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Pharmacokinetics of Daunorubicin after Administration as Free Drug or as DNA Complex in Leukemic Patients

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Summary. *An earlier whole-body autoradiographic study in mice revealed large differences between the tissue distribution of daunorubicin (D) after administration as free drug and as DNA-linked D. Therefore, the pharmacokinetics of D administered as free drug or linked to DNA was studied in 15 adult patients with acute non-lymphoblastic leukemia.*

The data obtained following infusion of free drug over either 45 or 240 min could be fitted to a two-compartment open-body model. With the D-DNA infusion considerably higher plasma concentrations were achieved, with a slower distribution and elimination from plasma than seen after the administration of free drug. This confirmed earlier animal data indicating a different pharmacokinetic behavior of D when it was administered linked to DNA. Furthermore, different pharamcokinetic parameters were obtained for D during infusion and in the post-infusion phase after administration of DNA-linked D ($P < 0.005$). This finding strongly indicates that the D-DNA acts as a slow-release preparation in humans, which might modify tissue distribution and toxic side-effects of the drug.

Introduction

The anthraquinone glycoside daunorubicin (D) is one of the most widely used drugs in the treatment of acute non-lymphoblastic leukemia (ANLL). D is generally administered as an IV injection or infusion of the free drug, but infusion of the drug linked to a macromolecular carrier, e.g., DNA, has been proposed by Trouet et al. [23], who suggested a selective accumulation of the drug-carrier complex in cells of high endocytic activity, preferentially certain tumor cells.

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Experimental and clinical results of complexed versus free drugs have been conflicting. Comparative studies of the cytostatic efficacy of DNA complexes of adriamycin (A), D, and actinomycin D and of the corresponding free drugs showed that a preferential incorporation of the DNA-linked drugs into human acute leukemia blast cells was not likely, since the DNA complexes appeared to dissociate already at the outer cell membrane [21].

A decreased cardiac toxicity of the drugs when they were bound to DNA was suggested by studies of perfused rat hearts [18]. A clinical observation indicates a decreased cardiac toxicity in children with acute leukemia treated with DNA-linked A [19].

In an earlier study on the tissue distribution of D as studied by the whole-body autoradiographic technique in mice [3], we found considerable differences in the tissue distribution of free D and D-DNA during the first 2 h after administration. This prompted us to perform the present study on the comparative pharmacokinetics of D in humans after administration of free and DNA-linked drug. Plasma levels of D were followed for 24 h by the use of a selective analytical method based on reversed-phase liquid chromatography [9].

Materials and Methods

Chemicals. D and daunorubicinol (DOH) were supplied by Pharma Rhodia (Stockholm, Sweden). Other chemicals used are listed in [9].

Preparation of the D-DNA Complex. Herring sperm DNA (type VII, Sigma, St Louis, Mo., USA) was dissolved in 0.9% NaCl to given a concentration of 2.34 mg/ml, autoclaved for 15 min at 120° C, and allowed to stand overnight at room temperature. D was dissolved in 0.9% NaCl to a concentration of 10 mg/ml and added to the DNA solution to give a final D concentration of 0.20 mg/ml.

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Table 1. Patient characteristics

Patient	Body weight (kg)	Dose (mg)	Diagnosis	Stage	Other therapy
W. L.	94	140	AML	C. R.	–
E. C.	58.5	88	AMoL	Relapse I	Allopurinol 100 mg 3 times daily Glibenclamid 5 mg 2 + 1 + 1 daily
F. P.	92	130	AML	Onset	Allopurinol 100 mg 3 times daily
E. J.[a]	58.5	100	AMoL	C. R.	–
A. E.	75	70	AML	Onset	Allopurinol 100 mg 3 times daily
T. J.	70	105	AUL	Onset	–
B. L.	71	100	AML	Onset	Estradiol valerate 2 mg once daily Bactrim 2 tablets twice daily Bacitracin 15,000 IU / Neomycin 300 mg } 2 tablets 3 times daily
M. R.	61.7	93	AUL	Onset	Allopurinol 100 mg 3 times daily
J. P.	52.6	80	AML	C. R.	Allopurinol 100 mg 3 times daily
B. B.	56	78	AUL	Relapse I	Nystatin 500,000 IU 4 times daily Bacitracin 15,000 IU / Neomycin 300 mg } 2 tablets 4 times daily Bactrim 2 tablets twice daily Haldol 1 mg twice daily
C. A.	67	100	AUL	Onset	Allopurinol 100 mg 3 times daily
I. H.	64.5	97	AML	Onset	Allopurinol 100 mg 3 times daily
M. H.	67	50	AML	C. R.	Dihydroergotamin 2.5 mg once daily
T. A.	84.5	120	AML	Relapse I	Allopurinol 100 mg 3 times daily
C. C.[b]	83.5	97	AML	C. R.	Allopurinol 600 mg once daily Rifampicin 600 mg once daily Isoniazid 300 mg once daily Pyridoxin 150 mg once daily Folic acid 10 mg once daily

[a] Intercurrent disease: diabetes mellitus
[b] Intercurrent disease: pulmonary tuberculosis

Patients. The study was performed in 15 adult patients with ANLL. The patients were free of clinical signs of renal or liver disease at the time of the investigation. They were at different stages of their disease (Table 1). When in complete remission the patients were maintained on monthly courses of D-ARA-C alternated with courses of ARA-C-6-thioguanine.

Chemotherapy Protocol. The patients were randomized to the treatment protocol [10] as follows:
A. Infusion for 45 min (1.0–1.5 mg/kg body weight), D dissolved in 200 ml 0.9% NaCl;
B. Infusion for 240 min (1.0–1.5 mg/kg body weight), D dissolved in 500 ml 0.9% NaCl;
C. Infusion as the DNA complex, 20 mg D in 100 ml/h (1.0–1.5 mg/kg body weight).
The infusion rate was controlled by the use of a Tekmar T 92 volumetric infusion pump.

Plasma Samples. Blood samples (5–7 ml) were collected in 10-ml glass test tubes (Vacutainer) containing 250 IU heparin (freeze-dried) during and after infusion. The samples were immediately cooled in an ice bath and centrifuged at 4,080 *g* for 10 min at 2–4° C. The plasma fraction was carefully removed and frozen at −20° C until assay. During the infusion, samples were taken at 15, 22, 45 min and, for the longer infusions, also at 90 min after the start. At the end of the infusion samples were taken at 0, 15, 30, 45, 60, and 90 min. Another four or five samples were taken at different times 2–20 h post-infusion.

Determination of Daunorubicin and Daunorubicinol. Plasma levels of D and DOH were assayed by an analytical method based on extraction and reversed-phase liquid chromatography [9]. Samples of plasma 2 ml in volume were used for the analysis. The analytical technique used gives the total amount of free and DNA-linked D.

Statistical Analysis. The statistical analyses were performed according to Student's *t*-test for independent means. All results are expressed as mean values ± SE (standard error of the mean).

Pharmacokinetic Analysis. As judged from the present data, the total plasma concentration of D declined biexponentially with time in the post-infusion phase (Fig. 1). The plasma concentration (C_P) vs time (t) data were fitted to the zero-order infusion two-compartment open model (Fig. 2), with drug distribution between a central (V_C) and a peripheral (V_P) compartment and with elimination (k_{el}) from the central compartment as the sum of renal (k_r) and metabolic (k_m) elimination.

The time course of the plasma concentration is given by Eq. 1 [11]:

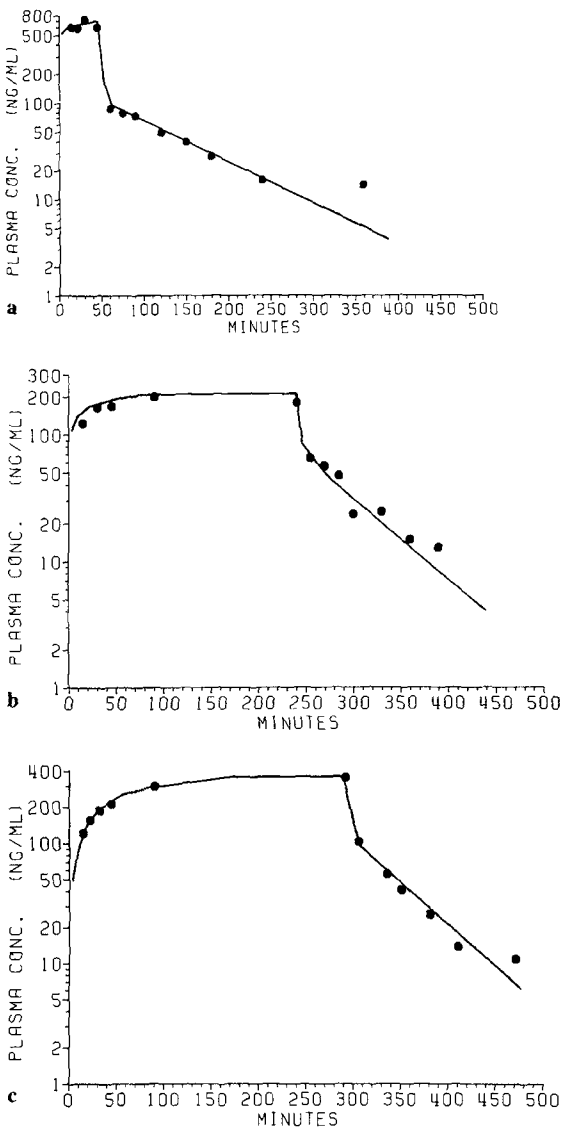

Fig. 1a–c. Plasma concentration curves for daunorubicin following administration of 1.50 mg D/kg body weight by infusion as free drug over 45 min to patient W. L. (**a**); by infusion as free drug over 240 min to patient T. J. (**b**); and by infusion as a DNA complex over 292 min to patient I. H. (**c**)

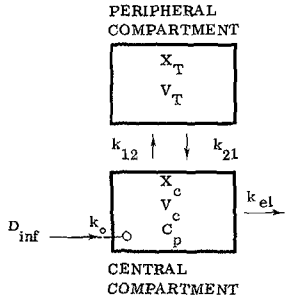

Fig. 2. Plasma concentration vs time data fitted to the zero-order infusion two-compartment model

$$C_P = \frac{k_0 (k_{21} - \alpha)(1 - e^{\alpha T})}{V_C \alpha (\alpha - \beta)} \cdot e^{-\alpha t} + \frac{k_0 (\beta - k_{21})(1 - e^{\beta T})}{V_C \beta (\alpha - \beta)} \cdot e^{-\beta t} \quad (1)$$

where T is the infusion time and k_0 is the zero-order infusion rate. During the infusion $T = t$ and varies with time.

The values for α, β, k_{21}, and V_C were determined by computer non-linear regression analysis from the plasma concentration-time data, by means of the NONLIN program [20]. The plasma concentrations were weighted according to the reciprocal of concentration ($1/C_P$). The microscopic rate constants k_{12} and k_{el} were calculated from the estimated values for α, β, and k_{21} [11].

The areas under the plasma concentration time curves (AUC) were calculated with reference to the trapezoidal rule and the residual area to infinite time (C_P/β).

Results

The resulting pharmacokinetic parameters relevant for the discussion are presented in Tables 2 and 3. The proposed model gave a very good agreement between experimental and calculated values for the infusion of free D both over 45 and over 240 min (Fig. 1). In this case the lines were calculated from plasma concentration-time data both during and after the end of infusion.

Experimental data for the infusion of DNA-linked D did not fit the model used. It was, however, possible to fit each part (infusion and post-infusion phase) of the time course of plasma concentration separately, resulting in different values of the estimated parameters (Table 3). In all patients the elimination constants, k_{el}, were higher during the post-infusion phase than during the infusion ($P < 0.005$).

Both during the infusion and during the post-infusion phase, k_{el} for D was smaller when the drugs were infused linked to DNA than when the free drug was infused (Tables 2 and 3) ($P < 0.01$). As expected, the maximum plasma concentration of D was much higher during the 45-min infusion than when it was administered over 240 min (Table 4) ($P < 0.0025$).

There was no significant difference in AUC for the administration of free drug over 45 and over 240 min (Table 4). This suggests that the pharmacokinetic is linear in the actual concentration range. The AUC for the D-DNA infusion was approximately three times higher than that for the administration of free D (Table 4) ($P < 0.0005$).

Discussion

The pharmacokinetics of D has previously been studied by a technique based on measurement of total fluorescence [1]. This technique is unselective since

Table 2. Pharmacokinetic parameters of the two-compartment open-body model following IV infusion of free daunorubicin

	Patient	$(t_{1/2})_\alpha$ (min)	$(t_{1/2})_\beta$ (min)	k_{el} (min^{-1})	k_{12} (min^{-1})
Infusion over 45 min	W. L.	1.73	71.7	0.2610	0.1340
	E. C.	1.36	102.8	0.2267	0.2744
	F. P.	3.55	60.6	0.1397	0.0511
	E. J.	0.89	52.5	0.5361	0.2327
	A. E.	5.41	28.5	0.1110	0.0133
	Mean	2.59	63.2	0.2549	0.1411
	SE	0.84	12.2	0.0755	0.0503
Infusion over 240 min	T. J.	3.42	51.9	0.1273	0.0677
	B. L.	4.03	64.6	0.1134	0.0531
	M. R.	2.30	53.2	0.2720	0.0274
	J. P.	3.04	51.6	0.1654	0.0572
	B. B.	3.03	77.2	0.1520	0.0724
	Mean	3.16	59.7	0.1660	0.0556
	SE	0.28	5.0	0.0280	0.0079

Table 3. Pharmacokinetic parameters of the two-compartment open-body model following infusion of daunorubicin as DNA complex (20 mg daunorubicin per hour)

Patient	Infusion time (min)	$(t_{1/2})_\alpha{}^a$ (min)	$(t_{1/2})_\alpha{}^b$ (min)	$(t_{1/2})_\beta{}^a$ (min)	$(t_{1/2})_\beta{}^b$ (min)	$k_{el}{}^a$	$k_{el}{}^b$	$k_{12}{}^a$	$k_{12}{}^b$
C. A.	300	9.66	6.49	114.9	74.6	0.0602	0.0524	0.0104	0.0447
I. H.	292	4.20	9.83	45.9	51.7	0.1210	0.0312	0.0386	0.0224
M. H.	150	12.1	8.89	59.7	88.9	0.0564	0.0468	0.00081	0.0260
T. A.	360	6.73	19.6	57.6	99.0	0.0772	0.0186	0.0217	0.0104
C. C.	290	7.23	5.66	96.9	76.0	0.0814	0.0537	0.0132	0.0570
Mean		7.98	10.09	75.0	78.0	0.0792	0.0405	0.0169	0.0321
SE		1.35	2.50	13.1	8.0	0.0115	0.0068	0.0064	0.0083

[a] Parameters calculated from the slope after the end of infusion
[b] Parameters calculated from data obtained during the infusion

Table 4. Area under plasma concentration by time curve (AUC) and maximum plasma concentration (C_{max}) for daunorubicin given as infusion over 45 min, 240 min, and as DNA complex

Subject	Infusion over 45 min		Subject	Infusion over 240 min		Subject	Infusion as DNA complex	
	$AUC \cdot K \cdot 10^{-3}$ $ng \cdot min \cdot ml^{-1}$	$C_{max} \cdot K$ $ng \cdot ml^{-1}$		$AUC \cdot K \cdot 10^{-3}$ $ng \cdot min \cdot ml^{-1}$	$C_{max} \cdot K$ $ng \cdot ml^{-1}$		$AUC \cdot K \cdot 10^{-3}$ $ng \cdot min \cdot ml^{-1}$	$C_{max} \cdot K$ $ng \cdot ml^{-1}$
W. L.	28.9	494.2	T. J.	33.1	134.7	C. A.	53.3	189.6
E. C.	11.6	162.2	B. L.	17.2	66.7	I. H.	63.1	240.7
F. P.	20.4	402.7	M. R.	10.7	47.8	M. H.	59.5	435.5
E. J.	12.9	220.6	J. P.	25.9	100.6	T. A.	74.7	246.5
A. E.	22.7	502.5	B. B.	15.0	59.6	C. C.	68.5	250.5
Mean	19.3	356.4		20.4	81.9		63.8	272.6
SE	3.20	70.2		4.0	15.9		3.7	42.2

K, body weight/dose

the metabolites daunorubicinol (DOH) and aglycones are co-determined. Analytical methods including a chromatographic step give a higher selectivity and have recently been used for the determination of anthraquinone glycosides in biological samples [6, 9, 12, 14]. Furthermore, by these techniques DOH, which proved to have some, albeit rather low, cytostatic activity [4, 7] can be determined simultaneoulsy. The plasma half-lives of D and DOH were estimated to be 18.5 h and 26.7 h, respectively, the plasma levels being determined fluorimetrically after separation by thin layer chromatography [12].

The plasma half-lives in the present study (Tables 2 and 3) are considerably shorter than reported in other publications [5, 12]. The discrepancy may be due to differences in the dosage schedules and the fact that five-fold lower doses were used in the present study. However, in our recent study in both normal and leukemic rats a ten-fold increase in dose of D did not significantly influence the plasma half-life of the drug either in normal or in leukemic animals (B. Andersson et al. 1981, unpublished work).

In a recently published study [16] on the pharmacokinetics of D in man, the plasma half-lives for D and DOH were estimated at 24.6 h and 23.8 h, respectively, when D was infused as free drug, and 12.1 h and 26.8 h, respectively, when it was infused linked to DNA.

We are aware of a possible triphasic decline in the plasma concentration with time, as indicated by the results of a preliminary study where D was administered as a bolus injection over 5 min (2 mg/kg). The data obtained in the present study could not be made to fit a three-compartment model, as proposed by Hulhoven et al. [16]. The data gave a very good fit with a two-compartment model and therefore this model was chosen to present the pharmacokinetics of D. No detectable level of D ($\geq$ 5 ng/ml) was found in any plasma sample 12 h after the start of infusion.

The lower values of k_{12} and k_{el} (Tables 2 and 3) after infusion as D-DNA ($P < 0.01$) indicate that the complex is distributed and eliminated more slowly than the free drug, probably due to a slow release from the complex. The different pharmacokinetics during and after the infusion might be due to different degrees of dissociation of the D-DNA. Its stability in the circulatory system is determined by several factors. The formation of the DNA complex can be regarded as an equilibrium process, as illustrated in Eq. 2.

$$m\ (\text{Daunorubicin}) + n\ (\text{DNA}) \rightleftarrows (\text{Daunorubicin})_m\ (\text{DNA})_n \qquad (2)$$

where m and n are the numbers of molecules of D and DNA, respectively, involved in the complex. The equilibrium constant K_c, can be defined by

$$k_c = \frac{[(\text{Daunorubucin})_m\ (\text{DNA})_n]}{[\text{Daunorubicin}]^m\ [\text{DNA}]^n}. \qquad (3)$$

The relative amounts of complexed to uncomplexed D are determined by the equilibrium constant and by the concentration of the different moieties. Decreasing the total concentration by dilution will result in a decrease of the relative amount of D-DNA. A decrease of free D, e.g., by elimination, will give an increase of the relative amount of the complex, assuming that DNA is eliminated more slowly than the free drug [13, 15].

The D-DNA may also be dissociated by DOH formed by metabolism of D, through competition of the binding sites on the DNA molecules. Similar DNA-binding characteristics have been found for anthraquinone glycosides differing by substitution in the C-9 side chain [8]. The high concentrations of DOH found in plasma during infusion of the D-DNA [2] most probably promote dissociation of the complex. The situation is further complicated by the fact that elevated DNA levels have been found in leukemia [17, 22].

Our earlier study in mice showed large differences in plasma as well as organ kinetics between free D and D-DNA. This resulted in a higher concentration in bone marrow and a lower cardiac concentration of D after administration of D-DNA. The present study in humans revealed corresponding differences in plasma kinetics of D and D-DNA, and probably there are differences in organ kinetics of free and complexed drug in humans as well.

Whether this leads to any therapeutic advantage of the D-DNA is uncertain, but preliminary data have failed to show any higher antileukemic effect of the complex [10]. The subtle early signs of the cardiotoxic side-effects of D make it even more difficult to evaluate whether the different pharmacokinetic behavior of D-DNA results in a lower cardiotoxicity. Careful clinical examination of large groups of patients will be required before this question can be definitely answered.

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