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Phase II studies with DaunoXome in patients with nonresectable hepatocellular carcinoma: clinical and pharmacokinetic outcomes

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Abstract A total of 14 Chinese patients with inoperable hepatocellular carcinoma received a liposomal formulation of daunorubicin (DaunoXome) at a dose equivalent to 100 mg/m^2 of the free drug every 3 weeks. Altogether, 12 patients were assessable for response; 2 patients had stable disease for 8 weeks, but all eventually developed progressive disease and there was no responder. The drug was well tolerated, with no evidence of cardiac toxicity being observed. Deterioration of liver-function tests was attributed to progressive tumors in the terminal stage of the disease. Pharmacokinetics studies revealed a biexponential decay for daunorubicin in association with mean initial and terminal half-lives of 1.8 and 7.4 h, respectively, and a mean total clearance of 15.0 ± 5.5 ml/min. The AUC ratio between the metabolite daunorubicinol and daunorubicin was 0.07. These data differ markedly from the pharmacokinetics of the free drug.

Key words DaunoXome · Hepatocellular carcinoma · Liposomal daunorubicin · Pharmacokinetics

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and most highly malignant cancers in China and the Far East. In Hong Kong it is the second most common malignancy [20]. Around 85% of such patients carry the hepatitis B virus (HBV), and the presence of cirrhosis in the majority of cases further worsens the prognosis, resulting in an overall median survival of less than 3 months [34]. Most cases are not suitable for surgical resection with curative intent.

Anthracyclines, especially doxorubicin, have shown activity against hepatocellular carcinoma [3, 5, 22, 23, 28, 30, 36]. A response rate of up to 20% has been achieved, but there is no evidence that overall survival has been prolonged [28]. Liposome-entrapped drugs are well known to accumulate in the liver and are taken up primarily by the reticuloendothelial system. This accumulation may be useful for targeted delivery of anthracyclines for treatment of HCC. Additionally, liposomal encapsulation of anthracyclines may reduce their systemic toxicity [15, 26] and modulate multidrug resistance [33]. On this basis the present phase I/II study was designed to evaluate the new, stable liposomal formulation of daunorubicin (DaunoXome) for the treatment of patients with this disease. Previously, limited pharmacokinetics of DaunoXome have been reported in a phase I setting involving small numbers of patients treated at different doses with limited time points and duration [17, 19]. Thus, in addition to the clinical evaluation, the present study reports the clinical pharmacokinetics of DaunoXome in 14 patients receiving a uniform-dose regimen.

Patients and methods

A total of 14 patients with inoperable HCC who were seen at the Prince of Wales Hospital (Shatin, N.T., Hong Kong) and had received no prior systemic therapy were entered into the study. The diagnosis of HCC was confirmed either by histology (biopsy) or by radiology (a space-occupying lesion on liver ultrasonography) and a raised alpha-fetal protein (AFP) value of ≥500 μg/l. The entry of these patients strictly followed the criteria established in protocol 103–17, which had been approved by the Independent Ethics Committee of the Chinese University of Hong Kong. Informed and signed consent was given in all cases. The patients' characteristics are described in Table 1. DaunoXome was supplied as a sterile, pyrogen-free, preservative-free product in a single-dose vial

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Table 1 Patients' characteristics for DaunoXome study

Patients' characteristics	Number of patients	
Total number of patients	14	
Sex: M	13	
F	1	
Median age (years)	53.5 (range 34–67)	
Hepatitis B surface antigen-positive	13	
Diagnosis confirmed by:		
Histology	2	
Raised alpha-fetoprotein (AFP) and ultrasound findings	12	
Median AFP (ng/ml, for the positive cases)	40,895 (range 893–721,700)	
Median Karnofsky performance score	100	
Extent of tumor:		
Massive primary HCC in one lobe	2	
Bilobed HCC	6	
Tumor invasion in major vessels	10	
Distant metastases	2	
Median total bilirubin (μmol/l):		
Pretreatment	16.5 (range 8–35)	($P = 0.001$)
Posttreatment	19.5 (range 6–234)	
Median albumin (g/l):		
Pretreatment	34.5 (range 24–38)	($P = 0.001$)
Posttreatment	27 (range 22–31)	
Median alanine transferase (IU/l):		
Pretreatment	64.5 (range 33–445)	($P = 0.02$)
Posttreatment	99 (range 25–1,894)	
Median number of courses	2 (range 1–6)	
Median survival (days)	74 (range 23–747)	

containing 50 mg of daunorubicin. The drug was given 3-weekly at a dose of 100 mg/m^2 (based on free daunorubicin in 150 ml of 5% dextrose) over 60 min. A minimum of two cycles was given to each patient unless there was obvious clinical deterioration. Response outcome was based on clinical and radiological (ultrasonography) assessment. WHO criteria for response and toxicity were used [37]. The cardiac ejection fraction was determined prior to chemotherapy and when the cumulative DaunoXome dose reached 300 and 500 mg/m^2, respectively. Survival was calculated from the initiation of treatment until the patient's death.

Laboratory studies

DaunoXome (VS103) and unformulated daunorubicin (Da), daunorubicinol (DaOH), and the internal standard daunorubicinol were supplied by Nexstar, Inc. All solvents were of reagent grade (Fisher Scientific, Pittsburgh) and were used without further purification. Precoated and prescored thin-layer chromatography (TLC) plates (Merck silica gel G, 20 × 20 cm) were obtained from Alltech Associates (Deerfild, Ill.). All glassware used in analyses was silanized with dimethyldichlorosilane (Pierce, Rockford, Ill.) before use.

An Aminco-Bowman spectrophotofluorometer equipped with a modified fluorescence scanner (Aminco-Bowman, Silver Spring, Md.) was used. The modification includes the replacement of the original motor-driven X-Y motion platform by an automated computer-controlled, variable-speed driver motor (RTC Instrument, Blacksburg, Va.). The output was recorded on a strip chart recorder (Perkin-Elmer, Beaconsfield, UK).

Assay methods

The requirement of freezing of samples for shipment allowed the measurement of only total plasma Da concentrations. Much of the Da leaks from the liposomal formulation during freezing. The total drug was analyzed using the modified thin-film fluorescence scanning method previously described elsewhere [19] and modified as described above. Sample preparation for the total Da assay can briefly be described as follows. To each 0.5 ml of plasma, 100 ng of doxorubicinol in methanol was added as the internal standard. The sample was extracted with 2 ml of ice-cold chloroform: isopropanol (1:1) by mixing for 1 min. Under this condition the liposomes were totally disrupted. The tubes were allowed to stand in an ice bath for 10 min and were then centrifuged at 2,500 *g* at 4 °C for 5 min. The top aqueous layer was removed by aspiration and the organic layer was transferred into a 12 × 75-mm silanized Pyrex tube. The organic layer was evaporated to dryness under a stream of nitrogen. The walls of the tube were washed with 75 μl of chloroform; methanol (1:1, v/v) and the solvent was gently evaporated. The residue was dissolved by the addition of 10 μl of chloroform:methanol (1:1) and the entire sample was spotted on a prewashed silica gel plate using a 2 μl Lange-Levy pipette. The spotted plate was placed in a lined tank and eluted ascendingly to the top of the plate with a solvent consisting of chloroform:methanol:glacial acetic acid (70:26:4, by vol). The developing time was about 2 h. After drying, the plate was scanned at an excitation wavelength of 457 nm and an emission wavelength of 580 nm. A calibration curve was performed on each plate under the same condition; this methodology has previously been widely used [5–7, 12, 14, 17, 25, 32]. No appreciable degradation of the analyte was detected under the condition used. The limit of the assay was 10 ng/ml. The assay was validated prior to sample analysis and showed a within-run reproducibility of 3.5%, 5.8%, and 4.3% coefficient of variance (CV), with corresponding accuracy values being 98%, 100%, and 100% at concentrations of 21, 63, and 84 ng/ml, respectively (all $n = 8$), and a between-run CV of 9.3% ($n = 6$). The validation for DaOH showed a within-run CV of 5.1%, 5.1%, and 3.1% at concentrations of 5, 15, and 25 ng/ml, respectively ($n = 8$), and a between-run CV of 7.1% ($n = 6$).

Sample collection

Blood samples were collected from an indwelling catheter before and following the start of infusion. A typical sampling schedule was 0 (before drug infusion), 10, 30, 45, 60, 75, 90, 105, 120, 240, 360, 1,440, 1,800, 2,880, and 4,320 min following the start of infusion. Plasma was separated from red blood cells by centrifugation and

was stored at −20 °C until shipment. Plasma samples were shipped in dry ice from Hong Kong to Columbus, Ohio, by international courier and the shipment was completed within 3 days. Upon receipt, samples were analyzed within 2–4 weeks, and no instability of Da in plasma was observed in control frozen plasma samples spiked with DaunoXome over a 1-month period.

Pharmacokinetic calculations

Since the data appeared to conform to a triexponential function, to obtain a more accurate assessment of the terminal slope with minimal bias the entire data set was fitted to a triexponential function and the terminal slope was estimated from the fit. According to convention, only data for the descending phase were selected to obtain a terminal slope, and this selection may have introduced bias. No model was proposed to describe the pharmacokinetics of liposomal Da under the current mode of drug administration. This fitting was accomplished using the standard computer pharmacokinetics software PCNONLIN. Similarly, the same treatment was performed on the DaOH metabolite data, except that in this case a one-compartmental model with first-order input was used. Terminal half-lives ($t_{1/2}$ s), areas under the curve (AUC), and areas under the first-moment curve were thus computed to infinity with appropriate extrapolation. The order-relevant pharmacokinetic parameters were estimated by the model-independent method [16], which is a more appropriate method under the current situation.

Results

Two patients were not assessable for response; one defaulted after the first cycle of treatment, and the second died 6 days after the initiation of treatment following rupture of the tumor. Two of the patients had stable disease for 8 weeks, but subsequently, both of them and the remaining ten subjects developed disease progression. One patient was alive at the end of the study. This patient, who has clear evidence of progressive disease, remains symptom-free at the time of writing and is taking a traditional herbal remedy. The median survival was 74 days (range 23–747 days).

Toxicity

A total of 32 courses of treatment were given to 14 patients. Toxicity of grade 3 or above comprised 19 episodes of leukopenia and 2 episodes of thrombocytopenia. Four patients had chest/back pain (a recognized reaction to liposomal Da whose mechanism is unknown), which resolved after discontinuation of chemotherapy and administration of hydrocortisone and chlorpheniramine. There was no abnormality in serial electrocardiograms, cardiac enzymes, or other aspects of cardiac or renal function. Nausea and vomiting and alopecia were minimal. There were two episodes of neutropenic fever. During the course of treatment, hepatic function deteriorated as reflected by a significant rise in total bilirubin and a significant fall in serum albumin levels (Table 1, Student's paired *t*-test). Although liposomal preparations may themselves be hepatotoxic [8], it is most likely that the observed deterioration in liver function was the result of progressive tumor.

Pharmacokinetics

The composite plots of total Da and DaOH generated for patients receiving DaunoXome at 100 mg/m^2 are shown in Figs. 1 and 2, respectively. A representative set of concentration-time (C-T) plots of total Da and DaOH determined for a patient with computer-fitted curves (see Patients and methods) is shown in Fig. 3.

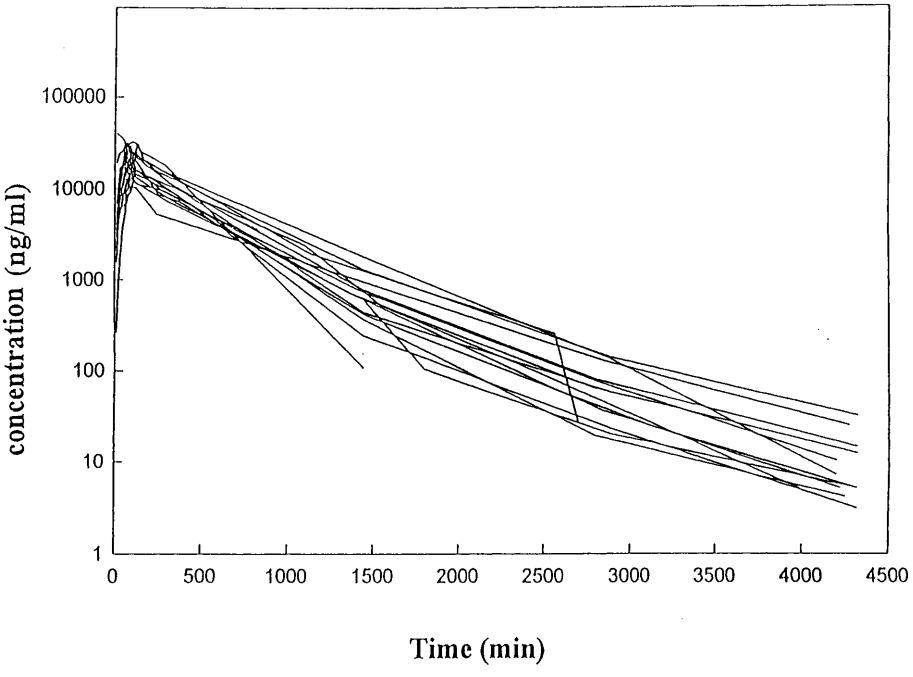

Fig. 1 Composite plots of plasma concentration-time profiles of Da for all 14 patients (with 1 patient having a repeated set of samples taken in the second cycle of DaunoXome)

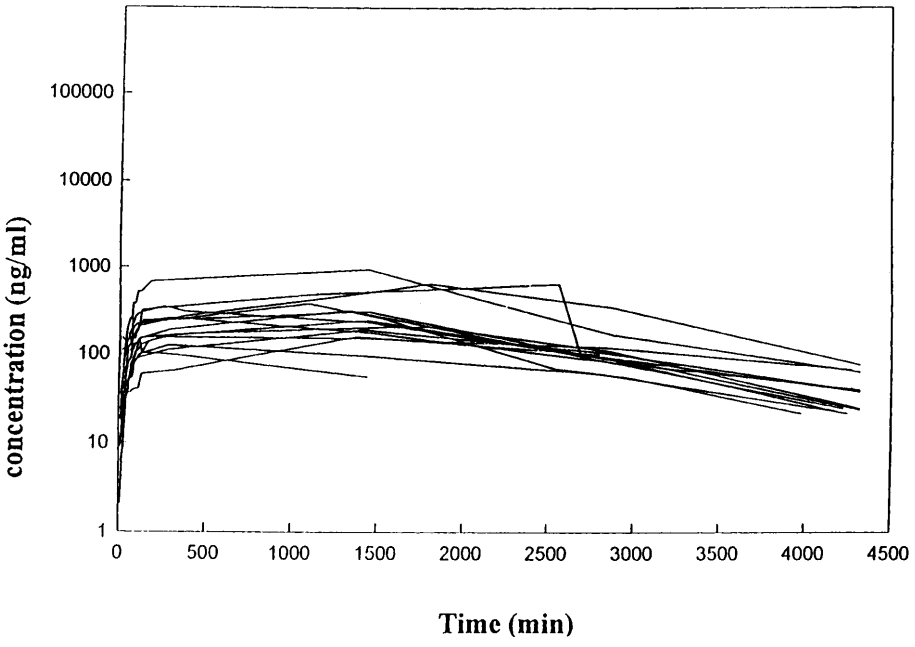

Fig. 2 Composite plots of plasma concentration-time profiles of DaOH for all 14 patients (with 1 patient having a repeated set of samples taken in the second cycle of DaunoXome)

The plasma concentrations of Da first ascended to a peak, which then declined biexponentially in all patients. The peak concentration (C_{max}) occurred between 0.22 and 2.12 h, the mean peak time being 1.4 h. The corresponding mean C_{max} was 25,127 ± 7,318 ng/ml. Thus, the peak concentration of total Da was rapidly achieved following intravenous infusion. The relevant pharmacokinetic parameters as estimated by the model-independent method and by regression ($t_{1/2}$) are shown in Table 2. Those estimated for DaOH are shown in Table 3. As shown, the mean initial $t_{1/2}$ of total Da was approximately 1.81 h (range 0.12–3.48 h) and the mean terminal $t_{1/2}$ was 7.36 h (range 2.19–13.68 h). Thus, there was substantial interpatient variation in the terminal $t_{1/2}$ of Da. The mean total clearance was 15.04 ± 5.49 ml/min and the mean volume of distribution at steady-state (V_{dss}) was 5.58 ± 2.96 l.

Generation of DaOH was observed in plasma, and peak concentrations were reached at time points ranging from 1.92 to 30 h, with the mean peak time being 17.67 h, thus indicating variable metabolism of DaunoXome in this patient population. The mean peak concentration was only 337 ng/ml, only slightly over 1% of the peak value recorded for the parent drug. Fol-

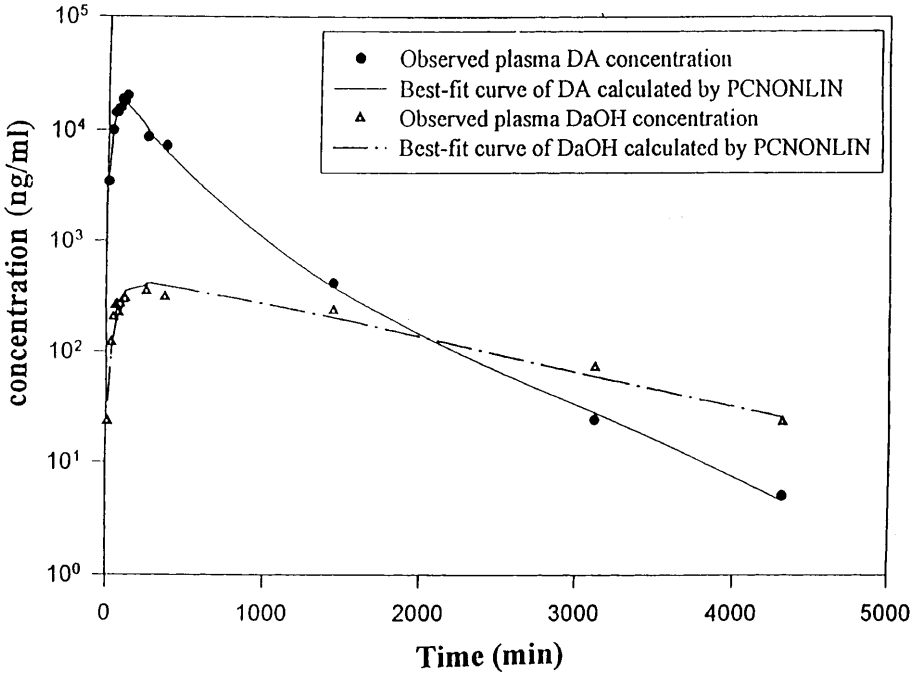

Fig. 3 Typical plasma concentration-time profile obtained for Da and DaOH (M) in patient 2 and fitted to a two-compartmental model with first-order input

Table 2 Relevant pharmacokinetic parameters recorded for total daunorubicin in patients given intravenous DaunoXome at 100 mg/m^2

PK/patients' parameters	AV ± SD	Range
C_{max} (ng/ml)	25,127 ± 7,318	12,640–39,580
T_{max} (min)	1.4 ± 0.47	0.22–2.12
$t_{1/2\alpha}$ (h)	1.81 ± 1.26	0.12–3.48
$t_{1/2\beta}$ (h)	7.36 ± 2.65	2.19–13.68
MRT (h)	6.17 ± 2.15	3.84–12.41
AUC × 10^7 (ng ml^{-1} min^{-1})	1.285 ± 0.481	0.631–2.301
CLT (ml/min)	15.04 ± 5.49	6.95–21.29
V_{dss} (l)	5.58 ± 2.96	2.283–7.394
Dose (mg)	163 ± 10.1	150–192

Table 3 Relevant pharmacokinetic parameters recorded for daunorubicinol in patients given intravenous DaunoXome at 100 mg/m^2 ($T_{1/2\lambda}$ $T_{1/2}$ terminal)

PK/patients' parameters	AV ± SD	Range
C_{max} (ng/ml)	337 ± 231	125–934
T_{max} (h)	17.67 ± 10.43	1.92–30.0
$t_{1/2\lambda}$ (h)	19.27 ± 12.94	7.33–59.78
MRT (h)	34.4 ± 15.69	22.3–42.6
AUC × 10^7 (ng ml^{-1} min^{-1})	1.285 ± 0.481	0.631–2.301
AUCm/AUCp	0.072 ± 0.039	0.011–0.152

lowing its peak the plasma DaOH concentration declined monoexponentially with a mean $t_{1/2}$ of about 19.27 h, substantially longer than that of the parent drug. This was further substantiated by the difference in median resident time observed between the metabolite and the parent drug (28.23 h) [4]. This also shows that the metabolite might be eliminated slower than the parent drug [4]. The ratio of the metabolite AUC to that of the parent drug also showed a rather low value of 0.07, indicating that only a small fraction of the parent drug was converted to the metabolite.

Discussion

This clinical trial demonstrates that DaunoXome has limited or transient activity when used as a single agent in patients with hepatocellular carcinoma (HCC). Although free anthracyclines have been reported to achieve response rates of up to 20%, there is no evidence that overall survival is improved, and to this extent the present results are similar to previous reports. The median survival of patients in our region is about 10 weeks, entirely in keeping with the results of the present study. The single patient who has remained alive and symptom-free for more than 2 years after the end of the treatment reflects the heterogeneity and effect of lead time in this disease, where he presumably represents one extreme of the spectrum of the natural history of the disease. However, this study does further support the phase I finding that DaunoXome is safe and well tolerated by the majority of patients. Clinical adverse effects were rare and mainly mild to moderate in severity. Treatment-related laboratory abnormalities were infrequent. The deterioration in liver-function tests during the course of the trial follows the expected natural history of the disease in its terminal phases and is unlikely to be related to the treatment.

Pharmacokinetics

In a previous phase I study the pharmacokinetics of DaunoXome (VS103) has been described at different doses of 10, 20, 40, 60, and 80 mg/m^2 [12, 13, 19]. The duration of the pharmacokinetics study mostly covered 24 h, and 10–12 plasma samples were drawn during this period. Monoexponential declines were observed in plasma concentration-time (C-T) profiles in most of the patients, with mean $t_{1/2}$s values ranging from 2.8 to 8.3 h. The mean total clearance values ranged from 8.9 to 15.7 ml/min. The results of the current study agree with these results remarkably well but probably provide a more accurate assessment of the pharmacokinetic data, since the number of time points and the duration covered were greater, typically to at least 72 h, 3 times as long as that of the previous study. Additionally, the number of subjects was larger and the patient population was more homogeneous (all had HCC). The larger number of data points allows critical evaluation of the model for curve fitting, which accords with the biexponential profile. The mean terminal elimination $t_{1/2}$ was in a higher range as compared with those previously observed. Some nonlinearity in kinetics was observed, though it was not strong. The slightly long $t_{1/2}$ observed in the present study may have been due to nonlinear pharmacokinetics or to better data acquisition because of the longer monitoring period. However, in the present case it was not possible to examine the nonlinear pharmacokinetics on the basis of the dose, since only one dose was used. However, it was possible to examine the shape of the C-T profile for evidence of Michaelis-Menton kinetics, and no convex shape was evident even at the initial portion of the C-T profile. Thus, we think that the slightly longer plasma terminal $t_{1/2}$ was a result of better assessment because of the prolonged duration and the more frequent sampling. The mean clearance value also agrees well with the above-mentioned studies.

These pharmacokinetic parameters also resemble those of entrapped DaunoXome [18] at 40 mg/m^2, which showed a mean clearance of 21 ml/min and a $t_{1/2}$ of 9.3 h. Thus, in the present case the pharmacokinetics of DaunoXome behaves rather consistently in patients from different studies but differ substantially from that reported for nonliposomal formulations of Da [2].

Metabolism and metabolite kinetics

Reduction of Da to DaOH has been shown to be the major metabolic pathway for free Da [1, 24, 31]. In patients treated intravenously with free Da, plasma

DaOH levels often exceeded those of Da at as early as 0.5 h, and a DaOH-to-Da AUC ratio exceeding 10:1 was found in patients treated with 30–45 mg/m^2 Da. A dramatic decrease in the reductive pathway was observed in patients receiving the present formulation. The AUC ratio between the metabolite and the parent drug was less than 0.1, thus giving at least a 100-fold reduction in the formation of this reductive metabolite. The apparent terminal $t_{1/2}$ of DaOH as estimated by curve fitting was 19 h, substantially longer than that estimated for DaunoXome.

Concern has arisen in recent years over the role of these reductive metabolites of anthracyclines. Whereas DaOH retains significant cytotoxicity of the parent drug, questions have been raised as to its potential contribution to anthracycline cardiac toxicity [9, 10, 27, 29, 35]. Liposomal Da and doxorubicin have previously been shown to produce lower degrees of cardiac toxicity in animals [11, 14]. It is generally thought that liposomal formulations alter drug distribution in organ tissues and that there is a general reduction in the exposure of cardiac tissue to high anthracycline concentrations. Although for the most part this is true, the alteration of anthracycline metabolism, especially the reduction in formation of the alcohol metabolite, may also contribute to the reduction in cardiac toxicity. Thus, in many of the clinical experiences reported thus far, no clinical manifestation of cardiotoxicity has been observed, even in patients receiving a cumulative dose in excess of 1 g/m^2 [19]. Additionally, a reduction in side effects such as alopecia has been achieved without a loss in the efficacy of anthracyclines [19].

Comparison of the pharmacokinetics of DaunoXome with that of the free drug and those of other similar formulations

It has been shown that following an intravenous bolus dose of free Da, plasma C-T profiles follow a biexponential decline, with typical initial and terminal $t_{1/2}$ values being 0.51 and 22.5 h, respectively (after reanalysis of data from Huffman et al. [21] and Forssen et al.'s [14] reanalysis of Alberts' data [2]). A rapid clearance, a large V_{dss}, and a low AUC are characteristics of free Da. On the other hand, the present pharmacokinetics study of DaunoXome showed a dramatic difference from the pharmacokinetics of nonliposomal formulations of Da. A detailed comparison of the difference in pharmacokinetics between free Da and DaunoXome has recently been made [12]. The present pharmacokinetics was essentially similar to those reported previously, despite the larger number of subjects investigated at a single dose level. The present data also suggest that the HCC has little effect on the pharmacokinetics of DaunoXome relative to the other disease sites examined in the phase I study. On the other hand, this study also showed that liposomal Da is inactive in HCC, and further study of this drug in patients with HCC is therefore not warranted.

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