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Human Pharmacokinetics of the Daunorubicin-DNA Complex

An Alternative View of the Lysosomotropic Theory

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Summary. *Plasma kinetics and urinary excretion of daunorubicin (DNR) and its active metabolite, daunorubicinol (DNR-ol) were studied in 15 leukemic patients after a 4-h infusion of 75 mg DNR/m² either as the free drug or as a complex with DNA. The data obtained after infusion of the DNR-DNA complex were compared with the data obtained after infusion of the free drug. The DNR plasma levels were found to be higher during the 2 h following the infusion of the complex; the levels of DNR-ol were only higher for a few minutes after infusion.*

Kinetic analysis showed that complexing with DNA does not fundamentally modify the three-compartment model described for DNR. Only quantitative modifications were observed: a marked lengthening of the α-phase and a shortening of the γ-phase. Urinary excretion of DNR and DNR-ol was increased after infusion of the complexed drug, in relation to the persistence of higher plasma levels.

The data recorded in this work do not confirm the lysosomotropic mechanism postulated for the DNR-DNA complex, but show a delayed distribution of DNR, which is progressively released by dissociation of the circulating DNR-DNA complex, as previously demonstrated in rabbits infused under same conditions.

Introduction

Daunorubicin (DNR), an anthracyclinic cytostatic antibiotic, has been found effective in the treatment of several hematological malignancies, but its use is limited by serious toxic reactions [4].

For better selectivity against malignant cells, Trouet et al. proposed its use as a complex with deoxyribonucleic acid (DNA). This complex postulated as a lysosomotropic agent was found to be active in the treatment of L-1210 leukemia of DBA_2 mice [17] and of human acute nonlymphocytic leukemia (ANLL) [5, 7, 15]. In previous reports on the pharmacokinetics of the DNR-DNA complex in rabbit, a dissociation in the plasma with slow release of DNR was shown [9, 12, 13]. Preliminary results in leukemic patients also showed the same trend [11].

The aim of this work is to compare the pharmacokinetics of DNR and its active reduced metabolite, daunorubicinol (DNR-ol) [3] in leukemic patients after the infusion of DNR either as the free drug or as a complex with DNA.

Materials and Methods

Preparation of the DNR-DNA Complex

The DNR-DNA complex was prepared as previously reported [17], with DNA from herring sperm (Sigma type VII) and DNR (Cérubidine®) for a molar ratio of 20 : 1 in a final volume of 1,000 ml NaCl 0.9%. For the free drug infusion, DNR (Cérubidine) was also brought to 1,000 ml by addition of NaCl 0.9%. The infused dose was 75 mg DNR/m² for both the free and the complexed drug.

Patients

This study included 15 patients of both sexes, with an age range of 18–63 years (mean 41 years), who were being treated for ANLL in the Hematology Department. All the patients' data were obtained during the period of induction (AML '75 protocol) [7]. The study was performed during the first drug infusion in nine patients, and in the other patients during the second or the third; infusions were given at 3–4 week intervals.

Both free and complexed DNR were infused over 4 h. A constant rate of drug delivery was obtained with a peristaltic infusion pump (Braun Infusomat 870202), through a needle placed in an antecubital vein. Another needle, placed into a vein of the contralateral arm, was used for blood sampling. Blood specimens were drawn in EDTA-di K tubes at given intervals (0, 5, 15, 30, 45, 60, and

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120 min, and in the last 11 cases also at 4, 8, 16, and 24 h) after the end of the infusion. Blood was immediately centrifuged. The plasma was removed and stored at −20° C until analysis.

Urines were collected on sodium azide during the infusion and during the 24 h following the infusion; 30-ml aliquots were stored at −20° C until analysis.

Chromatographic Analysis

DNR and DNR-ol in the plasma were measured by high-performance liquid chromatography (HPLC) and visible absorption at 490 nm, as previously described. At this wavelength no interference peaks were observed [10, 11]. Each extraction and determination was made in duplicate. If the calculated DNR plasma level was lower than 15 ng/ml, a check was made in extracts from 2 or 3 ml plasma to improve accuracy at these low levels.

For urine processing, 1 ml urine was mixed with 3 ml sodium borate-KCl 0.05 *M* buffer at pH 8.6. Aliquots (1 ml) of this mixture were extracted and analyzed by HPLC, the same procedure being used as for the plasma. DNR and DNR-ol urinary excretion is expressed as a percentage of the dose of DNR administered.

For some controls, thin layer chromatography (TLC) was performed according to system III of Takanashi and Bachur [16].

Binding of DNR and DNR-ol to the Plasma Proteins

DNR and DNR-ol binding to the plasma proteins was calculated according to a method used to estimate the binding of anthracyclines to DNA [2, 6].

Three dialysis bags of a length of 50 cm (Dialysis Tubing Visking 1–8/32″, Medicell International Ltd.) were filled, respectively, with 10 ml NaCl 0.9% to evaluate the absorption of DNR or DNR-ol into the bag membrane; 10 ml fresh human serum containing 4.84 g albumin and 2.78 g globulins/100 ml to estimate their total protein binding; and 10 ml SSPP (stable solution of pasteurized human plasma protein, Red Cross, Belgium) containing 4.5 g albumin/100 ml to estimate their binding to the albumin. The bags were placed under continuous agitation in the dark at 4° C with 80 ml solution of DNR or DNR-ol in NaCl 0.9%. One drop of toluene was added to prevent bacterial proliferation. After 90 h dialysis a sample of the dialysate was taken and dosed by fluorimetry [16].

Student's *t*-test for independent means was used for the statistical analysis. The results were considered significantly different if P was < 0.05. The results were expressed as the mean ± SE. The kinetic parameters were processed by computer (HP-97) for calculation of multiphasic decay curves.

Results

In vivo Studies

1) DNR and DNR-ol Plasma Levels During the 2 h After Infusion of DNR either as Free Drug or as DNR-DNA Complex. The DNR plasma levels found at the end of the infusion of the complexed drug were very significantly higher ($P < 0.005$) than the ones observed at the end of the free drug infusion (Fig. 1). The DNR-ol levels showed also a significant but less marked difference ($P < 0.05$).

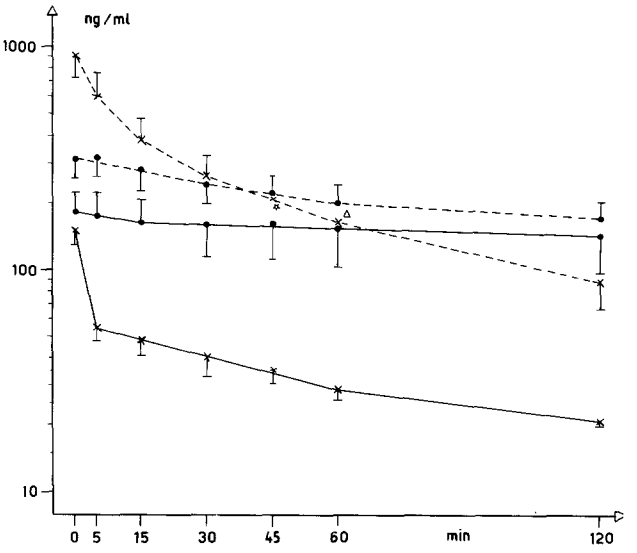

Fig. 1. DNR and DNR-ol plasma levels over 2 h after infusion of DNR either as free drug ($n = 6$) or as DNR-DNA complex ($n = 9$). *SE = 51 ng/ml; △ SE = 39 ng/ml. x——x, DNR after free DNR; ●——●, DNR-ol after free DNR; x– – –x DNR after DNR-DNA; ●– – –●, DNR-ol after DNR-DNA

Thereafter, the DNR plasma levels dropped quickly in the initial minutes after the infusion. The rate of decay diminished after minute 5 when free DNR was infused, and after minute 15 when DNR-DNA complex was infused. The fall in DNR-ol was less marked in both cases. Statistical analysis revealed significantly higher DNR levels during the 2 h after infusion and raised DNR-ol levels only during the initial 5 min following infusion of the complexed drug.

2) DNR and DNR-ol Plasma Kinetics Following the Infusion of DNR either as Free Drug or as DNR-DNA Complex. In both cases, the plasma decay curve (Fig. 2) of the DNR levels could be fitted as the sum of three exponential terms. From the fourth hour onwards, the DNR plasma levels were no longer significantly different in the two groups of patients. Comparison of the kinetic parameters (Table 1) revealed a highly significant longer α half-life ($P < 0.0005$) and a significantly shorter γ half-life ($P < 0.0125$) when the complex was administered.

After the DNR-DNA complex infusion, the plasma decay curve of the DNR-ol levels could be fitted to the sum of two exponential terms. In the case of the free drug infusion, analysis of the kinetic parameters of the DNR-ol decay curve was more difficult, because there were marked differences among patients during the initial period (Table 2). In the first patient a plateau in the DNR-ol plasma levels was observed during the first hour. In two other patients, the decay curve was bi-exponential; in the last one, a short α-phase was ob-

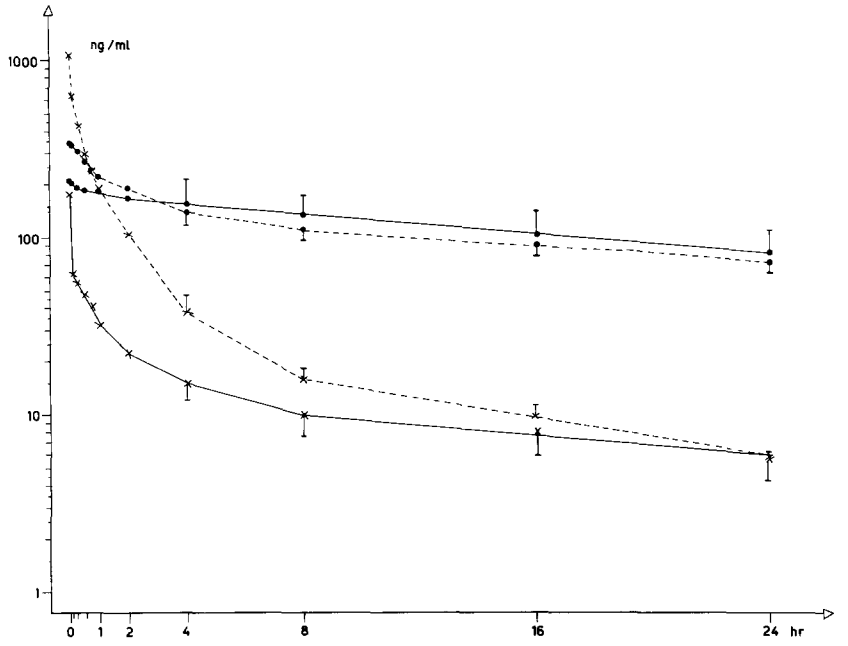

Fig. 2. DNR and DNR-ol plasma decay curves after the infusion of DNR either as free drug ($n = 4$) or as DNR-DNA complex ($n = 7$). ×——×, DNR after free DNR; ●——●, DNR-ol after free DNR; ×– – –× DNR after DNR-DNA; ●– – –● DNR-ol after DNR-DNA

Table 1. Kinetic parameters of the DNR plasma decay curves after the infusion of DNR either as free drug or as DNR-DNA complex

No. of patient	A (ng/ml)	$t_{1/2}\ \alpha$ (min)	r	B (ng/ml)	$t_{1/2}\ \beta$ (h)	r	C (ng/ml)	$t_{1/2}\ \gamma$ (h)	r
A. Free DNR infusion									
1	144	0.98	–[a]	51	1.10	0.998	7	27.4	1.000
2	72	0.73	–	55	0.38	0.998	29	16.3	0.991
3	119	0.96	–	75	0.79	0.993	9	38.5	0.999
4	107	1.07	–	29	1.87	0.978	8	16.0	0.995
Mean	111	0.94	–	53	1.04	0.992	14	24.6	0.996
SE	15	0.07	–	9	0.31	0.005	5	5.4	0.002
B. DNR-DNA complex infusion									
1	461	6.09	0.953	283	0.88	0.990	19	11.0	0.998
2	309	5.54	0.999	139	1.68	0.988	30	10.1	0.989
3	367	4.61	0.960	301	0.82	0.999	14	16.0	1.000
4	1367	6.52	1.000	922	0.95	0.999	63	7.6	1.000
5	525	4.88	0.951	395	0.79	0.998	40	6.4	1.000
6	547	5.29	0.977	308	0.85	0.997	14	18.3	0.962
7	482	3.75	0.998	259	0.86	0.987	24	15.2	0.987
Mean	580	5.24	0.977	372	0.98	0.994	29	12.1	0.991
SE	135	0.35	0.008	96	0.12	0.002	7	1.7	0.005

[a] No regression, because calculated on two time points

served, lasting only 15 min. On the other hand, the β-phase was very homogeneous in these four patients receiving the free drug. No significant difference was observed toward complexed drug infusion.

3) Urinary Excretion of DNR and DNR-ol During the Infusion and During the 24 h Following the Infusion of DNR either as Free Drug or as DNR-DNA Complex. Urinary excretion of DNR and DNR-ol during the infusion and during the 24 h following the infusion was significantly increased after administration of the DNR-DNA complex, the unchanged DNR accounting for most of this differences.

TLC of the urines also revealed trace amounts of

Table 2. Kinetic parameters of the DNR-ol plasma decay curves after the infusion of DNR either as free drug or as DNR-DNA complex

No. of patient	A (ng/ml)	$t_{1/2}\ \alpha$ (h)	r	B (ng/ml)	$t_{1/2}\ \beta$ (h)	r
A. Free DNR infusion						
1	–	–	–	393	17.0	0.992
2	64	0.60	0.911	195	26.4	0.968
3	52	0.41	0.940	77	23.6	0.996
4	37	0.12	0.998	57	28.6	0.998
Mean	–	–	–	181	23.8	0.989
SE	–	–	–	77	2.5	0.007
B. DNR-DNA complex infusion						
1	174	0.94	0.961	186	25.8	0.984
2	91	0.20	0.996	123	22.8	0.997
3	107	1.05	0.979	129	21.3	0.997
4	488	1.78	0.996	163	20.2	0.954
5	287	0.97	0.978	169	44.9	0.981
6	141	0.69	0.987	113	27.2	0.962
7	84	0.88	0.997	80	25.5	0.993
Mean	196	0.93	0.985	138	26.8	0.981
SE	55	0.18	0.005	14	3.2	0.006

Table 3. DNR and DNR-ol urinary excretion (percentage of the infused DNR dose) during the infusion and during the 24 h following the infusion of DNR either as free drug or as DNR-DNA complex

	Free DNR infusion ($n = 6$)	DNR-DNA complex infusion ($n = 6$)	P
DNR (%)	4.69 ± 0.59	8.00 ± 0.69	< 0.0025
DNR-ol (%)	3.78 ± 0.26	6.04 ± 0.77	< 0.01
Total (%)	8.47 ± 0.70	14.04 ± 1.33	< 0.0025

other metabolites: demethyl-daunorubicinol-deoxyaglycone-4 sulfate and -4 glucuronide [16], representing at most 5% of the total excretion [8].

In vitro Studies

Protein-Binding of DNR and DNR-ol. The absorption of DNR and DNR-ol in the dialysis membrane was 1% and 4%, respectively. The binding of DNR to albumin was 27% at the levels of 8 and 232 μg/ml, i.e., independent of its concentration between these two values. At 8 μg/ml, the binding to total serum proteins was 49%. At the same level of 8 μg/ml, the binding of DNR-ol to albumin was 44% and its binding to the total serum proteins, 46%. Hence, the total protein binding was the same for the drug and its metabolite, but their distribution between albumin and globulins was different.

Discussion

Before discussing the pharmacokinetic data obtained in the present work, they must be correlated to those in our previous published papers.

In the rabbit, the pharmacokinetics of infused DNR-DNA complex does not support the theory according to which the disappearance of the complex results from a lysosomotropic mechanism [17], since it has been demonstrated by simultaneous measurement of the cytostatic drug (DNR) and its labeled macromolecular vector (^{125}I.DNA) that the complex dissociates into the circulation [9]. This release of DNR from DNA occurs progressively. Moreover, the plasma decay curve of the cytostatic drug is tri-exponential, whereas that for DNA is bi-exponential. Furthermore, the reduced metabolite DNR-ol, which is produced intracellularly [3], can be detected in the circulating blood early after the infusion of the DNR-DNA complex is started [12]. When these kinetic data are considered it appears difficult to ascribe the DNR-DNA complex distribution to endocytosis.

In addition, the DNR and DNR-ol plasma levels during infusion of DNR or DNR-DNA in leukemic patients [11] have been found to be similar to those found in animals. A delayed distribution of the complexed drug has also been reported by other authors in a preliminary publication [1].

In the present study, only the cytostatic drug and its reduced metabolite have been measured in the plasma; but the pattern of the plasma decay in these leukemic patients is similar to the pattern observed in rabbits infused under same conditions. Indeed, the plasma decay curve of both the free and the complexed DNR have the same triphasic slope. The only differences resulting from infusion of the complex are a significant lengthening of the α-phase and a shortening of the γ-phase; these changes involve only quantitative aspects. Thus these results do not suggest a fundamental difference between use of DNR as free drug and its use as a complex with DNA, finding we would not have expected if the basic mechanism of distribution of the complex were by endocytosis.

Moreover, the plasma DNR-ol level in patients is higher at the end of the infusion of the complexed drug, although lower than the one observed in rabbits [9, 12, 13]. However, this metabolite is only produced after intracellular penetration [3]. Higher DNR-ol plasma levels are likely to result from interaction with circulating DNA, as demonstrated in the rabbit [9, 12, 13].

The low protein binding of both DNR and DNR-ol must presumably play a negligible role in their plasma transport.

We believe that modifications in plasma pharmacokinetics of the DNR complexed drug in patients must be attributed principally to a slow and progressive release of the cytostatic drug from the DNA, as supported by the lengthening of the α-phase. The shortening of the γ-phase is probably due to a reduced diffusion of the complexed drug into the tissues.

Finally, urinary excretion of the cytostatic drug and its metabolite is increased when the drug is infused as a complex with DNA. This is relevant with more sustained plasma levels of the cytostatic drug and its metabolite.

In conclusion, if the DNR-DNA complex is cleared more slowly from the blood stream, the three-compartment kinetic model of DNR infused IV is not basically modified by complexing the drug with DNA. Thus our results do not support the lysosomotropic mechanism postulated earlier. Furthermore, an in vitro study has also failed to confirm the lysosomotropic mechanism for DNR-DNA cellular uptake [14].

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