

Pharmacokinetics and Toxicity of Two Schedules of High Dose Epirubicin¹

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ABSTRACT

Epirubicin, a stereoisomer of doxorubicin, is reported to have equal antitumor activity with lower cardiac and systemic toxicity. Recently the maximum tolerated dose of this drug has been revised upwards with reported increased response rates. However, the pharmacokinetics of epirubicin at high doses have never been reported. Accordingly, this study was designed to evaluate the pharmacokinetics of epirubicin when administered as either a 15-min i.v. bolus or a 6-h i.v. infusion in a phase I study at high doses. Nineteen patients with a variety of malignancies were given a total of 52 cycles of epirubicin at doses of 90 to 150 mg/m² given once every 3 weeks. The maximum tolerated dose was 150 mg/m² epirubicin given either as a bolus or as an infusion. The major dose-limiting toxicity was neutropenia. Interpatient variation occurred in the pharmacokinetics at each dose level but overall there were dose-dependent pharmacokinetics. This was manifested as a disproportionate increase in plasma levels and areas under the curve as the epirubicin dose was increased from 90 to 150 mg/m². The pharmacokinetics of epirubicin could best be described by an open two-compartment model. Peak plasma concentrations were attained at a median of 12 min following the bolus injection and concentrations approached the steady state within a median of 55 min following the start of the 6-h infusion. Administration of the 150 mg/m² dose over the 6 h compared to the bolus administration was associated with a 92% decrease in peak concentration from 3088 ± 1503 to 234 ± 126 ng/ml. This was not associated with an appreciable change in hematological or nonhematological toxicities. The median distribution half-life was 10 min and the median elimination half-life was 42.0 h. The cumulative renal excretion of the parent compound accounted for less than 2% of the administered dose. The major metabolites in both plasma and urine samples were 4'-O-β-D-glucuronyl-4'-epidoxorubicin, 13-S-dihydro-4'-epidoxorubicin, and 4'-O-β-D-glucuronyl-13-S-dihydro-4'-epidoxorubicin. This study demonstrates that a 135 mg/m² bolus infusion given on a 3-weekly schedule is an appropriate initial dose for further clinical studies.

INTRODUCTION

Anthracycline drugs are effective against a wide range of human malignancies. Doxorubicin is the most commonly used agent in this class. The use of doxorubicin is limited by a number of side effects, which include the acute reversible toxicities of nausea, vomiting, stomatitis, and bone marrow suppression. A chronic dose-related cardiomyopathy limits the extension of the drug's use in clinical oncology (1, 2). New anthracycline analogues have been developed in an attempt to improve both the therapeutic index and the spectrum of activity.

One such analogue is 4'-epidoxorubicin (epirubicin), a stereoisomer of doxorubicin differing in the orientation of the hydroxyl group on carbon atom four of the hexopyranosyl sugar (3). Epirubicin has been extensively tested in a wide range of cancer types and, although it has an almost identical spectrum

of activity at similar doses and schedules of administration compared with doxorubicin, its therapeutic index is more favorable, with less hematological and cardiac toxicity at equimolar doses (4). Most phase I-II studies have utilized doses in the range of 60-90 mg/m² administered every 3 weeks, with leukopenia being the dose-limiting toxicity (5). Recently the maximum tolerated dose of epirubicin determined in these early studies has been questioned, with a number of investigators reporting higher MTDs⁵ and higher response rates. Case *et al.* demonstrated the safety and efficacy of epirubicin doses up to 180 mg/m² every 3 weeks in patients with lymphoma (6) and 105-120 mg/m² in previously treated patients with multiple myeloma (7), reporting acceptable toxicity and apparently higher response rates when compared to patients treated with lower doses of epirubicin.

Although the cardiomyopathy caused by anthracyclines may be decreased by increasing the duration of the infusions to 48 and 96 h (8), the use of new cardioprotectors such as ADR-529 (ICRF-187) may make very long infusions unnecessary (9). Moreover, these cardioprotectors may also allow the use of high doses of anthracyclines with either bone marrow protection or bone marrow reconstitution with hematopoietic growth factors (10). In an attempt to prevent cardiac arrhythmias, it has been suggested that high doses of anthracyclines should be administered slowly and that the administration of epirubicin as a 6-h infusion may be associated with less acute and possibly chronic toxicity because of lower peak levels (11). Thus this study was designed to establish the maximum tolerated dose and determine the pharmacokinetics of epirubicin when administered as an i.v. bolus and a 6-h infusion at higher doses.

MATERIALS AND METHODS

Patient Selection

Patients had histologically documented locally advanced or metastatic malignancy for which no treatments with a greater likelihood of benefit were available, had an ECOG performance status of 2 or better, had a life expectancy of at least 8 weeks, were aged 18 or more, had recovered from toxic effects of previous chemotherapy or radiotherapy, and had granulocyte count >1,500 mm⁻³, platelet count >100,000 mm⁻³, creatinine <0.2 mmol/liter, bilirubin <57 μmol/liter, and serum glutamate oxalate transaminase <86 IU/liter. Patients previously treated with anthracyclines were not eligible. This study was conducted in accordance with the ethical guidelines of the National Health and Medical Research Council (Australia) and all patients gave informed consent. Pretreatment evaluation included history, physical examination, performance status, height, weight, tumor measurements, complete blood count (including platelets and differential WBC count), biochemical profile (by Sequential Multichannel Analyzer and Computer) including electrolytes, creatinine, urea, and liver function tests, radionuclide cardiac LVEF, and appropriate X-rays and nuclear medi-

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⁵ The abbreviations used are: MTD, maximum tolerated dose; HPLC, high pressure liquid chromatography; C_{max}, maximum concentration; T_{max}, time to reach C_{max}; AUC, area under curve; ECOG, Eastern Cooperative Oncology Group; LVEF, left ventricular ejection fraction; A, B, C, α, β, and γ, pharmacokinetic terms in the general equation relating concentration of drug as a function of time, as per formulae: concentration (drug) = Ae^{-αt} + Be^{-βt} (two-compartment model) and concentration (drug) = Ae^{-αt} + Be^{-βt} + Ce^{-γt} (three-compartment model); UICC, Union Internationale Contre Cancer; V_d, volume of distribution.

cine imaging to evaluate tumor response.

While patients were receiving treatment, blood counts were performed weekly and biochemistry was evaluated every 3 weeks. Tumor evaluation was performed after each two cycles and at the completion of treatment or earlier if clinically indicated. LVEF was repeated after two cycles of treatment and then after a cumulative dose of 450, 600, 750, and 900 mg/m² epirubicin.

The criteria for tumor response and toxicity were those developed by the World Health Organization and UICC reported by Miller *et al.* (12). All entered patients were evaluable.

Dose Scheduling

For patients treated with i.v. bolus, epirubicin (Farmitalia Carlo Erba, Milan, Italy) was reconstituted with 0.9% sodium chloride solution and administered over 15 min. For patients treated with 6-h i.v. infusions, epirubicin was reconstituted in 400 ml of 0.9% sodium chloride solution and infused via an antecubital central venous catheter or Hickman catheter using a syringe pump.

The initial dose of epirubicin was 90 mg/m² by i.v. bolus every 3 weeks. Two patients were entered at this dose without significant toxicity. Subsequent groups of patients were entered at 120 mg/m², 135 mg/m², and 150 mg/m² until the MTD was determined. A total of 12 patients were entered at the MTD, 6 by infusion and 6 by bolus. The MTD was defined as the dose which resulted in UICC grade IV toxicity in two patients.

There was no dose escalation in individual patients. Patients with grade IV toxicity had the next dose reduced by 30 mg/m². Treatment was discontinued if disease progression occurred, if toxicity was unacceptable, if six cycles were given with stable disease, or if two treatments were given after maximal response. No patient experienced a fall in LVEF below 0.45 requiring cessation of treatment.

Pharmacokinetics

Blood and Urine Samples. Epirubicin was administered as either a 15-min bolus infusion or a 6-h extended infusion. The pharmacokinetics were studied during the first cycle of chemotherapy. Ten-ml blood samples were obtained prior to infusion and 10 min into the infusion. In the case of the bolus infusion, further 10-ml samples were obtained 5, 15, 30, 45, and 60 min and 2, 3, 4, 6, 8, 12, 24, and 48 h after the end of infusion. In the case of the 6-h infusion, further 10-ml samples were obtained 10 and 30 min and 1, 2, 4, and 6 h into the infusion and then 5, 15, 30, 45, and 60 min, 2, 3, 4, 6, 8, 12, and 24 h and 2, 3, 4, 5, and 6 days after the end of the infusion. Blood was collected in 10-ml heparinized polystyrene tubes and centrifuged at ambient temperature at 600 × *g* for 15 min. The plasma was transferred to labeled sterile polystyrene tubes. Urine samples were obtained over 6-h intervals for 24 h in the case of the bolus infusion and a 10-ml aliquot from the known volume was taken. In the case of the extended infusions, further 24-h urine samples (10 ml from known total volumes) were collected on days 2 to 6. All urine and plasma samples were stored at -20°C until analysis. After thawing, all samples were centrifuged (600 × *g* for 5 min) to remove fibrous material.

HPLC Instrumentation and Analytical Methodology. The HPLC system consisted of a Beckman System Gold programmable solvent module model 126 fitted with μ -Flow analytic liquid heads, interfaced to an IBM AT-compatible computer using a Beckman model 406 analog interface module. The software driving the system and collecting and analyzing the data was the System Gold software revision B (1988). Samples were injected using an AS2000 automatic injector (ICI Instruments, Australia) and the column was a μ Bondapak Phenyl 8-mm (i.d.) × 10-cm Radial-Pak cartridge mounted in a Z-module radical compression separation unit (Waters, Bedford, MA). Detection was by a F-1000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) set at time constant of 1 sec, sensitivity of 100, excitation wavelength of 480 nm, and emission wavelength of 550 nm. The chromatography was performed using an isocratic solvent system consisting of 70% 7 mM Na₂HPO₄, pH adjusted to 2.6 with formic acid/30% acetonitrile, HPLC grade (Mallinckrodt, Australia), at a constant flow rate of 3.0 ml/min.

Solid Phase Extraction. Two hundred ng of the internal standard,

daunorubicin (Farmitalia, Carlo-Erba), were added to 1.0-ml aliquots of plasma or urine samples and diluted with 2.0 ml of 0.9% NaCl solution (saline). The mixture was extracted using C-18 Sep-Pak cartridges (Waters-Millipore, Bedford, MA). The Sep-Paks were prepared with 3 ml of methanol followed by 3 ml of methanol/H₂O (1/1, v/v) and 10 ml of 0.05 M Na₂HPO₄, pH 8.9. Then 3 ml of diluted plasma (or urine) were added, followed by a wash with 3 ml of 0.05 M Na₂HPO₄, pH 8.9. The eluent was then obtained by adding four 0.5-ml aliquots of CHCl₃/methanol (2/1, v/v).

Sample Concentration. The extracted eluents were spun at ambient temperature under vacuum. The dried samples were dissolved in 200 μ l of 60% 7 mM Na₂HPO₄, pH 2.6/40% acetonitrile, gently vortexed, and spun down at 4000 rpm (3000 × *g*) for 15 min to remove coextracted proteins. The supernatant was transferred to labeled conical tip vials (suitable for analysis using the autosampler). The order of elution for the peaks of interest was the glucuronide metabolite 4'-*O*- β -D-glucuronyl-13-*S*-dihydro-4'-epidoxorubicin, followed by the 13-*S*-dihydro- metabolite, the second glucuronide metabolite (4'-*O*- β -D-glucuronyl-4'-epidoxorubicin), epirubicin, and then the internal standard daunorubicin. The identity of each peak was confirmed by comparison with standards (provided by Farmitalia, Carlo-Erba). These peaks were quantitated as described below. Other metabolites were also detected and separated, but no attempt was made to quantitate their levels.

The minimum quantifiable concentration of epirubicin and its metabolites was defined as that which could be reproducibly detected at 5 times the baseline noise level. This was 0.6 ng/ml. At this level the variability within duplicate samples (prepared sequentially) was 2.7%.

Calibration and Quality Control of Assay Precision and Accuracy. Assay precision was determined within the same day and between different days by extracting batches of 8 replicates. All were derived from a single prepared lot in conjunction with a storage stability study (which showed less than 9% nonspecific loss for all materials stored under these conditions for 43 days). The coefficient of variation between samples on the same day was 7.5% and the variation in samples measured 43 days later was 10.4%. Calibration curves were obtained for each of the components of interest and the coefficients of correlation always exceeded 0.99. Quality control was assured by assaying three plasma samples, representing low, medium, and high concentrations of epirubicin and its metabolites, before and after a batch of patient samples to check for any deviation in detector response or a possible shift in retention times for the components. No significant deviations were noted within a given day and detector response was not observed to differ within the entire study period.

Pharmacokinetic Data Analysis. Initial estimates of *A*, *B*, α , and β were made using a stripping program (JANA version 2.1; Statistical Consultants, 1987) (13), using both two and three exponents. The coefficient of correlation for the two-exponential parameters (*A*, *B*, α and β) was in all cases slightly higher than for three exponents (*A*, *B*, *C*, α , β , and γ) and thus all modelling was done using two exponential terms.

The modelling of the data was performed with a nonlinear pharmacokinetic modelling program (PCNONLIN version 3.0; Statistical Consultants, 1989) using the initial estimates acquired from JANA. Models 8 and 10 for the bolus and infusion cases, respectively (*i.e.*, single-dose two compartment with bolus or constant i.v. input, respectively, and first-order output, having macro-constants as the primary parameters), were used. Because this was a new program to our laboratory, all the data obtained was compared with values calculated manually using the residual stripping method and the AUCs that were calculated using the trapezoidal rule (14). There was no significant difference between the two sets of values obtained and the values presented are those obtained using the program.

RESULTS

Clinical Study

Nineteen patients (11 male, 8 female) were entered into the study and received a combined total of 52 cycles of epirubicin. A wide variety of neoplasms was represented. The median age

was 49 years (35–67 years) and ECOG performance status was 1 (0–2). Four patients had received prior chemotherapy and three patients prior radiotherapy (Table 1). There were no deaths attributable to drug toxicity. There were no adverse local side effects attributed to epirubicin administration.

Toxicity

The major toxicity in this study was leukopenia (Table 2). Grade IV granulocytopenia was noted in two of three patients at 120 mg/m² bolus, in one of two patients at 135 mg/m² bolus, in five of six patients at 150 mg/m² bolus, and in six of six at 150 mg/m² infusion. Febrile neutropenic episodes requiring hospital admission and i.v. antibiotic treatment occurred in one of two patients at 135 mg/m² bolus, in three of five at 150 mg/m² bolus, and in one of six at 150 mg/m² infusion. No grade III or IV thrombocytopenia was observed. Neutropenia resulted in deferral of treatment in five patients.

Nonhematological side effects were less marked (Table 3). One patient at a level of 150 mg/m² bolus experienced grade IV nausea and vomiting, requiring hospital admission and ultimately withdrawal from further treatment. All patients experienced minor (grade I or II) nausea and vomiting. One patient developed a small bowel perforation after the first dose of treatment at the 150 mg/m² bolus level. This patient had metastatic melanoma with small bowel involvement and was thought to have tumor necrosis as the mechanism of perfora-

tion. The second cycle was deferred but subsequently three more cycles were completed prior to disease progression being noted. Another patient developed a pulmonary embolus after the second dose. Coumadin was administered but a left subclavian venous thrombosis developed requiring heparin. Three further cycles of treatment were given and a partial response was obtained. The pulmonary embolus was not believed to be due to administration of epirubicin. One patient at 150 mg/m² bolus experienced grade III stomatitis requiring hospital admission. No adverse cardiac toxicity was noted in this study. All baseline LVEFs were in the normal range. Five patients received more than two cycles of epirubicin and had serial LVEFs performed. No patient showed a significant decline in LVEF. There were no arrhythmias or episodes of heart failure.

Responses

There were three partial responses and one complete response. The partial responses were noted in two patients with adenocarcinoma of unknown primary site and in one patient with colorectal adenocarcinoma. The complete response was noted in a man with a chondrosarcoma involving the left parietal bone and extensive bone marrow involvement confirmed with a bone marrow aspirate and trephine biopsy. The primary site was treated with surgery and radiotherapy. After two cycles of treatment, repeat bone marrow biopsies showed no tumor infiltration and the blood film returned to normal. At the completion of six cycles, bone marrow biopsies were completely normal. The response lasted for 11 months.

Pharmacokinetics

Bolus Dose Studies. Pharmacokinetic studies with bolus administration of epirubicin were conducted on 13 patients. There was considerable interpatient variability in drug disposition, probably due to hepatic involvement or other uncharacterized factors in patients with extensive malignant disease. The major pharmacokinetic parameters are summarized in Table 4.

Following bolus administration, maximum concentrations of epirubicin (C_{max}) occurred between 8 and 14 min from the beginning of the 15-min "bolus" injection. Plasma concentrations then fell sharply due to rapid tissue distribution (mean $t_{1/2\alpha}$, 0.17 h; range, 0.13 to 0.21 h), followed by a slower rate of decline associated with drug elimination (mean $t_{1/2\beta}$, 56.5 h; range, 16.95 to 79.31 h). The extent of tissue distribution was highly variable between patients, and mean values for V_d ranged from 39.61 to 136.78 liters.

The pharmacokinetics of one of the two patients given 135 mg/m² differed considerably from those seen in all other patients. This patient had extensive hepatic disease and this probably was the cause of these abnormal pharmacokinetic

Table 1 Patient characteristics

No. of patients	19
Sex	
Male	11
Female	8
Median age (range)	49 (35–67)
ECOG performance status	
0	6
1	12
2	1
Prior therapies	
Chemotherapy	4
Radiotherapy	3
Diagnosis	
ACUP ^a	5
Colon	3
Gastric	3
NSCLC	2
Sarcoma	2
Renal	2
Breast	1
Melanoma	1

^a ACUP, adenocarcinoma of unknown primary; NSCLC, non-small cell lung cancer.

Table 2 Hematological toxicity of high doses of epirubicin (90–150 mg/m²)

Dose level (mg/m ²)	Bolus or infusion	No. of patients	Mean WBC nadir (range) (median) ^a	Mean granulocyte nadir (range) (median) ^a	Mean platelet nadir (range) (median) ^a	Delay of 2nd course or dose reduction (no. of patients)	Fever and neutropenia (no. of patients)
90	Bolus	2	4.95 (3.1–8.2) (4.2)	2.85 (1.2–4.9) (2.6)	295 (250–324) (284)	0	0
120	Bolus	3	2.90 (1.2–4.3) (3.2)	1.10 (0.3–2.3) (0.48)	318 (216–416) (310)	0	0
135	Bolus	2	2.67 (1.9–3.2) (2.9)	0.66 (0.4–0.9) (0.65)	261 (215–330) (235)	1 ^b	1 ^b
150	Bolus	6	1.87 (0.5–3.9) (2.0)	0.79 (0.1–2.5) (0.70)	205 (68–376) (223)	3	3
150	Infusion	6	1.54 (0.7–2.7) (1.5)	0.22 (0.1–0.6) (0.20)	213 (84–416) (201)	3	1

^a Units for these parameters are absolute numbers × 10⁹/liter.

^b One patient had both delay and dose reduction.

Table 3 Nonhematological toxicity of high doses of epirubicin (90–150 mg/m²)

Dose level (mg/m ²)	No. of patients at each dose level	No. of patients with respective UICC graded toxicities							
		Stomatitis		Diarrhea		Nausea and vomiting		Alopecia	
		Gr 1–2 ^a	Gr 3–4	Gr 1–2	Gr 3–4	Gr 1–2	Gr 3–4	Gr 1–2	Gr 3–4
90	2	0	0	0	0	1	0	1	1
120	3	0	0	0	0	3	1	3	0
135	2	0	0	1	0	1	0	1	0
150 bolus	6	3	1	4	0	3	1	5	1
150 infusion	6	1	0	1	0	6	0	5	0

^a Gr, UICC graded level toxicity.

Table 4 Pharmacokinetic parameters of epirubicin, its glucuronide, its dihydro-metabolite, and the glucuronide of the dihydro-compound

Values shown are the mean and SD except where indicated.

Dose (mg/m ²)	C _{max} (ng/ml)	T _{max} (min)	AUC (0–infinity) [(ng/ml)·h]	% of dose recovered in urine	t _{1/2α} (h)	t _{1/2β} (h)	V _d (liter)
Epirubicin							
90	817 ± 206	13 ± 11	813 ± 793	0.35 ± 0.19	0.16 ± 0.05	16.95 ± 6.16	136.78 ± 101.82
120	1184 ± 57	8 ± 3	1670 ± 1413	1.06 ± 0.94	0.18 ± 0.1	78.32 ± 120.9	91.06 ± 32.23
135	1756 ± 127	10 ± 0	1267 and 33903 ^a	1.57 ^b	0.21 ± 0.13	79.31 ± 44.33	70.56 ± 38.89
150 bolus	3088 ± 1503	14 ± 5	3280 ± 1789	0.44 ± 0.33	0.13 ± 0.06	51.44 ± 90.03	39.61 ± 32.77
150 infusion	233.5 ± 126 ^c	55 ± 34 ^d	2537 ± 1338	1.65 ± 0.75	0.17 ± 0.13	10.2 ± 6.91	52.98 ± 46.44
				0.96 ± 0.8			
4'-O-β-D-Glucuronyl-4'-epidoxorubicin							
90	181 ± 255	100 ± 134	1380 ± 1948	0.29 ± 0.22			
120	177 ± 117	75 ± 0	1546 ± 740	0.63 ± 0.5			
135	169 ± 135	52 ± 40	2015 ± 2045	0.78 ^b			
150 bolus	180 ± 118	55 ± 25	1122 ± 703	0.21 ± 0.08			
150 infusion	316 ± 138	396 ± 28	3306 ± 1790	0.75 ± 0.34			
13-S-Dihydro-4'-epidoxorubicin							
90	383 ± 197	18 ± 18	2277 ± 2180	0.43 ± 0.29			
120	180 ± 91	13 ± 6	3413 ± 2376	0.96 ± 0.71			
135	444 ± 493	10 ± 0	1026 ± 1370	1.99 ^b			
150 bolus	344 ± 455	15 ± 6	2500 ± 1876	0.33 ± 0.2			
150 infusion	195 ± 103	407 ± 50	5227 ± 2796	1.27 ± 0.51			
4'-O-β-D-Glucuronyl-13-S-dihydro-4'-epidoxorubicin							
90	170 ± 135	128 ± 11	1758 ± 2145	0.28 ± 0.22			
120	98 ± 47	160 ± 94	1466 ± 1530	0.5 ± 0.48			
135	27 ± 31	52 ± 40	236 ± 255	0.46 ^b			
150 bolus	216 ± 196	49 ± 51	2678 ± 2923	0.21 ± 0.07			
150 infusion	208 ± 74	383 ± 27	1983 ± 703	0.45 ± 0.18			

^a One patient with major infiltration of the liver had unusually high plasma levels of epirubicin.

^b Analysis of the urine of one of the two patients at the dose of 135 mg/m² was not possible.

^c C_{plasma} (ng/ml).

^d T_m (min).

parameters. For example, the value of AUC (33,903 ng·h/ml) was in excess of 10 SD greater than the highest mean value for this parameter (AUC at 150 mg/m², 3,280 ± 1,789 ng·h/ml). This patient was, therefore, not included in further pharmacokinetic analysis.

Fig. 1 shows the relationship between epirubicin dose and AUC and C_{max}. Linear regression analysis demonstrated a significant linear increase in C_{max} ($r^2 = 0.808$; $P < 0.05$; $n = 12$) but not in AUC ($r^2 = 0.510$; $P > 0.05$; $n = 12$) over the dose range 90 to 150 mg/m². By contrast, conducting linear regression analysis of a double-reciprocal plot of these parameters (*i.e.*, assuming nonlinear Michaelis-Menten kinetics prevailed) produced improved correlations which were significant for both C_{max} ($r^2 = 0.981$; $P < 0.05$; $n = 12$) and AUC ($r^2 = 0.956$; $P < 0.05$; $n = 12$). (Fig. 2).

Fig. 3 shows a highly significant inverse linear correlation between V_d and dose ($r^2 = 0.995$; $P < 0.05$; $n = 12$). The urine recovery was in the range of 1% of the total administered dose. Three major metabolites were detected, as well as a number of much smaller peaks on the chromatograms. Two of the three major metabolites were glucuronides, being 4'-O-β-D-glucuronyl-4'-epidoxorubicin, the glucuronide of the parent drug, and 4'-O-β-D-glucuronyl-13-S-dihydro-4'-epidoxorubicin, the

glucuronide of the 13-S-dihydro-4'-epidoxorubicin metabolite formed in the liver. The peak levels of both glucuronides were detected between 1 and 2 h after the parent peak appeared, while the third major metabolite, 13-S-dihydro-4'-epidoxorubicin, appeared rapidly, approximately 5 min after the parent peak.

In an effort to determine whether a direct relationship existed between the hematological or nonhematological toxicities and the C_{max} or AUC, these parameters were analyzed in the 13 patients who received a bolus dose of epirubicin. The relationships between the percentage of change in both granulocytes and neutrophils and C_{max} and AUC were plotted as individual points (linear regression analysis) and by analysis of the data grouped at each dose (Student's *t* test), but no correlation could be identified. Similarly, a correlation was sought between the severity of stomatitis, nausea, vomiting, and diarrhea and the AUC or C_{max}, where these latter two parameters were grouped as cohorts based on sequential changes of C_{max} and AUC (data not shown). There was no significant correlation (Student's *t* test) between the toxicities and either the C_{max} or AUC.

Six-h Infusion Studies. Epirubicin was administered as a 6-h infusion in an additional six patients at 150 mg/m². Plasma concentrations approached the steady state (234 ± 126 ng/ml)

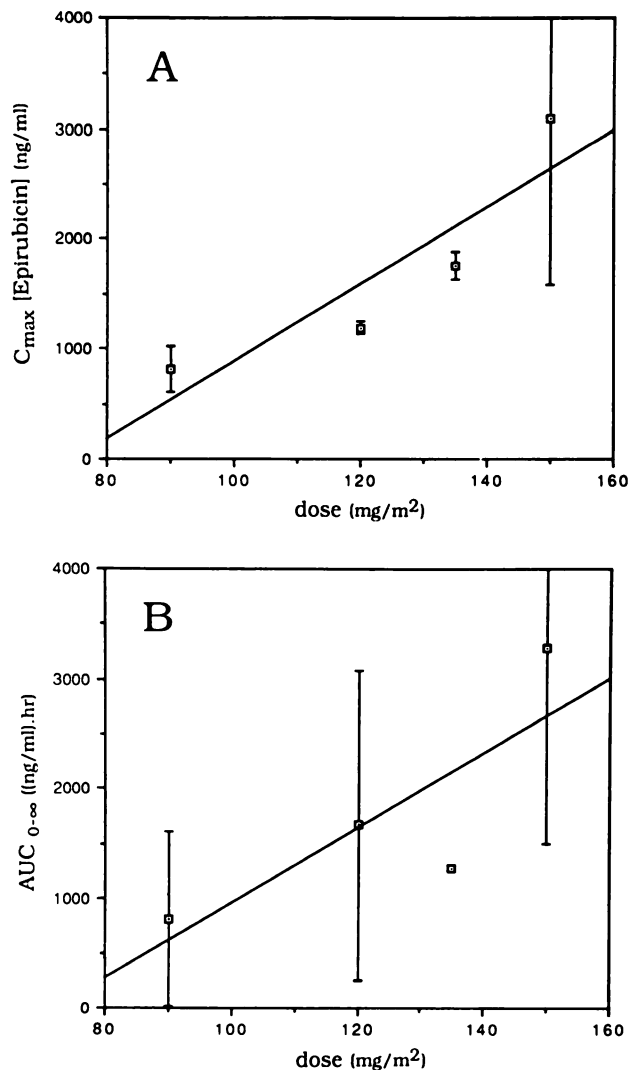


Fig. 1. Relationship between the bolus dose of epirubicin administered (mg/m²) and the C_{max} (A) and between the dose administered and AUC, (B) are plotted for the four dose levels studied. The number of patients studied at each point is listed in Table 2. Linear regression analysis performed for the data revealed a significant linear increase in C_{max} (A) ($r^2 = 0.808$; $P < 0.05$; $n = 12$) but not AUC (B) ($r^2 = 0.510$; $P > 0.05$; $n = 12$) over the dose range 90 to 150 mg/m².

approximately 1 h into the 6-h infusion (Fig. 4). C_{max} values were, therefore, reduced by 92% in comparison with the same dose delivered by bolus administration. There was no significant difference in AUC following 6-h infusion (2537 ± 1338 ng·h/ml) compared with bolus administration (3280 ± 1789 ng·h/ml), indicating that overall drug elimination was unaffected by rate of drug administration.

Peak levels of the 4'-O-β-D-glucuronyl-4'-epidoxorubicin metabolite were higher than those of the parent compound, while the 4'-O-β-D-glucuronyl-13-S-dihydro-4'-epidoxorubicin and 13-S-dihydro-4'-epidoxorubicin metabolites were of similar magnitude with either schedule. The proportion of each metabolite recovered from the urine was approximately the same as that of the parent compound.

AUCs in Responding Patients. All patients who had responses were treated at the maximum dose of 150 mg/m². Of the three partial responders, two received the epirubicin as a 6-h infusion and one as a bolus. The AUCs for the two patients who received epirubicin as a 6-h infusion were 1755 and 706 ng/ml·h. The two patients who responded and received epirubicin as a bolus had AUCs of 2327 and 3737 ng/ml·h, the first being the patient

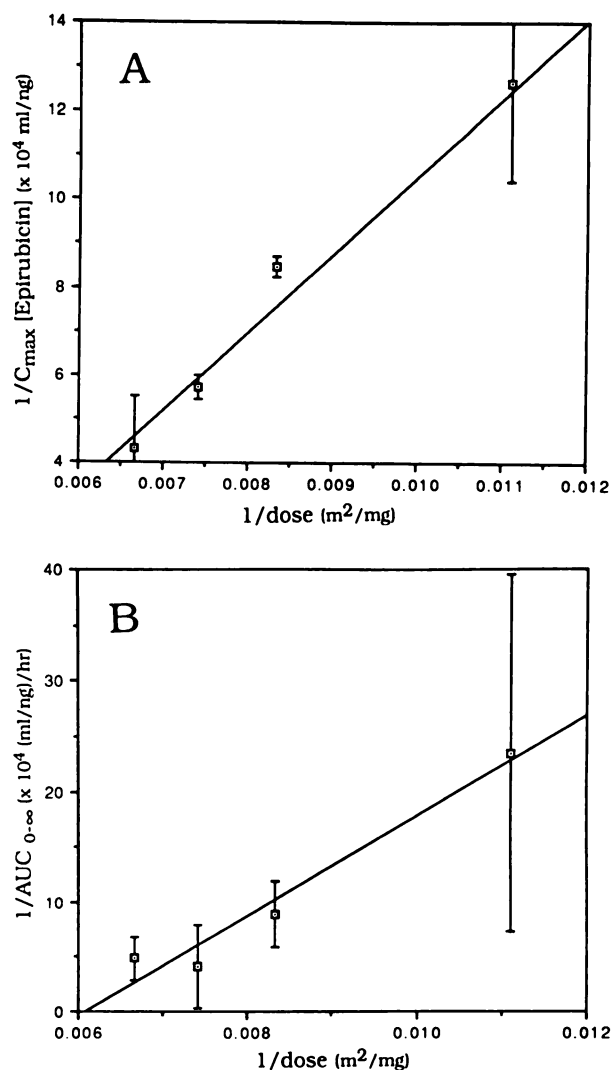


Fig. 2. Double-reciprocal plots showing the relationship between the reciprocal of the bolus dose of epirubicin administered (mg/m²) and the reciprocal of the C_{max} (A) and between the reciprocal of the dose administered and the reciprocal of the AUC (B). This type of plot assumes nonlinear Michaelis-Menten pharmacokinetics prevails. Linear regression analysis of the double-reciprocal plot of these parameters produced an improved correlation for both C_{max} ($r^2 = 0.981$; $P < 0.05$; $n = 12$) and AUC ($r^2 = 0.956$; $P < 0.05$; $n = 12$).

who had a complete response. By grouping the AUCs and comparing responders to nonresponders, no significant difference was found ($P > 0.05$, Student's *t* test).

DISCUSSION

Although the concept of a dose-response relationship for anthracyclines in humans is disputed, there are several cancer models and phase I/II studies (15-17) which indicate a dose-response relationship for these drugs. Jones *et al.* (15) reported a phase II study of doxorubicin in breast cancer in which a median dose of 99 mg/m² per month was delivered, with an overall response rate of 86%. The dose-limiting toxicity was neutropenia. Similarly, Carreo-Pereira *et al.* (17) compared 35 mg/m² with 70 mg/m² doxorubicin given every 3 weeks to patients with breast cancer and found a significant difference in response rate and time to disease progression in the higher dose arm. The favorable toxicity profile of epirubicin as compared to doxorubicin (4) suggests that it is an appropriate candidate for dose escalation studies. In view of recent advances in myeloprotection by colony-stimulating factors (18) and pre-

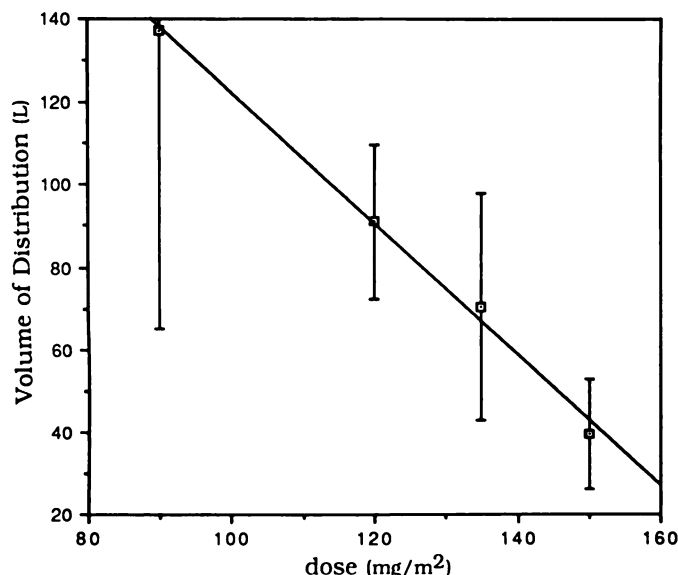


Fig. 3. Relationship between the dose of epirubicin administered as a 15-min bolus (mg/m^2) and the V_d ($r^2 = 0.995$; $P < 0.05$; $n = 12$).

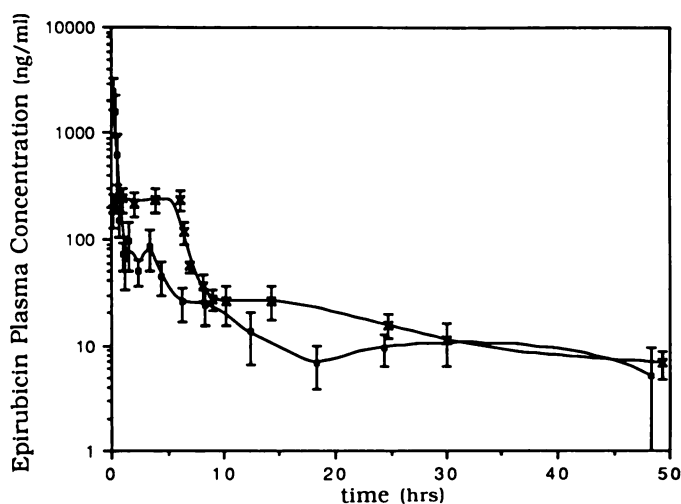


Fig. 4. Mean plasma concentrations of epirubicin following bolus administration (\blacksquare) and 6-h infusion of epirubicin (\times) at a dose of $150 \text{ mg}/\text{m}^2$ plotted against time.

vention of cardiac toxicity by new agents (9), there is renewed interest in establishing the toxicity and pharmacokinetic profiles of epirubicin given at high doses.

Most phase I/II studies of high dose anthracyclines have used a 3-weekly schedule. Case *et al.* recently demonstrated the safety and efficacy of epirubicin in doses up to $180 \text{ mg}/\text{m}^2$ in patients with lymphoma (6) and $105\text{--}120 \text{ mg}/\text{m}^2$ in previously treated patients with multiple myeloma (7). In these studies the dose-limiting toxicity was neutropenia, with a trend towards more leukopenia at the higher dose. Similarly, Feld *et al.* (19) performed a phase II study of epirubicin in previously treated non-small cell lung cancer and defined an MTD of $165 \text{ mg}/\text{m}^2$ every 3 weeks, with an apparent high response rate. In the current study, the MTD was found to be $150 \text{ mg}/\text{m}^2$ by i.v. bolus every 3 weeks, with neutropenia being the dose-limiting toxicity.

Four phase I studies of high dose epirubicin using an identical i.v. bolus schedule administered every 3 weeks have been published in abstract form (20–23). In patients with non-small cell lung cancer, doses of up to $180 \text{ mg}/\text{m}^2$ were administered every 3 weeks (20). The maximum tolerated dose was $165 \text{ mg}/\text{m}^2$,

with the dose-limiting toxicity being neutropenia. A similar study was conducted in patients with breast cancer who had received prior chemotherapy either with or without an anthracycline (21). In those who had not received prior anthracyclines, the MTD was $150 \text{ mg}/\text{m}^2$, with the dose-limiting toxicity being febrile neutropenia in three of six patients. In a phase I study of patients with a mixture of tumor types, the MTD was found to be $150 \text{ mg}/\text{m}^2$ (22). The dose-limiting toxicity was febrile neutropenia occurring in 6 of 10 patients receiving $150 \text{ mg}/\text{m}^2$. In the fourth abstract, doses up to $180 \text{ mg}/\text{m}^2$ were administered to patients with a variety of tumor types (23). Leukopenia, thrombocytopenia, and febrile episodes were the dose-limiting toxicity. However, hospitalization was required for febrile episodes in two of five patients at $165 \text{ mg}/\text{m}^2$, leading the authors to suggest a dose of $135 \text{ mg}/\text{m}^2$ given every 3 weeks as a safe phase II dose.

In previous studies (6, 7) a lack of correlation between dose and nonhematological toxicity was observed. This was not noted in the current study, in which nausea, vomiting, diarrhea, and stomatitis were observed more frequently at higher doses. This may be an artifact due to the small number of patients entered at the lower dose levels or it may indicate that a more direct correlation between dose and nonhematological toxicity exists than was previously thought.

Administration of anthracyclines by continuous infusion has been investigated in an attempt to reduce toxicity (24, 25). These studies are based on the hypothesis that hematological and nonhematological toxicities (in particular cardiac toxicity) may be related to peak drug concentrations and that the anti-tumor effect is related to the total dose given (24). Green *et al.* (25) compared 6-h with 24-h infusions of doxorubicin and found a trend towards reduced nausea, vomiting, and hematological toxicity in the patients receiving the 24-h infusion. In the current study no significant cardiac toxicity was observed. Nausea and vomiting were more common in the infusion group and stomatitis and diarrhea more common in the bolus group, but the number of patients in both groups was small. Importantly, in this study we could not identify any direct relationship between the degree of myelosuppression or the severity of nonhematological toxicities and C_{max} or AUC.

This study suggests that the pharmacokinetics of epirubicin may not be linear over the dose range studied ($90\text{--}150 \text{ mg}/\text{m}^2$). This was implied by the improved linear correlation obtained with regression analysis of double-reciprocal plots of dose versus C_{max} and AUC. The consequence of this nonlinearity was manifest as a disproportionate (*i.e.*, greater than) increase in both C_{max} and AUC, which translated into a greater than predicted systemic exposure, as epirubicin dosage was increased from 90 to $150 \text{ mg}/\text{m}^2$ (Fig. 1). These results extend observations from previous studies at lower doses ($<50 \text{ mg}/\text{m}^2$) (26–28) and imply the presence of Michaelis-Menten kinetics at the higher dose levels administered.

The presence of a significant inverse linear correlation in the volume of distribution as dose is increased suggests saturation of tissue distribution with epirubicin. As a consequence of higher epirubicin doses, proportionally higher levels of epirubicin were localized in the systemic circulation (*i.e.*, rapidly exchangeable body water). Previous studies have reported considerably higher values for V_d , ranging from 200 to 2000 liters (26–28), although here too these studies used lower doses of epirubicin.

Two glucuronides and a dihydro- compound were the major metabolites of the parent compound and appeared in the same relative proportions in both the bolus and infusion studies

(Table 4). The T_{max} values for the 13-S metabolite occurred rapidly following that of the parent drug, suggesting rapid and extensive hepatic metabolism of epirubicin. This was also true of one patient, at the dose of 135 mg/m², with substantial tumor involvement of the liver. The AUC for epirubicin in this patient was unusually high (33,903 ng·h/ml), while the AUC for the 13-S-dihydro-4'-epidoxorubicin metabolite was unusually low, as would be expected in patients with severe liver impairment. The distribution half-life ($t_{1/2\beta}$) was approximately 10 min in all cases; however, the elimination half-life ($t_{1/2\alpha}$) was highly variable between the patients. The mean value for elimination half-life at higher epirubicin doses obtained in this study (Table 4) was slightly higher than the values reported by Camaggi *et al.* (26), Roberts *et al.* (27), and Mross *et al.* (28). In their studies, the values for $t_{1/2\alpha}$ were 31.2 ± 9.4, 18.3 ± 2.4, and 19.0 ± 2.4 h at the lower doses of 40–50, 50, and 40 mg/m², respectively. The shorter values for $t_{1/2\alpha}$ in these other studies, compared to that reported here, are consistent with the proposal that drug disposition is saturated at elevated doses of epirubicin.

The pharmacokinetic data obtained from the 6-h infusion part of the study suggest that, although peak levels obtained are reduced by 92% compared with bolus administration, the distribution and elimination half-lives and the AUC and volume of distribution are similar. The fact that the acute toxicities were similar in the bolus and 6-h infusion groups strongly suggests that peak levels of the drug are not related to these toxicities.

This study supports and extends other observations which indicate that high doses of epirubicin can be given relatively safely, especially to patients previously untreated with cytotoxic drugs or radiation. The pharmacokinetics of epirubicin appear to be dose related but these parameters are not predictive of the more severe toxicities.

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