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Cellular pharmacokinetics of doxorubicin in patients with chronic lymphocytic leukemia: comparison of bolus administration and continuous infusion

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Abstract. The purpose of this study was to determine whether administration of doxorubicin (DOX) as a continuous infusion or a bolus injection resulted in similar leukemic cell drug concentration in patients with refractory chronic lymphocytic leukemia (CLL). This study was carried out on five patients with refractory CLL, with DOX administered either as a bolus injection (35 mg/m^2; CHOP protocol) or as a constant-rate infusion for a period of 96 h (9 mg/m^2 per day; VAD protocol). The two types of drug administration were used alternatively with the same patient. Plasma and cellular DOX concentration were determined using high-performance liquid chromatography. Peak plasma DOX levels were higher after the bolus injection than after continuous administration (1509 ± 80 ng/ml vs 11.6 ± 1.8 ng/ml, respectively), whereas the plasma area under the curve (AUC) levels were similar. Maximum DOX cellular concentrations were 8629 ± 2902 ng/10^9 cells (bolus injection) and 2745 ± 673 ng/10^9 cells (96 h infusion). The cellular AUC after the bolus injection was 2.85 times greater than that observed after continuous administration. This difference was due to a higher cellular peak level followed by a relatively prolonged retention of the drug, with a loss of only 25% in the first 24 h following. These findings demonstrated that in CLL the cellular DOX exposure can be notably modified by the method of drug administration, with higher drug intracellular concentrations being achieved after bolus administration than with the infusion schedule.

Introduction

Doxorubicin (DOX) is widely used in the treatment of lymphoid neoplasias. In non-Hodgkin malignant lymphomas (NHML), DOX-based regimens significantly improved both response and survival rate as compared to first-generation COP (cyclophosphamide, vincristine, prednisone) or COP-derived protocols (see [4] for review). In advanced chronic lymphocytic leukemia (CLL), DOX remains a potentially useful drug even if it is now challenged by new drugs such as fludarabine [11] and 2-chlorodeoxyadenosine (2DCA, [16]). DOX is usually administered as a bolus injection over a 1–5 min period in doses ranging from 25–75 mg/m^2, in association with vincristine, cyclophosphamide, and prednisone, according to CHOP or CHOP-derived regimens [6, 7, 13].

It has been argued, however, that some DOX-related side effects (including cardiotoxicity) could be related to the peak plasma concentrations [9, 12]. Therefore, some investigators have suggested that DOX be preferentially administered as a continuous infusion [12]. Subsequently, a number of protocols based on DOX continuous infusion have been developed. Among these protocols, VAD (vincristine-doxorubicin-dexamethasone) or VAD-derived regimens have been used in multiple myeloma [2] as well as in refractory CLL and NHML [14, 21].

Despite the clinical evidence that VAD represents an efficient protocol, it remains uncertain whether continuous infusion represents the most appropriate method of DOX administration in terms of DOX-induced anti-tumor cytotoxicity. Indeed, previously reported in vitro studies emphasized that both the magnitude of drug cellular exposure and peak concentration are essential for DOX cytotoxicity [1, 8, 15]. These findings strongly suggest that, in vivo, the modality of drug administration may not only profoundly influence the cellular pharmacokinetic parameters but could also modify its anti-tumor activity.

The purpose of this study was to determine whether continuous infusion (VAD) and bolus injection (CHOP) resulted in similar leukemic cell DOX exposure in refractory CLL patients with DOX being administered in the same total dose.

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Table 1. Patient characteristics[a]

Patient	Age	Hb (g/dl)	Lymphocytes ($\times 10^9$/l)	Platelets ($\times 10^9$/l)	Generalized lymphadenopathy	Previous treatment
1	62	12	140100	146000	+	CLB, COAP
2	70	9.5	35000	102000	+	CLB, COAP
3	66	11.6	95000	112000	+	CLB+PDN, COAP
4	50	11.2	96700	160000	+	CLB, COAP
5	67	10.2	140000	104000	+	CLB+PDN, COAP

CLB, Chlorambucil; PDN, prednisone; COAP, cyclophosphamide, vincristine, aracytine, prednisone
[a] At time of the initiation of therapy

Patients, materials and methods

Patients. This study was carried out on five patients with B-CLL (Table 1). Patients were in advanced stages of the disease, corresponding to group B or C according to the Workshop classification [3]. They were refractory to prior therapy with chlorambucil, COP (cyclophosphamide, vincristine, and prednisone), or COAP (COP + cytosine-arabinosine) protocols. None of the patients had received DOX prior to the study.

Exclusion criteria were: age above 70 years, performance status (WHO grading) above 2, isotopic ventricular ejection fraction less than 60%, leukocytes <20000 mm^3, and platelets <100000 mm^3. Informed consent was obtained from each patient in accordance with institutional policy.

Drugs and drug administration. For the VAD protocol, DOX (9 mg/m^2 per day) was administered over 96 h by means of an indwelling central venous catheter connected to an external pumping device. Additional treatment consisted of vincristine (0.4 mg/m^2 per day) and 40 mg dexamethasone orally, both administered from day 1 to day 4.

For the CHOP protocol, DOX (36 mg/m^2) was adminstered on day 1 over a 5-min period using an indwelling central venous catheter connected to an electric syringe. Additional therapy consisted of cyclophosphamide (750 mg/m^2); vincristine (2 mg/m^2 on day 1), and prednisone (40 mg/m^2 from day 1 to day 5).

The treatment always began with a CHOP course and courses were repeated at 40-day intervals. Each patient underwent at least two courses of treatment, and treatment was interrupted if there was no response or if major toxic effects were observed. A total of 14 cycles (7 CHOP, 7 VAD) were performed.

Blood sampling. Venous blood was drawn at regular intervals from 5 min up to 216 h in ethylenediaminetetraacetate – containing tubes and placed on ice immediately to prevent further DOX loss. After centrifugation, plasma was collected and frozen at –20° C until analysis. Mononuclear cells were obtained by separation on Ficoll-Hypaque, and the pellet of mononuclear cells was diluted in 1.1 ml 0.9% saline solution. The number of cells was determined and the sample was frozen at –20° C until analysis. The entire procedure was carried out at 4° C. After Ficoll separation, the mononuclear cells of CLL patients consisted of B lymphocytes (more than 95%) with a restricted CD5/CD19 membrane phenotype. This allowed the determination of DOX concentration in a homogenous tumor cell population using high-performance liquid chromatography (HPLC).

Chromatographic determination of the plasma and cellular DOX concentration. Plasma or cellular DOX and doxorubicinol (its main metabolite; DOXOL) concentrations were determined by the HPLC method with fluorescence detection as described elsewhere [5]. The chromatographic system consisted of a Waters model M590 solvent pump, a WISP 710B sample injector (Waters) packed with a μBondapak Waters C18 column (300 by 3.9 mm inside diameter), a guard column identical to the loop column, and a Shimadzu RF 535 fluorescence detector. After the addition of daunorubicin as an internal standard and 1.5 ml of 0.05 M Borate buffer (pH 9.8), 1 ml of plasma or 1 ml of cell suspension was extracted by 5 ml of chloroform-methanol (4/1). After centrifugation at 1000 *g*, the organic phase was removed and evaporated to dryness under nitrogen stream. The residue was reconstituted with 200 μl of mobile phase and 20 or more μl were injected into the column eluted by a mobile phase (1 ml/min) consisting of: 0.05 *N* H_3PO_4, acetonitrile, tetrahydrofurane, and triethylamine (59.8/35/5/0.2) pH 2.5. The peaks of DOX (retention time = 4.8 min), DOXOL (retention time = 3.9 min), and daunorubicin (retention time = 5.8 min) were detected by fluorescence (1 exc: 478 nm; 1 em: 550 nm). In these conditions, the limit of detection (signal-to-noise ratio of 3 : 1) of the various products was 1 ng/ml. Extraction ratios ranged from 75% to 85% and the coefficients of variation for a 1-ng/sample were 10.4% and 12.6% for doxorubicin and doxorubicinol, respectively. For a 10-ng/sample, the coefficients of variation were 6.9% and 8.7% for doxorubicin and doxorubicinol, respectively.

Cellular DOX concentration was expressed in nanograms per 10^9 cells. For comparison with plasma concentrations, it was assumed that 10^9 cells = 1 ml. The two cycles of chemotherapy were analyzed during the same analysis for the same patient.

Computer modeling of the decline of DOX in plasma was performed with Siphar, a computer program which performs iterative, weighted, non-linear, least-squares regression. The cellular area under the concentration curves of DOX was estimated by the trapezoidal method for 216 h and was not extrapolated to infinity. The pharmacokinetic parameters obtained according to the modality of administration for each patient were compared by means of the ANOVA two-way test.

Results

Plasma pharmacokinetics

The pharmacokinetic profiles of the concentration-time curves of DOX are shown in Fig. 1 for the two methods of administration.

After the bolus injection, the mean peak plasma concentration reached 1509 ± 80 ng/ml. The concentration declined triexponentially with a terminal half-life of 39.5 ± 10.4 h (Table 2).

After continuous infusion, steady-state DOX concentrations were reached between 24 and 48 h and averaged 11.6 ± 1.8 ng/ml, wich corresponds to 0.7% of the peak plasma value obtained after bolus injection. After the end of the infusion, the plasma disappearance of DOX was biexponential with a terminal half-life of 44.6 ± 8.5 h.

As shown in Table 2, except for the peak plasma concentrations the plasma pharmacokinetic parameters showed no significant differences (P <0.05) as a function of the duration of infusion. Similarly, the plasma pharmacokinetic parameters of doxorubicinol (including plasma AUC values) were not influenced by the modality of DOX administration (data not shown).

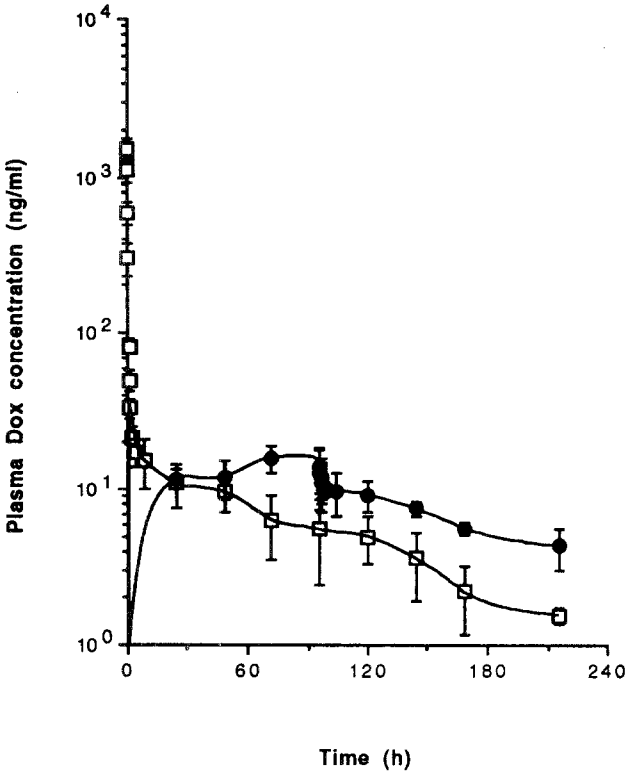

Fig. 1. Plasma DOX concentrations in five patients (14 cycles) treated with bolus injection (□) or continuous infusion (●). Mean and SD values are given

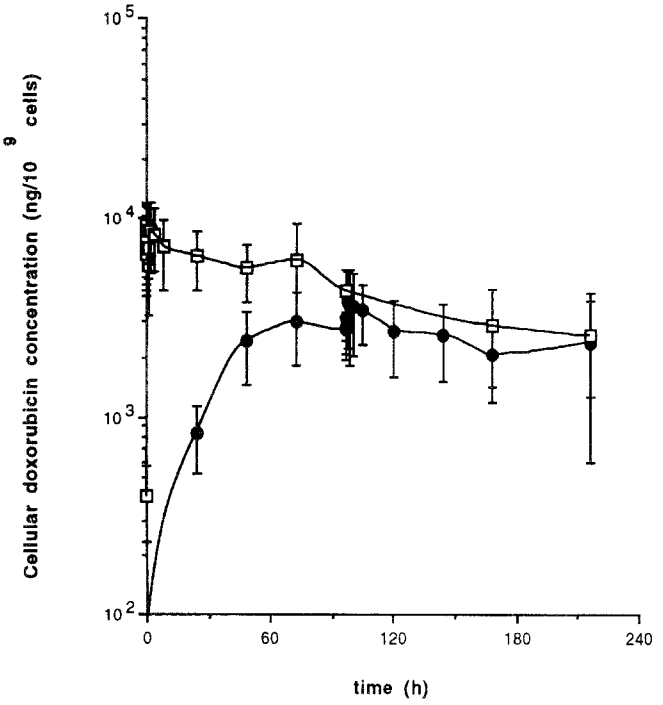

Fig. 2. Cellular DOX concentrations in five patients (14 cycles) treated with bolus injection (□) or continuous infusion (●). Mean and SD values are given

Table 2. Plasma pharmacokinetic parameters

Protocol	$t_1/2\gamma$ (h)	AUC (μg/l × h)	Cl (l/min/m2)	V_d ss (l/m^2)
CHOP	39.5 ± 10.4	1596 ± 509	22.4 ± 7.1	1250 ± 486
VAD	44.6 ± 8.5	1466 ± 507	24.5 ± 7.1	1360 ± 547

Values expressed as mean ± SD.
No statistically significant differences (P <0.05) were observed between the differnt routes of DOX administration (Anova two-way test).
$t_{1/2\gamma}$, Terminal half-life; AUC, area under the curve; Cl, total body clearance; V_d ss, volume of distribution at steady state

Cellular pharmacokinetics

Maximum cellular DOX concentrations of 8629 ± 2902 ng/10^9 cells were observed at the end of the bolus injection (Fig. 2). This high intracellular DOX concentration was maintained over 75% of the initial concentration for up to 24 h afterwards. The mean cellular DOX half-life was 114 ± 57 h.

During the 96-h injection, DOX cellular concentrations increased continuously (Fig. 2) to over 325 times the plasma concentrations, and amounted to a maximal concentration of 3779 ± 1577 ng/ml. The final DOX half-life was 103 ± 38 h. No doxorubicinol was detected (limit of quantification: 1 ng/10^9 cells).

As shown in Table 3, the mean cellular AUC after bolus injection was 2.85 ± 2.35 times higher than that measured after continuous infusion. This difference was even greater when a second sequence of treatment was administered (see below). While there were no differences in the plasma AUC, the ratio of cellular to plasma AUC was greater for the bolus injection (P <0.05).

As described in earlier works [18–20], our study revealed wide interindividual variations in plasma AUC and cellular AUC. However, as shown in Table 3, the cellular AUC achieved in CHOP cycles was significantly greater than that achieved in VAD cycles in each of the patients studied.

Evolution of cellular pharmacokinetic parameters during time-course administration of the two protocols

As shown in Table 3, the cellular and plasma pharmacokinetic parameters have been studied in two patients for one additional CHOP/VAD cycle ($CHOP_2/VAD_2$).

For the two patients studied [2, 4], the ratio between cellular AUCs ($CHOP_2/VAD_2$) was 7.86 and 3.64, respectively, in favor of bolus injection. These values were higher than those measured in the $CHOP_1/VAD_1$ cycle (1.77 and 1.56, respectively). The increase in the $CHOP_2/VAD_2$ cellular AUC ratio compared with the $CHOP_1/VAD_1$ cellular AUC ratio was mainly due to a decrease in the cellular AUC in VAD_2 as compared to VAD_1, especially for patient 2 for whom the diminution was almost eightfold. However, while the plasma AUCs (VAD_1/VAD_2) tended to increase, in both patients the ratio of cellular to plasma AUC dramatically decreased for the second cycle of VAD administration.

Table 3. Cellular and plasma pharmacokinetic parameters

Patient	Protocol	Cell AUC µg/10⁹cell × h	Plasma AUC µg/l × h	Cell AUC/plasma AUC (%)	AUC CHOP/AUC VAD
1	$CHOP_1$	189200	4727	40	
	VAD_1	105500	1520	69	1.79
2	$CHOP_1$	1822000	1540	1183	
	VAD_1	1026000	1401	732	1.77
	$CHOP_2$	1022000	926	1103	
	VAD_2	130000	2355	55.2	7.86
3	$CHOP_1$	769300	1660	463	
	VAD_1	709800	1275	556	1.08
4	$CHOP_1$	379600	964	393	
	VAD_1	243400	808	260	1.56
	$CHOP_2$	522600	1633	320	
	VAD_2	143520	1807	79.4	3.64
5	$CHOP_1$	390620	2381	164	
	VAD_1	175040	2007	87.2	2.23
Mean ± SD	CHOP	740086±542661	1596±509	523±446	
	VAD	361886±360151	1466±507	262±274	2.85±2.35
Anova two-way		$P<0.05$	NS	$p<0.05$	

Cell, cellular; AUC, area under the curve; SD, standard deviation

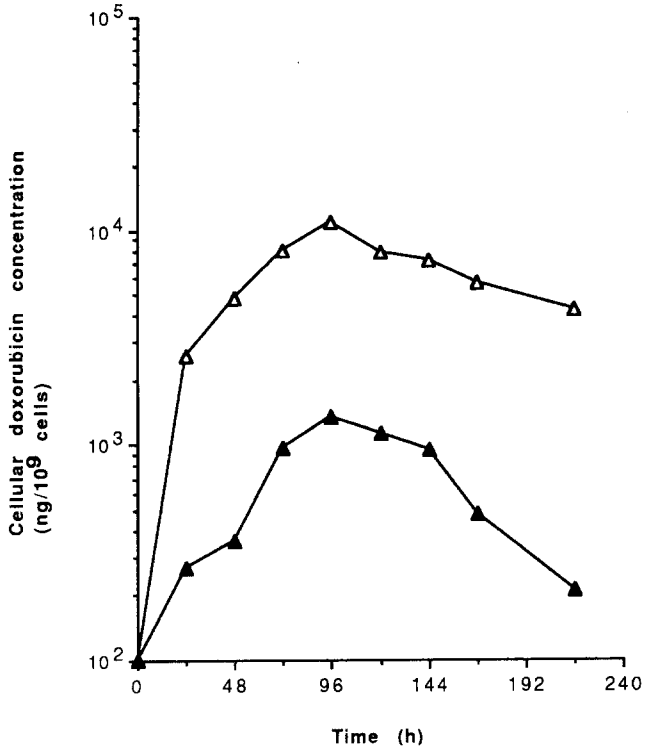

Fig. 3. Cellular DOX concentrations in patient 2 treated with (△) VAD_1, (▲) VAD_2

As emphasized in Fig. 3 for patient 2, the comparison between VAD_1 and VAD_2 revealed both a decrease in intracellular drug accumulation during the infusion time and an increase in intracellular drug elimination at the end of the infusion (terminal half-life: 94 h vs 45 h).

Discussion

Continuous infusion of DOX is generally applied to reduce the toxicity of the drug (i.e., nausea, vomiting, cardiotoxicity) [9, 12]. However, clinical and pharmacokinetic studies are needed to determine whether continuous injection and bolus injection result in similar activity regarding the tumor cells. In this study, we investigated the cellular concentrations of DOX in the leukemic cells of refractory CLL patients as a function of the exposure time and the exposure dose according to the CHOP (bolus) or the VAD (continuous) regimen. The results presented here are in favor of bolus injection in preference to continuous administration. Indeed, the comparison of the cellular AUC values after bolus injection and continuous infusion revealed significant differences, with greater AUC values after bolus administration, and with DOX administered in the same total dose. The higher intracellular AUC values observed after bolus administration appeared to be related both to elevated maximal cellular DOX concentration and to prolonged intracellular retention of the drug, with a loss of only 25% in the 24 h following. The high intracellular accumulation observed immediately after bolus injection can be related to the plasma peak concentration. Indeed, in vitro studies [15] have already established that DOX intracellular concentration is a linear function of DOX extracellular drug concentration up to 5 µg/ml, with DOX entering the cells by a passive mechanism. On the other hand, DOX elimination is a low-action efflux process which can explain the long-lasting high cellular DOX concentration. In continuous infusion, the elimination process remained similar to that observed after bolus injection. However, the intracellular AUC was lower due both to slow intracellular accumulation and to low maximum intracellular concentration.

Our study indicates that bolus administration sustained higher drug intracellular concentrations than the infusion schedule. It should be pointed out that in keeping with the study design, all patients were treated sequentially with a bolus injection during the first administration and with an infusion during the second. Therefore, we cannot rule out the possibility that the elimination of drug-sensitive cells after the first chemotherapy cycle ($CHOP_1$) may have led to an increase in drug-resistant cells, which could explain the decreased cellular AUC values observed after VAD_1. However, this hypothesis seems unlikely. Indeed, DOX-resistance is most probably supported by increased elimination. As shown in Fig. 2, the mean slope of DOX-kinetic efflux after bolus injection was similar to that observed after the infusion schedule. Moreover, in the two patients who received the $CHOP_2$-VAD_2 sequence, the cellular AUC measured after $CHOP_2$ was higher than or similar to that observed after VAD_1. These observations argue against a possible selection by $CHOP_1$ of DOX-sensitive cells and strongly suggest that the difference in AUC between $CHOP_1$ and VAD_1 is predominantly influenced by the modality of DOX administration, even if drug selection occasionally occurs later during the course of chemotherapy, as was illustrated in patients 2 (see below).

Our study apparently contradicts the results of other authors who reported a similar cellular AUC after bolus or continuous administration of doxorubicin and related anthracyclines when administered in the same dose [18–20]. In these studies, the kinetics of cellular drug elimination after bolus injection was found to be much more rapid than we found in our study, with a cellular drug loss of up to over 40% of the initial concentration immediately after the end of the bolus administration. In earlier studies, this rapid decline in the cellular DOX concentration counterbalanced the high initial peak level and rendered the cellular AUC values similar to those observed after continuous infusion. Different parameters may explain the discrepancies observed between these previous reports and our data. First of all, in Speth's studies bolus and continuous DOX administration were compared in two distinct groups of patients. As emphasized in our study (Table 2) and as in previous reports [18–20], the measurement of plasma and cellular AUCs revealed large interindividual variations. In our study, the presence of an internal control (comparison of the two types of drug administration in the same patient) may correct misinterpretations due to individual pharmacokinetic profiles.

Furthermore, it should be mentioned that in our study DOX was given in combination with other drugs, while in Speth's study DOX was given as a single chemotherapy agent. Whether or not the different companion drugs used in the two protocols may have influenced the cellular pharmacokinetic profile of DOX in the CHOP and VAD regimen remains to be answered. Finally, the tumor cell type, e.g., chronic lymphocytic leukemia cells (this study) vs leukemia blasts [18] or myeloma cells [20] may account for these conflicting findings. Indeed, it is quite reasonable to speculate that the kinetics of drug elimination may be different from one tumor cell population to the other, depending on the ability of the cells to actively eliminate the drug. If this is true, our study suggests that the elimination of DOX after bolus injection into CLL cells is less active than in other malignant blood cells.

Interestingly, we observed that the cellular AUC may vary during repeated courses of drug administration. In the two patients studied, the second sequence of treatment ($CHOP_2$/VAD_2) led to an even greater increase in the ratio between CHOP and VAD cellular AUC, in favor of the bolus administration. For one patient, we were able to establish clearly that the decrease in the cellular AUC observed in VAD_2 as compared to VAD_1 was due both to a decrease in drug accumulation during the time infusion and to an increase in drug elimination after the end of the infusion. Since tumor burden and plasma drug concentration were almost unchanged, these findings suggest that modifications of the influx-efflux process of DOX may occur occasionally in CLL cells during chemotherapy. Since P-glycoprotein has been detected in some CLL cells [10, 17], the modifications of cellular DOX pharmacokinetics that we observed in this patient could be due to the selection, during the first chemotherapy cycles, of CLL cells which displayed a highly active Pgp-mediated outward process.

Of the five patients, only two displayed a minor and transient response after the first $CHOP_1$-VAD_1 sequence (patients 2 and 4). We were unable to draw any conclusion in terms of the clinical superiority of CHOP vs VAD. Broader well-designed studies are clearly needed to learn whether such variations of the cellular AUC influence the in vivo DOX cytotoxicity. However, our study may have some clinical implications. Indeed, it has been shown in vitro that DOX toxicity is a function of intracellular drug accumulation [8]. Moreover, previous studies showed that with a similar intracellular exposure dose a greater cytotoxicity effect was achieved after incubation with a high drug concentration for a short time than after incubation with a lower concentration for a longer time [1, 15].

Finally, although VAD has been proved to be an effective regimen in myeloma and in refractory CLL, our study showed that continuous infusion, compared with bolus injection, may lead to a significantly lower in intracellular drug exposure, which in turn may decrease the antileukemic efficacy of DOX. Whether these findings can be extended to other neoplastic disorders remains to be determined.

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