_1$.
[b] 2nd $t_{1/2}$, $\ln 2/k_2$.

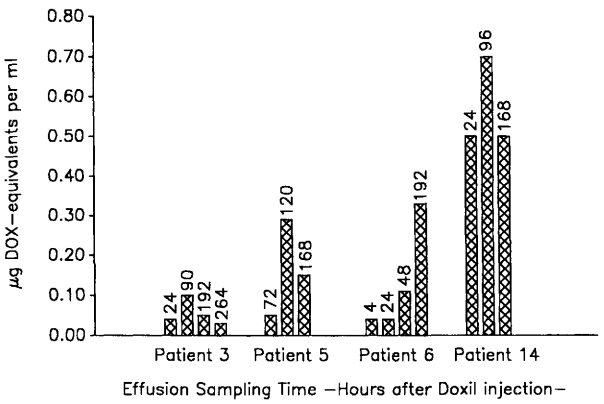

Fig. 3. Accumulation of doxorubicin in pleural fluid after Doxil treatment. Sampling h for each patient are shown above respective *columns*. Patients *3* (breast cancer), *5* (non-small cell lung cancer), and *6* (breast cancer) received 25 mg/m² Doxil. Patient *14* (non-small cell lung cancer) received 50 mg/m² Doxil.

of doxorubicin in which a terminal elimination phase similar to free DOX was clearly identified (21). It should also be noted that the data were almost superimposable when the pharmacokinetic analysis was based on measurements of total doxorubicin or liposome-associated doxorubicin. No significant changes were observed in various pharmacokinetic parameters, such as elimination half-life, CL, and V_{ss}, when the dose was increased from 25 to 50 mg/m² (Table 1).

As seen in Table 1, the distribution half-life for free DOX was very fast, in the order of min. The terminal elimination half-life was approximately 10 h, which is shorter than the 25 to 30 h commonly reported (22). This observation may be due to the fact that our method of analysis was unable to detect drug concentrations below 0.025–0.05 μg/ml, possibly leading to an underestimation of the tail of the clearance curve.

Striking differences were observed in key parameters when Doxil and free DOX are compared (Table 1). Thus, clearance and volume of distribution figures for Doxil were lower than those for free DOX by more than two orders of magnitude, while the mean residence time was 5- to 10-fold greater.

Drug Levels in Malignant Effusions. The accumulation of Doxil in malignant effusions is a slow process peaking between 3 to 7 days after injection. This phenomenon is depicted in Fig. 3, which shows data obtained in four patients (two of them undergoing repeated pleurocentesis and two of them bearing a drainage chest tube) indicating that peak concentrations in the pleural fluid are obtained several days after Doxil injection. This is consistent with the long distribution phase of Doxil. The median values (n = 4) of doxorubicin levels in malignant effusions increased from 0.20 to 0.45 μg/ml fluid and from 0.16 to 0.41 μg/g cells when the dose of Doxil was raised from 25 to 50 mg/m².

In three patients, the pleural effusion drug levels were examined after free DOX and Doxil treatments and found to be 4- to 16-fold greater in Doxil-treated patients (Fig. 4). The differences between free DOX and Doxil were more striking for supernatants than for cells. Based on the short distribution phase of free DOX, sampling was done between 4 to 24 h after injection of free DOX, while, in the case of Doxil, the data presented are from samples obtained several days after injection.

Metabolism and Excretion of Doxil. In plasma (Fig. 5*A*), we could not detect any significant amounts of metabolites by HPLC analysis, suggesting that the rate of metabolite production is slower than the rate of metabolite clearance from plasma. In urine (Fig. 5*B*), three metabolites were identified: two polar metabolites with short retention times (sulfate and glucuronide conjugates of the 4-demethyl, 7-deoxy-aglycones) and doxorubicinol. The presence of all three metabolites was confirmed by TLC analysis. Identification of the two polar aglycones (sulfate and glucuronide) was based on the R_f values reported by Takanashi and Bachur (14). There were as well other minor spots of unidentified metabolites. The urinary excretion of doxorubicin and metabolites peaked during the first or second day after injection of Doxil. The fraction of injected dose recovered in urine (median values, n = 9) using the fluorescence assay was 2.5% in urine collected for 24 h and 5.5% in urine collected for 72 h after injection. In contrast, after free DOX (n = 4), 11% of the dose was recovered in the urine within only 24 h after injection. The reduced renal clearance of Doxil is probably due to the fact that liposome-

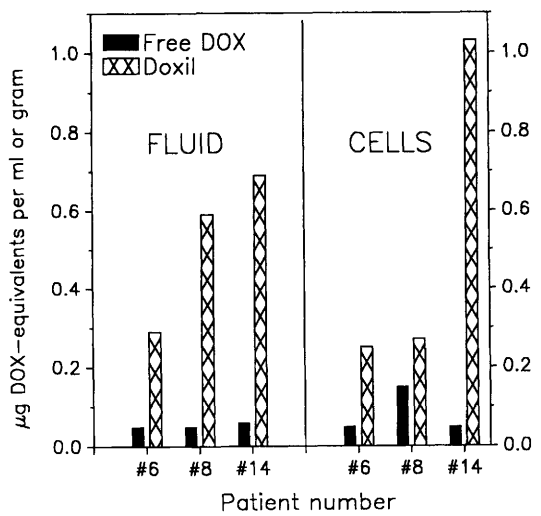

Fig. 4. Doxorubicin levels in malignant pleural effusions in patients treated successively with free DOX and Doxil with a 3-week interval. Sampling was done between 4 to 24 h after injection of free DOX and between 5 to 6 days after injection of Doxil. Patient *6* (breast cancer) received 25 mg/m². Patients *8* (ovarian cancer) and *14* (non-small cell lung cancer) received 50 mg/m².

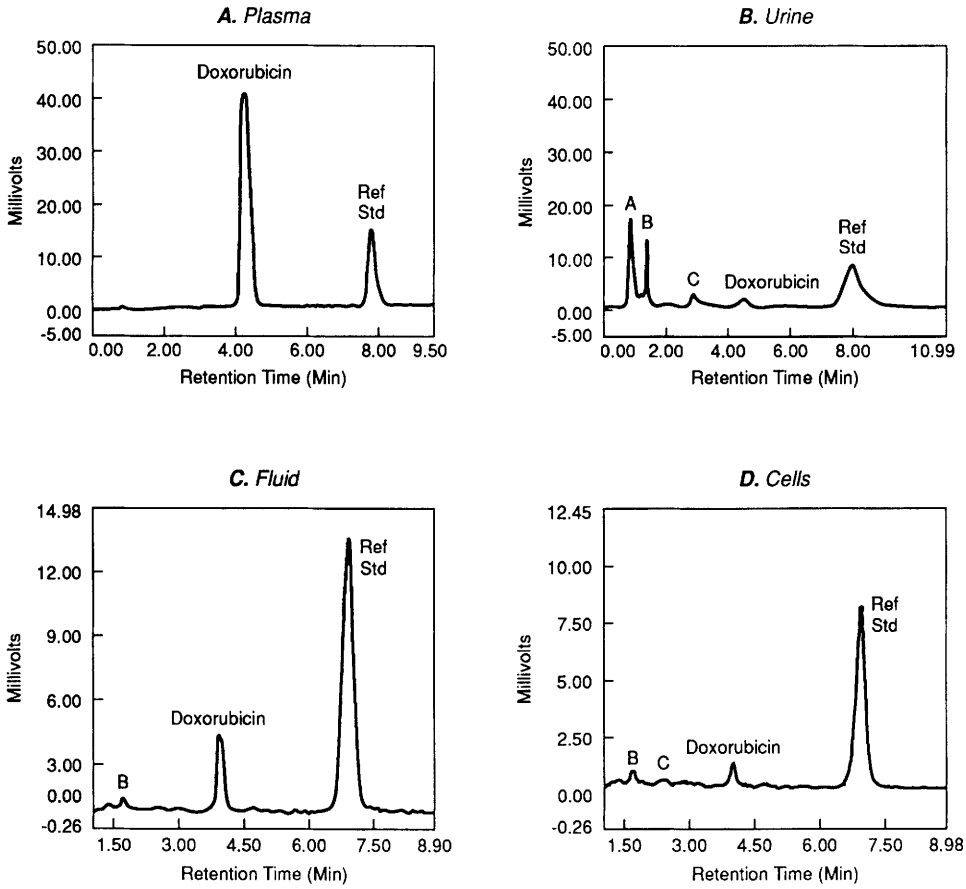

Fig. 5. HPLC chromatograms of *A*, plasma; *B*, urine; *C*, pleural effusion supernatant; and *D*, pleural effusion cell pellet. The reference standard is daunorubicin. Metabolites *A* and *B* are the polar conjugates (glucuronide and sulfate) of the 4-demethyl,7-deoxyaglycones. Metabolite *C* is doxorubicinol. Data from Patient *8* (ovarian cancer) who received 50 mg/m^2 Doxil. Plasma was sampled 24 h after injection. Urine is from 24-h collection postinjection; effusion was obtained 6 days after injection.

encapsulated doxorubicin cannot be filtered by the glomeruli because of the particle size.

Small amounts of metabolites were found in exudates, typically more in cell pellets and less in fluid supernatants (Fig. 5, *C* and *D*), suggesting that the liposomal drug becomes bioavailable in peripheral tissues.

DISCUSSION

Liposomes are an attractive carrier system for intravenous use because of their biocompatibility and versatility of formulation. As witnessed by recent publications, liposomes have been or are being tested for i.v. delivery of cytotoxic drugs, antifungal agents, and biological response modifiers in humans (1). One of the drugs most extensively tested in liposomes is doxorubicin. Phase I (23–25) and breast cancer Phase II studies (26) with liposomal doxorubicin have been reported. These studies hint at a slight reduction in toxicity for liposomal drug over free drug with doubtful clinical significance. However, the most disturbing point of many of these formulations is the rapid and dominant uptake of these liposomes by the RES, as indicated by the short distribution phase of liposome-associated drug (21, 24) and by imaging with radiolabeled liposomes (21, 27). This suggests that the drug distribution is shifted in favor of the RES and away from neoplastic tissue.

The observation that long-circulating liposomes of small size (<100 nm) accumulate in the interstitial fluid of transplanted tumors at levels comparable to those in RES-rich organs, such as liver (28–31), is the basis for a renewed momentum in the search of liposomal drug formulations with potential applications in cancer therapy. When anthracyclines are encapsulated in these long-circulating liposomes, a superior therapeutic index has been demonstrated in various experimental animal tumor models (6, 7, 32, 33). One of the factors with major impact on the circulation time of liposomes is the inclusion of a small fraction of a PEG-derivatized phospholipid. The resulting coating of the liposome surface with PEG increases surface hydrophilicity, decreases opsonization and RES uptake, and prolongs liposome circulation time (2, 3). We have recently reported that the pharmacokinetic properties of doxorubicin encapsulated in PEG-coated liposomes are consistent when examined in rodents and in large mammalian species such as dogs (5). The present study confirms that the same pharmacokinetic observations are extrapolable to humans using a PEG-containing formulation, Doxil. Thus, for doxorubicin encapsulated in PEG-coated liposomes, the elimination half-life is approximately 20 h in mice, 30 h in dogs, and, as shown in this study, 45 h in humans. In all cases, very small clearance rates and small volumes of distribution, slightly above the species plasma volume, are observed. The latter observation suggests that Doxil is restricted to a great extent to the intravascular space. In fact, 5 min after injection, the plasma concentration of doxorubicin accounted for the whole injected dose in most of the patients (see Fig. 2). These observations also rule out the possibility of a sudden burst of drug release after injection, in contrast to what has been observed with other formulations of liposomal doxorubicin (21, 34). This is a relevant issue since preclinical studies have shown that the stability of liposomal doxorubicin in circulation is an important factor in its toxicity (35). In terms of changes in AUC and clearance, the results reported here point at differences of one to two orders of magnitude with respect to previous reports with other formulations of liposomal doxorubicin (21, 24, 25).

We were not able to detect any circulating free drug after injection of Doxil. There are two possible explanations for this which may complement each other. One is that most of the drug is cleared from plasma as liposome-associated drug that distributes slowly into the peripheral tissue compartment. The other one is that the efflux rate of

drug from circulating liposomes is slower than the rate of clearance of free drug from plasma, thus preventing any accumulation of free drug in plasma.

Regarding metabolites, the pattern observed in urine suggests that Doxil undergoes metabolism by similar pathways to those of free DOX, although, in contrast to the latter, the rate of metabolite formation is slower than the rate of excretion, thus preventing a significant accumulation in plasma. Since liposome-encapsulated drug is not available to enzymes, the source of these metabolites must be drug that has leaked from liposomes in plasma and interstitial fluid or drug released from endocytosed liposomes.

From the point of view of mechanism of antitumor activity, the most relevant observation is the enhancement of drug concentration in malignant effusions when Doxil is compared to free DOX. The sampling of these fluids was done as the best possible approximation to the drug concentration in the tumor interstitial fluid using a relatively noninvasive method. The slow increase in drug concentration, which reaches a peak several days after injection, is consistent with a liposome extravasation process, similar to what has been described in preclinical models (7, 31). There are two likely mechanisms of drug delivery to tumors using liposomes: release of drug from circulating liposomes followed by free distribution into all body compartments and extravasation of liposomes into the tumor interstitial fluid followed by *in situ* release of drug. Pharmacologically, the latter possibility is the most interesting one since it represents first order targeting. The ability of long-circulating liposomes to extravasate into human malignant effusions is clearly supported by this study. Yet, we cannot rule out a concomitant process of slow drug release from circulating liposomes. In fact, the occurrence in several patients of stomatitis, a common side effect when DOX is given by continuous infusion (36), suggests that a slow release process in the intravascular compartment is also involved in drug clearance.

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