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Leukemic cell and plasma daunomycin concentrations after bolus injection and 72 h infusion*

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Summary. The effect of the duration of daunomycin (DNM) infusion on leukemic cell drug concentrations was evaluated. Cellular and plasma DNM concentrations were measured in 20 patients with acute non-lymphocytic leukemia. DNM 45 mg/m^2 was administered either as a bolus injection or as a 4-, 8- or 72-h constant-rate infusion during 3 consecutive days. Peak plasma DNM levels amounted to 227 ± 116 ng/ml after bolus injection and were only 16 ± 6 ng/ml after 72-h DNM infusions. Terminal plasma DNM half-lives were 14 ± 4 h. Peak leukemic cell DNM concentrations at the 3rd day of administration were 16810 ± 2580 ng/10^9 cells (bolus injections) and 10310 ± 5510 ng/10^9 cells (72-h infusions). The areas under the cellular curve were similar and independent of the duration of the DNM infusion. Peak leukemic cell daunomycinol (DNMol) concentrations were respectively 3500 ± 1600 ng/10^9 cells and 2850 ± 1720 ng/10^9 cells. Cellular DNM terminal half-life was 13 ± 4 h. DNM concentrations in nucleated blood and bone marrow cells correlated well (r = 0.93, n = 26). Long-term infusion produced less severe side effects. Therapeutic efficacy was maintained during long-term infusion.

Introduction

Daunomycin (DNM) and cytosine arabinoside (Ara-C) have been the main drugs used in the treatment of acute leukemia [13, 19]. DNM has usually been administered as a bolus injection [13], whereas Ara-C has mainly been given as a prolonged infusion. Plasma pharmacokinetic studies have had a limited impact on the way anticancer drugs are administered [11]. In pharmacokinetic studies, plasma DNM and daunomycinol (DNMol) concentrations have not been found useful in estimating the target tissue concentrations [6, 10, 16]. Recent clinical reports suggested improvements of the DNM administration schedule with reduction of the side effects [3, 9]. In this pharmacokinetic study, the effect of bolus injections compared to constant-rate infusions on leukemic cell DNM concentrations was evaluated. Furthermore, drug uptake in different cell types (leukemic blasts, lymphocytes, granulocytes) was determined using laser flow cytometry.

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Patients, materials and methods

Patients. Pharmacokinetic studies were done in 20 patients with acute non-lymphocytic leukemia at first presentation, at relapse or during remission. Patients gave informed consent for participation in these studies. The mean age was 43 ± 8 years (range 17–67 years); seven were female, 13 male. All had normal liver and renal function. Treatment consisted of DNM (45 mg/m^2) on days 1, 2 and 3; Ara-C 200 mg/m^2/day on days 1–7; and vincristine 1 mg/m^2 on day 2. DNM was administered as a bolus injection (< 5 min) in four patients, a 4-h infusion in two patients, an 8-h infusion in three patients and a 72-h infusion in 11 patients. For long-term infusions a Hickman catheter (Evermed, Kirkland, Wa.) and a Syringe Driver MS 26 (Graseby Medical, Watford, UK) were used.

Blood and bone marrow sampling. Venous blood was drawn at regular intervals from 5 min up to 216 h in heparinized polypropylene tubes (Greiner, Nürtingen, FRG) on ice. After 10 min centrifugation (900 *g*, 0° C) plasma was removed and stored at −20° C. After lysis of the erythrocytes with cold ammonium chloride [15] the remaining leukocytes were resuspended in phosphate buffered saline and counted. One aliquot was stored at −20° C until analysis with high-performance liquid chromatography. Another aliquot was kept on ice until flow cytometric determination of cellular DNM + DNMol concentrations in the blast and other cells.

Bone marrow samples were collected in acid citrate dextrose solution (ACD formula A, pH 7.4) on ice. A peripheral blood sample was always taken at the same time to enable comparison of bone marrow and blood cell DNM concentration. Bone marrow samples with more than 20% admixture of peripheral nucleated blood cells were excluded from the study [7]. After lysis of erythrocytes and normoblasts as described above, cells were resuspended, counted and stored until analysis.

Chemicals. DNM was obtained from Specia (Paris, France). Pure DNM, the metabolite DNMol and adriamycin (ADM; internal standard) for chromatographic purposes were generously supplied by Professor F. Arcamone, Farmitalia Carlo Erba (Milan, Italy). All other chemicals

Table 1. Pharmacokinetic characteristics of plasma and cellular daunomycin concentrations

Mode of administration	No. of patients	A Measured (ng/ml)	B Calculated (ng/ml)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (h)	AUC (mg · h/l)	V_f (l)
Plasma							
Bolus	4	227±116	18± 7	20±13	18±7	0.9±0.1	2690±1690
4 h	2	120± 30	28±13	16± 9	9±3	2.0±0.5	
8 h	3	96± 15	26±19	18±10	15±6	2.1±0.9	
72 h	11	16± 6	31±15	12± 6	16±4	1.5±0.3	
Cells		(ng/10^9 cells)	(ng/10^9 cells)				
Bolus	4	16810±2580	10820±3020	35±20	10±4	568± 12	
4 h	2	15240±2070	14681±1800	6± 4	9±3	630± 19	
8 h	3	13740±3040	12180±1770	16± 3	12±2	657±121	
72 h	11	10310±5510	9490±2100	15±12	16±6	616± 22	

Values expressed as mean ± SD
Except for the peak plasma concentrations, no statistically significant ($P < 0.01$) differences were observed between the different ways of DNM administration (two-tailed Student's *t*-test). A, measured peak (5 min after bolus) or maximum (infusion) DNM concentration; B, calculated concentration constant; $t_{1/2\alpha}$, $t_{1/2\beta}$, half-lives; AUC, area under the curve up to 120 h; V_f, distribution volume

used for extraction and chromatographic analysis were of analytical grade and were obtained from Merck (Darmstadt, FRG).

High-performance liquid chromatography. The high-performance liquid chromatography (HPLC) method has been described previously [15]. Briefly, 500 µl plasma or 250 µl sonicated cell suspension was extracted twice with chloroform/methanol 9:1 (v/v). After evaporation of the organic phase, the dry residue was dissolved in 750 µl chloroform/methanol. Then 500-µl aliquots were injected into a straight-phase HPLC system with a 100×3.0 mm column packed with LiChrosorb Silica60. Drug recovery from plasma and cells was approximately 92% [15]. Detection limit was 1 ng. Cellular DNM concentrations were expressed in nanograms per 10^9 cells. For comparison with plasma concentrations, it was assumed that 10^9 cells are equal to 1 ml.

Flow cytometry. As described previously [14] the population of the leukemic blasts can be discriminated by flow cytometry (FCM) on the basis of their scatter characteristics. Simultaneously, cellular DNM + DNMol concentrations can be quantified from the cellular fluorescence. A Cytofluorograf 50H (Ortho Diagnostic Systems, Westwood, Mass.) equipped with a 5 W argon laser was employed, using the 488-nm line running at 500 mW.

Pharmacokinetics. The DNM and DNMol cellular and plasma concentration-time curves were fitted according to a two-compartment open model, and pharmacokinetic data were calculated according to the conventional procedures [18]. The actual measured peak plasma and cell drug concentrations have been given, instead of the calculated values.

Results

Plasma concentrations

The pharmacokinetic characteristics of the concentration-time curves are given in Table 1. The mean peak plasma concentration of DNM after three bolus injections (Fig. 1) was 227±116 ng/ml. A short distribution phase with a plasma half-life of 20±13 min was followed by a disappearance phase with a terminal half-life of 18±7 h. The metabolite DNMol was observed in plasma almost immediately, and at 30 min exceeded the DNM concentrations. DNM and DNMol hardly accumulated at subsequent injections.

In the case of 72-h infusions (Fig. 3), steady-state DNM concentrations were reached at 24 h, whereas plasma DNMol continuously increased during the infusion time. The disappearance rate of DNMol was similar to that of DNM. Mean maximum plasma concentrations during prolonged infusion (4, 8, 72 h respectively, Figs. 2, 3) were reduced to 48% (120±30 ng/ml), 33% (96±15 ng/ml) and 9% (16±6 ng/ml) of the peak plasma value after bolus injection.

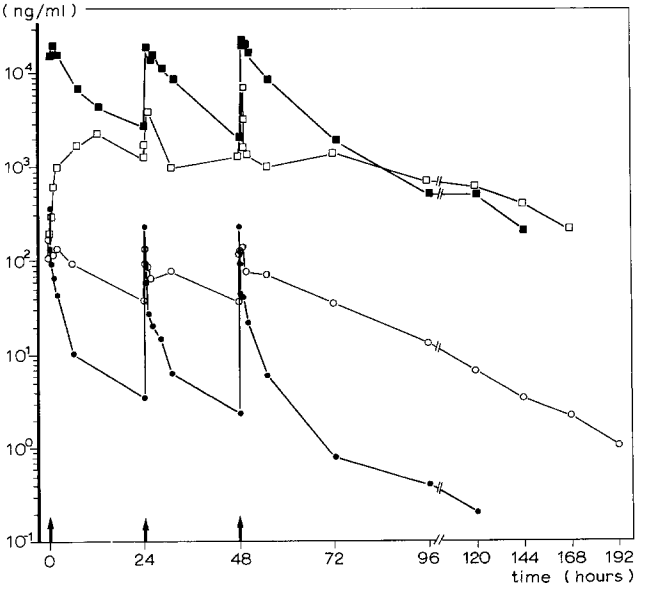

Fig. 1. Cellular (□, ■) and plasma (○, ●) DNM (*closed symbols*) and DNMol (*open symbols*) concentrations in four patients treated with three bolus injections. *Arrows* indicate administration. Mean values are given

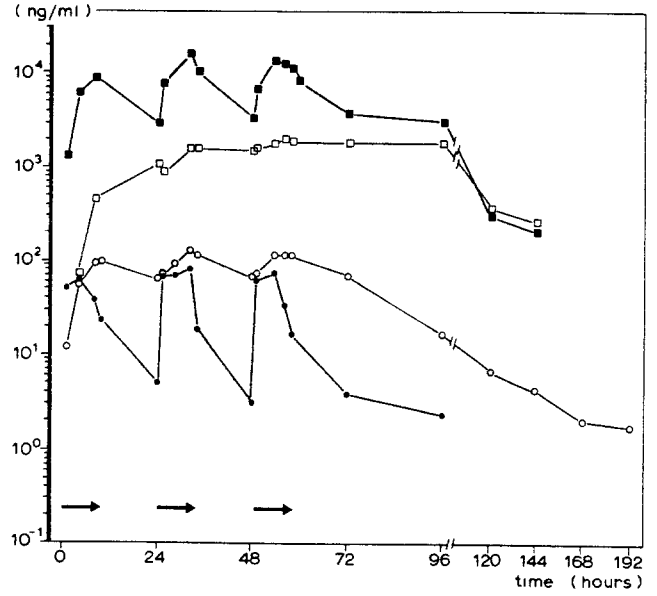

Fig. 2. Cellular and plasma DNM and DNMol concentrations in three patients treated with three 8-h infusions. Symbols as in Fig. 1

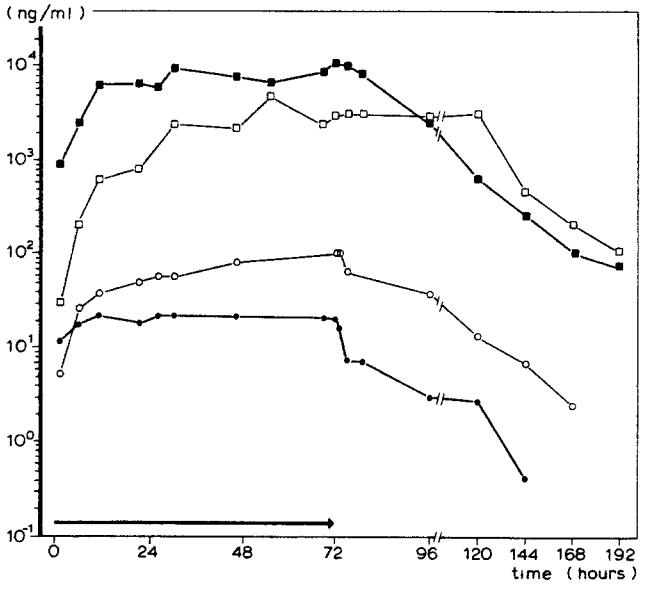

Fig. 3. Cellular and plasma DNM and DNMol concentrations in 11 patients treated with 72-h infusions. Symbols as in Fig. 1

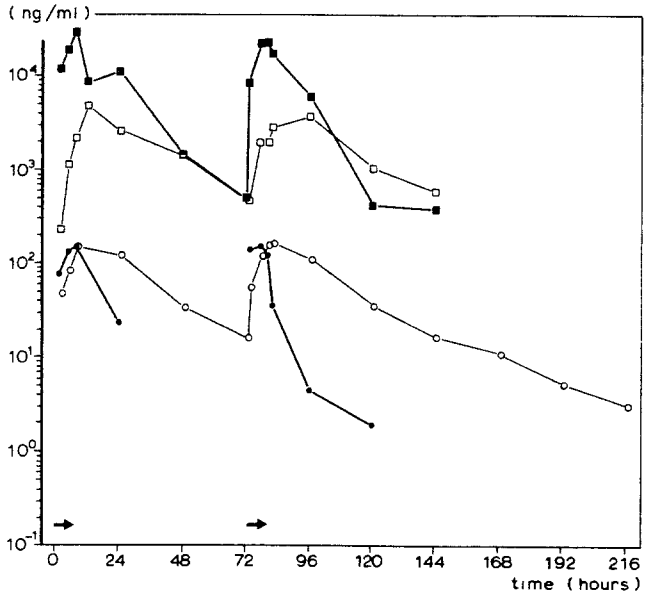

Fig. 4. Cellular and plasma DNM and DNMol concentrations in two patients in whom DNM was administered as an 8-h infusion on days 1 and 4. Symbols as in Fig. 1

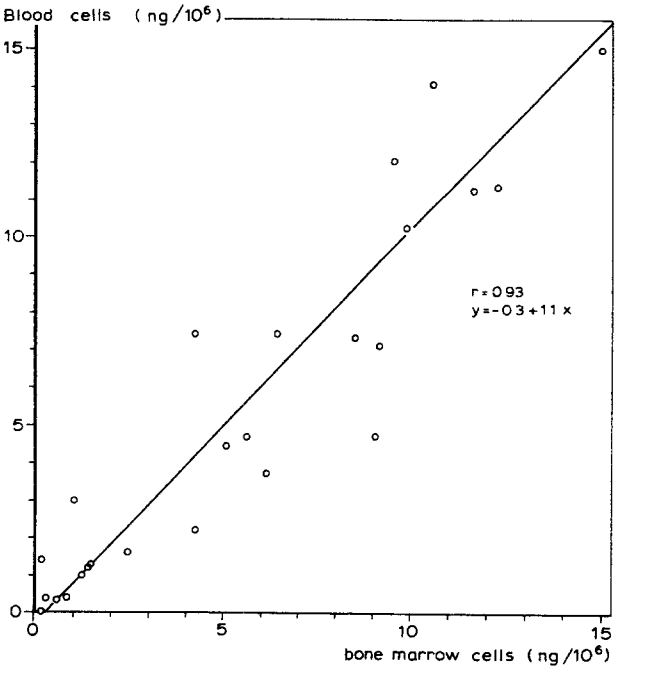

Fig. 5. Correlation between cellular DNM concentrations in nucleated blood and bone marrow cells. Bone marrow and blood samples were collected from the end of the administration to 144 h ($n = 26$, $P < 0.001$)

Cellular concentrations

Maximum cellular DNM concentrations of 16810 ± 2580 ng/10^9 cells were observed at 5 min after the bolus injections. The peak cellular DNM concentrations did not substantially increase after the second and third injections. In the first 2 h after the injection, cellular DNM is released only slowly from the cells. In the next hours the efflux of DNM increased, at a rate similar to the DNM plasma disappearance rate. The DNM efflux pattern was independent of the mode of administration.

DNMol appeared less rapidly intracellularly than did DNM (in contrast to its rapid appearance in plasma) and reached less high cellular concentrations than the parent drug did. Maximum concentrations were 3500 ± 1600 ng/10^9 cells at 6–8 h after bolus injection. DNMol efflux from the cells was slower than that of the parent drug.

The ratio of cellular to plasma concentration was ca. 40 at the end of the bolus injection due to rapid cellular DNM accumulation. This ratio increased to over 500 at 24 h thereafter due to the rapid initial plasma DNM clearance and the slower DNM release from the cells.

The plasma and cellular area under the curve (AUC) values of bolus injections and prolonged infusions showed

no significant differences in relation to the duration of the infusion (Table 1).

Cellular DNM concentrations were determined in two patients to whom DNM was administered at longer intervals. Cellular and plasma DNM concentrations after administration of DNM as 8-h infusion on days 1 and 4 are given in Fig. 4. Although plasma DNM concentrations fell below the detection limit at 24–48 h, cellular DNM concentrations could be demonstrated at up to 72 h after infusion. DNMol persisted both in plasma and in cells.

Bone marrow cell DNM concentrations

Concentrations of DNM in bone marrow cells and peripheral blood cells were determined by HPLC in simultaneously drawn pairs of blood and bone marrow samples. The concentrations observed in nucleated bone marrow cells correlated well with those observed in peripheral blood cells ($r = 0.93$ $n = 26$; Fig. 5). No differences in cellular DNM concentration were observed between leukemic bone marrow cells and normal bone marrow cells of patients in remission.

Leukemic cell DNM and DNMol concentration

Flow cytometry allowed rapid and quantitative determination of DNM and DNMol concentrations in individual leukemic cells. From the fluorescence signal DNM cannot be discriminated from DNMol. Figure 6 gives an example of the course of cellular DNM + DNMol concentrations in a patient with circulating blast cells treated with three bolus injections. DNM + DNMol concentrations were higher in myeloid cells and the two drugs were retained longer than in lymphocytes.

Clinical course

Complete remissions were obtained in seven of 11 patients treated with 72-h infusions. These results were comparable with the complete remission rate observed in a far larger number of patients treated in our center with bolus injections. The time to granulocytopenia ($<0.5 \times 10^9$/l) was 3 ± 2 days and the time to recovery ($>0.5 \times 10^9$/l) was 21 ± 6 days. These findings are also similar to those after bolus injections. In the case of continuous infusion nausea and vomiting were considerably less frequent and intense. No mucositis was observed.

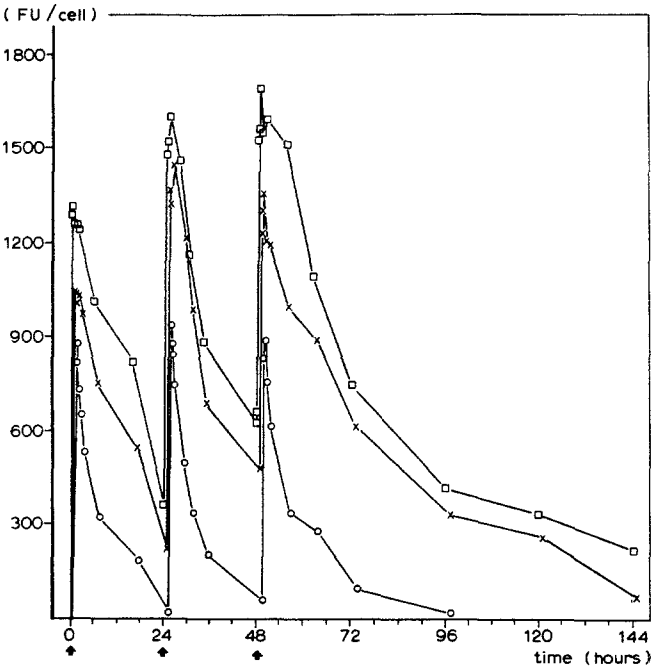

Fig. 6. FCM-determined cellular DNM + DNMol concentrations in subpopulations of blood cells of a patient with acute leukemia treated with DNM bolus injections. ×, blast cells; □, mature granulocytes; ○, lymphocytes. Blood and bone marrow cell concentrations were similar at 24 h and 48 h (not depicted)

Discussion

In this and other studies [6, 10], differences between cellular and plasma disappearance rates of anthracyclines were observed, illustrating the limited value of plasma pharmacokinetic studies.

After a 72-h infusion the maximum plasma value was 8% of the bolus peak concentration; nevertheless, the final cellular concentrations attained were in the same order of magnitude (Table 1). This supports the observed similar antitumor effect after continuous infusion and bolus injection chemotherapy [9]. These results contradict the conclusions, from in vitro experiments, that peak plasma concentrations are essential for the cytotoxic effect [1]. They confirm the prediction that in vivo the total cytotoxic effect of DNM is independent of the schedule of administration [8]. In mice, an improved antileukemic activity was found, dependent on the schedule of treatment. Administration on days 1, 3 and 6 resulted in an increase of life span, compared with DNM monotherapy administered on days 1, 2 and 3 [4]. We found persisting cellular DNM concentrations in the case of administration at larger intervals, on days 1 and 4. This may support the suggestion that also in man, a higher therapeutic index can be obtained by better spacing of the drug administration. Whether long-term infusion selects for increasingly resistant cell types, as has been proposed [12], remains to be determined from the duration of the remission in man.

As in other studies [16, 17], patients treated with prolonged infusions felt better (less or no vomiting). Whether chronic (cardiac) side effects [17] are also reduced as with ADM, remains to be investigated.

Although DNM is administered at 150% of the ADM dose (45 mg/m^2 and 30 mg/m^2 respectively) [5], plasma DNM concentrations were at least 50% lower than peak plasma ADM concentrations due to more rapid DNM distribution into the tissues. The DNM distribution volume is approximately 2 times larger than that of ADM (1450 ± 84 l, $n = 7$). Despite these lower peak plasma concentrations, the observed maximum cellular concentrations of DNM were almost twice as high as those of ADM. Similar results were found in vitro, where cellular concentrations of DNM exceeded those of ADM even at lower medium drug concentrations. Cellular DNM uptake is faster since DNM has higher lipophilicity than ADM [2].

Another remarkable difference between ADM and DNM was the fact that cellular DNM concentrations stayed at their maximum level for 1–2 h, whereas meanwhile the plasma concentrations steeply decreased. From 3 h after the end of the infusion, cellular DNM concentrations decreased, at a faster rate than that observed for ADM. Peterson et al. [10] concluded that DNM has a low-

er affinity for DNA than does ADM, and therefore was retained in the cell for a shorter period than ADM. Drug polarity also influenced the cellular kinetics of DNMol compared to DNM. Due to the lower partition coefficient of DNMol [2], cellular DNMol concentrations were lower than cellular DNM concentrations, whereas in plasma this relation was inverse. The AUC of plasma and of cellular DNMol was approximately 3–5 times larger (plasma) and 3–4 times smaller (cells) than the respective AUC of the parent drug.

In contrast to other authors, who reported a limited number of observations [16], we found a good correlation between DNM concentrations in nucleated bone marrow and those in peripheral blood cells. This might justify sampling of peripheral blood cells to monitor concentration-time curves in bone marrow cells.

It has to be investigated whether "on line" determination of DNM concentrations in blood or bone marrow cells during continuous infusion of DNM can be used to adapt the DNM dose to the patient's individual needs. The maintained efficacy and reduced toxicity of long-term DNM infusion justify further exploration of this mode of administration.

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Note added in proof:
For a comparable pharmacokinetic study of adriamycin concentrations in plasma and cells after bolus injection and 72-h infusion, see accompanying paper: Speth PAJ, Linssen PCM, Boezeman JBM, Wessels HMC, Haanen C (1987) Cellular and plasma adriamycin concentrations in long-term infusion therapy of leukemia patients. Cancer Chemother Pharmacol 20: 305–310

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