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Liposomal daunorubicin plasmatic and renal disposition in patients with acute leukemia

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Abstract Liposomal formulations of anthracyclines have been developed to increase their delivery to solid tumors while reducing toxicity in normal tissues. DaunoXome (DNX, NeXstar) is a liposomal-encapsulated preparation of daunorubicin registered for treatment of Kaposi's sarcoma that during prior in vitro studies showed a toxicity to leukemic cells at least comparable to that of free daunorubicin. The aim of our study was to determine DNX pharmacokinetics in 11 poor-risk patients with acute leukemia treated with DNX 60 mg/m^2 IV on days 1, 3, and 5. Blood and urine samples were collected at appropriate intervals after each of the three DNX administrations. The total amount of daunorubicin (free and entrapped) (t-DNR) and of its metabolite daunorubicinol (DNRol) was assayed by HPLC. The main pharmacokinetic parameters ($t_{1/2\alpha}$ 4.54 ± 0.87 h; Vd_{ss} 2.88 ± 0.93 l/m^2; Cl 0.47 ± 0.26 l/h/m^2) showed that in patients with acute leukemia liposomal-entrapped daunorubicin pharmacokinetics greatly differed from that observed for the conventional formulation. In fact, DNX produced mean plasma AUC levels (t-DNR $AUC_{0-\infty}$ 456.27 ± 182.64 μg/ml/h) about 100- to 200-fold greater than those reported for the free drug at comparable doses due to a very much lower total body clearance. Volume of distribution at steady state was 200- to 500-fold lower than for the free drug. Plasma AUC of DNRol (17.62 ± 7.13 μg/ml · h) was similar to or even greater than that observed with free daunorubicin for comparable doses. Cumulative urinary excretion showed that about 6% and 12% of the total dose of DNX administered was excreted in urine as daunorubicin and daunorubicinol, respectively. No major toxicity was encountered. Therefore, pharmacokinetic characteristics suggest that DNX may be more convenient than free daunorubicin in the treatment of acute leukemia. In fact, liposomal formulation may allow a reduction of daunorubicin captation in normal tissues, thus minimizing toxicity at least for the parent drug, and guarantee an unimpeded access to leukemic cells in the bloodstream and bone marrow, thus theoretically improving efficacy.

Key words Liposomal daunorubicin · Pharmacokinetics · Renal excretion · Leukemia

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Introduction

Anthracycline antibiotics have been considered reference drugs for the treatment of leukemia, lymphoma and other tumors for many years. Among these, daunorubicin (DNR) has had a major role in the treatment of acute leukemia and is still competing for this with newer compounds such as idarubicin and the anthracenedione derivative mitoxantrone. In fact, several clinical studies have indicated that combination therapy with cytarabine is very effective for the treatment of newly diagnosed acute leukemia [11, 29, 53, 55, 58]. However, two major factors affect optimal effectiveness of DNR: on one hand, its dose-dependent cumulative cardiotoxicity limits the maximum tolerated dose, as it may lead to irreversible congestive cardiomyopathy [9, 51, 54], and on the other hand, the development of resistance by leukemia cells may impair its efficacy [8, 27]. Liposomal encapsulation of narrow therapeutic index drugs has been shown to be an optimal drug delivery system for cancer chemotherapy of solid tumors [5]. In fact, these drug carriers may make it possible to increase tumor captation of cytotoxic drugs

while at the same time reducing damage to normal tissues [4] and perhaps to overcome multidrug resistance [35, 37, 48, 52]. DaunoXome (DNX, NeXstar) is a liposomal-encapsulated preparation of DNR registered for treatment of Kaposi's sarcoma in AIDS patients that was developed to allow a selective distribution of DNR to tumor tissues in an attempt to improve its therapeutic index [16, 17]. DNX is a liposomal targeting system in which DNR is entrapped within the aqueous core of small lipid vesicles whose mean diameter ranges from 35 to 65 nm. These vesicles are composed of a single bilayer membrane of distearoylphosphatidylcholine and cholesterol (DSPC/chol) (2:1 molecular ratio) that shows a remarkable physical stability at body temperature which protects the entrapped drug from rapid and extensive uptake by the reticuloendothelial system and from diffusion to the majority of normal tissues [3]. Biodistribution experiments in animals showed that this liposomal formulation delivered about tenfold more DNR to solid tumors than to normal tissues when compared with the free drug [15]. The principle responsible for this selective accumulation in tumor tissues is actually considered to be the abnormal disposition of endothelial cells in neoformed tumor vessels which allow liposome passage through large fenestrations [57]. DNX may also be considered an attractive agent for the treatment of leukemia, as in vivo target cells are localized in sites where the liposomal-entrapped drug may have unimpeded access, such as bone marrow and bloodstream. Moreover, DNX may help in limiting the development of resistance related to P-glycoprotein (also known as P-gp or P170) [30, 31, 32]. This protein is frequently overexpressed in acute nonlymphocytic leukemia (especially in the elderly and in high-risk cases), and its overexpression is significantly related to treatment failure [13, 28, 33]. Our previous in vitro studies documented that DNX is accumulated to a greater extent in multidrug-resistant cells exhibiting a greater toxicity [34].

On this basis, we planned a pilot study with DNX as a single agent in poor-risk patients with acute leukemia. During this study its disposition and toxicity were evaluated in view of the fact that, to our knowledge, none of the studies on DNX published so far have evaluated its pharmacokinetics and cumulative renal excretion of DNR and its major metabolite, daunorubicinol (DNRol), in hematological patients during multiple administrations.

Patients and methods

Study design

The pharmacokinetics of DNX was investigated in 11 patients (5 male, 6 female; aged 61.60 ± 12.49 years, range: 39.4–77.2 years; body weight: 70.30 ± 9.38 kg, range: 60.2–85.4 kg) with a diagnosis of poor-risk leukemia [9 cases of acute nonlymphocytic leukemia (ANLL), 1 case of acute lymphocytic leukemia (ALL), and 1 case of advanced blastic phase of chronic myeloid leukemia (CML)]. The drug was provided by NeXstar Pharmaceuticals Italia on a compassionate basis, and informed consent was obtained from each subject prior to the beginning of the study. The patients received a DNX regimen of 60 mg/m^2 (mean 58.10 ± 2.92 mg/m^2) administered as a 1-h intermittent IV infusion on days 1, 3, and 5. DNX was the primary treatment in five cases at onset, while the other six patients had previously been treated with combination chemotherapy including cytarabine and idarubicin, as they were in second or subsequent relapse. No patient received other cytotoxic drugs for at least 4 weeks after DNX treatment. During the pharmacokinetic evaluation, all patients received uniform concomitant treatment (allopurinol; oral ciprofloxacin or levofloxacin for antibacterial and itraconazole or fluconazole for antifungal prophylaxis, respectively), except for antibacterial chemotherapy (cotrimoxazole in one case, amoxicillin plus clavulanic acid in one case, and amikacin plus ceftazidime in one case) and antiemetic drugs (serotonin receptor antagonists in three cases) administered when required.

No patient presented major renal or hepatic impairment as indicated by plasma creatinine and bilirubin concentrations within normal ranges in all cases. Plasma albumin was decreased in three cases while a more than twofold increase of AST/sGOT and ALT/sGPT was recorded in one and two cases, respectively. Fluid balance was normal in all cases with a diuresis of 1300–2500 ml/day while no effusions or edema were present. Response was determined according to standard criteria as follows: a complete remission was defined as a return to normal bone marrow and normal blood count for more than 4 weeks after chemotherapy; a partial remission was defined as a reduction to less than 20% of marrow blast cells, but without a complete hematologic recovery (either a platelet count $< 50 \times 10^9$/l or an absolute neutrophil count $< 1.5 \times 10^9$/l); no response was defined as no modification or an increase in the blasts. Toxicity and side effects were classified according to WHO standard criteria [56].

Blood sampling

In order to assess DNX pharmacokinetics and to evaluate properly DNRol production rate, blood samples were drawn at closer intervals, that is before and 0.08, 0.25, 0.5, 0.75, 1.5, 3, 5, 7, 9, 12, 18, 24, 36, and 47 h after each of the three 60-min IV DNX infusions and 72 and 96 h after the last DNX dose. After centrifugation plasma samples were stored at −80 °C until assayed.

Urine sampling

Urine samples were collected for the 0–4 h, 4–8 h, 8–12 h, 12–24 h, 24–36 h, and 36–48 h intervals after each DNX administration and for the 48–72 h and 72–96 h intervals after last dosing. The total volume of each urine sample was measured, and a 20-ml aliquot was removed and stored frozen (−80 °C) until assayed.

Assay method

The determination in plasma and urine of total DNR (t-DNR) and of its main metabolite, daunorubicinol (DNRol), was performed by using the HPLC method of Camaggi and co-workers with few modifications [10].

Preparation of samples

Briefly, to 1 ml plasma samples 20 µl internal standard stock solution (idarubicin, 10 µg/ml), 1 ml 10 mM phosphate buffer (pH 8) supplemented with 0.6 µM tetrabutylammonium bromide, and 1 ml methanol were added for plasma extraction. The solution was applied to a 6-cm^3-C_{18} Bondelut cartridge previously washed with 3 ml methanol and 3 ml phosphate buffer:methanol (2:1). The cartridge was washed with 4 ml water:methanol (3:1) and then the analytes were eluted with 3 ml 0.03 mM phosphoric acid in methanol. After the addition of 100 µl 0.1 M KH_2PO_4, the extract was evaporated under vacuum at 25 °C. A 100-µl aliquot of the

residue (200–400 µl) was injected into the liquid chromatograph. For urine extraction 10 µl internal standard stock solution (idarubicin, 10 µg/ml) was added to 1-ml urine samples. After centrifugation the supernatant was injected directly into the liquid chromatograph.

Chromatographic analysis

The eluate analytes were detected by means of a fluorimetric detector (excitation $\lambda = 470$ nm; emission $\lambda = 580$ nm) and eluted with a mixed solution {A: 78% KH_2PO_4 (10 mM) + 22% CH_3CN; B: 30% [KH_2PO_4 (10 mM) + H_3PO_4 (6 mM)] + 70% CH_3CN changing during analysis linearly from A 65% and B 35% to A 60% and B 40% in 14 min} on a 5-µm-C_{18}-CN precolumn linked to a 5-µm-C_{18}-CN column at room temperature.

This assay separates the parent drug from the metabolite, but does not allow of distinguishing the free drug from the entrapped drug. However, we chose to determine the total amount of DNR in plasma (free plus liposomal-entrapped) (t-DNR) as previous studies showed that only 2%–5% of DNX was found as free DNR in plasma due to a very high stability of liposomes at body temperature [15].

Calibration curves for plasma samples ranged from 0.05 to 30 µg/ml for t-DNR and 0.05 to 0.6 µg/ml for DNRol, respectively. Quality control samples of DNX (0.2, 1, 8, 20 µg/ml) and DNRol (0.1, 0.2, 0.4, 0.8 µg/ml) in plasma were routinely assayed with intra- and inter-assay coefficients of variation (CV) always less than 10%. The within-day CV for the respective concentrations was 7.86%, 5.08%, 2.68%, 2.65% for DNX and 7.03%, 5.52%, 2.71%, 5.06% for DNRol. The between-day CV was 9.76%, 2.59%, 2.13%, 5.93% for DNX and 6.39%, 2.82%, 6.90%, 7.51% for DNRol. Calibration curves for urine samples ranged from 0.5 to 15 µg/ml for t-DNR and 0.5 to 4.0 µg/ml for DNRol, respectively. Quality control samples of DNX (1.0, 4.0, 10.0, 15.0 µg/ml) and DNRol (0.5, 0.8, 1.0, 3.0 µg/ml) in urine were routinely assayed with a within-day CV for the respective concentrations of 5.36%, 5.37%, 4.26%, 3.67% for DNX and 5.73%, 6.62%, 5.42%, 6.30% for DNRol, and a between-day CV of 4.59%, 5.92%, 3.40%, 2.68% for DNX and 4.11%, 4.38%, 5.87%, 6.38% for DNRol. DNX, DNR and DNRol were gifts from NeXstar Pharmaceuticals Italia (Milan, Italy), while idarubicin was supplied by Pharmacia & Upjohn (Milan, Italy). The lower limits of quantification both in plasma and urine were 0.05 µg/ml either for t-DNR or for DNRol.

Pharmacokinetic evaluations

We estimated individual patient's concentration-versus-time profile using a one-compartment open model with zero order absorption and first order elimination using the WinNonlin pharmacokinetic software package (Pharsight Corporation, Mountain View, CA., USA). The pharmacokinetic parameters explored included: maximum plasma concentration (C_{max}), distribution rate constant (α), volume of distribution at the steady state (Vd_{ss}), distribution half-life ($t_{1/2}\alpha$), total body clearance (Cl), and area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$). The $AUC_{0-\infty}$ of t-DNR and DNRol were calculated by the trapezoidal method. Cl and Vd_{ss} were calculated as dose/$AUC_{0-\infty}$ and (dose × $AUMC_{0-\infty}$)/$AUC^2_{0-\infty}$, respectively (where $AUMC_{0-\infty}$ is the area under the first moment curve from zero to infinity). The elimination rate constant of DNRol (DNRol λ) was obtained by log-linear regression of the terminal portion of the plasma concentration vs time curve, and the elimination half-life (DNRol $t_{1/2\lambda}$) was calculated as $\ln 2/\lambda$. Cumulative amounts of t-DNR and DNRol excreted in urine were calculated. Data are expressed as mean ± SD or median and range.

Results

Plasma pharmacokinetics

Plasma t-DNR and DNRol concentration-versus-time profiles of patients treated according to the above schedules are shown in Fig. 1, while DNX pharmacokinetic parameters are summarized in Table 1.

As far as the parent drug is concerned, maximum plasma concentration (C_{max}) of t-DNR was 23.64 ± 9.43 µg/ml, 21.36 ± 7.34 µg/ml, and 19.18 ± 5.89 µg/

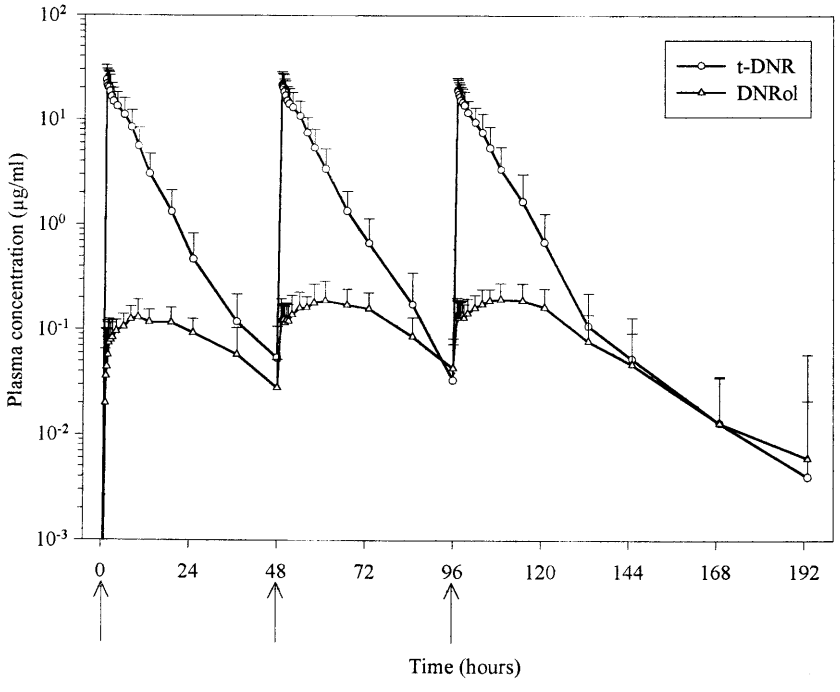

Fig. 1 Plasma total daunorubicin (t-DNR) and daunorubicinol (DNRol) concentration-versus-time profiles following 1-h IV infusion of DaunoXome 60 mg/m^2 on days 1, 3, and 5 (↑) in leukemia patients. Values are expressed as mean ± SD

Table 1 DaunoXome pharmacokinetic parameters: comparison versus free daunorubicin. $AUC_{0-\infty}$ area under the plasma concentration-time curve from zero to infinity, C_{max} peak plasma level, *Cl* total body clearance, *DNRol* daunorubicinol, *n* number of patients, *t-DNR* total amount of daunorubicin, $t_{1/2\alpha}$ distribution half-life, Vd_{ss} volume of distribution at steady state, α distribution rate constant

Parameter	DaunoXome				Free daunorubicin					
	Our study 60 mg/m^2 Leukemia (n = 11)	Guaglianone [22], Gill [21] 60 mg/m^2 Solid tumors – Kaposi's sarcoma (n = 2)	Yeo [59] 100 mg/m^2 Hepato-Carcinoma (n = 14)	Zucchetti [60] 50 mg Glioblastoma (n = 7)	Rahman [40] 60 mg/m^2 Solid tumors (n = 4)	Paul [39] 60 mg/m^2 Leukemia (n = 12)	Alberts [2] 80 mg/m^2 Solid tumors (n = 7)	Speth [49-50] 45 mg/m^2 Leukemia (n = 20)	Robert [42] 30 mg/m^2 Leukemia (n = 7)	Galettis [20] 50 mg/m^2 Leukemia (n = 12)
t-DNR C_{max} (μg/ml)	23.64 ± 9.43	36.20	25.13	4.8–21.8	0.80–1.10[h]	0.48[h]	0.45[h]	0.23	0.50[h]	0.22[h]
Vd_{ss} (l/m^2)	2.88 ± 0.93	2.9[f]	5.58[f]	1.35	1297	39.2[a]	619	2690[f]	1725	
α (h^{-1})	0.14 ± 0.02				7.7				9.04	
$t_{1/2\alpha}$ (h)	4.54 ± 0.87	8.30	1.81	4.8	0.08		0.77	0.30	0.11	
Cl (l/h/m^2)	0.47 ± 0.26	0.40[g]	0.90[g]	0.20	199.8[g]	2.32[b]			38.6	212[g]
t-DNR $AUC_{0-\infty}$ (μg/ml · h)	456.27 ± 182.64[d]	301.10	214.17		0.63[c]	0.81		1.60	0.79	0.56[c]
	155.40[e]									
DNRol C_{max} (μg/ml)	0.131 ± 0.062		0.337	0.200[h]	0.25–0.80[h]			0.180[h]	0.50–0.60[h]	0.085[h]
DNRol λ (h^{-1})	0.022 ± 0.008				0.03				0.019	
DNRol $t_{1/2\lambda}$ (h)	34.95 ± 11.79		19.27		23.4				37.3	
DNRol $AUC_{0-\infty}$ (μg/ml · h)	17.62 ± 7.13[d]		15.42		2.5[c]	2.27		6.80	3.72	1.54[c]
	6.00[e]									
AUC ratio DNRol/ t-DNR	0.041 ± 0.015		0.072	<0.05	4	2.80		4	4.74	2.76
Normalized t-DNR $AUC_{0-\infty}$ (μg/ml · h per mg/m^2)	2.59 ± 0.98									
Normalized DNRol $AUC_{0-\infty}$ (μg/ml · h per mg/m^2)	0.10 ± 0.04									

[a] Vd_{ss} in l/kg
[b] Cl in l/h/kg
[c] AUC from zero to 24 h
[d] Cumulative AUC for three doses
[e] AUC normalized to a DaunoXome dose of 60 mg/m^2
[f] Total Vd_{ss} in liters
[g] Total Cl in liters per hour
[h] Evaluated by means of visual inspection of concentration-time curves

ml immediately after each 1-h DNX IV infusion on days 1, 3, and 5, respectively. Plasma concentration of t-DNR seemed to decline monoexponentially showing a mean distributional half-life of 4.54 h, even if a change in the slope of the t-DNR profile suggesting a second half-life may be noted 48–72 h after the last DNX administration. No accumulation was found after the three 48-h interval DNX administrations as only six patients showed quantifiable trough levels of t-DNR ($C_{min} \geq 0.05$ μg/ml) just before each subsequent drug administration. On the other hand, as far as the metabolic conversion to DNRol is concerned, a slight accumulation of this metabolite was observed after multiple administration with DNRol peak plasma levels reached more or less 10 h after each dose (0.131 ± 0.062 μg/ml, 0.189 ± 0.103 μg/ml, and 0.196 ± 0.082 μg/ml on days 1, 3, and 5, respectively) and a mean elimination half-life of 34.95 h (range: 18.73–57.75 h). The AUC ratio between metabolite and parent drug was 0.041.

Dose-normalized results

To avoid bias due to interindividual differences in dose per m^2, the dose-related pharmacokinetic parameters ($AUC_{0-\infty}$) were normalized with respect to DNX dose per m^2 (cumulative dose on days 1, 3, and 5 = 174.30 ± 8.77 mg/m^2) and consequently to a 1 mg/m^2 DNX dose. The mean dose-normalized $AUCs_{0-\infty}$ guaranteed by each mg/m^2 of DNX were 2.59 μg/ml · h for t-DNR and 0.10 μg/ml · h for DNRol, respectively.

Urinary excretion

Mean cumulative urinary excretions of t-DNR and DNRol during the observational period (0–192 h) (Fig. 2) showed that about 15%–20% of the total administered dose of DNX was excreted in urine as DNR (5.70% ± 0.73%) and DNRol (11.98% ± 2.12%), respectively.

Excretion was almost completed at the end of the observational period (4 days after the last dose). DNR and DNRol percentage excretion after the first dose of DNX were 5.00% ± 1.61% and 5.90% ± 1.85% over 24 h, and 5.67% ± 1.31% and 9.05% ± 2.56% over 48 h, respectively (Fig. 3).

Efficacy and tolerability

A complete remission lasting 4 months was achieved in the patient with ALL. A partial response (with a hypocellular bone marrow and mixed normal and leukemic recovery) was obtained in five cases (including the case of CML in blastic phase) while the other five patients were refractory. Toxicity and side effects are listed in Table 2.

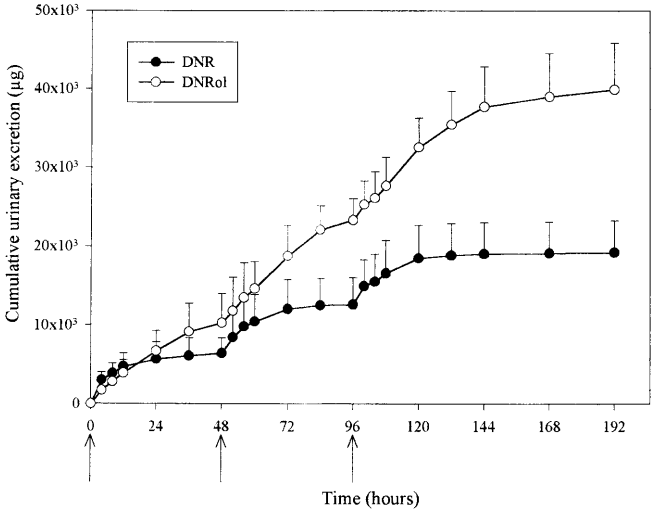

Fig. 2 Cumulative urinary excretion of daunorubicin (DNR) and daunorubicinol (DNRol) following 1-h IV infusion of DaunoXome 60 mg/m^2 on days 1, 3, and 5 (↑) in leukemia patients

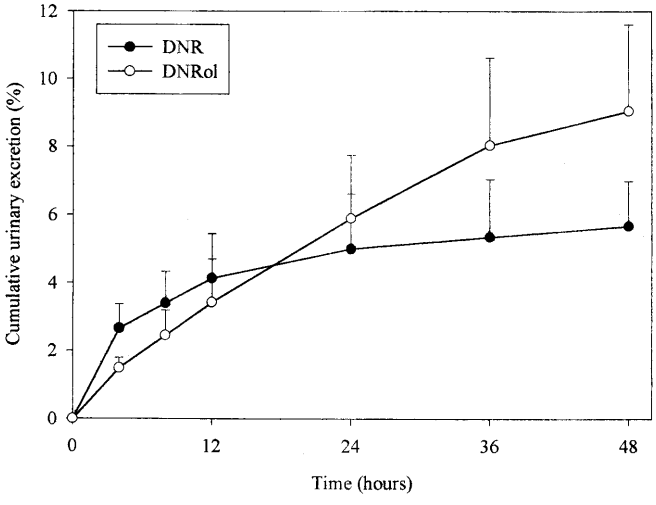

Fig. 3 Percentage cumulative urinary excretion of daunorubicin (DNR) and daunorubicinol (DNRol) following the first 1-h IV infusion of DaunoXome 60 mg/m^2

Table 2 Toxicity and side effects

	Median (range)	*n*
Time of hospitalization (days)	23 (16–27)	
Time to neutrophil recovery $>0.5 \times 10^9$/l[a] (days)	17 (11–20)	
Time to platelet recovery $>20 \times 10^9$/l[a] (days)	20 (10–24)	
No of days with fever	5 (0–18)	
No of days with antibiotics i.v.	9 (0–18)	
Red cell transfusion (units)	10 (2–20)	
Platelet transfusion (apheresis unit)	4 (0–11)	
Cases with infection		6[b]
Cases with antiemetics (nausea grade 1)		3
Cases with diarrhea		1[c]
Cases with mucositis (grade 2)		1

[a] Calculated on the basis of six cases
[b] Five bacterial infections, one case of aspergillosis
[c] One day in duration

No patient died during induction or within 4 weeks from DNX. Infections developed in six cases. A slight nausea (grade 1, without vomiting) developed in three patients and was controlled with serotonin receptor antagonists. Mucositis was observed in one case (grade 2). No patient required parenteral nutrition. The time of hospitalization (from DNX treatment to discharge) ranged between 16 and 27 days (median 23). A slight increase of plasma bilirubin concentration (1.7–2.1 mg/dl) was recorded in three cases. In the six responders, neutrophil and platelet recovery time ranged between 10 and 24 days.

Discussion

Our findings suggest that following IV administration liposomal DNR presented a deeply different plasma disposition when compared to free DNR (Table 1) [2, 20, 39, 40, 42, 43, 49, 50]. In fact, as far as the parent drug is concerned, liposomal DNR showed much higher peak plasma concentration than free DNR with an apparently monoexponential decline. A single 60-mg/m^2 dose of DNX produced mean plasma AUC levels (calculable from dose-normalized data) about 100- to 200-fold greater than those reported for the free drug at comparable doses due to a very much lower total body clearance [39, 40]. Volume of distribution at steady state was 200- to 500-fold lower than for the free drug [2, 40, 42, 50].

Indeed, this plasma profile may be explained by considering that as long as DNR is entrapped in the liposomal carrier it assumes the pharmacokinetics of the carrier. In fact, the kind of composition (DSPC/chol 2:1 mol ratio) and the size (35–65 nm) of these liposomes entrapping DNR were demonstrated to be properties which guarantee a remarkable stability in plasma at body temperature leading to a prolonged circulation time [3, 16, 17, 18, 19]. Moreover, entrapment of DNR in the aqueous interior of the liposomes, such as in DNX formulation, has increased its stability inside liposomes reducing the opportunity to exchange out through binding to plasma proteins [15]. In fact, in vivo studies on fetal calf plasma documented that only 2%–5% of total DNR was present as free drug in plasma after 50 h [15]. Indeed, the slower the release from liposomes, the more the pharmacokinetics of the encapsulated drug is expected to be similar to that of the liposomes themselves [3, 5]. This makes it possible to explain the peculiar pharmacokinetic characteristics of DNX such as a low volume of distribution (approximating plasma volume) with a reduced diffusion into normal tissues (except for the mononuclear phagocyte system, hepatocytes, bone marrow and blood cells), a slow rate of clearance with augmented AUC, and a long distribution half-life which probably masks the true terminal half-life of the free drug. In fact, the change in the slope of the t-DNR profile observed 48–72 h after the last DNX administration is probably due to the fact that when liposomal-entrapped daunorubicin has been completely cleared from plasma, the true pharmacokinetic profile of the elimination phase of the free daunorubicin may become visible.

As far as the metabolic conversion to the 13-dihydro derivative is concerned (Table 1), the AUCs ratio between DNRol and the parent drug was 70- to 100-fold lower than for free DNR (0.041 vs 2–5, respectively) [39, 40]. However, these data have to be interpreted cautiously as they are not mainly related to a reduced metabolite production, but to a greater plasma exposition to the liposomal-entrapped parent drug related to its lower total body clearance. In fact, the absolute AUC plasma value of DNRol appeared to be at least similar to or even larger than the one observed with free daunorubicin for comparable doses [20, 39, 40, 42], indicating that the total body exposition to this metabolite is at least of the same degree over the free drug. This could have some importance in the incidence of long-term toxicity for cumulative doses since DNRol may be more cardiotoxic than DNR [12, 14, 36, 44] and its extended elimination half-life might expose myocardial cells to its toxicity over a long period of time.

This production of DNRol in a comparable amount over the free drug is not surprising considering that DNR conversion to its 13-dihydro derivative is due to ubiquitous aldoketoreductases and that DNX has unimpeded access and can be internalized by those cells which play a major role in this metabolic conversion, such as hepatocytes and blood cells [1, 26]. In fact, Scherphof et al. [45, 47] demonstrated that liposomes sufficiently small to pass through fenestrations of the hepatic sinusoids may be internalized and then processed by the hepatocytes with a distribution rate of 80%:20% (for liposomes of 85 nm) between hepatocyte and Kupffer's cell, respectively [46]. Bachur et al. [6,7] and Huffman et al. [23, 24, 25] showed that DNR reductase was present in normal red and white blood cells with a very high level in myeloblasts. Moreover, Rahman et al. [40] showed that a rapid metabolic conversion of DNR to DNRol occurred in isolated whole blood, DNRol becoming the main species present in plasma 4 h after exposition.

Indeed, despite a DNRol AUC value similar to that observed with free daunorubicin for comparable doses found in our study, the concentration-versus-time profile of DNRol showed a lower accumulation rate and therefore a different shape. This is probably related to a lower rate of metabolite production because of a slow release of DNR from liposomes after their internalization into cells. In fact, while after free DNR administration DNRol plasma concentrations exceeded the parent drug concentrations very soon with a C_{max} at 0.5–1 h [20, 40, 42, 50], in our study DNRol C_{max} was reached only 8–10 h after each DNX administration.

As far as the urinary disposition of DNX is concerned, the percentage of the cumulative excretion both as parent drug and metabolite is similar to that observed with a comparable dose of the free drug (about 6% as

DNR and 12% as DNRol) [2, 38, 41]. However, excretion as parent drug occurred mainly during the first 12–24 h after each DNX administration, while metabolite excretion increased progressively according to a reduced rate of production and to a long elimination half-life.

Our data are in agreement with other authors' findings on DNX pharmacokinetics (Table 1) [21, 22, 59, 60]. However, while some authors [21, 22] showed that DNX at a dose of 60 mg/m^2 presented a dose-dependent kinetic manifested by a convex shape in the profile with longer half-life, in our study DNX presented an apparently monoexponential decline as suggested by the log-linear plasma concentration-time profile. Nevertheless, this observation cannot be considered conclusive, as only one dose level was studied.

In conclusion, these pharmacokinetic data support the expectation that DNX may improve the therapeutic index of free DNR in the treatment of acute leukemia. In fact, its formulation may make it possible both to reduce DNR captation in normal tissues, thus minimizing toxicity at least for the parent drug, and to guarantee a significantly greater plasma availability for uptake by leukemic cells and bone marrow, thus theoretically improving efficacy. Increasing the exposure of leukemic cells to DNX should increase the amount of DNR that is accumulated into the cells, and hence its cytotoxicity. This has been clearly shown in vitro, especially when multidrug-resistant cells are exposed to DNX [34], but has not been proven in vivo as yet. Therefore, our subsequent pharmacokinetic studies on DNX are being planned to evaluate simultaneously disposition in plasma and penetration in the leukemic cells.

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