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ORIGINAL ARTICLE

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Pharmacokinetics of liposomal doxorubicin (TLC-D99; Myocet) in patients with solid tumors: an open-label, single-dose study

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Abstract *Purpose*: Liposomal encapsulation of doxorubicin is designed to increase safety and tolerability by decreasing cardiac and gastrointestinal toxicity through decreased exposure of these tissues to doxorubicin, while effectively delivering drug to the tumor. We conducted an open-label phase I study to determine the pharmacokinetic profile of a single dose of liposome-encapsulated doxorubicin (Myocet) in patients with various solid tumors. Safety and tolerability were monitored. *Experimental design*: Patients received a single intravenous infusion of Myocet 75 mg/m^2. Plasma samples were analyzed for concentration of liposome-encapsulated doxorubicin, total doxorubicin, and doxorubicinol. *Results*: A total of 18 patients aged 20–73 years (median 60 years) participated; 17 were evaluable for pharmacokinetic analysis. The most common primary tumor was soft tissue sarcoma (22%). Total body clearance for total doxorubicin was 5.6 l/h/m^2 while the volume (V_{ss}) was 82 l/m^2. The terminal half-life was 52.6 h. Based on the AUC and C_{max} values for total doxorubicin and encapsulated doxorubicin, an estimated 85% of circulating doxorubicin was encapsulated. Doxorubicinol was detected in all patients; the mean AUC was 2.03 ± 1.10 μmol/l/h. The mean 48-h urinary excretion of doxorubicin was 6.44% of the dose. The most common adverse events were nausea (94%), fatigue (78%) and vomiting (67%). Cardiotoxicity (measured as ten-point fall in LVEF to $< 50\%$) was observed in one patient. Pharmacokinetic values did not correlate with hematological, laboratory or demographic variables. *Conclusions*: The pharmacokinetic profile of Myocet suggests that the liposomal formulation results in a longer half-life with less free drug available for tissue distribution than conventional doxorubicin, consistent with the enhanced therapeutic index observed in clinical studies.

Keywords Liposomal doxorubicin · Pharmacokinetics · Safety · Tolerability

The Liposome Company is now Medeus Pharmaceuticals, Inc.

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Introduction

Doxorubicin is a highly active anthracycline antibiotic used widely in the treatment of patients with breast cancer, ovarian cancer, sarcomas, leukemias and lymphomas. Anthracyclines are associated with an increased risk of cardiomyopathy. The risk is directly proportional to the lifetime cumulative dose of anthracycline administered. Clearly, this limits the total dose of doxorubicin a given patient can receive and, in particular, its use in patients at increased risk of cardiotoxicity. Risk factors include age over 70 years, combination therapy, mediastinal radiotherapy, previous cardiac disease and hypertension [1].

Myocet (TLC-D99; Medeus Pharmaceuticals) consists of doxorubicin complexed with citrate inside nonpegylated liposomes. Encapsulation of doxorubicin within liposomes is designed to minimize distribution of the active drug to healthy tissues such as the heart while increasing preferential distribution of drug to the tumor site. The disposition of doxorubicin when administered as Myocet reflects that of the liposome and differs substantially from conventional doxorubicin, as illustrated by the different pharmacokinetic profile [2]. Thus, Myocet preserves the antitumor activity of doxorubicin while reducing the risk of cardiotoxicity. These benefits have been demonstrated in phase III trials in the treatment of patients with metastatic breast cancer [3, 4]. The clinical development of Myocet which ended with a drug approval was strictly focusing on breast cancer; all other indications for doxorubicin have not been clinically

evaluated so far. Preclinical results in a limited number (five) of murine tumor models has shown antitumor activity at least equivalent to that observed with conventional doxorubicin.

The clinical pharmacology of doxorubicin has been well characterized [5–10]. In humans, doxorubicin undergoes extensive hepatic metabolism. The major metabolite is doxorubicinol, which has cytotoxic activity. Doxorubicin is extensively (79–85%) protein bound and biliary excretion is the primary excretion route with about 40% of the dose recovered in the bile and feces. Less than 15% of a dose is recovered in the urine over 6 days.

The primary objective of our study was to evaluate the pharmacokinetic properties of doxorubicin administered as Myocet. Both encapsulated and total doxorubicin in plasma were quantified following administration of a single dose of Myocet 75 mg/m^2 in patients with a documented solid tumor. We also monitored the safety and tolerability of Myocet in these patients.

Methods

Patients

Patients with histologically or cytologically proven new or advanced solid organ cancer who could benefit from anthracycline therapy were eligible. Inclusion criteria included Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, age ≥ 18 years, adequate bone marrow function, serum creatinine < 1.5 mg/dl, documented adequate liver function (total bilirubin ≤ 1.2 times the upper limit of normal), and resting left ventricular ejection fraction (LVEF) $\geq 50\%$ as determined by echocardiography. All patients were required to provide written informed consent.

Exclusion criteria were pregnancy or lactation; a history of allergy to anthracyclines, eggs, or egg products; expected survival < 3 months; and a documented history of congestive heart failure (CHF). Patients with documented brain metastases, as well as those concurrently being treated with other anticancer drugs or hormonal therapy, were also excluded.

Drug and drug regimen

Myocet, a liposome-encapsulated doxorubicin–citrate complex, is a sterile, non-pyrogenic, aqueous dispersion of egg phosphatidylcholine and cholesterol liposomes which must be prepared from a three-vial system: "Myocet for injection" (50 mg Dox/vial), "Myocet liposomes for injection" (100 mg total lipid/ml) and "Myocet buffer". After preparation, the drug is a red–orange, opaque liposomal dispersion with a pH of 6.5–8.5. The drug-to-lipid ratio is approximately 0.25:1 (w/w). The median size of these liposomes is approximately 150 nm. For the first dose, patients received a single, 60-min infusion of 75 mg/m^2 of Myocet as a single agent. Patients had the opportunity to continue to receive Myocet on 21-day cycles until progressive disease was documented or other stopping criteria were met.

Granulocyte colony-stimulating factor (G-CSF) could be administered to patients requiring supportive therapy for myelosuppression. G-CSF was discontinued at least 24 h before the next administration of chemotherapy and could not be commenced until 24 h after it. Antiemetics were permitted as needed, and prophylactic treatment for fever associated with Myocet (preferably paracetamol 500 mg) could be given before and 4 h after each infusion.

Clinical setting

The study was conducted in the oncology department at a single center (Tumor Biology Center at the Albert-Ludwigs University, Freiburg). Patients received inpatient care during the period of monitoring (2 days following the initial dose of Myocet) and those receiving subsequent cycles were treated on an inpatient or outpatient basis, as appropriate.

Study endpoints

The following pharmacokinetic variables were measured or derived: maximum observed concentration in plasma (C_{max}), time of C_{max} (T_{max}), terminal half-life ($T_{1/2\lambda}$), area under the concentration–time curve from the time of dosing extrapolated to infinity ($AUC_{0-\infty}$), total body clearance (Cl), and volume of distribution at steady state (V_{ss}). Each variable was recorded for total doxorubicin, encapsulated doxorubicin and doxorubicinol. Free doxorubicin was not quantified. Urinary concentrations of doxorubicin and doxorubicinol were measured over time.

Cardiotoxicity was assessed by measuring LVEF by echocardiography and by electrocardiograms. Safety and tolerability were evaluated by recording all adverse events and laboratory testing of blood samples for hemoglobin, hematocrit, red blood cell, platelet, white blood cell and absolute neutrophil counts, sodium, potassium, chloride, blood urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, aspartate transaminase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH).

Efficacy in terms of objective response rates determined by CT or MR imaging was not assessed in this study. Patients were followed up clinically and with minimal technical expenditure (radiography, sonography and laboratory tests) until clear signs of progression. Thus, no objective response rate can be given.

Study design

This was a single-center, single-dose, open-label study with 18 patients. All patients received a single

intravenous infusion of 75 mg/m^2 of Myocet. Following the first dose, patients could continue Myocet therapy on a compassionate basis in an extension period. During this period, other anticancer agents could be combined with Myocet at the investigator's discretion, and patients were monitored for safety only. Samples for pharmacokinetic analysis were collected during the first cycle of treatment only.

Statistical analysis

Pharmacokinetic variables were determined by noncompartmental methods using the computer program WinNonlin Pro version 3.0 (Pharsight Corporation, Cary, N.C.) [11]. Area under the plasma concentration–time curve was estimated using the trapezoidal rule from time 0 to peak concentration and the log-trapezoidal rule from the peak concentration to the last measurable plasma concentration (AUC_{last}). $AUC_{0-\infty}$ was then calculated from the time of dosing and extrapolated to infinity. Mean residence time and V_{ss} were corrected for infusion time [12].

For the pharmacokinetic analysis, doses, plasma and urine concentrations were converted from nanograms per milliliter to micromoles per liter using the following molecular weights: doxorubicin HCl, 580; doxorubicin free base, 543.3; doxorubicinol HCl, 582.0; doxorubicinol free base, 545.55.

For the pharmacodynamic analysis, the correlation between pharmacokinetic variables (not normalized for body surface area, BSA) and each of the baseline laboratory values or hematological values was evaluated by calculating the Pearson correlation coefficient, *r*, a dimensionless index that ranges from −1.0 to 1.0 inclusive and reflects the extent of a linear relationship between two data sets. MS Excel 7.0 was used for these calculations.

Sampling schedule

Following the first cycle of treatment with Myocet, blood samples were collected in tubes containing EDTA. A 10-ml sample of blood was collected from a superficial vein in the arm contralateral to the arm used for infusion. Samples were collected just before the infusion of Myocet (0-h time point or preinfusion), at the midpoint of the infusion (30-min time point), and immediately before the end of infusion (1-h time point). Additional samples (10 ml each) were obtained at 1.5, 2, 4, 6, 12, 18, 24, 48, 72, 96, 120, 144, and 168 h after the beginning of the intravenous infusion. Patients remained at the institution until the 48-h sample was collected.

Immediately following collection, samples were centrifuged at approximately 2500 *g* for at least 5 min at 4°C to separate plasma. Plasma (4.0 ml) was transferred to a vial containing 0.4 ml 55% glucose as a cryoprotectant. The vials were placed immediately at −20°C or lower in a freezer. The processing time between collection and freezing was less than 30 min. Samples placed in a −20°C freezer were transferred to a −70°C freezer within 24 h of the sample draw. Samples were shipped to the analytical facility for assay.

Urine was collected for 24 h preinfusion and over the first and second 24-h periods following study-drug infusion. The volume was recorded and two 10-ml aliquots were taken from each collection and frozen at −70°C before analysis.

Assay procedure

Plasma samples were analyzed at Covance Laboratories (Madison, Wis.). High-performance liquid chromatographic (HPLC) techniques were used to quantify the concentration of liposome-encapsulated doxorubicin, total doxorubicin and doxorubicinol in human plasma. Two assays were used, one to measure total doxorubicin and one to measure liposome-encapsulated doxorubicin and doxorubicinol (Fig. 1). In the first assay, for total doxorubicin (liposome-encapsulated and/or free doxorubicin), 0.5-ml samples were spiked with 100 µl of the internal standard (daunorubicin) and treated with 500 µl reduced Triton X-100 detergent (RTX-100) to release any encapsulated doxorubicin in the plasma. The total doxorubicin was then separated from contaminating materials using a solid-phase extraction procedure with C18 SEP-PAK cartridges. Upon sample application, total doxorubicin and the internal standard bound to the C18 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.

In the second assay, liposome-encapsulated doxorubicin, doxorubicinol and the internal standard were analyzed in a separate plasma sample. A 1-ml aliquot of plasma was applied to a C18 SEP-PAK column and the first fraction (liposome-encapsulated doxorubicin) was collected in a tube containing 500 µl RTX-100 and 100 µl of the internal standard (daunorubicin). After washing the SEP-PAK column, the remaining fraction (released or free doxorubicin and doxorubicinol) was collected into another tube. The first fraction (liposome-encapsulated doxorubicin) eluant was then applied to a second C18 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, redissolved in methanol and separated on a HPLC column (150×4.6 mm; Phenomenex IB-Sil Cyano, 5 µm particle size) with a mobile phase containing 35 m *M* NH_4OAc, triethylamine pH 4.0 and acetonitrile (72.5:27.5) using a FS 980 spectrofluorimeter at an excitation wavelength of 233 nm and emission filter at 550 nm for the detection of the anthracyclines. Peak

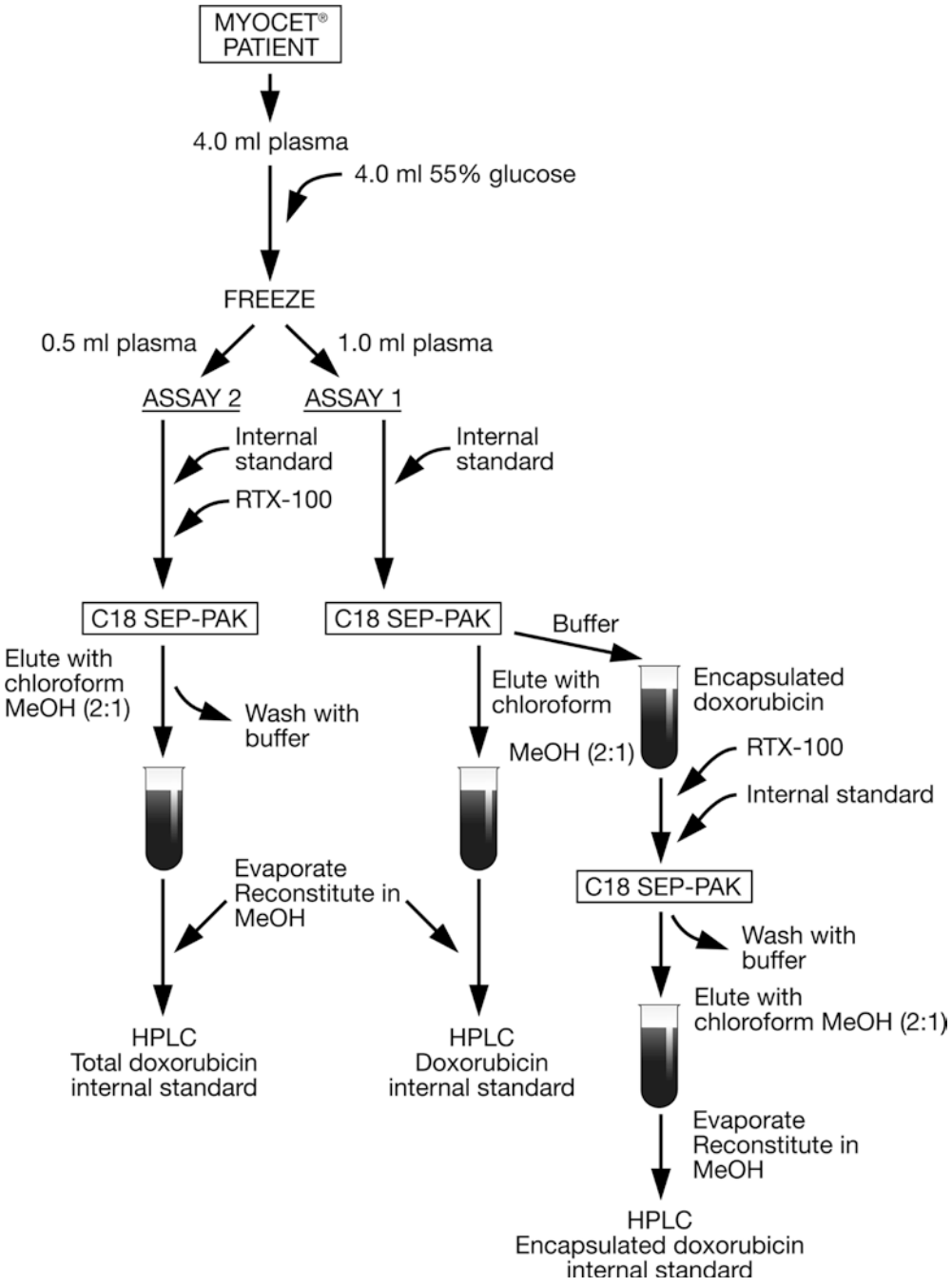

Fig. 1 Assay scheme for plasma

height ratios were used for quantification and concentrations were calculated using a 1/concentration^2 weighted linear regression.

Subject samples were assayed in batches or analysis groups, consisting of calibration standards, quality control (QC) samples and subject samples prepared for concurrent analysis. Acceptance and reporting of data from each analysis group were based on the following criteria: a calibration curve with correlation coefficient ≥0.950, a sufficient number of calibration standard samples with back-calculated concentrations within ±15% of theoretical concentration (±20% for the lower limit of quantification standard), and at least half of the QC samples at each of three concentrations (four for total doxorubicin) and two-thirds of all QC samples with back-calculated concentrations within ±20% of theoretical concentration. At least half of the partial volume QC samples in an analysis group must have had back-calculated concentrations within ±20% of theoretical in order to accept data for subject samples assayed at partial volume in that group. If the acceptance criteria were not met in an analysis group, the results were not reported and subject samples were reassayed. The lower limit of quantification for all analytes was 2.0 ng doxorubicin free base per milliliter or doxorubicinol HCl per milliliter.

The method for the analysis of urine samples was based on that of Mross et al. [7]. Briefly, human urine (1.0 ml) containing doxorubicin, doxorubicinol and the internal standard was treated with aqueous sodium phosphate buffer (pH 4.0)/acetonitrile (72:28) and 1.0 *M* HCl. An aliquot was injected on to the HPLC system. Separation was achieved using a MicroSphere 3 C-18, 100×4.6 mm, 3 μm particle size analytical column. Fluorescence detection was employed to quantify the analytes using an excitation wavelength of 233 nm and an emission filter wavelength of 550 nm. Peak ratios were used for quantification and concentrations were calculated using a 1/concentration^2 weighted linear regression. Urine was also analyzed for the presence of possible conjugated metabolites of doxorubicin. Urine samples were treated with β-glucuronidase in sodium acetate buffer (pH 5.0) for 20 h at 37°C. Doxorubicin, doxorubicinol and the internal standard were extracted from the hydrolyzed urine using solid-phase (C18 cartridges) extraction and injected onto the HPLC for fluorescence detection.

Results

Patient demographics

A total of 18 patients, 9 men and 9 women, participated in this study (Table 1). Their ages ranged from 20 to 73 years (median 60 years). All patients were Caucasians. All 18 patients were assessed for safety (adverse events and laboratory tests) and 17 were assessed for pharmacokinetics. The most common primary tumor was soft tissue sarcoma (22%). The mean baseline LVEF was 62%. The median time from first diagnosis to first dose of Myocet was 28.8 months. Five patients (28%) had received prior doxorubicin before starting this study; the mean total lifetime dose of prior doxorubicin for these patients was 212 mg/m^2 (range 60–480 mg/m^2).

Of the 18 patients, 11 dropped out after one or two cycles due to tumor progression, and 7 were treated with more than two cycles because of stable disease after two cycles. The latter seven patients received a mean of four treatment cycles until they progressed. Therapy was discontinued in one patient owing to an adverse event. Among the remaining four patients, one died, one attained the maximum dose, one had stable disease and one withdrew consent.

Extent of exposure

A total of 47 cycles of Myocet were administered to 18 patients. Four patients received a single cycle, seven received two cycles, four received three cycles, one received five cycles, and two received six cycles. A mean of three cycles were administered per patient.

The mean duration of treatment was 55 days (median 49, range 7–132 days), and the mean cumulative dose of Myocet administered was 190 mg/m^2 (median 150, range 75–450 mg/m^2). The mean dose

Table 1 Individual and group demographics and tumor characteristics of the patients

Gender	Age (years)	Primary site	ECOG performance status	LVEF (%)	Prior DOX (mg/m^2)	Height (cm)	Weight (kg)	BSA (m^2)
Female	61	Soft tissue sarcoma	1	78	0	167	58.0	1.67
Female	58	Rectum	1	64	0	158	69.4	1.71
Female	59	Soft tissue sarcoma	1	63	60	168	69.1	1.80
Female	56	Cervix	1	59	0	172	75.0	1.88
Male	20	Bone sarcoma	2	64	100	190	55.8	1.78
Female	60	Gallbladder/bile ducts	1	71	0	158	45.5	1.42
Female	35	Neuroendocrine	1	76	300	161	65.0	1.67
Female	60	Soft tissue sarcoma	1	50	0	167	57.8	1.65
Female	55	Breast	0	65	0	153	63.0	1.61
Male	71	Kidney	1	60	120	175	63.4	1.77
Male	61	Small intestine	0	80	0	178	56.3	1.70
Male	31	Soft tissue sarcoma	2	67	480	179	60.9	1.78
Male	71	Mesothelioma	2	55	0	174	74.2	1.90
Male	48	Gallbladder/bile ducts	1	67	0	182	86.9	2.06
Male	66[a]	Thymus	0[a]	55[a]	0[a]	166[a]	65.5[a]	1.74[a]
Female	73	Ovary	1	50	0	163	57.4	1.60
Male	66	Non-small-cell lung	1	50	0	176	74.5	1.91
Male	64	Pancreas	1	50	0	184	94.2	2.18
All patients ($n=17$)								
Mean	55.82		1.06	62.9	62.4	170.9	66.3	1.77
SD	14.63		0.56	9.8	132.8	10.3	12.1	0.18
%rsd	26.21		52.5	15.7	213.0	6.0	18.3	10.20
Median	60		1	64	0	172	63.4	1.77
Minimum	20		0	50	0	153	45.5	1.42
Maximum	73		2	80	480	190	94.2	2.18

[a]Patient excluded from statistics as he was not evaluable for plasma pharmacokinetics

intensity was 22.8 mg/m^2 per week (median 23.9, range 16.6–25.0 mg/m^2 per week), and the mean dose administered per cycle was 72.7 mg/m^2 (median 75.0, range 50.0–75.0 mg/m^2).

Of the 47 cycles, 29 (62%) were administered beyond the first cycle. Two cycles were delayed by at least 7 days due to myelosuppression. The dose was reduced in five cycles due to mucositis ($n=3$) and myelosuppression ($n=2$).

Pharmacokinetics

One patient had an interfering endogenous peak on chromatography, making it impossible to analyze drug concentrations. Thus, 17 patients were evaluable for pharmacokinetic analysis.

Figures 2, 3, and 4 show the kinetics of total doxorubicin, encapsulated doxorubicin and doxorubicinol for each patient. Mean concentrations are shown in Fig. 5.

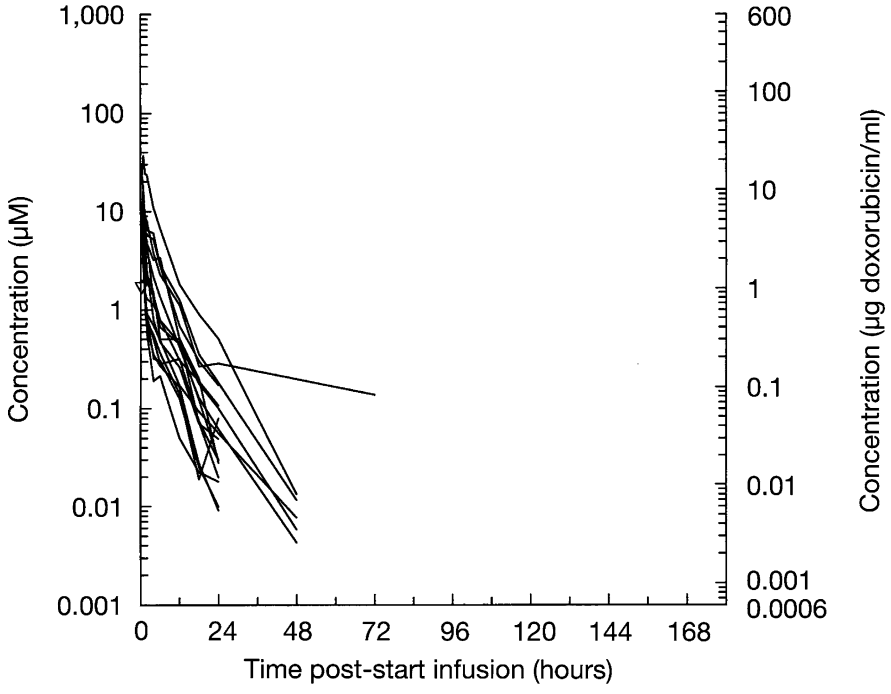

Fig. 2 Plasma levels of encapsulated doxorubicin in patients given Myocet ($n=17$)

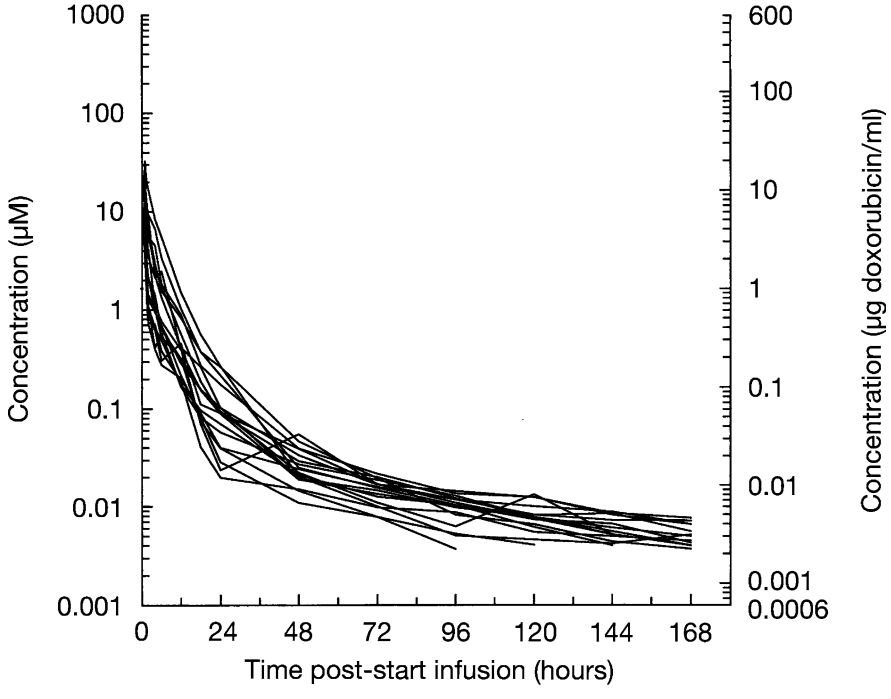

Fig. 3 Plasma levels of total doxorubicin in patients given Myocet ($n=17$)

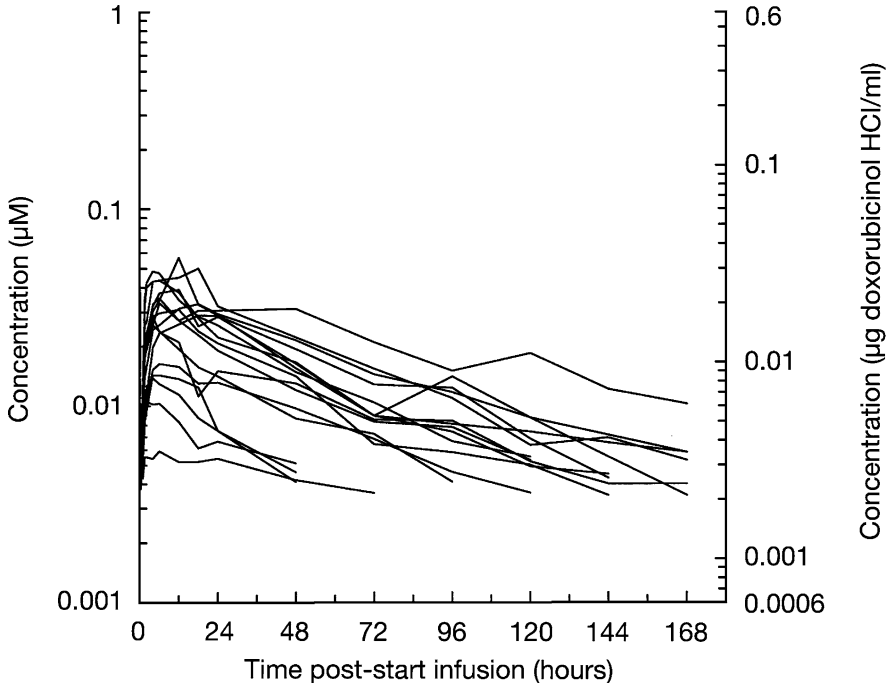

Fig. 4 Plasma levels of doxorubicinol in patients given Myocet ($n = 17$)

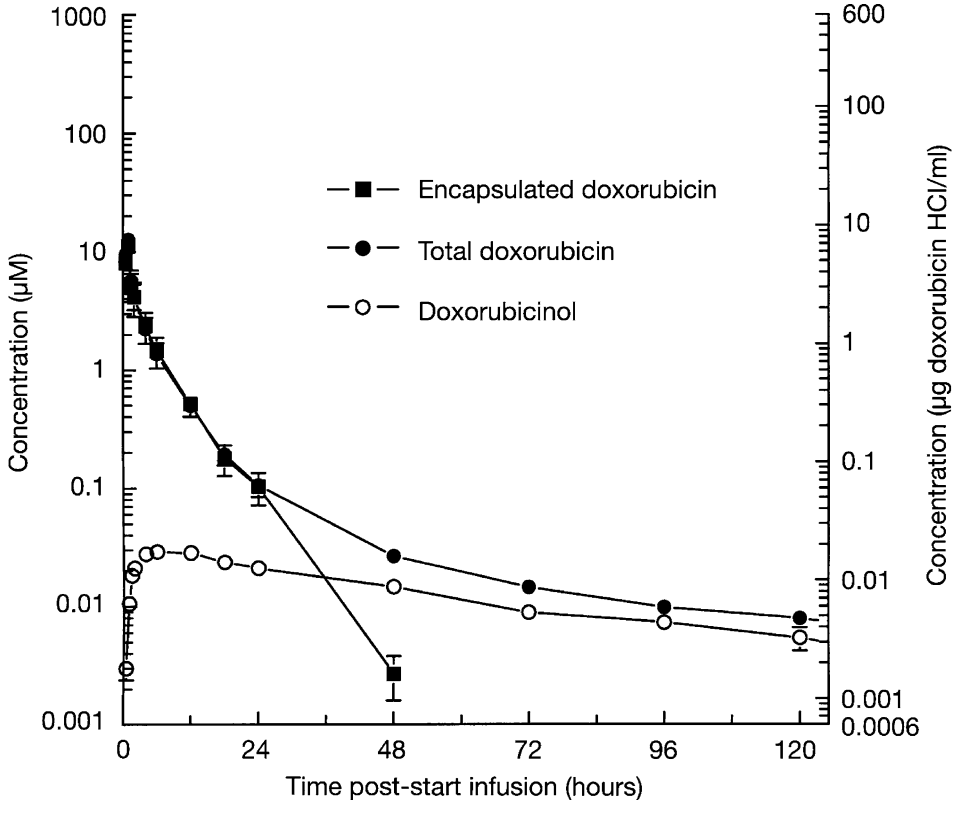

Fig. 5 Mean (±SEM) plasma levels of total doxorubicin, encapsulated doxorubicin and doxorubicinol in patients given Myocet ($n = 17$)

Table 2 presents the mean observed and calculated pharmacokinetic variables for all patients. The mean clearance for total doxorubicin in patients receiving Myocet was 5.6 ± 3.0 l/h/m^2 while V_{ss} was 81.7 ± 72.5 l/m^2. The interpatient variability in pharmacokinetic variables for total doxorubicin was fairly large. The coefficient of variation (CV) for clearance (normalized to BSA) in this study was 53%. Doxorubicinol was detected in all patients. The mean AUC of doxorubicinol was 2.03 ± 1.10 μmol/l/h.

Table 2 Pharmacokinetic variables for patients ($n = 17$ for all variables; C_{max} maximum observed concentration, $AUC_{0-\infty}$ area under the curve from the time of dosing extrapolated to infinity, $T_{1/2}$ terminal half-life; Cl total body clearance, V_{ss} estimate of the volume of distribution at steady state, T_{max} time of maximum observed concentration)

	Variable	Mean ± SD	Median (range)	%CV
	Age (years)	55.82 ± 14.63	60 (20–73)	26.2
	BSA (m^2)	1.77 ± 0.18	1.77 (1.42–2.18)	10.2
Total doxorubicin	C_{max} (μmol/l)	13.48 ± 7.52	11.59 (5.72–32.75)	55.8
	$AUC_{0-\infty}$ (μmol/l h)	35.61 ± 26.28	21.59(13.27–109.43)	73.8
	$T_{1/2}$ (h)	52.63 ± 20.67	50.95 (12.79–81.43)	39.3
	Cl ($l/h/m^2$)	5.58 ± 2.95	6.36 (1.24–10.72)	53.0
	V_{ss} (l/m^2)	81.73 ± 72.51	58.08 (6.79–282.5)	88.7
Encapsulated doxorubicin	C_{max} (μmol/l)	11.86 ± 8.41	8.96 (3.77–37.16)	70.9
	$AUC_{0-\infty}$ (μmol/l h)	33.30 ± 33.66	20.96 (7.41–139.02)	101.1
	$T_{1/2}$ (h)	7.68 ± 11.41	4.92 (2.43–51.51)	148.6
	Cl ($l/h/m^2$)	7.83 ± 5.40	6.57 (0.97–18.65)	69.0
	V_{ss} (l/m^2)	34.70 ± 23.79	33.28 (3.90–79.54)	68.6
Doxorubicinol	C_{max} (μmol/l)	0.03 ± 0.015	0.03 (0.01–0.06)	48.5
	T_{max} (h)	8.422 ± 5.65	6.05 (1.67–19.58)	67.1
	$AUC_{0-\infty}$ (μmol/l h)	2.03 ± 1.10	1.98 (0.55–4.25)	54.1
	$T_{1/2}$ (h)	53.21 ± 26.16	45.98 (22.06–131.85)	49.2

The mean urinary excretion of doxorubicin during 48 h after a single dose was 6.4% (±2.4%) of the dose. Very little doxorubicinol was present in the urine, with a mean of only 1.4% (±0.7%) excreted in this form over 48 h. The amount of doxorubicin detected after glucuronidase treatment was slightly greater in most patients (mean of 7.1 ± 2.1% after 48 h) which cannot be used as a proof for glucuronidation. No additional peaks indicative for DOX glucuronides have been observed in the original chromatograms.

Pharmacodynamics

The C_{max}, AUC, Cl, V_{ss} of total doxorubicin, encapsulated doxorubicin and doxorubicinol were correlated with the nadirs of hemoglobin, hematocrit, and erythrocyte, white blood cell, absolute neutrophil and platelet counts, and with creatinine, bilirubin, alkaline phosphatase, AST, ALT, LDH, LVEF, height, weight and BSA. The absolute value of the Pearson correlation coefficient was less than 0.7 in all cases, suggesting that there are no clear correlations between the pharmacokinetic values for total doxorubicin, encapsulated doxorubicin, or doxorubicinol after Myocet and hematological variables, baseline laboratory values, or demographic factors.

Cardiotoxicity

No patient had congestive heart failure or a type 1 change (defined as a decrease of ≥20 points from the baseline LVEF to a final value ≥50%). One patient had a type 2 change (defined as a decrease of ≥10 points from the baseline LVEF to a final value < 50%). This 66-year-old man was withdrawn from the study 22 days after his sixth cycle of Myocet. The cumulative doxorubicin dose was 450 mg/m^2, all administered as Myocet. His LVEF had decreased 10 points, from 55% at baseline to 45% during the sixth cycle. The patient had a thymus carcinoma with a large tumor in the upper front of the mediastinum compressing the superior vena cava. The tumor was irradiated with 50 Gy. Besides this, no other cardiac risk factors were known. The pharmacokinetic values of this patient showed no significant deviation from the results of all other patients.

Safety and tolerability

The most common adverse events considered to be related (possibly, probably, or definitely) to study drug were nausea (94%), fatigue (78%) and vomiting (67%). Grade 3 adverse events considered to be drug related were fever, leukopenia, nausea and vomiting (11% each) and anemia, fatigue and stomatitis (6% each). No patient had a grade 4 nonhematological toxicity. Four patients had grade 3 nonhematological toxicity: three had increased LDH, and the other had hyperbilirubinemia.

Two patients discontinued treatment because of adverse events, one because of grade 3 nausea and vomiting as well as significant fatigue after the third cycle of treatment (this patient was treated 6 months after study treatment ended with DTIC), and one patient who died as a result of sepsis secondary to tumor progression occurring after the first cycle.

Five patients had serious adverse events: sepsis and ureteral disorder (one patient), dyspnea and respiratory insufficiency (one patient), sepsis (one patient), bronchitis (one patient), and stomatitis (one patient).

Grade 4 hematological toxicity included neutropenia (four patients), lymphopenia (two patients) and leukopenia (one patient). Grade 3 hematological toxicity included leukopenia (three patients), neutropenia (three patients), and anemia (two patients). No patient was withdrawn from the study because of an abnormal laboratory test result.

Discussion

Conventional doxorubicin is associated with a rapid distribution phase and slow elimination phase. Robert and Gianni [13] estimated that successive half-lives (α, β, γ) for doxorubicin were 5 min, 1 h and 30 h, based on a range of studies at therapeutic doses. Its total plasma clearance is about 30 l/h/m^2 and its total volume of distribution at steady state is approximately 23 l/kg, although higher volumes of distribution of around 25–30 l/kg have been reported [7, 8, 14]. We found the mean clearance for total doxorubicin in patients receiving Myocet 75 mg/m^2 was 5.6 l/h/m^2 while the volume (V_{ss}) was 82 l/m^2 (approximately equivalent to 2.2 l/kg). Thus, the clearance of doxorubicin in patients receiving Myocet is about fivefold lower and the volume about tenfold lower than in patients receiving conventional doxorubicin. Similar fold differences have been reported previously in a direct comparison of Myocet 60 mg/m^2 and conventional doxorubicin 60 mg/m^2 in patients with metastatic breast cancer who were also receiving cyclophosphamide (Table 3) [2].

We observed a fairly large interpatient variability in pharmacokinetic variables for total doxorubicin, with a coefficient of variation (CV) for clearance of 53%. Such variability is not surprising in cancer chemotherapy [15]. In one study of 35 patients receiving conventional doxorubicin administered over 1 h, the CV for clearance was 65% [16]. In a study of another liposomal formulation of doxorubicin (Caelyx), the CV for clearance among 42 patients was 119–161% [17].

The terminal half-life of Myocet 75 mg/m^2 in this study was over 50 h, longer than the value of 16 h reported for Myocet 60 mg/m^2 by Swenson et al. [2]. In that study, the area under the curve for Myocet was approximately double the value obtained in the present study, while clearance values were similar. Although the reasons for the differences are unclear, confounding factors such as interpatient variability, small sample sizes, the presence of hepatic dysfunction due to liver metastases in some of the cohort studied by Swenson et al. and the fact that the study was conducted in the context of a combination regimen are likely to account for this variation [2].

A terminal half-life of 50 h of Myocet is approximately half the value recently reported for a pegylated liposomal formulation of doxorubicin, Caelyx [18] (Table 3). Pegylation is designed to prevent detection and uptake of the liposomes by the reticuloendothelial system and, with a volume of distribution approximating to the plasma compartment (3 l), tissue uptake of pegylated liposomes is very low. This difference in half-life between Myocet and Caelyx is likely to contribute to the difference in toxicity profiles between the two formulations. The pegylated formulation is associated with a high incidence of palmar-plantar erythrodysesthesia (hand-foot syndrome), often dose limiting, which is characterized by redness, blistering and pain in the hands and feet. This is likely to result from distribution of the long-lasting pegylated liposomes to the skin capillaries [19–21]. Stomatitis has also been reported as a dose-limiting side effect of pegylated liposomal doxorubicin [21, 22]. Excessive prolongation of plasma stability may not be clinically desirable if it is associated with increased toxicity and reduced achievable dose intensity.

In this study, doxorubicinol was detected in all patients. The mean AUC of doxorubicinol was 2.03 ± 1.10 μM/l/h, not markedly different from that previously observed (1.82 ± 0.41 μM/l/h) in patients receiving conventional doxorubicin [2]. The longer time of maximum plasma concentration—2 h vs 8 h for conventional doxorubicin—reflects the delayed release of free doxorubicin from within the Myocet liposomes.

Myelosuppression is the dose-limiting toxicity of anthracyclines [13, 23]. Neutropenia was the most common grade 4 hematological toxicity in our study, but occurred in only 3 of 18 patients (17%). The only adverse event related to the study drug leading to discontinuation of treatment was nausea in one patient. Serious stomatitis was experienced by one patient. In a large phase III randomized clinical trial it was shown that Myocet in combination with cyclophosphamide is significantly less likely to cause grade 4 neutropenia and mucositis (all grades) than conventional doxorubicin [3].

Anthracycline-induced cardiotoxicity is thought to be related to peak plasma concentrations of free drug following intravenous administration. Based on the values for AUC and C_{max} obtained for total doxorubicin and

Table 3 Pharmacokinetic data for doxorubicin and other liposomal doxorubicin formulations (Caelyx, Myocet, DL-1)

Variable	Caelyx 60–70 mg/m^2 [a]	Caelyx 50 mg/m^2 [b]	Doxorubicin 50 mg/m^2 [b]	Myocet 60 mg/m^2 [c]	Doxorubicin 60 mg/m^2 [c]	DL-1 80 mg/m^2 [d]	Myocet 75 mg/m^2
C_{max} (μM)[e]	58.6–79.3	36.6	10.2	16.0	1.67	8.56	13.5
$AUC_{0-\infty}$ (μM/h)	–	1555.2	6.03	79.2	3.85	72.3	35.6
Terminal $t_{1/2}$ (h)	76–99	45.9	10.4	16.4	42.9	18.1	52.6
Clearance (ml/min/m^2)	0.22–0.25	0.88	248	50.8	452.3	59.2	93.0
V_{ss} (l/m^2)	1.7–1.8	3.5[f]	215[f]	34.2	851	51.3	81.7

[a]Patients with metastatic breast cancer, $n = 15$ [18]
[b]Patients with solid tumors; Caelyx $n = 14$, doxorubicin $n = 4$ [25]
[c]Patients with metastatic breast cancer received Myocet ($n = 10$) or doxorubicin ($n = 10$) in combination with cyclophosphamide 600 mg/m^2 [2]
[d]Patients with metastatic breast cancer, $n = 7$ [26]
[e]Micromolar values were calculated from nanograms per milligram values given in the original papers by use of the molecular weight of doxorubicin HCl
[f]Normalized by BSA (dividing by 1.7 m^2)

encapsulated doxorubicin, levels of free doxorubicin were estimated, showing that at least 85% of the circulating doxorubicin in patients receiving Myocet was encapsulated. While the C_{max} values in this study were higher than observed previously with conventional doxorubicin, the measurement is of encapsulated or total (85% encapsulated) doxorubicin, hence reducing the risk for cardiotoxicity. There was only 1 out of 18 patients with an LVEF below the normal threshold of 50% and this patient had received a fairly high cumulative doxorubicin dose of 450 mg/m^2. This is consistent with previous clinical studies that have demonstrated a significant reduction in risk of cardiotoxicity with Myocet compared with the same doses of conventional doxorubicin (with or without concomitant cyclophosphamide) [3, 4].

In conclusion, the pharmacokinetics of Myocet appear to be characterized by a reduced volume of distribution, a prolonged half-life and low plasma clearance compared with what would be expected in patients receiving conventional doxorubicin. This profile, together with the fact that liposomes of the size of Myocet can generally only escape from the circulation to tissues with leaky capillaries such as areas of inflammation, disorderly tissue structure due to tumor metastasis and in organs rich in reticuloendothelial system (liver, spleen and bone marrow) supports the hypothesis of distribution of Myocet to target tumor tissue rather than to healthy tissues such as the heart. This is consistent with the improved therapeutic index of Myocet compared with conventional doxorubicin that has been observed in clinical studies.

Liposomes as a drug-targeting system have obviously a potential for the improvement of "old" cytotoxic drugs with respect to selectivity and effectiveness by increasing the therapeutic index [24]. Myocet has a different pharmacokinetic profile from doxorubicin, resulting in an improved therapeutic index (less cardiotoxicity and equal anticancer activity) which offers new possibilities to combine, e.g., Herceptin and Myocet in HER2-positive breast cancer patients. Herceptin in combination with conventional doxorubicin has far too high a risk for the development of congestive heart failure.

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