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Systemic Administration of Doxorubicin-containing Liposomes in Cancer Patients: a Phase I Study

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Abstract—*A clinical study was designed to evaluate the tolerance of cancer patients to liposome-associated doxorubicin (L-DXR). The liposomes used contain phosphatidylglycerol, phosphatidylcholine, cholesterol, and DXR intercalated in the lipid bilayer, and have a mean size in the range of* 0.3–0.5 μm. *Thirty-two patients, most of them with primary or metastatic liver cancer refractory to conventional therapy, were entered into the study. A total of* 69 *courses of therapy was administered by intravenous infusion of a suspension of L-DXR* (0.5–2.0 mg *DXR*/ml) *in physiologic saline at an approximate rate of* 2 ml/min *given on a 3-week intermittent schedule. The L-DXR and phospholipid doses were escalated from* 20 mg/m^2 *and* 0.3 g/m^2 *to* 120 mg/m^2 *and* 3.2 g/m^2 *respectively. Treatment was generally well tolerated and acute toxic effects such as nausea and vomiting were mild and infrequent. Chills and fever* (>38.0°C) *were observed in three patients during infusion of L-DXR and in seven patients* 6–12 h *after the end of infusion. Median WBC nadir counts were* 2700, 2300 *and* 700/μl *at* 85, 100 *and* 120 mg/m^2 *respectively. All three patients receiving* 120 mg/m^2 *developed grade* 4 *leukopenia and fever requiring intravenous antibiotics, and, in two of them, severe stomatitis (grades* 3 *and* 4) *was observed. Significant hair loss was apparent in all patients receiving doses higher than* 50 mg/m^2. *The maximal tolerated dose of L-DXR appears to be* 120 mg/m^2, *with leukopenia and stomatitis being the dose-limiting factors. While the subacute toxicity of L-DXR appears to be qualitatively similar to that of free DXR, its tolerance exceeds the recommended dose of free DXR* (75 mg/m^2) *in the standard* 3-*weekly schedule.*

INTRODUCTION

One of the most encouraging areas of preclinical work with liposome-associated chemotherapeutic agents deals with the use of anthracyclines. Several groups of investigators have reported that the systemic toxicity and specifically, the cardiotoxicity of doxorubicin (DXR) are significantly diminished by liposome-mediated delivery in rodent and dog models [1–5]. At equitoxic doses, the therapeutic activity of liposome-associated DXR (L-DXR) is equal or greater than that of free DXR in a variety of experimental tumor models either locally implanted or systemically disseminated [6, 7]. At milligram-equivalent doses, the antitumor activity of L-DXR is superior to that of free DXR in various murine models of liver metastases [8–10], while free DXR appears to be more efficacious against SC- and IP-inoculated tumors [11]. Administration of DXR in liposome-associated form results in significant changes in the drug tissue distribution and pharmacokinetics [12, 13] which probably account for the observed differences in the pattern of toxicity and antitumor activity. Essentially, the experimental preclinical work set up three pharmacological bases upon which a clinical study of L-DXR appeared justified:

a. Attenuation of cardiotoxicity due to decreased uptake of L-DXR by the heart muscle [3, 4, 12–15].
b. Increased drug exposure and tumoricidal activity on metastases infiltrating liver and spleen [16,17]. Although liposomes are primarily taken up by Kupffer cells and other cells of the mononuclear phagocyte system (MPS) in direct contact with the bloodstream [18], it has been reported that DXR or its active metabolites can be accumulated by macrophages and

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subsequently released into neighboring cells or extracellular space [19], thus enabling indirect drug delivery to tumor cells.

c. Slow-release effect depending on the rate of dissociation of DXR from the liposomes during circulation and/or after uptake by the MPS.

Herein, we describe a Phase I study of a formulation L-DXR in cancer patients. The results reported here indicate that treatment is generally well tolerated with regard to acute side-effects and that the maximal tolerable dose exceeds the recommended dose of free DXR. These clinical results are consistent with the toxicity advantage of L-DXR over free DXR in preclinical studies. Bone marrow suppression and stomatitis were identified as the most likely single-dose limiting factors.

MATERIALS AND METHODS

The methods, procedure of preparation and experimental clinical use of L-DXR are in accordance with a protocol approved by the Pharmaceutical Division of the Israel Ministry of Health and by the Committee on Research Involving Human Subjects of the Hadassah Medical Center.

Formulation

The formulation contains the following lipids: egg phosphatidylcholine (PC); egg-derived phosphatidylglycerol (PG) (both from Avanti, Birmingham, AL); cholesterol; and *d*-α-tocopherol succinate (both from Sigma, St. Louis, MO), at a molar ratio of 7:3:4:0.2 respectively. Briefly, multilamellar liposomes were prepared by hydration of a thin dry lipid film with a solution of DXR (Adriamycin®, Farmitalia-Carlo Erba, Milano, Italy) and subsequent gradual extrusions through polycarbonate membranes (0.4 and 0.2 μm pore diameter, Nucleopore, Pleasanton, CA). The dispersions of DXR-containing liposomes were prepared in sterile, pyrogen-free, physiologic 0.9% sodium chloride (Travenol, Ashdod, Israel), to which deferoxamine mesylate (Desferal, Ciba-Geigy, Basel, Switzerland) was added to a final concentration of 50 μM. The role of Desferal and α-tocopherol succinate is to improve the stability of the drug and phospholipid components (authors' unpublished results). Entrapment of DXR varied between 60 and 80% of the initial drug input. Most of the entrapped DXR (>90%) is intercalated in the liposome bilayer [12]. Unlike our liposome preparations for preclinical use [5], free DXR was not removed by gel filtration, to avoid the risk of endotoxin contamination during this step. In the preparations injected to the last 15 patients (dose levels, 85–120 mg/m^2), free DXR was removed by a quick step of filtration through a cation-exchange resin coverted to the sodium form (Dowex 50W × 4, 200–400 mesh, from either Sigma, or Serva, Heidelberg, F.R.G.) [20]. Final Concentration of phospholipids (PC + PG) and DXR was in the range of 10–60 μmole/ml and 0.5–2.0 mg/ml respectively. The resin step accounted for most of the variability in the drug to phospholipid ratio of the final product (25–50 μg/μmol). All batches were stored in the dark at 4°C in sterile glass containers. A detailed description of the liposome preparation will be reported separately (Amselem *et al.*, submitted for publication).

Quality control analysis

The following assays were used for quality control analysis:

— Phospholipid concentration according to the procedures of Bartlett [21] and Stewart [22].
— Chemical purity of phospholipids assessed by thin layer chromatography (TLC).
— Lipid peroxidation by determining either the content of conjugated dienes from the absorbance at 233 nm [23] or the fatty acid composition by gas chromatography. Phospholipid fatty acids were transmethylated using Meth Prep II (Alltech, Deerfield, IL).
— DXR concentration determined either fluorometrically as previously described [12] or spectrophotometrically from the absorbance at 480 nm (molar extinction coefficient: 12,500) after liposomes were fully dissolved in 90% isopropranol containing 0.075 N HCl).
— DXR purity analyzed by TLC [24] and high performance liquid chromatography (HPLC) [25].
— Percentage of DXR associated with liposomes as determined by gel exclusion chromatography on Sephadex G-50 columns, or by filtration of the liposomes through a small Dowex resin column.
— Sterility tests using aerobic and anaerobic tryptic soy broth blood culture bottles (Bactec Johnson, Lane, MD).
— Rabbit pyrogenicity tests: a sample of each batch (0.5–2 mg/kg L-DXR) was tested according to USP specifications [26]. This test was not done in the preparations injected to the two first patients.
— Acute toxicity in mice: five BALB/c male mice were injected i.v. with 20 mg/kg L-DXR. Death of more than one animal within 1 week after injection would exclude the batch from human use. Using this test, free DXR is invariably lethal to more than 50% of the animals [5].
— Liposome size, measured by photon correlation spectroscopy, also referred as dynamic light scattering [27].

All batches were submitted to quality control analysis immediately after preparation. In addition, randomly selected batches were retested after 3–6 months of storage. The results of quality control analysis are briefly described below. Batch size

ranged between 150 and 1200 mg DXR. The pH was in the range of 6.0 ± 0.5. Phospholipid peroxidation did not exceed 1%. Formation of lyso-PC and lyso-PG was below detection limits. Traces of two other unidentified fluorescent compounds, one with R_f (distance of compound from origin/distance of solvent front from origin) lower than doxorubicinol, and the other with R_f higher than the aglycone or its 7-deoxy derivative, were detected in the original DXR material, their level increasing during storage of L-DXR but not exceeding 5%. The level of DXR unassociated with liposomes varied between batches but did not increase significantly throughout storage. In batches used up to the 70 mg/m^2 dose level in this study, free DXR accounted for 15–35% of total DXR. The additional resin treatment in the material injected to patients receiving 85 to 120 mg/m^2 resulted in a reduction of the level of free DXR to less than 10%.

All batches were sterile. The second batch, which caused a severe pyrogenic reaction with hypotension in the second patient of this study, was found retrospectively to contain endotoxin. All other batches were negative in the rabbit test. Only one batch was discarded because of excessive toxicity in mice.

The vesicle Gaussian mean as measured by dynamic laser scattering was in the range of 0.3–0.5 μm with a standard deviation between 15 and 40% of the mean.

Method of administration and schedule

L-DXR at a concentration of 0.5–2.0 mg/ml in physiologic saline was administered at a rate of 2–3 ml/min through a peripheral arm vein using an ordinary i.v. set and with the patient in a recumbent position. Infusion time ranged between 30 and 90 min. The infusion bag was protected from light throughout the administration. No prophylactic anti-emetic therapy was given. Additional courses were administered at 3-week intervals if blood counts were adequate (WBC ≥ 3900/μl, platelets ≥ 99,000/μl). Treatment was postponed for patients with lower counts until these returned to normal. In patients with nadir counts of less than 2000 WBC/μl or less than 50,000 platelets/μl, the dose of an additional course was reduced by 20–30%. Treatment was discontinued if there were signs of disease progression or when patients wished to withdraw.

Patients were injected with L-DXR batches stored for 1 week to 3 months.

Patient selection and evaluation

The criteria of eligibility of patients were those generally adopted for Phase I clinical trials, including: primary or secondary neoplasia refractory to conventional therapy; no clinical evidence of heart failure and normal left ventricle ejection fraction (≥ 55%) as determined by multigated radionuclide angiocardiography; serum transaminase levels under 3-fold normal values; bilirubin < 35 μmol/l; creatinine < 200 μmol/l; no evidence of brain metastases; informed consent.

Pretreatment evaluation and follow-up included physical examination and Karnofsky's performance status, chest X-ray, ECG, and left ventricle ejection fraction, complete blood counts before each treatment course and biweekly thereafter, complete blood biochemistry panel before and after each treatment course, and tests to document the extent of malignant disease. Patients were admitted to the ward for the first course of treatment and remained in hospital until the next day for observation of any acute side-effects. Subsequent courses were given on an ambulatory basis. Grading of acute and subacute toxicity was according to WHO recomendations [28].

In patients completing two or more courses of therapy, the antitumor effect was evaluated according to response criteria previously described [29].

RESULTS

Patient characteristics

Patient characteristics are presented in Table 1. Thirty-two patients were admitted to the study. A total of 69 courses was administered. The number of courses given per patient was distributed as follows: one course, 13 patients; two courses, 10 patients; three courses, five patients; four courses, two patients; six and seven courses, one patient each. One patient was not evaluable for subacute toxicity because of discontinuation of the infusion of L-DXR due to an acute febrile reaction. As seen in Table 1, the two largest groups of patients suffered from metastatic colorectal carcinoma and hepatocellular carcinoma. Primary or secondary liver involvement was present in all but five patients. Most of the patients (26/32) had been previously exposed to chemotherapy, and two of them had also received irradiation to the pelvis. Baseline performance status ranged between 50 and 100% in the Karnofsky scale, except for two of the patients at the initial dose levels of 20 and 30 mg/m^2 whose performance status was between 30 and 40%.

Dose escalation

The design of the study called for dose escalation to proceed until grade 3 or 4 dose-limiting toxicity was encountered in three consecutive patients. The patient distribution in relation to dose per course and cumulative dose is shown in Table 2. The dose of L-DXR was gradually escalated from 20 to 120 mg/m^2 over eight steps. The corresponding increase in phospholipid dose was approximately

Table 1. Patient characteristics

Number of patients treated (male/female)	32	(17/15)
Total number of courses	69	
Number of courses per patient — median (range)	2	(1–7)
Age in years — median (range)	58	(22–75)
Karnofsky peformance status — median (range)	70%	(30–100)
Primary tumor site — Colon and rectum	16	
Liver (hepatocellular Ca)	9	
Soft tissue sarcoma	2	
Other (melanoma, small cell lung, breast, pancreas, stomach)	5	
Primary or secondary liver involvement, yes/no	27/5	
Previous chemotherapy, yes/no	26/6	
Previous therapy with DXR, yes/no	8/24	

Table 2. Patient distribution by single and cumulative doses

Dose per course*		Cumulative dose	
Dose (mg/m²)	No. of patients	Dose (mg/m²)	No. of patients
20	2		
30	2		
40	2	<100	10
50	5	100–199	16
60	3	200–299	3
70	3	300–399	1
85	7	400–550	2
100	5†		
120	3		

*Patients classified according to the initial dose received.
†One patient not evaluable for subacute toxicity due to discontinuation of the infusion following severe pyrexia.

0.3–3.2 g/m². The difference in the escalation factor of drug (×6) and phospholipid (×10.6) is due to changes in the final drug to phospholipid ratio of the formulation (see Materials and Methods). Two patients were entered in each of the first three dose levels which were practically devoid of toxicity. Thereafter, dose escalation proceeded at stepwise increments of approximately 20%. Depending on the toxicity observed, three to seven patients were entered at each dose level. Escalation proceeded without dose-limiting toxicity until the 50 mg/m² dose. At this level, two patients developed granulocytopenia associated with fever. Dose escalation was resumed after three additional patients received 50 mg/m² and developed no hematologic toxicity beyond grade 2. The study was completed after reaching the 120 mg/m² dose level due to severe myelosuppression and/or stomatitis in three consecutive patients. This suggests that the maximal tolerated dose (MTD) of L-DXR, as given here, is 120 mg/m².

Toxicity

Acute toxicity. Most of the patients tolerated the treatment well. One of the most striking observations was the low incidence and severity of gastrointestinal (nausea, vomiting) toxicity (Table 3), which was limited to transient nausea and sporadic cases of vomiting, with no anti-emetic treatment required. The only relevant acute side-effect related to the administration of L-DXR was fever (>38°C) accompanied by chills occuring in 10 patients and affecting a total of 17 courses of treatment. In seven patients, fever developed between 6 and 12 h after the end of the infusion. In repeated courses, with different batches of L-DXR, the same patients developed similar episodes of pyrexia, suggesting that this side-effect was related to individual sensitivity. Furthermore, injection of the same batch of L-DXR would cause fever in some but not in other patients. In three cases, the pyretic reaction developed during infusion of L-DXR and was controlled with administration of corticosteroids and morphine sulphate. One of these patients, however, developed also hypotension apparently as a result of endotoxin contamination of the batch (see Materials and Methods). Except for this case, no other hemodynamic alterations occurred and rabbit pyrogenicity tests were consistently negative. Blood cultures obtained during these episodes were negative.

Although no accident of extravasation was reported, it is worth noting that no local effects, such as erythema, tenderness or ulcers, were ever seen at the site of i.v. injection of L-DXR. Two patients complained of sudden back pain during the infusion. The pain subsided after slowing the rate of infusion.

Subacute toxicity. Of all subacute manifestations of toxicity (Table 3), myelosuppression appears to be the most severe dose-limiting side-effect of L-DXR.

Table 3. Toxicity of L-DXR

Dose per course (mg/m²)	Acute toxicity				Subacute toxicity					
	Fever (>38°C)		Nausea-vomiting		Leukopenia (<4000/μl)		Stomatitis		Alopecia	
	No. of cases/ No. of courses	Maximal grade	No. of cases/ No. of courses	Maximal grade	No. of cases/ No. of courses	Median grade (range)	No. of cases/ No. of courses	Median grade (range)	No. of cases/ No. of patients*	Partial/ extensive†
20	1/3	4	1/3	2	1/3	0 (0–1)	1/3	0 (0–1)	0/2	—
30	0/6	—	1/6	1	1/6	0 (0–1)	0/6	—	0/1	—
40	0/4	—	0/4	—	1/4	0 (0–1)	0/4	—	0/1	—
50	3/9	2	0/9	—	9/9	2 (1–4)	3/9	0 (0–2)	3/5	2/1
60	0/6	—	2/6	2	5/6	1 (0–3)	3/6	0 (0–1)	3/3	0/3
70	2/11	2	2/11	2	7/11	1 (0–4)	5/10	0 (0–3)	1/3	1/0
85	7/18	2	4/18	2	12/18	1 (0–4)	9/18	1 (0–3)	7/7	3/4
100	4/9	3	2/9	2	8/8	2 (1–4)	4/8	1 (0–3)	3/4	1/2
120	0/3	—	1/3	2	3/3	4 (4–4)	2/3	3 (0–4)	3/3	1/2

*Evaluable patients classified according to the initial dose received.

†The definitions of partial and extensive alopecia are roughly equivalent to WHO grades 2 and 3, respectively.

Leukopenia (<4000 WBC/μl) occurred in most patients receiving 50 mg/m^2 and higher doses. In seven patients, leukopenia was accompanied by fever, requiring hospitalization and i.v. broad spectrum antibiotics. Median nadir counts (Table 4) were above 2000 WBC/μl up to the 100 mg/m^2 dose level. As with free DXR, the WBC nadir time was in all instances between day 10 and day 14 after treatment. Thrombocytopenia (<100,000 cells/μl) occurred less frequently, always accompanying leukopenia. No bleeding episodes occurred. Grades 2 and 3 stomatitis were observed with increased frequency in patients receiving 85 mg/m^2 or higher doses.

All three patients receiving 120 mg/m^2 required hospitalization and i.v. antibiotics due to agranulocytosis and fever. In addition, protracted and severe (grades 3 and 4) stomatitis were seen in two of them.

Repeated courses of treatment did not result in increased myelosuppression, suggesting that bone marrow toxicity was not cumulative (Fig. 1). A downward trend in the hemoglobin concentration was noticed in most patients receiving high doses, as depicted in Fig. 1, although it was of minor significance.

Significant hair loss was seen in most of the patients receiving doses of 50 mg/m^2 or higher, especially when two or more courses were administered (Table 3).

There were no indications of any acute hepatotoxic effect according to liver function tests. However, two patients suffering from hepatoma and cirrhosis, in whom injection of radiolabeled colloid for liver–spleen scan showed splenomegaly and bone marrow uptake, developed severe myelosuppression at 50 and 85 mg/m^2, with nadir values accounting for the lowest range points observed (see Table 4, nadir ranges of dose levels 50 and 85 mg/m^2), suggesting an increased lability of cirrhotic patients to this form of treatment.

There were no clinical signs indicative of cardiotoxicity, nor any significant decrease in the left ventricle ejection fraction of patients receiving cumulative doses of more than 200 mg/m^2. No treatment related deaths occurred in this study.

Table 4. Nadir blood counts in relation to dose

Dose per course (mg/m^2)	Median nadir (range)	
	WBC × 10^3/μl	Platelets × 10^3/μl
30–40	10.2 (3.2–18.3)	223 (180–510)
50	2.4 (0.5–3.5)	125 (8–200)
60	2.8 (1.5–4.2)	169 (90–230)
70	3.9 (0.5–12.1)	192 (39–420)
85	2.7 (0.8–8.0)	172 (18–442)
100	2.3 (0.6–3.2)	106 (44–471)
120	0.8 (0.6–0.9)	42 (14–69)

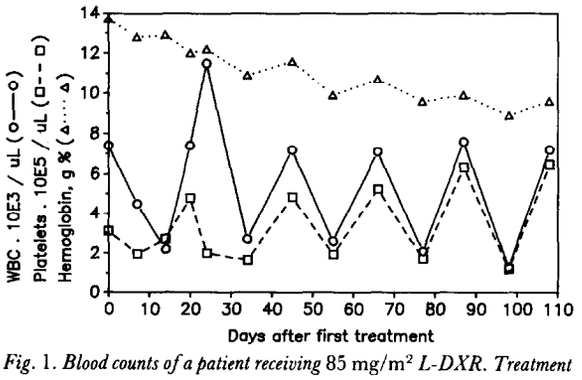

Fig. 1. Blood counts of a patient receiving 85 mg/m^2 *L-DXR. Treatment was given on days* 0, 24, 45, 66, *and* 87. *The patient is a* 65*-year old female suffering from liver metastases of colon cancer who had previously been treated with* 5*-fluorouracil (total of* 30 g *during* 9 *months).*

Antitumor effect

Among 18 patients evaluable for antitumor response, we observed one partial response in a hepatoma patient, and five minor responses (three hepatomas, one pancreatic carcinoma with liver metastases and one leiomyosarcoma with liver metastases). These responses were characterized by reduction of the size of liver and lung metastases, disappearance of pleural effusion, improvement in liver function tests, and 2–5-fold decrease in the α-fetoprotein titer in two of the responding hepatoma patients. Response duration was short, ranging between 3 and 8 months. Two of the responses were observed in patients previously treated with free DXR who had responded and relapsed before being admitted to this study. Two additional patients suffering from metastatic colorectal carcinoma had stable disease for 2–4 months as judged by chest X-ray, bone scan, liver CT scan, and carcinoembryonic antigen levels. In the remaining 10 patients (four cases of hepatoma, five cases of colorectal carcinoma, and one case of melanoma), there was evidence of progressive disease after two courses of L-DXR.

DISCUSSION

The first clinical use of liposomes dates to 1974, when the fate of radiolabeled liposomes was examined in three cancer patients [30]. More extensive studies in humans with radiolabeled fluid-phase sonicated liposomes indicated that the major sites of uptake are the liver and spleen [31]. In the earliest report in the literature on the therapeutic use of liposomes as drug carriers in a sizable group of patients, administration of amphotericin-B in liposomes resulted in antifungal activity without apparent toxicity at doses in which serious toxic effects of the free drug would be expected [32]. The feasibility of liposome-based systemic therapy is supported by

additional reports in experimental anticancer [33] and antifungal studies [34].

This study shows that administration of DXR in a liposome formulation is feasible at higher than conventional dose levels of free drug (60–75 mg/m^2) [35] and appears to be well tolerated. Based on this phase I study, the recommended starting dose for phase II studies would be 100 mg/m^2. It should be noted that most of the patients entered in this study suffered from heavy neoplastic involvement of the liver, and most of them had been previously treated with cytotoxic agents. Since there is suggestive evidence that DXR-induced myelosuppression is more severe when liver involvement is present [36, 37], and that liposome localization in the bone marrow is increased when neoplastic infiltration of the liver is present [38], it is possible that a higher dose could be administered to other patient populations with acceptable toxicity.

Regarding acute toxicity, nausea and vomiting appear to be substantially attenuated when compared to the common clinical experience with free DXR at standard 3-weekly doses [35]. Fever, a rather uncommon side-effect of free DXR therapy, was observed in a relatively high percentage of cases. Although the immediate reaction may still be attributable to a low amount of endotoxin undetectable in the rabbit test, the delayed reaction appears to be a specific feature of L-DXR. One possible explanation is that this treatment induces interleukin-1 secretion by DXR-activated macrophages [39] following L-DXR localization in tissue macrophages and breakdown of endocytosed liposomes.

The subacute toxicity of L-DXR appears to be qualitatively similar to that of free DXR with leukopenia and stomatitis being the most significant dose-limiting factors. A similar finding has been obtained in a clinical study with DXR in liposomes composed of cardiolipin, PC, stearylamine and cholesterol [40], although the MTD and recommended dose for Phase II studies reported (90 and 75 mg/m^2, respectively) are lower than in this study.

The fraction of drug causing myelosuppression may reach the bone marrow in several ways. The ability of liposomes to localize in the bone marrow, a tissue rich in sinusoidal capillaries, has been demonstrated in animals [41] and in humans [38]. Alternatively, drug leakage from circulating liposomes may account for the myelosuppressive effect. Another possible explanation is the release of DXR into the circulation from liver and spleen macrophages after liposome endocytosis and breakdown. Although human pharmacokinetic studies with L-DXR have been reported [42, 43], they do not permit discrimination between these various possibilities since they do not provide separate measurements of the free drug (bioavailable) and liposome-associated drug (non-bioavailable) in plasma. The availability of recently developed methods to conduct separate pharmacokinetic analysis of free and liposome-associated DXR [44] should provide a valuable insight into these questions.

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REFERENCES

1. Rahman A, Kessler A, More N *et al.* Liposomal protection of Adriamycin®-induced cardiotoxicity in mice. *Cancer Res* 1980, **40**, 1532–1537.
2. Forssen EA, Tokes ZA. Use of anionic liposomes for the reduction of chronic doxorubicin-induced cardiotoxicity. *Proc Natl Acad Sci USA* 1981, **78**, 1873–1877.
3. Olson F, Mayhew E, Maslow D, Rustum Y, Szoka F. Characterization, toxicity, and therapeutic efficacy of Adriamycin® encapsulated in liposomes. *Eur J Cancer Clin Oncol* 1982, **18**, 167–176.
4. Van Hoesel QG, Steerenberg PA, Crommelin DJ *et al.* Reduced cardiotoxicity and nephrotoxicity with preservation of antitumor activity of doxorubicin entrapped in stable liposomes in the Lou/M Wsl rat. *Cancer Res* 1984, **44**, 3698–3705.
5. Gabizon A, Meshorer A, Barenholz Y. Comparative long-term study of the toxicities of free and liposome-associated doxorubicin in mice after intravenous administration. *J Natl Cancer Inst* 1986, **77**, 459–469.
6. Mayhew E, Rustum Y. The use of liposomes as carriers of therapeutic agents. In: Rein R, ed. *Molecular Basis of Cancer. Part B: Macromolecular Recognition, Chemotherapy and Immunology*. New York, Alan R. Liss, 1985, 301–310.
7. Rahman A, Fumagalli A, Barbieri B, Schein PS, Casazza AM. Anti-tumor and toxicity evaluation of free doxorubicin and doxorubicin entrapped in cardiolipin liposomes. *Cancer Chemother Pharmacol* 1986, **16**, 22–27.
8. Mayhew E, Rustum Y, Vail WJ. Inhibition of liver metastases of M5076 tumor by liposome-entrapped Adriamycin®. *Cancer Drug Deliv* 1983, **1**, 43–58.

9. Gabizon A, Goren D, Fuks Z, Meshorer A, Barenholz Y. Superior therapeutic activity of liposome-associated Adriamycin® in a murine metastatic tumor model. *Br J Cancer* 1985, 51, 681–689.
10. Mayhew EG, Goldrosen MH, Vaage J, Rustum YM. Effects of liposome-entrapped doxorubicin on liver metastases of mouse colon carcinomas 26 and 38. *J Natl Cancer Inst* 1987, **78**, 707–713.
11. Gabizon A, Goren D, Barenholz Y. Investigations on the antitumor efficacy of liposome-associated doxorubicin in murine tumor models. *Isr J Med Sci* 1988, **24**, 512–517.
12. Gabizon A, Dagan A, Goren D, Barenholz Y, Fuks Z. Liposomes as *in vivo* carriers of Adriamycin®: reduced cardiac uptake and preserved antitumor activity in mice. *Cancer Res* 1982, **42**, 4734–4739.
13. Rahman A, Carmichael D, Harris M, Roh JK. Comparative pharmacokinetics of free doxorubicin and doxorubicin entrapped in cardiolipin liposomes. *Cancer Res* 1986, **46**, 2295–2299.
14. Forssen EA, Tokes ZA. Improved therapeutic benefits of doxorubicin by entrapment in anionic liposomes. *Cancer Res* 1983, **43**, 546–550.
15. Rahman A, White G, More N, Schein PS. Pharmacological, toxicological and therapeutic evaluation in mice of doxorubicin entrapped in cardiolipin liposomess. *Cancer Res* 1985, **45**, 796–803.
16. Gabizon A, Goren D, Fuks Z, Dagan A, Barenholz Y, Meshorer A. Enhancement of Adriamycin® delivery to liver metastatic cells with increased tumoricidal effect using liposomes as drug carriers. *Cancer Res* 1983, **43**, 4739–4735.
17. Gabizon A, Goren D, Ramu A, Barenholz Y. Design, characterization and antitumor activity of Adriamycin®-containing phospholipid vesicles. In: Gregoriadis G, Senior J, Poste G, eds. *Targeting of Drugs with Synthetic Systems*. London, Plenum, 1986, 229–238.
18. Poste G. Liposome targeting *in vivo*: problems and opportunities. *Biol Cell* 1983, **47**, 19–38.
19. Martin F, Caignard A, Olson O, Jeanin JF, Leclerc A. Tumoricidal effect of macrophages exposed to Adriamycin® *in vivo* and *in vitro*. *Cancer Res* 1982, **42**, 3851–3855.
20. Storm G, Van Bloois L, Brouwer M, Crommelin DJ. The interaction of cytostatic drugs with adsorbents in aqueous media. *Biochim Biophys Acta* 1985, **818**, 343–351.
21. Bartlett GR. Phosphorus assay in column chromatography. *J Biol Chem* 1959, **234**, 466–468.
22. Stewart JC. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal Biochem* 1980, **104**, 10–14.
23. Klein RA. The detection of oxidation in liposome preparations. *Biochim Biophys Acta* 1970, **210**, 486–489.
24. Takanashi S, Bachur NR. Adriamycin® metabolism in man—evidence from urinary metabolites. *Drug Metab Disp* 1976, **4**, 79–87.
25. Beijnen JH, Rosing H, deVries PA, Underberg WJ. Stability of anthracycline antitumor agents in infusion fluids. *J Parent Sci Technol* 1985, **39**, 220–223.
26. Pearson FC. *Pyrogens: Endotoxins, LAL Testing and Depyrogenation*. New York, Marcel Dekker, 1985.
27. Goll J, Carlson FD, Barenholz Y, Litman BJ, Thompson TE. Photon correlation spectroscopic study of the size distribution of phospholipid vesicles. *Biophys J* 1982, **38**, 7–13.
28. Miller AB, Hoogstraten B, Staquet M *et al.* Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
29. Eagan RT, Fleming TR, Schoonover V. Evaluation of response criteria in advanced lung cancer. *Cancer* 1979, **44**, 1125–1128.
30. Gregoriadis G, Swain CP, Wills EJ, Tavill AS. Drug-carrier potential of liposomes in cancer chemotherapy. *Lancet* 1974, **1**, 1313–1316.
31. Richardson VJ, Ryman BE, Jewkes RF *et al.* Tissue distribution and tumor localization of technetium-99M labeled liposomes in cancer patients. *Br J Cancer* 1979, **40**, 35–43.
32. Lopez-Berestein G, Fainstein V, Hopfer R *et al.* Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study. *J Inf Dis* 1985, **151**, 704–710.
33. Sculier JP, Coune A, Brassine C *et al.* Intravenous infusion of high doses of liposomes containing NSC 251635, a water-insoluble cytostatic agent. A pilot study with pharmacokinetic data. *J Clin Oncol* 1986, **4**, 789–797.
34. Sculier JP, Coune A, Meunier F *et al.* Pilot study of amphotericin B entrapped in sonicated liposomes in cancer patients with fungal infections. *Eur J Cancer Clin Oncol* 1988, **24**, 527–538.
35. Blum RH, Carter SK. Adriamycin®. A new anticancer drug with significant clinical activity. *Ann Intern Med* 1974, **80**, 249–259.
36. McKelvey EM, Gottlieb JA, Wilson HE *et al.* Hydroxyldaunomycin (Adriamycin®) combination chemotherapy in malignant lymphoma. *Cancer* 1976, **38**, 1484–1493.
37. Gundersen S, Kvinnsland S, Klepp O, Kvaloy S, Lund E, Host H. Weekly Adriamycin® versus VAC in advanced breast cancer. A randomized trial. *Eur J Cancer Clin Oncol* 1986, **22**, 1431–1434.
38. Perez-Soler R, Lopex-Berestein G, Kasi LP *et al.* Distribution of technetium-99m labeled multilamellar liposomes in patients with Hodgkin's disease. *J Nucl Med* 1985, **26**, 743–749.

39. Mace K, Mayhew E, Mihich E, Ehrke MJ. Alterations in murine host defense functions by Adriamycin® or liposome-encapsulated Adriamycin®. *Cancer Res* 1988, **48**, 130–136.
40. Treat J, Greenspan AR, Rahman A. Liposome encapsulated doxorubicin — preliminary results of phase I and phase II trials. In: Lopez-Berestein G, Fidler IJ, eds. *Liposomes in the Therapy of Infectious Diseases and Cancer*. New York, Alan R. Liss, 1989, 353–365.
41. Gregoriadis G, Senior J. Liposomes *in vivo*: a relationship between stability and clearance? In: Gregoriadis G, Senior J, Poste G, eds. *Targeting of Drugs with Synthetic Systems*. London, Plenum, 1986, 183–192.
42. Gabizon A, Sulkes A, Peretz T *et al.* Liposome-associated doxorubicin: preclinical pharmacology and exploratory clinical phase. In: Lopez-Berenstein G, Fidler IJ, eds. *Liposomes in the Therapy of Infectious Diseases and Cancer*. New York, Alan R. Liss, 1989, 391–402.
43. Rahman A, Roh JK, Treat J. Preclinical and clinical pharmacology of doxorubicin entrapped in cardiolipin liposomes. In: Lopez-Berestein G, Fidler IJ, eds. *Liposomes in the Therapy of Infectious Diseases and Cancer*. New York, Alan R. Liss, 1989, 367–389.
44. Druckmann S, Gabizon A, Barenholz Y. Separation of liposome-associated doxorubicin from nonliposome-associated doxorubicin in human plasma: implications for pharmacokinetic studies. *Biochim Biophys Acta* 1989, **980**, 381–384.