Pharmacokinetics of doxorubicin administered i.v. as Myocet (TLC D-99; liposome-encapsulated doxorubicin citrate) compared with conventional doxorubicin when given in combination with cyclophosphamide in patients with metastatic breast cancer

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Myocet (TLC D-99) is a liposomal formulation of the antineoplastic drug doxorubicin with an improved therapeutic index compared with conventional doxorubicin. The objective of this study was to assess the plasma disposition of doxorubicin when administered i.v. as TLC D-99 and to compare this to conventional drug. Metabolite (doxorubicinol) plasma levels were also quantitated in both treatment groups. Plasma was collected during the first course of treatment from 10 patients receiving TLC D-99 60 mg/m^2 and 10 receiving conventional doxorubicin 60 mg/m^2, each with cyclophosphamide 600 mg/m^2. Samples were assayed for total doxorubicin (all doxorubicin regardless of whether it is encapsulated or not), encapsulated doxorubicin (TLC D-99 group only) and doxorubicinol using high-performance liquid chromatography. Plasma concentrations of total doxorubicin were higher in patients receiving TLC D-99 than in patients receiving conventional doxorubicin. The clearance of total doxorubicin after administration of TLC D-99 was lower (approximately 9-fold) and the volume of distribution at steady state was less (25-fold) than that of doxorubicin after conventional drug. Doxorubicinol was detected in the plasma of all patients in both treatment groups. The mean $AUC_{0-\infty}$ of doxorubicinol for patients receiving TLC D-99 ($1.5 \pm 0.4\ \mu M \cdot h$) was not statistically different than that in patients receiving conventional doxorubicin ($1.8 \pm 0.4\ \mu M \cdot h$), although the appearance of the peak doxorubicinol concentration occurred later and was lower in patients receiving TLC D-99. There was a correlation between the plasma $AUC_{0-\infty}$ of total doxorubicin and the degree of myelosuppression in patients receiving conventional doxorubicin, but this correlation was not found in patients receiving TLC D-99. *Anti-Cancer Drugs* 14:239–246 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:239–246

Keywords: breast cancer, doxorubicin, liposomes, Myocet, TLC D-99

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Received 3 December 2002 **Accepted** 14 January 2003

Introduction

Doxorubicin is one of the cornerstones in the management of breast cancer, despite the recent introduction of several new therapies for this disease [1]. The clinical utility of anthracycline antineoplastics such as doxorubicin has been limited by a cumulative cardiac toxicity that may begin with the first dose of drug. Initially, this toxicity may be subclinical, but the risk of developing clinically relevant, and even life-threatening cardiac toxicity, such as congestive heart failure (CHF), increases with increasing cumulative doxorubicin doses. Preclinical studies have shown that encapsulation of doxorubicin inside liposomes decreases the cumulative dose-limiting cardiotoxicity (as well as some of the acute toxicities such as ulceration on extravasation, alopecia and gastrointestinal-related effects) associated with the conventional form of the drug while maintaining or, in some cases, increasing the anti-tumor potency [2].

TLC D-99 is a non-pegylated, liposomal form of doxorubicin in which doxorubicin is complexed with citrate inside the vesicle. The aggregation state of the internalized doxorubicin and a pH gradient across the liposomal membrane are responsible for the slow rate of drug release from the liposome in plasma [3]. Recently, the clinical efficacy and reduced toxicity of TLC D-99 compared with conventional doxorubicin in the treatment of patients with metastatic breast cancer has been

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000060626.61556.da

demonstrated. The pivotal randomized, controlled, phase III trial compared TLC D-99 (60 mg/m^2 given i.v. over 1 h) and cyclophosphamide (600 mg/m^2 i.v.) to conventional doxorubicin and cyclophosphamide at the same doses as first-line therapy in patients with metastatic breast cancer [4]. A second trial compared single-agent therapy (75 mg/m^2) with either TLC D-99 or conventional doxorubicin in the same patient population [5]. Patients on the TLC D-99 arms of both studies experienced significantly less cardiac toxicity ($p \leq 0.0001$), mucositis ($p \leq 0.001$) and diarrhea ($p \leq 0.001$) than did those on the doxorubicin arms. There was no significant difference in the response rate of the two drugs in either of these studies. The activity of TLC D-99 has also been compared to epirubicin in the first-line treatment of metastatic breast cancer [6].

The actual disposition of doxorubicin after administration of TLC D-99 as a single agent has been reported in several previous phase I studies in patients with advanced cancers [7–9]. The doses and schedules evaluated varied, ranging from 20–30 mg/m^2 daily for 3 days to 20–37.5 mg/m^2 weekly to 20–90 mg/m^2 once every 3 weeks. Although the number of patients evaluated at each dose and schedule was small, and the types of cancer varied, the general conclusion from all three studies was that treatment with TLC D-99 resulted in higher plasma levels and $AUC_{0-\infty}$ for total doxorubicin (representing both 'free' and encapsulated drug) than that which was predicted or found with conventional doxorubicin.

In the present study, we evaluated the pharmacokinetics of doxorubicin and doxorubicinol in patients receiving 60 mg/m^2 of TLC D-99 (in combination with cyclophosphamide 600 mg/m^2) as first-line treatment for metastatic breast cancer. Patients receiving the same dose and schedule of conventional doxorubicin were also evaluated for comparison. These patients were part of the phase III, multicenter trial (protocol 94CE32-0652), the clinical results of which are reported separately [4].

Patients and methods

Study design

This was a multicenter, randomized, parallel, open comparative study to compare the efficacy and safety of TLC D-99 and doxorubicin–HCl administered once every 3 weeks in combination with cyclophosphamide to patients with metastatic breast cancer. Plasma samples were collected from patients at selected sites prior to, during and following the first course of study drug only. This study was approved by the Institutional Review Boards of all participating centers, and the study was conducted in accordance with the Declaration of Helsinki and its amendments. All patients signed informed consent forms.

Drug dosage and schedules

After preparation, TLC D-99 (Elan Pharmaceuticals, Princeton, NJ) contains doxorubicin–HCl (2 mg/ml) entrapped in liposomes composed of egg phosphatidylcholine (5.2 mg/ml) and cholesterol (2.4 mg/ml) in a citric acid/sodium carbonate buffer. Each vial also contains lactose 10 mg/ml and may contain methylparaben (0.2 mg/ml). On the first day of the first dosing cycle, cyclophosphamide was administered at a dose of 600 mg/m^2 as a continuous i.v. infusion over 15 min, followed by adequate hydration with normal saline. This rate of infusion and hydration schedule could be extended as clinically indicated, and/or substituted by infusion of cyclophosphamide into a flowing saline line for patients who did not tolerate infusion over 15 min. On the same day following cyclophosphamide and hydration, TLC D-99 or standard doxorubicin was administered at a dose of 60 mg/m^2, as a continuous i.v. infusion over 1 h into a free-flowing line. Concomitant use of other anti-neoplastic agents was not permitted during the trial. Granulocyte colony stimulating factor (G-CSF) was permitted (but not less than 48 h prior to chemotherapy), as were antiemetics.

Patients

Patients with histologically or cytologically proven breast carcinoma with radiographic or physical examination evidence of distant metastatic disease were eligible. Inclusion criteria also included ECOG performance status ≤ 2 ECOG, age ≥ 18 years, adequate bone marrow function, bilirubin $\leq 1.2\times$ the upper limit of normal, SGOT and SGPT ≤ 4 times the upper limit of normal and creatinine < 1.5 mg/dl. Patients were allowed to have received up to a maximum of 300 mg/m^2 of prior anthracycline (except any liposomal anthracycline formulation). Patients were also excluded if they had previous therapy involving chronic administration of high doses of phenobarbital or other cytochrome P450 inducing agents within 4 weeks of study entry.

Pharmacokinetic sampling

For drug concentration assays, 10 ml of blood was collected in tubes containing EDTA from a superficial vein in the arm contralateral to that used for infusion. Samples were collected after cyclophosphamide and hydration just prior to the infusion of either doxorubicin or TLC D-99 (0 h time point or pre-infusion), at the midpoint of the infusion (30 min time point) and immediately prior to the end of infusion (1 h time point). Additional samples were also obtained at 1.5, 2, 4, 6, 12, 18, 24, 48, 72, 96 and 120 h after the beginning of the i.v. infusion. Patients remained at the institution until the 48-h sample was collected. If the patient required G-CSF therapy during this cycle, the 48-, 72-, 96- and 120-h samples were collected prior to the administration of G-CSF for that day.

Immediately following collection, samples were centrifuged at approximately 2500 r.p.m. for at least 5 min at 4°C to separate plasma. Exactly 4 ml of plasma was transferred to a vial containing 0.4 ml of 55% glucose as a cryoprotectant. Samples were placed in a –20°C freezer and were transferred to a –70°C freezer within 24 h.

Analytical method

Reference standards for doxorubicin and daunorubicin (internal standard) were obtained from the USP. The reference standard for doxorubicinol was obtained from Oread Laboratories (Lawrence, KS). The analytical method used to separate and quantitate the concentration of liposome-encapsulated doxorubicin, total doxorubicin (all doxorubicin present, regardless of whether or not it is liposome encapsulated) and doxorubicinol in human plasma utilized solid-phase extraction followed by high-performance liquid chromatography (HPLC). For patients receiving TLC D-99, two assays were used—one to measure total doxorubicin, and one to measure liposome-encapsulated doxorubicin and doxorubicinol. Samples containing liposome-encapsulated and/or free doxorubicin were spiked with the internal standard (100 ng/ml daunorubicin) and treated with detergent [reduced Triton X-100; 2% (v/v) final concentration] to release any encapsulated doxorubicin in the plasma. The total doxorubicin was then separated from contaminating materials using a solid-phase (C_{18} Sep-Pak cartridges; Waters, Milford, MA) extraction procedure. The C_{18} cartridges (100 mg) were preconditioned with methanol and washed with 10 mM phosphate-buffered saline (pH 7.4) before applying 0.5 ml of sample. Both total doxorubicin and the internal standard bound to the C_{18} cartridge. After washing the cartridge with buffer, the doxorubicin and internal standard were eluted from the cartridge with chloroform:methanol (2:1). The eluant was evaporated to dryness under nitrogen, reconstituted with methanol and analyzed by HPLC with fluorescence detection. From a separate plasma sample, liposome-encapsulated doxorubicin and doxorubicinol were analyzed. An aliquot of 1 ml of plasma was applied to a C_{18} Sep-Pak column and the first fraction (liposome-encapsulated doxorubicin) was collected into a tube containing reduced Triton X-100 and the internal standard. After washing the Sep-Pak column, the remaining fraction (released or free doxorubicin and doxorubicinol) was collected in another tube. The first fraction (liposome-encapsulated doxorubicin) eluant was then applied to a second C_{18} Sep-Pak column, which was subsequently washed prior to elution of the final liposomal fraction and internal standard into a test tube. Both fractions were dried, re-dissolved in methanol and analyzed by HPLC.

For patients receiving conventional doxorubicin, only a single assay was required. A 1-ml aliquot of plasma was spiked with the internal standard and then applied to a C_{18} Sep-Pak cartridge. The doxorubicin, doxorubicinol and internal standard bound to the cartridge and contaminants were washed off with buffer. The doxorubicin, doxorubicinol and internal standard were then eluted with chloroform:methanol (2:1), evaporated, reconstituted in methanol and analyzed by HPLC.

HPLC was performed with a IB-sil 5 cyano, 150 mm × 4.6 mm, 5 μm particle size Phenomenex column (Phenomenex, Torrance, CA). The mobile phase consisted of ammonium acetate buffer (pH 4.0):acetonitrile (72.5:27.5 v/v) at a flow rate of 1.5 ml/min. Fluorescence detection was accomplished with an excitation wavelength of 233 nm and an emission wavelength above 550 nm. With this method, good separation of doxorubicin, doxorubicinol and internal standard was achieved in all fractions, although the actual retention times varied as the presence of Triton shifted the chromatography in those samples containing the detergent. No significant peaks eluted at the retention times of the analytes in blank human plasma from six subjects. Cyclophosphamide (10 μg/ml) did not co-elute with any of the analytes. The peak height ratios of doxorubicin and doxorubicinol versus the internal standard and concentrations (using a weighted 1/concentration^2 linear regression) were linear in the range of 2–500 ng/ml (0.003–0.75 μmol/l) with correlation coefficients of ≥ 0.995 for all analytes. The relative standard deviation for quality control samples analyzed on three separate days was $\leq 6.4\%$ for encapsulated doxorubicin, $\leq 7.0\%$ for total doxorubicin and $\leq 5.0\%$ for doxorubicinol. Samples were stable at –70°C for at least 4.5 months.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined by non-compartmental methods using the computer program WinNonlin Pro (Pharsight, Cary, NC). Area under the plasma concentration–time curve was estimated using the trapezoidal rule from 0 time to peak concentration and the log-trapezoidal rule from the peak concentration to the last measurable plasma concentration. $AUC_{0-\infty}$ was calculated by extrapolating to infinity from the last measurable data point using the terminal exponential rate constant. With infusion doses of conventional doxorubicin or liposomal doxorubicin, mean residence time and volume of distribution at steady state were determined by appropriate methods [10]. The following symbols are used: BSA = body surface area (m^2), C_{max} = maximum plasma concentration (observed), T_{max} = time of maximum plasma concentration (observed), CL = clearance (dose/$AUC_{0-\infty}$), V_{ss} = steady-state volume of distribution, AUC = area under the plasma concentration–time curve and MRT = mean residence time.

Pharmacodynamic analysis

A statistical evaluation was conducted to: (i) determine if any particular baseline (before the first infusion of study drug) laboratory value effects the disposition of doxorubicin when administered as TLC D-99 or conventional drug; (ii) determine if the presence of liver metastasis prior to treatment effects the disposition of doxorubicin when administered as TLC D-99 or conventional drug; (iii) determine if any pharmacokinetic parameter(s) observed in patients during the first cycle of TLC D-99 or conventional doxorubicin correlated with any measure of toxicity [e.g. white blood cells (WBCs), ANC or platelet nadir during the first cycle, incidence or severity of mucositis, alopecia, etc., during the first cycle, percent change in left ventricular ejection fraction (LVEFR) at the first post-treatment evaluation, etc.]; and (iv) determine if any pharmacokinetic parameter(s) observed in patients during the first cycle of TLC D-99 or conventional doxorubicin correlated with the best overall response during the patient's full course of therapy. The correlation between the pharmacokinetic parameters (for encapsulated doxorubicin, total doxorubicin and doxorubicinol), and each of the laboratory and safety parameters was evaluated by calculating the Pearson correlation coefficient. For the toxicities and responses, mean pharmacokinetic parameters for patients who did and did not experience the toxicity or response were compared to determine whether there was a relationship between the parameter and response.

Results

Patient characteristics

Twenty female patients (10 in the TLC D-99 group and 10 in the conventional doxorubicin group) were enrolled in the pharmacokinetic portion of this study. Most patients in both groups were Caucasian (eight of 10 in the TLC D-99 group and seven of 10 in the conventional doxorubicin group), and three in the TLC D-99 group and one in the conventional doxorubicin group had received prior adjuvant therapy with doxorubicin. Four patients in the TLC D-99 group and seven patients in the conventional doxorubicin group had liver metastases. The weights and ages of the pharmacokinetic patients in each group are shown in Table 1.

Pharmacokinetics

Figure 1 shows the mean concentrations of each analyte in each treatment group. Both total doxorubicin and encapsulated doxorubicin plasma levels in patients receiving TLC D-99 greatly exceeded the plasma levels of doxorubicin in patients receiving conventional doxorubicin. Most of the circulating doxorubicin in patients receiving TLC D-99 is encapsulated (at least about 80% and probably greater than 95%). Levels of 'unencapsulated' doxorubicin in patients receiving TLC D-99 cannot be reliably measured for technical reasons. In addition, since both total and encapsulated levels are so high relative to the unencapsulated level, calculating the 'unencapsulated' fraction by subtraction is unreliable.

The observed and calculated pharmacokinetic parameters for all patients in each treatment group are shown in Table 1. One patient in the TLC D-99 group had a C_{max} for total doxorubicin of 125.29 μM and was excluded from the calculation of the mean for this parameter on the basis of the Q-test [11]. Similarly, one patient in the conventional doxorubicin group had a C_{max} of 40.66 μM and an $AUC_{0-\infty}$ of 44.29 μM·h, and was not included in the calculation of the means for these and related parameters. The pharmacokinetic parameters obtained in this study for patients receiving conventional doxorubicin are similar to those previously reported [12]. The clearance of total doxorubicin after administration of TLC D-99 is markedly lower than the clearance of conventional doxorubicin (approximately 9-fold lower). The volume of distribution at steady state for total doxorubicin after administration of TLC D-99 is markedly less (25-fold) than that for conventional doxorubicin.

The terminal $T_{1/2}$ for TLC D-99 shown in Table 1 probably represents the half-life for uptake of liposomes into tissues rather than the true disposition half-life of doxorubicin from TLC D-99.

Doxorubicinol was detected in all patients in both treatment groups. The mean $AUC_{0-\infty}$ of doxorubicinol for patients receiving TLC D-99 (1.51 μM·h) was not markedly different than that in patients receiving conventional doxorubicin (1.82 μM·h), although the appearance of the peak doxorubicinol concentration occurred somewhat later in patients receiving TLC D-99. The mean terminal exponential half-life of doxorubicinol in patients in the TLC D-99 group was 50.7 ± 11.7 h, while that in the conventional doxorubicin group was 43.7 ± 3.5 h.

Pharmacodynamics

As this was an exploratory study, a large number of parameters were evaluated in a relatively small number of patients and some statistical correlations were found. Whether any of these correlations are meaningful cannot be fully assessed with this limited database. Selected correlations between pharmacokinetic and hematologic parameters measured during the first cycle of treatment are shown in Table 2. The most obvious correlation found was between the extent of exposure ($AUC_{0-\infty}$) and the surviving fraction of WBCs [nadir WBCs during cycle/baseline WBCs] during the first cycle in patients receiving conventional doxorubicin (Fig. 2). This correlation has been noted for conventional doxorubicin previously [13] and is confirmed in this study.

Table 1 Plasma pharmacokinetic parameter estimates in patients receiving 60 mg/m^2 of TLC D-99 or conventional doxorubicin (in combination with cyclophosphamide)

Patient ID	Treatment	Age (years)	BSA (m^2)	Weight (kg)	Total dose (μmol)	Total doxorubicin					Doxorubicinol		
						C_{max} (μM)	$AUC_{0-\infty}$ (μM·h)	Terminal $T_{1/2}$ (h)	Clearance (l/h·m^2)	V_{ss} (l/m^2)	C_{max} (μM)	T_{max} (h)	$AUC_{0-\infty}$ (μM·h)
4107-1010	D-99	49.6	1.72	67.7	178	20.24	217.37	10.95	0.48	6.32	0.045	6.00	0.96
4108-1003	D-99	67.2	1.70	70.5	174	15.13	137.75	11.21	0.74	9.56	0.036	4.00	2.07
4108-1010	D-99	57.2	1.72	73.0	178	24.65	122.73	11.43	0.84	6.74	0.023	6.00	1.42
4108-1014	D-99	45.6	1.54	49.7	159	4.07	12.77	19.33	8.09	122.00	0.020	6.25	1.63
4108-1015	D-99	60.2	1.76	65.7	183	10.93	26.61	15.57	3.91	42.29	0.027	4.00	1.49
4108-2001	D-99	62.7	1.84	79.3	190	5.81	13.65	20.60	7.56	73.91	0.017	4.05	1.35
4108-2003	D-99	39.8	1.54	52.0	159	8.85	31.09	16.29	3.32	39.02	0.012	6.00	1.18
4112-1012	D-99	64.3	1.44	47.3	148	125.29[a]	63.41	21.43	1.62	5.51	0.023	1.50	1.69
4112-1013	D-99	44.7	1.42	46.4	147	23.18	134.19	11.21	0.77	8.61	0.027	12.00	2.04
4112-2002	D-99	54.8	1.75	75.9	186	30.91	33.19	26.26	3.20	28.03	0.030	4.00	1.29
	N	10	10	10	10	9	10	10	10	10	10	10	10
	Mean	**54.6**	**1.60**	**62.8**	**170**	**15.97**	**79.27**	**16.43**	**3.05**	**34.20**	**0.026**	**5.38**	**1.51**
	SD	9.3	0.1	12.6	15.7	9.29	69.60	5.35	2.80	38.05	0.009	2.75	0.35
4107-1009	doxorubicin	69.2	1.62	61.0	168	1.58	3.56	40.77	29.17	948	0.026	2.00	1.41
4108-1004	doxorubicin	59.5	1.70	69.0	176	1.82	3.82	34.49	27.11	734	0.057	1.50	2.40
4108-1006	doxorubicin	64.2	1.52	52.0	157	1.51	4.35	47.57	23.73	933	0.037	1.50	1.88
4108-1009	doxorubicin	36.9	1.70	64.0	176	1.38	3.14	33.34	33.00	811	0.025	2.00	1.30
4108-1011	doxorubicin	48.0	1.70	68.4	176	1.64	3.99	41.60	25.93	891	0.027	1.50	1.30
4108-1013	doxorubicin	45.8	1.56	56.5	162	1.57	4.02	53.04	25.83	1107	0.035	6.00	1.74
4108-2002	doxorubicin	44.7	1.62	63.7	167	2.15	4.35	34.89	23.68	568	0.063	1.78	2.39
4112-1009	doxorubicin	46.6	2.20	121.8	228	1.40	3.40	39.50	30.45	810	0.062	2.00	1.95
4112-1011	doxorubicin	74.4	1.70	65.5	176	40.66[a]	44.29[a]	45.27	2.34[a]	11[a]	0.057	0.52	2.13
4112-1014	doxorubicin	61.0	1.79	69.8	184	1.95	4.05	58.83	25.37	859	0.023	1.50	1.71
	N	10	10	10	10	9	9	10	9	9	10	10	10
	Mean	**55.03**	**1.71**	**69.2**	**177**	**1.67**	**3.85**	**42.93**	**27.14**	**851**	**0.041**	**2.03**	**1.82**
	SD	12.28	0.19	19.33	19.61	0.26	0.42	8.35	3.15	150	0.017	1.46	0.41

[a]Value excluded from descriptive statistics.

Hematologic monitoring was fairly intense in the first cycle (every 3–4 days in most patients). Furthermore, although G-CSF was permitted, only one patient (patient 4112-1011, receiving conventional doxorubicin) in this study received G-CSF during the first treatment cycle, beginning on day 13 post-treatment. This patient was excluded from the pharmacodynamic correlation studies as the very high C_{max} and AUC values obtained appeared to be outliers. It is worth noting, however, that her WBC surviving fraction was the lowest (0.056) of all the patients in the pharmacokinetic portion of this study. There appeared to be no clear correlations between pharmacokinetic parameters for total doxorubicin in patients receiving TLC D-99 and myelosuppression, although in all cases the trend was toward less myelosuppression with higher C_{max} or AUC for total doxorubicin in patients receiving TLC D-99.

The three patients in the TLC D-99 group with the highest AST values (slightly above the normal range of 9–25 U/l for females) had increased T_{max} and AUC for doxorubicinol. The results indicate that T_{max} and AUC of doxorubicinol are positively correlated with AST with $r = 0.730$ and $r = 0.697$ respectively. The correlation is significant at the 5% level of significance. There may also be a correlation between higher alanine aminotransferase and a longer MRT ($r = 0.714$) for total doxorubicin in patients receiving TLD D-99.

Among those patients receiving TLC D-99, four had liver metastases and this appeared to be associated with an increased AUC for doxorubicinol ($p = 0.020$). Seven of the patients receiving conventional doxorubicin had liver metastases, and this appeared to correlate with an increased AUC ($p = 0.028$) and decreased clearance ($p = 0.018$) of doxorubicin.

None of the patients in this study experienced significant diarrhea. None of the patients receiving TLC D-99 had mucositis. Six patients receiving conventional doxorubicin experienced mucositis and this was correlated with a higher C_{max} for doxorubicin ($p = 0.025$). Two patients receiving conventional doxorubicin had significant infections and one of these patients had a high C_{max} for doxorubicin.

There was no direct correlation between the overall tumor response and any pharmacokinetic parameter in either treatment group.

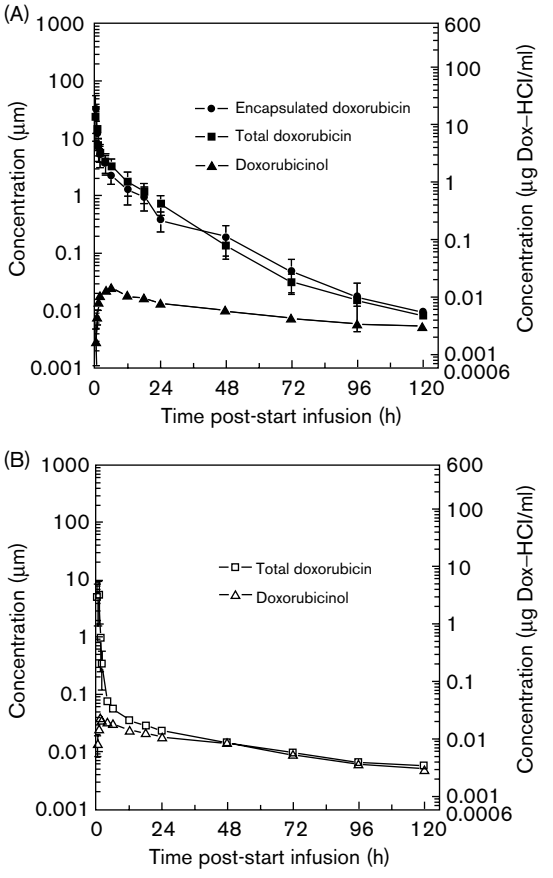
Discussion

TLC D-99 is administered at the same dose and interval as conventional doxorubicin to provide equal efficacy with reduced cardiac and gastrointestinal toxicity. TLC D-99 is given as a 1-h infusion to patients rather than as a bolus to minimize the infusion-related side effects that may

occur with i.v. lipid infusions. The resulting plasma levels of total doxorubicin after TLC D-99 infusion are substantially higher than those after the administration of the same dose of conventional doxorubicin. The clearance of total doxorubicin after TLC D-99 is lower and the volume of distribution at steady state is less than after conventional doxorubicin. The majority of the circulating doxorubicin in patients treated with TLC D-99 remains associated with the liposome for 48–92 h or more (based on the detection of encapsulated doxorubicin in patient samples) after infusion. Doxorubicin appears in the plasma later with TLC D-99 than with conventional doxorubicin; however, the AUC of doxorubicinol after TLC D-99 is not markedly different than that of conventional doxorubicin. These data suggest that the doxorubicin in TLC D-99 is bioavailable and metabolized in a manner similar to conventional doxorubicin, but at a slower rate.

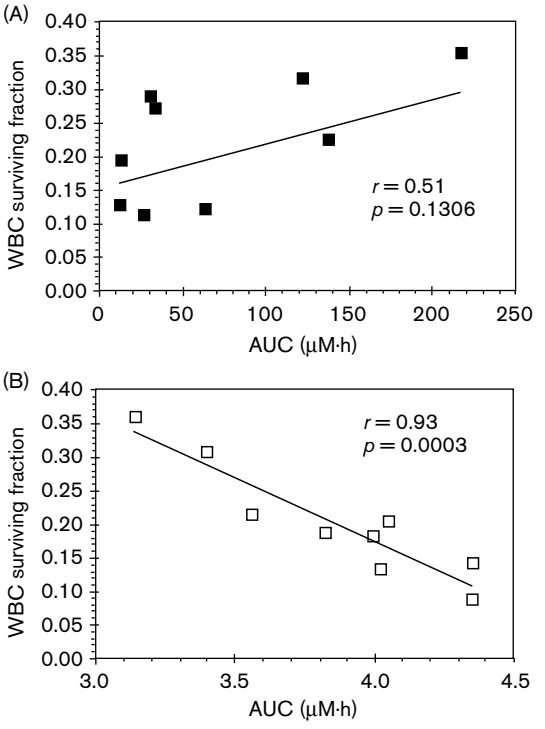
Analysis of plasma levels indicates that liposome encapsulation alters the pharmacokinetics of the encapsulated drug. However, the plasma AUC alone may not be a

Fig. 1



Mean (±SEM) plasma concentrations versus time for doxorubicin and doxorubicinol in patients receiving TLC D-99 (A) or conventional doxorubicin (B).

Fig. 2



$AUC_{0-\infty}$ for total doxorubicin versus WBC surviving fraction for individual patients receiving TLC D-99 (A) or conventional doxorubicin (B).

Table 2 Correlation statistics between hematologic and pharmacokinetic parameters during the first cycle of treatment with TLC D-99 or conventional doxorubicin

Pharmacokinetic parameter	Hematologic parameter	TLC D-99[a]			Doxorubicin[b]		
		N	*r*	*p*	*N*	*R*	*p*
C_{max} (total)	platelet SF[c]	9	0.4664	0.2057	9	−0.5857	0.0974
	ANC SF	9	0.6485	0.0589	9	−0.4806	0.1903
	WBC SF	9	0.3855	0.3055	9	−0.4490	0.2253
$AUC_{0-\infty}$ (total)	platelet SF	10	0.5135	0.1289	9	−0.4467	0.2281
	ANC SF	10	0.4698	0.1707	9	−0.8506	0.0037
	WBC SF	10	0.5115	0.1308	9	−0.9296	0.0003

[a]Excludes patient 4112-1012 for all correlations with C_{max}.
[b]Excludes patient 4112-1011 for all correlations.
[c]SF = surviving fraction.

reliable indicator of exposure since most of the drug is encapsulated and not bioavailable or active. Even consideration of the AUC of unencapsulated drug (if it could be reliably measured) may not give an accurate picture of exposure since it does not take into account tissue exposure resulting from direct liposome-mediated transport and release of drugs in tissues. The most useful information on the disposition of doxorubicin after administration of TLC D-99 is that obtained in animal studies where tissue levels can be directly measured. In animals, the levels of doxorubicin in the circulation and in tumors were higher, while the levels in the heart and gastrointestinal tract were lower after TLC D-99 than after conventional doxorubicin [14–16]. It is hypothesized that the decreased cardiac and gastrointestinal toxicity seen with TLC D-99 is related, at least in part, to decreased exposure of these tissues to doxorubicin, while the efficacy is at least comparable due to efficient delivery of the drug to tumors.

Liposomes and other macromolecular delivery systems have been shown to have 'enhanced penetration and retention' in tumor tissue [17] and less access to normal tissues. This is generally thought to be due to the leaky vasculature and poor lymphatic drainage of most tumors, which allows large particles access and prevents or delays their removal. Normal tissues, with tight capillary junctions, generally prevent access of particulate material. Clinical trials of 96-h infusions or weekly treatment versus standard 21-day regimens of conventional doxorubicin support the hypothesis that chronic cardiotoxicity is related primarily to peak drug concentration, whereas the antitumor effect is more dependent on the total drug exposure (AUC) [18]. Liposomes may provide a slow release mechanism (with release occurring directly in the tissues as well as in the plasma), which would mimic in some respects prolonged infusions or weekly regimens. Finally, it has been hypothesized that the increased phospholipase activity in infected or inflamed sites plays a role in the selective targeting of certain amphotericin-containing liposomes *in vivo* [19]. The increased phospholipase activity that has been noted in some tumor types [20] might also serve to release doxorubicin from liposomes at the site of the tumor.

It has been theorized that liposomes that circulate for *very* extended times would have enhanced chances of passive extravasation into tumors [21]. Recently, it has been shown that polyethylene-glycol (PEG)-coated liposomes circulate longer than similar, uncoated liposomes, but that there was no improvement in delivery of the encapsulated agent (doxorubicin) to tumors or enhanced therapeutic activity in a murine tumor model for the PEG-coated liposomes. Both formulations were equally effective [22].

Pharmacodynamic data from this study support previous findings that myelosuppression is related to plasma AUC of doxorubicin in patients receiving conventional drug. This correlation was not found with TLC D-99. The data also suggest that liver function may affect the disposition of TLC D-99 as well as conventional doxorubicin and additional studies in hepatically impaired patients are ongoing. No other clear correlations were seen.

The pharmacokinetics of doxorubicin after the administration of TLC D-99 are strikingly different than the pharmacokinetics of doxorubicin after administration of conventional doxorubicin. They are also markedly different from those obtained with the other marketed liposomal formulation of doxorubicin, Doxil. Doxil is a pegylated-liposomal formulation composed of doxorubicin (HCl) in liposomes made of methoxypolyethylene glycol-distearoylphosphatidylethanolamine, fully hydrogenated soy phosphatidylcholine and cholesterol. Although no direct, comparative studies between the two liposomal formulations have been conducted, pharmacokinetic data in the package insert for Doxil [23,24] indicate that the clearance for total doxorubicin after administration of Doxil is approximately 70-fold lower than the clearance for total doxorubicin following TLC D-99 (0.041 l/h·m^2 for Doxil versus 3.05 l/h·m^2 for TLC D-99). Assuming linear kinetics for Doxil, the $AUC_{0-\infty}$ for the pegylated-liposome at a dose of 60 mg/m^2 would be approximately 40 times that of TLC D-99. The difference in pharmacokinetic profiles may explain the use of lower-dose intensity regimens and a higher incidence of skin toxicity (palmar plantar erythrodysthesia) with Doxil [25], compared with TLC D-99.

Understanding the pharmacokinetics of i.v. administered liposomal preparations is a challenge. In the case of TLC D-99, additional studies should be conducted to (i) improve the bioanalytical assay such that a better estimate of the amount of released or 'free' doxorubicin in the circulation can be obtained, (ii) understand the source of the inter-individual variation in the pharmacokinetics of total and free drug, and (iii) understand the effect of hepatic function on clearance.

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