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Cellular and plasma adriamycin concentrations in long-term infusion therapy of leukemia patients*

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Summary. To determine whether long-term adriamycin (ADM) infusions resulted in cellular ADM concentrations at least comparable to those observed after bolus injections, ADM cellular and plasma concentrations were measured in 18 patients with leukemia. ADM was administered at 30 mg/m^2 per day for 3 days, either as bolus injections or as 4-, 8-, or 72-h infusions. Negligible accumulation of plasma ADM was observed. Peak plasma ADM concentrations after bolus injections were 1640 ± 470 ng/ml (n = 7). Maximum levels were 176 ± 34 ng/ml during 4-h infusion (n = 5); 85 ± 50 ng/ml during 8-h infusion (n = 4); and 47 ± 5 ng/ml (n = 2) after 72-h infusion. ADM concentrations in nucleated blood and bone marrow cells correlated well (r = 0.82, n = 47). ADM accumulated in leukemic cells up to 30–100 times the plasma concentrations. The shorter the administration time-span, the higher the peak leukemic cell concentration and the greater the loss of drug immediately after the end of the administration. The final cellular ADM half-life was approximately 85–110 h. After long-term infusion and bolus injection of the same dose, similar areas under the curve for plasma or leukemic blast cell ADM concentrations were attained. Since comparable therapeutic efficacy was observed in all regimens, the antileukemic effect appeared not to be related to the peak plasma concentrations, while acute toxicity phenomena decreased with increasing duration of the infusion. Long-term ADM infusion deserves more attention in the treatment of patients with anthracyclines.

Introduction

In the treatment of acute leukemia and many other malignancies, adriamycin (ADM) plays an important role as a potent cytotoxic anthracycline antibiotic [8]. ADM is usually administered as a bolus injection over 1–5 min. Some of its toxic effects (nausea, vomiting and cardiotoxicity) have been related to this rapid administration [13]. In particular, the risk of cardiotoxicity is a therapy-limiting side effect [9] which seems to be related to peak plasma concentrations [13]. Administration of ADM as a continuous infusion reduces the maximum plasma concentration, which has allowed treatment up to higher cumulative dosages [13].

The purpose of this study was to determine whether continuous infusion and bolus injection resulted in similar leukemic cell ADM concentrations. Cellular and plasma ADM concentrations were studied as a function of the duration of continuous infusion on 3 subsequent days in patients with leukemia.

Patients, materials, and methods

Patients. In all, 18 patients entered this pharmacokinetic study, 15 with acute nonlymphocytic leukemia, 2 with blastic transformation of chronic myeloid leukemia, and 1 with end-stage Hodgkin's disease. The mean age was 44 ± 17 years (range 17–67 years); there were 8 female and 10 male patients. All had normal liver and renal function. All gave informed consent for participation in these studies.

The patients were treated with a remission induction regimen consisting of ADM (30 mg/m^2) on days 1, 2 and 3, vincristine 1 mg/m^2 at day 2, and cytosine arabinoside 200 mg/m^2 per day on days 1–7. ADM was administered as a bolus injection in 7 patients, a 4-h infusion in 5 patients, an 8-h infusion in 4 patients, and a 72-h infusion in 2 patients. In case of 72-h infusions, a central venous catheter was used to avoid the risk of extravasation. A Syringe Driver MS 26 (Graseby Medical, Watford, UK) was used for infusion.

Since in animal studies an increase in the life-span of tumor-bearing animals had been observed following ADM administration at wider intervals than in the conventional schedule of ADM administration on days 1, 2 and 3 [3], the cellular ADM concentration-time curve was also determined in patients to whom ADM was administered at longer intervals. In one patient with acute leukemia in relapse ADM was administered on days 1, 5 and 9 as bolus injections and in another, as 8-h infusions.

Blood and bone marrow sampling. From 5 min up to 240 h after the start of administration venous blood was drawn into heparinized polypropylene tubes (Greiner, Nürtin-

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gen, FRG) on ice. Blood was sampled frequently from at least 2 patients treated according to each regimen. From the other patients samples were only drawn at the expected times of peak and nadir concentrations. A bone marrow aspirate was obtained at one of these times. After 10 min centrifugation (900 *g*, 0° C) plasma was removed and stored at −20° C. Erythrocytes in the pellet were lysed with cold ammonium chloride [19]. Recovery of the leukocytes after the lysis step was 80%–95%. The leukocytes were resuspended in phosphate-buffered saline and counted. One aliquot of the suspension was stored at −20° C until analysis with high-performance liquid chromatography (HPLC). Another aliquot was kept on ice until determination of the cellular ADM concentration with flow cytometry.

Bone marrow samples were collected in acid citrate dextrose solution (ACD formula A, pH 7.4). At the same time a blood sample was also taken to compare bone marrow and blood cell ADM concentrations. The admixture of peripheral nucleated blood cells in the bone marrow samples was calculated [12] to allow exclusion from the study of samples with more than 20% blood admixture. After lysis of erythrocytes and normoblasts as described above, cells were resuspended, counted, and stored until analysis.

Chemicals. ADM was obtained from Laboratoire Roger Bellon S. A. (Neuilly-sur-Seine, France). Pure ADM, the metabolite adriamycinol (ADMol), and daunomycin (internal standard) for chromatographic purposes were generously supplied by Professor F. Arcamone, Farmitalia Carlo Erba (Milan, Italy). All other chemicals used for extraction and chromatographic analysis were of analytical grade and were obtained from Merck (Darmstadt, FRG).

HPLC. The method has been described previously [20]. Briefly, 100 µl Tris buffer (pH 8.8, 1 *M*) containing daunomycin as internal standard was added to 500 µl plasma or 250 µl sonicated cell suspension. Following two extractions with chloroform/methanol 9:1 (v/v), the organic phase was evaporated under air at 35° C. The dry residue was dissolved in 750 µl chloroform/methanol. Aliquots (500 µl) were injected into a straight-phase HPLC system with a 100 mm × 3.0 mm column packed with LiChrosorb Silica60 (Chrompack, Middelburg, The Netherlands). Drug recovery from plasma and cells was approximately 92%. The detection limit of ADM and ADMol was 1 ng. Cellular ADM 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. Cellular ADM uptake in different subpopulations of hematopoietic cells was determined with flow cytometry (FCM), as described previously [19]. In brief, cells in suspension passed the 488-nm laser beam of a Cytofluorograf 50H flow cytometer (Ortho Diagnostic Systems, Westwood, Mass), running at 500 mW. Forward and perpendicular light scatter signals enabled discrimination of lymphocytes, blast cells, monocytes and granulocytes. Simultaneously, ADM concentrations in individual cells could be quantified owing to its fluorescent property.

Pharmacokinetics. The ADM and ADMol 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 [22]. However, especially after ADM infusions and bolus injections, the calculated A-value overestimated the real peak concentrations or could not be calculated very reliably. Therefore, measured peak ADM concentrations are given instead of calculated A-constant values. In a pilot study the concomittant drug treatment with vincristine and cytosine arabinoside did not appear to influence the pharmacokinetics of ADM.

Table 1. Cellular and plasma pharmacokinetic parameters after the third of three administrations of adriamycin

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	7	1640±470	27±14	4±2	22± 9	2.3±0.5	1450±84
4 h	5	176± 34	13± 3	2±1	45± 3	3.2±0.8	
8 h	4	85± 50	31±10	2±1	31±11	2.0±1.3	
72 h	2	47± 5	38± 9	5±2	58± 8	2.5±0.7	
Cells							
		(ng/10^9 cells)	(ng/10^9 cells)				Plateau-conc. ng/10^9 cells
Bolus	7	11070±1490	7520±1490	10±8	111±32	718± 51	8605± 675
4 h	5	9350±4260	8040± 120	14±3	72± 3	755±128	7430±2820
8 h	4	8820±1580	7720± 270	15±3	90±20	771±128	5890±1581
72 h	2	8840± 820	8457± 720	6±2	55±12	752±191	8920±1340
							7710±1190

All values given are means ± SD

A, concentrations measured at 5 min after administration; B, calculated concentration constant; $t_{1/2\alpha}$ and $t_{1/2\beta}$, half-lives; AUC, cumulative area under the curve up to 120 h; V_f, distribution volume. "Plateau concentration" refers to the cellular ADM concentration, measured at approximately 1 h after the end of the third administration, when the rapid first phase of the half-life has ended. Peak plasma values differ significantly ($P < 0.01$) for the different routes of administration (two-tailed Student's *t*-test). Peak cellular concentrations and plasma and cellular AUC do not differ significantly. $t_{1/2\beta}$ is different for cells and plasma ($P < 0.01$), except at 72 h (too few values)

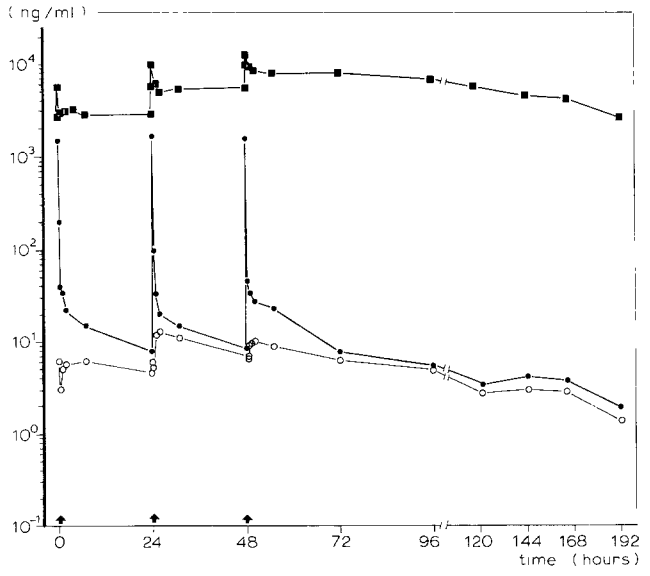

Fig. 1. Cellular (■) and plasma (●) ADM concentrations in 7 patients treated with three subsequent bolus injections. (○) Plasma adriamycinol. *Arrows* indicate time of administration. Mean values are given. SD was 5%–35%, with large interindividual differences

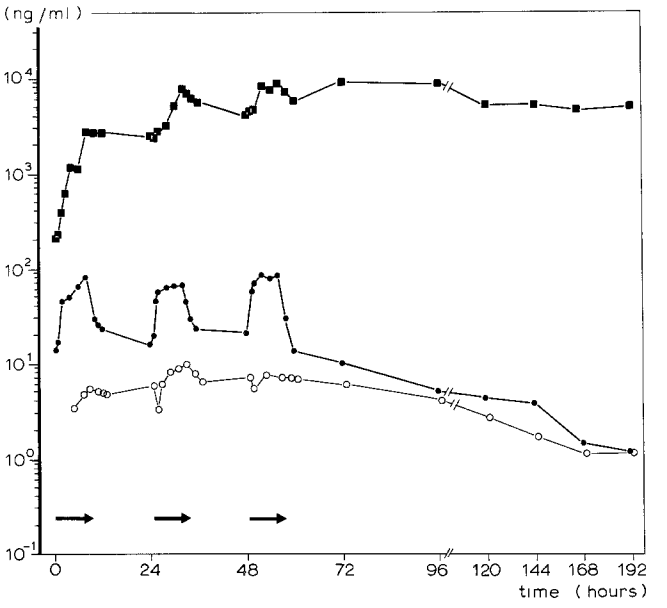

Fig. 2. Cellular and plasma ADM concentrations in 4 patients treated with 8-h infusions on 3 subsequent days. Symbols as in Fig. 1

Results

Plasma concentrations

Plasma concentrations measured at 5 min after the bolus injections (Fig. 1) were already declining and amounted to 1640 ± 470 ng/ml. In one patient, from whom blood was sampled every 30 sec during and shortly after a 1 min bolus injection, a peak plasma value of 9980 ng/ml at 90 s was observed. The first half-life phase was approximately 5 min. The second half-life phase was 22 ± 19 h, with plasma concentrations of less than 50 ng/ml a few hours after administration. Peak and nadir plasma ADM concentrations did not increase substantially on subsequent days of administration. The metabolite ADMol was soon detectable in plasma, was always less than 40 ng/ml, and seldom exceeded the concentration of ADM. Pharmacokinetic characteristics of the concentration-time curves after three injections are given in Table 1.

In the case of long-term infusions (4, 8, 72 h), the maximum plasma concentrations were reduced to approximately 12% (172 ± 34 ng/ml; data not shown), 5% (85 ± 50 ng/ml: Fig. 2) and 3% (47 ± 5 ng/ml: Fig. 3), respectively, of the mean peak plasma values after bolus injection. The plasma AUC-values were comparable for the bolus injection versus the different infusion-times. The plasma ADMol-AUC was approximatelly 20%–35% of the ADM-AUC.

Cellular concentrations

At 5 min after the first bolus injection (Fig. 1), peak cellular ADM concentrations were 5740 ± 2100 ng/10^9 cells. During the next 15–30 min a rapid efflux was observed, until a plateau was reached at 3080 ± 830 ng/10^9 cells. The final cellular ADM half-life was approximately 100 h. Peak cellular ADM concentrations after the second and the third injections were 7680 ± 1900 and 11070 ± 1490 ng/10^9 cells, respectively. Similar patterns of stepwise increase were observed with 4-h, 8-h (Fig. 2) and 72-h (Fig. 3) duration of administration, although peak concentrations were lower with longer infusion times.

With increasing duration of infusion, loss of cellular ADM after the end of the infusion was diminished. Compared with the peak cellular concentrations, the initial loss after bolus injection was 37% ± 19%; after 4-h infusion, 18% ± 14%; and after 8-h infusion, 12% ± 22%; almost no immediate loss was observed after the 72-h infusion. Shortly after the end of the treatment, cellular ADM concentrations were 7710 ± 1190 ng/10^9 cells, regardless of the duration of the infusion. Considerable interindividual differences were noted. Cellular ADMol was only rarely observed at concentrations just above the detection limit,

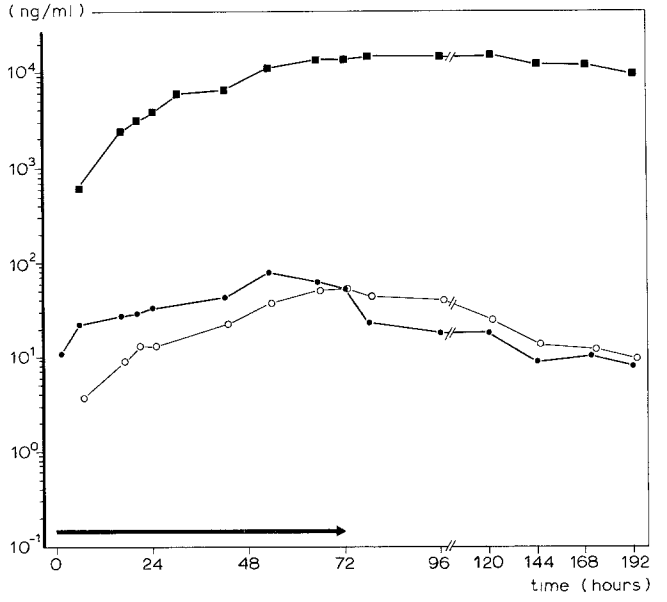

Fig. 3. Cellular and plasma ADM concentrations in 2 patients treated with 72-h infusions. Symbols as in Fig. 1

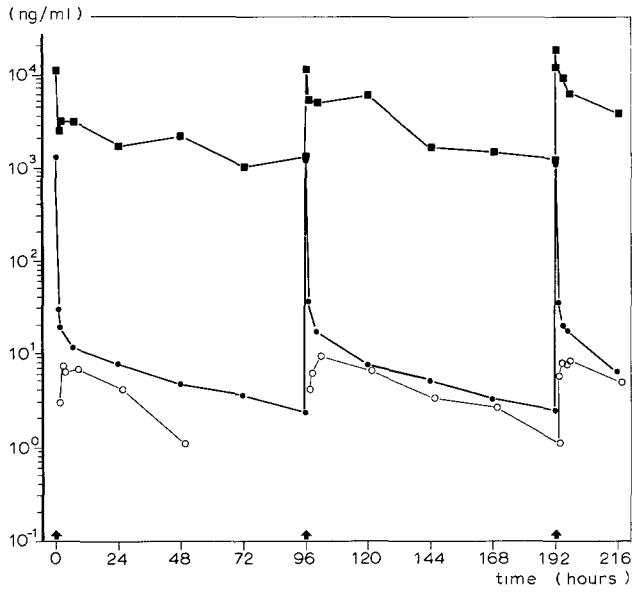

Fig. 4. Cellular and plasma ADM concentrations in a patient treated with adriamycin bolus injections on days 1, 5 and 9. Symbols as in Fig. 1

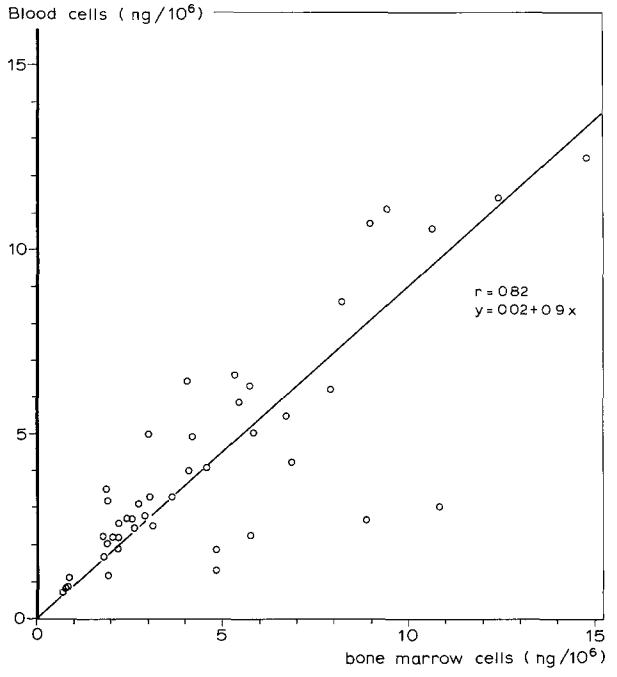

Fig. 5. Correlation between cellular ADM concentrations in nucleated blood and bone marrow cells. Bone marrow and blood samples were collected simultaneously from 5 min to 240 h after administration ($n = 47$, $P < 0.001$)

despite the fact that the plasma AUC of ADMol reached 20%–35% of the AUC of ADM. The last measured point was at least 2–4 days after the last injection in all patients. No other fluorescent metabolites were ever observed.

The ratio of cellular to plasma concentrations increased from 3.7 at the peak after the bolus injection up to over 1000 by 72–96 h. During the 72-h infusion cellular ADM concentrations continously increased to over 150 times the plasma concentration. Thereafter, ADM was retained to a considerable extent, while the plasma concentrations rapidly dropped.

When ADM was administered on days 1, 5 and 9 (Fig. 4), ADM was retained intracellularly and also accumulated during each 4-day interval.

Comparison of the AUC values after bolus injections and continuous infusions for the patients from whom blood was sampled frequently revealed wide interindividual differences in plasma AUCs or cellular AUCs. However, no important differences between the different modes of administration were noted.

Cellular ADM concentrations in bone marrow cells

Cellular ADM concentrations were measured by HPLC in bone marrow and peripheral blood cells of simultaneously obtained samples taken at various times after administration. The ADM concentrations for bone marrow and peripheral blood nucleated cells correlated well ($r = 0.82$, $n = 47$; Fig. 5). Bone marrow cells contained approximately 10% more ADM than nucleated blood cells. No differences in ADM concentrations were observed between bone marrow from patients with overt leukemia or from patients in complete remission (data not shown). Comparable ADM concentrations were measured in bone marrow and blood cells obtained at 5 min, 1 h, and 2 h after ADM injection. This indicates a rapid penetration of ADM in the bone marrow cells.

ADM in subpopulations

FCM determination of cellular ADM concentrations in subpopulations of blood and bone marrow cells revealed high concentrations in granulocytes, monocytes and blast cells, whereas lymphocytes contained less ADM per cell (Fig. 6). From the limited amount of data available thus far, no trend could be detected between successful remission induction and blast cell ADM concentrations at the end of the infusion (parameter for influx) or at the trough 24 h thereafter (as a parameter for efflux).

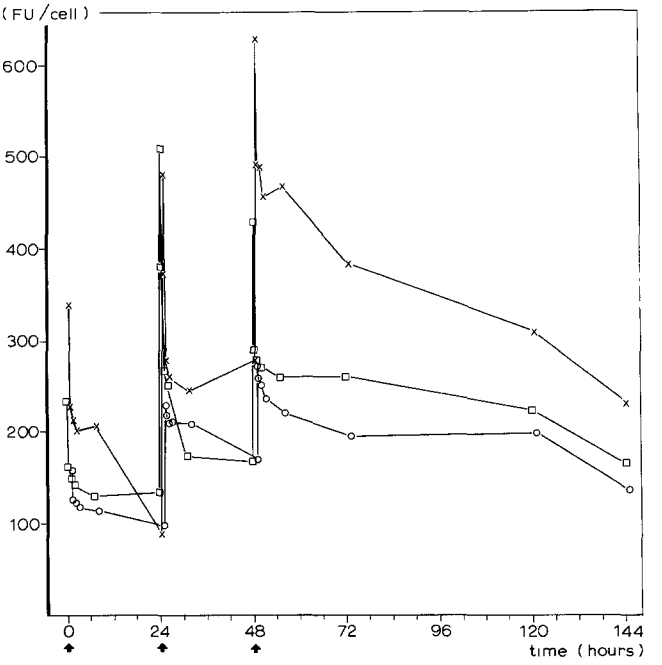

Fig. 6. FCM-determined cellular ADM concentrations in subpopulations of blood cells of a patient treated for acute non-lymphocytic leukemia. ×, blast cells; □, mature granulocytes; O, lymphocytes. Concentrations in blood and bone marrow cells (not depicted) were similar at 24 h and 48 h

Clinical outcome

In this small group of patients complete remissions were obtained in 4 of 5 patients treated with bolus injections and in 7 of 9 evaluable patients with acute leukemia treated with constant-rate infusion. We observed less nausea and vomiting in the case of long-term infusions. Mucositis was observed in 1 patient treated with 72-h infusion. Alopecia was always complete. Leucocyte and thrombocyte nadirs, as well as recovery of the cell counts were similar in case of bolus injections and long-term infusions.

Discussion

The study of plasma pharmacokinetics of antineoplastic drugs has improved our understanding of their mechanism of action, but only in a few cases has it contributed to the design and improvement of drug scheduling, which is still mostly empirically based. Concentrations in the leukemic cells are considerably different from the plasma concentrations [4, 7] and certainly more relevant to the cytotoxic effect. ADM concentrations have not been determined in the target cells in the evaluation of new therapy schedules in which continuous infusions are applied to reduce (cardio)toxicity or enhance the efficacy [2, 15]. In the design of better tolerated therapeutic schedules, our data are in favor of long-term ADM infusion instead of bolus injection. The usage of bolus injections originates mainly from logistic arguments and is not based on (cellular) pharmacokinetic data. The plasma ADM concentrations reported here are in good agreement with the low plasma ADM concentrations predicted in the case of long-term infusions [7, 11].

Although during 72-h infusion the maximum plasma value is only 3% of the peak concentration after bolus injection, the final cellular concentrations attained were in the same range, irrespective of the mode of administration. This may explain why the antitumor efficacy was independent of the mode of administration, as was observed in the case of continuous infusion chemotherapy for leukemia [14] and slowly proliferating tumors [2, 9, 18].

The absence of fluorescent cellular ADM metabolites may in part be attributed to the fact, that the known metabolites are more hydrophilic than ADM. As we observed in experiments in vitro, their penetration into cells is considerably less at equimolar concentrations in medium.

An intriguing phenomenon in the cellular ADM pharmacokinetics is the short-lasting high cellular ADM concentration immediately after bolus injections, followed by a considerable loss of up to over 40% in the first hour thereafter. This is a great contrast to the near-absence of loss of cellular ADM after the end of long-term infusions. It also is considerably different from the efflux process observed for daunomycin (21), although the chemical structures of the two compounds hardly differ. Although the nuclear membrane is not considered to be a barrier to nuclear ADM uptake [5], intercalation in DNA is apparently a time-consuming process. When plasma concentrations dropped below the cytoplasmatic concentration a few minutes after injection, the loosely bound and probably cytoplasmatic ADM fraction leaked out of the cell. DOX fraction ($\pm 40\%$) leaked out of the cell. As the duration of the exposure to (lower plasma concentrations of) ADM increased during infusions, proportionally more of the drug was rapidly tightly bound to DNA and the loss of ADM from the cell was considerably less (zero to 18%) after the end of the exposure. These observations may lead to the hypothesis, that ADM uptake is a process with different rates with which the drug crosses the cellular and the nuclear membrane, subsequently. We observed similar efflux processes in experiments in vitro (data not shown). Peak medium ADM concentrations appeared not to be essential for the cytotoxic effect, as was also observed in different cell lines [6, 10]. This, however, is in contrast with what has been concluded from other in vitro experiments with ADM [16] or daunomycin [1].

The correlation between ADM concentrations in nucleated bone marrow and peripheral blood cells seems to justify monitoring of ADM concentrations in peripheral blood cells, as a substitute for the more invasive method of determination of ADM concentration in the bone marrow cells.

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