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ORIGINAL ARTICLE

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Pharmacokinetics of liposomal daunorubicin (DaunoXome) during a phase I-II study in children with relapsed acute lymphoblastic leukaemia

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Abstract *Purpose*: The pharmacokinetics of DaunoXome were studied during a multicentric phase I-II study performed in children suffering from relapsed acute lymphoblastic leukaemia and treated on a weekly schedule. *Patients and methods*: A group of 18 patients were studied during the first course of treatment at dose levels between 40 and 120 mg/m^2. Blood samples were obtained up to 72 h after infusion. The liposomal and free forms of daunorubicin, as well as daunorubicinol, were separated and quantified by HPLC using fluorometric detection, and data were analysed using a model-independent approach. *Results*: Unchanged liposomal daunorubicin disappeared from plasma following a monoexponential decay. Its AUC represented 95.8% of the total fluorescent species found in plasma and increased linearly with the dose administered. The elimination half-life was 5.23 h, total plasma clearance 0.344 l/h per m^2, and volume of distribution at steady state 2.08 l/m^2. Free daunorubicin and daunorubicinol were detected in plasma at all time-points studied. Their AUCs represented, respectively, 2.53% and 1.70% of total fluorescent species and their elimination half-lives were, respectively, 16.6 h and 22.3 h. The daunorubicinol/daunorubicin AUC ratio was 0.82%. *Conclusions*: This study is the first to demonstrate that free daunorubicin is present in plasma after DaunoXome administration and that it originates from in vivo release from the liposomes. The pharmacokinetics of free daunorubicin appeared to be comparable to those observed after conventional administration. However, the concentration of daunorubicinol appeared to be lower than that found after conventional administration of daunorubicin.

Key words Liposomal daunorubicin · Phase I-II study · Children · Acute lymphoblastic leukaemia · Pharmacokinetics

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Introduction

Daunorubicin has for many years been one of the most commonly used drugs for the treatment of acute lymphoblastic leukaemia, especially in children. In association with vincristine, cyclophosphamide and prednisone, complete remissions are obtained in about 85–95% of patients, with more than 70% achieving prolonged survival [24]. However, in patients who do not respond to the remission-induction treatment, or who relapse after complete remission, the clinical results are strongly compromised, either by emerging drug resistance or cumulative toxicity. The latter is true for daunorubicin, which is known to induce a congestive cardiomyopathy

of very poor prognosis when the cumulative dose reaches 500 mg/m^2 [18, 33].

There is, therefore, major interest in developing new anthracyclines or new anthracycline formulations without these cardiotoxic side effects, but which retain activity against resistant leukaemia cells. Liposomal encapsulation was proposed long ago as a means for decreasing the toxicity of anthracyclines, particularly their cardiotoxic potential [4, 25, 26], probably because these particulate formulations do not enter normal tissues to the same extent as free drugs. A liposomal formulation of daunorubicin, DaunoXome, was developed by NeXstar and is marketed in most Western countries for the treatment of Kaposi's sarcoma [38]. Kaposi's sarcoma is an angiosarcoma characterized by the presence of the tumour cells in blood networks, with no endothelial barrier between blood and tumour. This situation has encouraged the evaluation of DaunoXome in the treatment of leukaemia, where the same situation occurs. DaunoXome has been shown in preclinical models to be much less cardiotoxic than the reference anthracyclines, daunorubicin and doxorubicin [23]. This formulation has also been shown to be able circumvent the P-glycoprotein-mediated mechanism of resistance to anthracyclines [21], as do some [29, 36] but not all [19] formulations of liposomes. A phase I-II trial was undertaken in France for the evaluation of the potential of DaunoXome in the treatment of acute lymphoblastic leukaemia. Preliminary results have recently been presented [2] and the complete study will be published separately.

During the course of this clinical trial, a pharmacokinetic study of this new formulation of daunorubicin was conducted. We were particularly interested in evaluating the relative plasma concentrations of unchanged liposomal daunorubicin and free daunorubicin, as well as daunorubicinol, the major metabolite of daunorubicin. It is well known that liposomes are taken up by the reticuloendothelial system, especially in the liver, which may release the liposome content into the circulation. Evaluating the relative concentration of free and liposomal drug may shed some light on the behaviour of DaunoXome and help understand its specificities as compared to daunorubicin.

Several studies have been published on the pharmacokinetics of DaunoXome [16, 17, 39], in each of which only total plasma daunorubicin and daunorubicinol was evaluated without attempting to separate free and liposomal forms of daunorubicin. These studies have generally shown a one-compartment behaviour with a half-life of 4 to 8 h and a volume of distribution rarely exceeding the plasma volume. We showed in the study reported here that free daunorubicin can be evaluated separately from liposomal drug, and that its pharmacokinetic behaviour is quite different, resembling that of conventional daunorubicin rather than that of liposomal daunorubicin.

Materials and methods

Drug

DaunoXome was provided by NeXstar Pharmaceuticals as a suspension of daunorubicin-containing liposomes at a concentration of 50 mg in 25 ml. It was diluted to 250 ml in 5% glucose solution and used within 6 h according to the recommendations of the manufacturer. The liposomes containing daunorubicin are small unilamellar vesicles less than 100 nm in diameter, constituted of a mixture (2:1, mol/mol) of distearoylphosphatidylcholine and cholesterol, with high stability in aqueous solutions at physiological temperature [11].

Patients

The pharmacokinetic study included 20 patients suffering from relapsed acute lymphoblastic leukaemia. Their characteristics are presented on Table 1. They were treated by intravenous infusion of DaunoXome, using a central or peripheral access, connected to a programmable syringe. The duration of the infusion was set at 60 min. The starting dose of the phase I was set at 40 mg/m^2 and interpatient escalation was performed by 20 mg/m^2 steps. Only drugs used for supporting care were allowed concomitantly with the treatment (antibiotics, analgesics, antiinflammatory drugs). Neither lipid parenteral nutrition, nor any cytotoxic drug or liposomal formulation of any drug were allowed during the study.

Blood sampling

Blood was sampled from a peripheral line distinct from the one used for infusion. Blood samples were obtained before infusion, at the end of the 1-h infusion, and at 2, 4, 7, 24, 48 and 72 h after the onset of infusion. A reduced sampling protocol was used for the second and following courses: before infusion, end of infusion and 24 h after the onset of infusion.

Drug extraction

The liposomal and free forms of daunorubicin, as well as daunorubicinol, could be quantified separately thanks to a technique developed by Touinsi and Catalin (unpublished results) for lipo-

Table 1 Characteristics of the patients included in the pharmacokinetic study (number of patients, except age in years)

Gender	
Male	10
Female	10
Age (years)	
Median	10.4
Range	4–17
Patients included per course	
First	20
Second	16
Third	12
Fourth	7
Patients per dose level	
40 mg/m^2	6
60 mg/m^2	3
80 mg/m^2	2
100 mg/m^2	5
120 mg/m^2	4
Patients excluded due to improper handling	
40 mg/m^2	1
120 mg/m^2	1

somal doxorubicin. It is based upon the solid extraction technique of anthracyclines which was developed earlier in our laboratory [31]. Other authors had already used solid extraction procedures for the separation of liposomal drug from free drug [9, 37].

Fresh patient plasma (1 ml) was spiked with a known amount of doxorubicin (internal standard) and run through a Sep-pak C-18 cartridge (Waters Associates, Milford, Mass.) which had previously been reconstituted with methanol and equilibrated with phosphate-buffered saline (PBS). The eluate and subsequent aqueous washings with PBS were collected in a tube containing 100 µl 20% Triton X-100 (Sigma, Saint Louis, Mo.). This eluate contained the liposomal form of daunorubicin (unchanged DaunoXome). A second eluate was obtained by running 2 ml methanol through the cartridge. This eluate contained the free forms of daunorubicin, as well as daunorubicinol, and the internal standard, doxorubicin. The first eluate, in which liposomes were disrupted by Triton X-100, was spiked with a known amount of doxorubicin and was run through a second Sep-pak cartridge. The eluate was discarded as well as the washings with PBS, and methanol was then run through the Sep-pak, allowing the recovery of the daunorubicin fraction originally encapsulated in liposomes and of the internal standard secondly added, doxorubicin.

High-performance liquid chromatography (HPLC)

Both methanolic extracts were evaporated to dryness, reconstituted in a small volume of the chromatography mobile phase (see below) and injected into a HPLC system consisting of an AS1000 automatic sampler (Thermo Quest, Les Ulis, France), a P1000XR pump (Thermo Quest), a C18 Radial Pak column (Waters Associates) and a laser-induced fluorescence detector (Zeta Technology, Toulouse, France). The mobile phase was delivered isocratically and consisted of a mixture of 1% ammonium formate and acetonitrile (68/32, v/v). Peaks were recorded and analysed automatically using PC-1000 software (Thermo Quest).

The performance of the method was evaluated after spiking known amounts of DaunoXome in blank plasma. The recovery of daunorubicin (liposomal plus non-liposomal) was $80.8 \pm 8.9\%$. The limit of detection for both forms was 1 ng and the limit of quantification 1 ng/ml. The extraction was linear between 10 ng and 10 µg DaunoXome per ml plasma. The within-day precision varied between 3% and 10%, being smaller for the liposomal form than for the non-liposomal form, and decreasing as a function of the amount of DaunoXome spiked. Similarly, between-day precision varied between 5% and 17%. The complete description of the method and its validation for the separate estimation of liposomal and free daunorubicin will be published separately.

Data analysis

For unchanged DaunoXome, the data were fitted to a one-compartment model using a weighted nonlinear regression method [20]. This was performed with the software APIS (Miips, Marseilles, France). In addition, areas under the curves ($AUC_{0-\infty}$) were calculated using the trapezoidal rule and extrapolated to infinity.

For nonliposomal daunorubicin and for daunorubicinol, the data were analysed using a model-independent approach. Areas under the curves were calculated using the trapezoidal rule and extrapolated to infinity. Elimination half-lives ($T_{1/2}el$) were evaluated by linear regression of the log-transformed concentration values of the three to five last points of the kinetics.

Results

Figure 1 illustrates the average plasma concentration vs time curves following administration of 40 and 100 mg/m^2 of DaunoXome. Liposomal daunorubicin (unchanged DaunoXome) disappeared from plasma following a monoexponential decay. The pharmacokinetic parameters obtained for liposomal daunorubicin, free daunorubicin and daunorubicinol are presented in Table 2. The $AUC_{0-\infty}$ of liposomal daunorubicin increased linearly with the dose administered ($r = 0.787$, $P < 0.001$; Fig. 2). It represented 95.8% of the total fluorescent species found in plasma after administration. Using a one-compartment model, the total plasma clearance of liposomal daunorubicin was 0.344 ± 0.216 l/h per m^2. The elimination half-life of liposomal daunorubicin was 5.23 ± 1.01 h and the volume of distribution at steady state was 2.08 ± 0.70 l/m^2. The highest plasma concentration (C_{max}) did not always coincide with the end of the infusion, as expected following an i.v. administration. Indeed, in 6 cases out of 18, the C_{max} occurred 1 to 3 h after the end of the infusion, suggesting the occurrence of an adsorption-release process from the reticuloendothelial system.

Free daunorubicin was detected in plasma at all time-points studied (Table 2). Its $AUC_{0-\infty}$ represented $2.53 \pm 2.19\%$ of the total fluorescent species. It was significantly related to the dose of DaunoXome administered ($r = 0.566$, $P < 0.02$; Fig. 2). The maximum plasma concentration (C_{max}) occurred at the end of DaunoXome infusion in all but one case. There were large individual differences in the proportion of free daunorubicin in

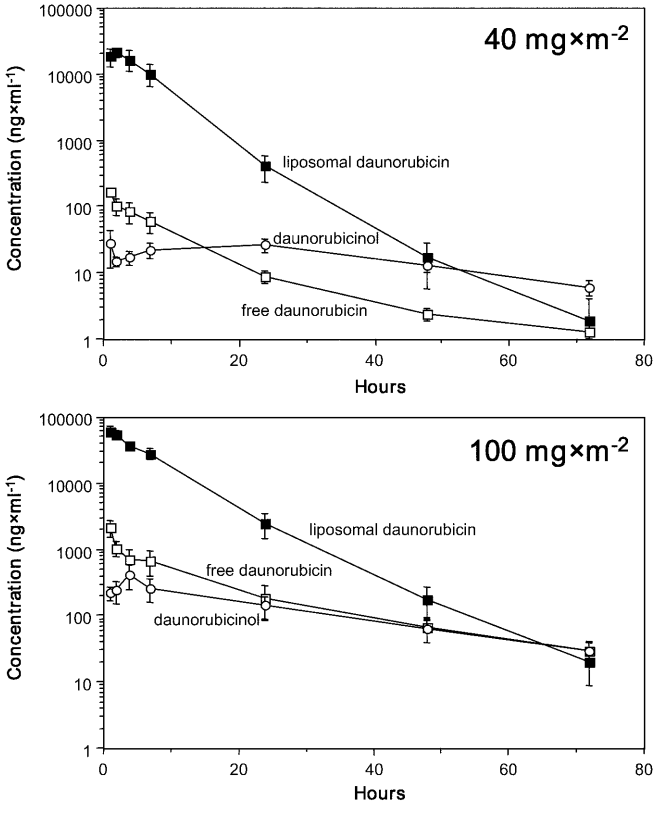

Fig. 1A, B Average concentrations (±s.d.) of liposomal daunorubicin, free daunorubicin, and daunorubicinol after administration of 40 mg/m^2 (**A** five patients) and 100 mg/m^2 (**B** five patients) of DaunoXome

Table 2 Pharmacokinetic parameters of liposomal daunorubicin, free daunorubicin and daunorubicinol at each dose level (*TPlCl* total plasma clearance)

Dose (mg/m^2)		Liposomal daunorubicin				Free daunorubicin		Daunorubicinol	
		$AUC_{0-\infty}$ ($\mu g/ml \cdot h$)	TPlCl ($l/h/m^2$)	$T_{1/2}$ el (h)	Vd_{ss} (l/m^2)	$AUC_{0-\infty}$ ($\mu g/ml \cdot h$)	$T_{1/2}$ el (h)	$AUC_{0-\infty}$ ($\mu g/ml \cdot h$)	$T_{1/2}$ el (h)
40 (5)	Mean ± s.d.	224 ± 70	0.353 ± 0.111	4.43 ± 0.62	2.23 ± 0.65	1.40 ± 0.82	16.3 ± 7.1	1.43 ± 0.69	22.9 ± 3.8
	Range	150–337	0.246–0.367	3.74–5.21	1.70–3.26	0.73–2.69	6.85–28.3	0.90–2.79	16.7–27.8
60 (3)	Mean ± s.d.	357 ± 54	0.624 ± 0.436	5.49 ± 1.66	2.01 ± 1.12	9.97 ± 6.85	11.8 ± 4.7	6.92 ± 4.50	25.4 ± 8.4
	Range	295–391	0.217–1.084	3.97–7.26	1.13–3.27	2.88–16.5	6.71–16.1	1.82–10.4	18.0–34.6
80 (2)	Mean	511	0.246	5.50	1.97	26.5	8.76	22.2	21.3
	Range	365–656	0.160–0.332	5.37–5.63	1.24–2.70	21.3–31.7	7.44–10.1	14.5–29.8	14.5–28.1
100 (5)	Mean ± s.d.	565 ± 202	0.240 ± 0.070	5.64 ± 0.98	1.89 ± 0.37	18.5 ± 13.9	18.5 ± 5.3	9.77 ± 7.51	21.5 ± 2.5
	Range	360–873	0.149–0.296	4.66–6.94	1.35–2.38	5.58–38.4	16.0–24.3	3.87–21.9	18.3–24.6
120 (3)	Mean ± s.d.	634 ± 111	0.285 ± 0.084	5.45 ± 0.96	2.29 ± 1.04	18.8 ± 15.8	24.0 ± 14.0	9.61 ± 6.07	20.5 ± 6.7
	Range	514–733	0.199–0.288	4.71–6.54	1.47–3.46	7.99–36.9	9.65–37.6	4.34–16.2	13.6–27.0
Overall	Mean ± s.d.	–	0.344 ± 0.216	5.23 ± 1.01	2.08 ± 0.70	–	16.6 ± 8.3	–	22.3 ± 5.2

plasma, its AUC ranging between 0.4% and 7.4% of the total fluorescence. Daunorubicin was eliminated from plasma in most cases following a two-exponential decay, with an elimination half-life of 16.6 ± 8.3 h.

Daunorubicinol was also detected in plasma all along the kinetics (Table 2). Its $AUC_{0-\infty}$ represented 1.70 ± 1.60% of the total fluorescent species and was only weakly related to the dose of DaunoXome administered ($r = 0.420$, $P = 0.08$; Fig. 2), due to wide individual variations in the proportion of this metabolite (range 0.34–7.0%). However, there was a highly significant correlation between the AUC of free daunorubicin and that of daunorubicinol ($r = 0.879$). The C_{max} of daunorubicinol occurred in most cases between 4 and 7 h after the onset of infusion. Daunorubicinol was cleared from plasma with an elimination half-life of 22.3 ± 5.2 h. The relative importance of daunorubicinol was compared with that of free daunorubicin, the parent compound. The daunorubicinol/daunorubicin AUC ratio was found to be 0.815 ± 0.412. Surprisingly, in nine patients a small amount of daunorubicinol was found in the liposomal fraction. This could correspond to a passive adsorption of the metabolite on the liposome.

From several patients, 1-h and 24-h samples were obtained during the subsequent courses of treatment. These were compared with the corresponding samples obtained during the first course. The percentage variations in the plasma levels of DaunoXome, free daunorubicin and daunorubicinol were not significantly different from zero.

Discussion

The pharmacokinetics of DaunoXome have been already described in several reports, but without distinguishing liposomal and free daunorubicin. The pharmacokinetic parameters for unchanged DaunoXome were close to those reported in the literature for total daunorubicin after DaunoXome infusion (Table 3). This similarity was expected since liposomal daunorubicin represents 95% of the total fluorescent species

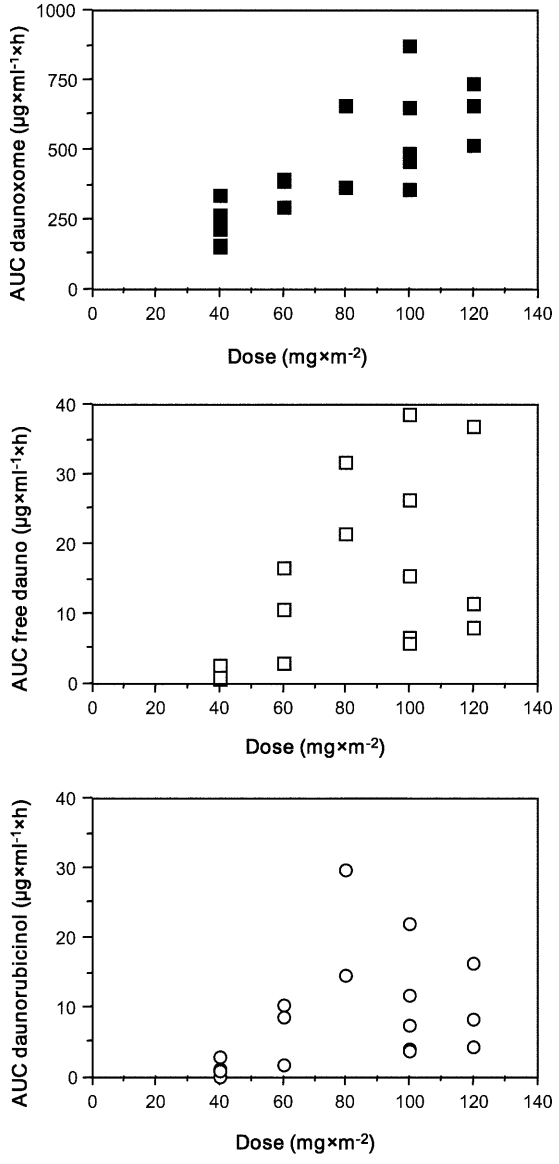

Fig. 2A–C Relationship between the dose of DaunoXome administered and the $AUC_{0-\infty}$ of liposomal daunorubicin (**A** $r = 0.787$, $P < 0.001$), free daunorubicin (**B** $r = 0.566$, $P < 0.02$) and daunorubicinol (**C** $r = 0.420$, $P = 0.08$)

Table 3 Pharmacokinetic parameters of DaunoXome and conventional daunorubicin in the literature and in our study

Reference	Dose administered (mg/m^2)	Total plasma clearance ($l/h/m^2$)	Elimination half-life (h)	Volume of distribution at steady-state (l/m^2)
DaunoXome				
This study	40–120	0.344	5.23	2.08
16	10–80	0.337	4.82	1.81
17	10–60	0.363	4.67	1.79
39	100	0.467	7.36	2.82
Conventional daunorubicin				
30	75	67.5	20.6	–
32	30	38.6	47.4	1725
15	30–50	137	11.2	–
35	45	50.0	18	1582

present in plasma. These parameters are quite different from those obtained after administration of conventional daunorubicin (Table 3). The total plasma clearance of DaunoXome is 100- to 200-fold lower, the half-life 5-fold lower and the volume of distribution at steady-state 500- to 1000-fold lower than those of conventional daunorubicin.

The low clearance and volume of distribution of unchanged DaunoXome indicate that the liposomes remain mostly in the blood stream and that only a minority leave the central compartment and bind to tissues. Indeed, we have observed that DaunoXome infusion to isolated rat hearts leads to minimal daunorubicin accumulation in the tissue in contrast to conventional daunorubicin [23]. The peculiarities of DaunoXome pharmacokinetics are characteristic of stable liposomal preparations. The lipid components of DaunoXome liposomes are distearoylphosphatidylcholine and cholesterol. The liposomes are stable at 37ºC due to the gel-phase structure of these lipids at this temperature. A doxorubicin liposomal formulation (Doxil), which is made of other lipids but which is protected from disruption by a polyethylene glycol (PEG) coating, shows the same behaviour [6, 12]. Doxil has also been shown to have very low plasma clearance (0.1 l/h) and volume of distribution (2–4 l), although it has a longer elimination half-life [1, 14, 22]. This contrasts with standard liposomal formulations of doxorubicin which are not stable in the body and display a pharmacokinetic behaviour closer to that of free doxorubicin [5, 7, 10, 13, 28].

We were able to evaluate the plasma concentration of free daunorubicin after DaunoXome administration. This free daunorubicin may have several possible origins: it may be present in small amounts in the DaunoXome formulation, it may be generated by the extraction process, or it may be produced from the release of daunorubicin from the liposomes in the body. When spiking a known amount of DaunoXome in a blood sample ex vivo, we found after extraction 2–4% of free daunorubicin in the extracts. The contribution of free daunorubicin possibly injected with DaunoXome can be easily estimated in terms of AUC using the pharmacokinetic parameters we have already determined for conventional daunorubicin [32]. A simple computation shows that the presence of 5% free drug in the liposomal preparation would only contribute a maximum of 8–10% of the AUC of free daunorubicin obtained in the plasma of patients treated with DaunoXome. In addition, we found a very highly significant correlation between the levels of free daunorubicin and those of daunorubicinol. Since daunorubicinol cannot be synthesized during the extraction process, this indicates that free daunorubicin observed in plasma is not artifactually produced during extraction. We can conclude that most of the free daunorubicin we measured in plasma originated from release from the liposomes within the body after DaunoXome administration.

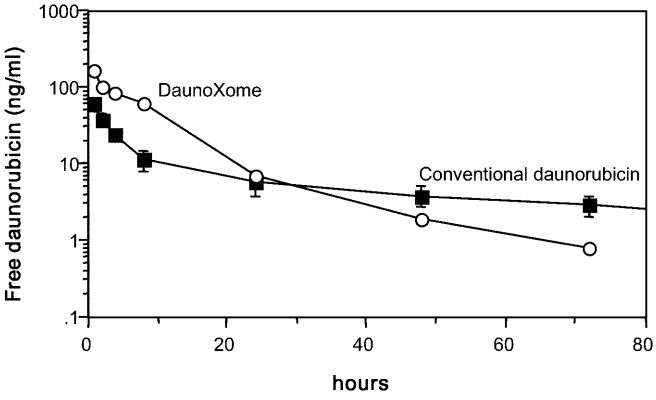

Fig. 3 Comparison of the plasma concentrations of free daunorubicin after administration of DaunoXome (40 mg/m^2, five patients, this study) and of conventional daunorubicin (30 mg/m^2, seven patients, reference 30)

The pharmacokinetic behaviour of free daunorubicin after DaunoXome administration resembles that obtained after administration of conventional daunorubicin (see Table 3). However, there are some differences that can be observed when comparing the plasma decays of free daunorubicin obtained after DaunoXome or after conventional daunorubicin administrations. Figure 3 shows the respective concentrations of free daunorubicin in plasma after administrations of DaunoXome (40 mg/m^2) and conventional daunorubicin (30 mg/m^2) (from reference 32). We are presently elaborating a model which could explain the respective behaviours of liposomal and free daunorubicin in plasma.

Daunorubicinol pharmacokinetics were also similar to those observed after administration of conventional daunorubicin. The elimination half-life we obtained (22.3 h) is comparable to that obtained by Riggs [30] (27.9 h), Robert et al. [32] (37.3 h) and Rahman et al. [27] (23.4 h). However, the ratio of the $AUC_{0-\infty}$ of daunorubicinol to that of free daunorubicin found in this study (0.815) was much lower than usually observed (2.66–4.74 in the studies mentioned in Table 3). This indicates a low conversion of daunorubicin to daunorubicinol when daunorubicin is administered in liposomal form. This may have important clinical consequences, since daunorubicinol displays no cytotoxicity [3, 34], while it may contribute to the cardiac toxicity of daunorubicin [8].

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