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Tissue Distribution and Myelotoxicity of Daunomycin in the Rat: Rapid Bolus Injection vs Continuous Infusion*

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Abstract—*The pharmacokinetic and cytotoxic behaviour of daunomycin in rats* (7.5 *mg/kg*) *when administered as an i.v. bolus injection were compared with those of a* 3-hr *infusion. After an i.v. bolus injection, the plasma drug levels decreased triphasically* ($t_{1/2}\alpha$ = 0.8 min, $t_{1/2}\beta$ = 29.6 min *and* $t_{1/2}\gamma$ = 9.9 hr). *The organs showed two types of concentration/time curves. One was found in the lungs, liver, kidneys and heart and had an initial high drug concentration followed by a relatively rapid elimination. The other was shown by the hemopoietic tissues* (*spleen and bone marrow*): *the drug uptake phase lasted longer* (*about* 1.5 hr) *and the elimination was slower. In general, daunomycin infusion led to substantially lower plasma and tissue levels, including in the heart, liver and kidneys. Again the hemopoietic tissues behaved in a different way: in spleen and bone marrow almost equal drug levels were obtained after daunomycin infusion as compared to a bolus injection. The myelotoxicity after daunomycin treatment was assessed by CFU-S* (*colony forming units-spleen*) *survival: the bolus injection killed as many CFU-S as did infusion* (*mean* ± *S.D:* 11.8 ± 5.3 *CFU-S survival versus mean* ± *S.D.*: 13.5 ± 2.4). *Thus, it can be predicted that daunomycin infusion will lead to less cardiotoxicity but to an equal bone marrow suppression.*

INTRODUCTION

ANTHRACYCLINE antibiotics are widely used in the treatment of cancers of different types, among which are solid tumors and leukemias (For a recent review on anthracycline drugs, see Ref. [1].) The best known anthracyclines are adriamycin and daunomycin. The clinical use of these drugs is restricted by their cumulative cardiotoxicity [2] and myelosuppression [3]. Attempts have been made to decrease the toxicity of anthracyclines by 'specific' drug targeting by, e.g. conjugation of the drugs to macromolecules such as DNA [4], non-specific proteins [5], specific antibodies [6] or by liposome encapsulation [7]. In these experiments, it is hypothesized that the complexed drug uptake is restricted to cells having a high endocytic activity. Although in animal tumor models this approach seemed to be promising in the sense of reduced cardiotoxicity [8], the results of the recently completed clinical trials are still a matter of dispute [9]. Another approach to the toxicity problem of anthracycline drugs could be a rational treatment schedule based on the pharmacokinetic and toxic behaviour of the drug. It has been shown in the literature that daunomycin or adriamycin i.v. infused during a prolonged period of time leads to lower plasma and tissue levels as compared with an i.v. bolus injection in both animals [10,11] and man [12] and most likely to a subsequent lower incidence of cardiomyopathy. Indeed, recent studies in man have shown that continuous infusion of daunomycin as compared with a rapid bolus injection resulted in a reduced incidence of cardiotoxicity [13–15]. However, knowledge of the pharmacokinetic behaviour of anthracyclines after i.v. infusion with regard to bone marrow toxicity is lacking.

The present study was designed to compare the pharmacokinetic and myelosuppressive behaviour of daunomycin administered either as a rapid bolus injection or as a continuous infusion.

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MATERIALS AND METHODS

Experimental procedures

Daunomycin, daunomycinol and adriamycin were kindly supplied by Farmitalia (Milan, Italy). Daunomycin (7.5 mg/kg body wt) was administered

i.v. either as a single bolus injection with a maximal duration of 15 sec or as a 3-hr continuous infusion in the tail vein of 12-week-old female Brown Norway (BN) rats. The rapid bolus injection was carried out under light ether anaesthesia. The infusion needle was also installed under light ether anaesthesia. During the infusion period, the animals were fixed in tube-like cages without anaesthesia. For the bolus injection, the drug was dissolved in 0.5 ml physiological saline. For the infusion the drug was dissolved in physiological saline supplemented with sodium citrate (0.2%). The continuous infusion was given at a rate of 0.75 ml/hr. At specific time intervals, the animals were sacrificed by exsanguination under ether anaesthesia.

Plasma obtained from aorta blood samples was prevented from coagulation by addition of EDTA. Organs of interest were removed and rapidly cooled by liquid nitrogen and thereafter stored at −20°C until further processing. Urine and bile were collected in 1-hr aliquots and stored at −20°C. Bile was obtained by canulation of the bile duct. The canulation was carried out under ether anaesthesia and thereafter the successfully canulated animals were used for the experiments.

Drug determination

Daunomycin and daunomycinol concentrations were determined by high performance liquid chromatography as described previously [16]. Tissue concentrations were determined in the liver, spleen, bone marrow, lungs, kidneys, skeletal muscle, heart and brain. Urine and bile samples were extracted in the same way as plasma [16]. Tissues were extracted as 10% homogenates in phosphate buffer (0.05 M; pH 8.3) with a chloroform–methanol (4 : 1) mixture. One hundred microliter aliquots of the organic phases were injected directly onto the column. For plasma, urine and bile, the recoveries from the extraction procedures were 90–95%. For tissues and bone marrow, the recoveries were 85–90%. Adriamycin was used as an internal standard for drug quantification. Each point in the plasma, urine, bile and tissue concentration/time curves represents the mean ± S.E. of 6–8 animals. For anthracycline determinations in bone marrow, the femoral contents were extracted with chloroform–methanol (4 : 1) at high pH by addition of borate buffer (0.5 M, pH 9.8). The results are expressed as ng/ml biological fluid (plasma, urine or bile), as μg/g wet weight for the tissues and as μg/10^9 nucleated cells for the bone marrow.

Pharmacokinetic calculations

For the pharmacokinetic modeling of drug distribution and elimination, the equations describing an open two- or three-compartment model with excretion from the central compartment only were used [17]. The data points were regarded as being part of the triexponential function $C(t) = A.e^{-\alpha.t} + B.e^{-\beta.t} + C.e^{-\gamma.t}$ (bolus injection) or biexponential function $C(t) = A.e^{-\alpha.t} + B.e^{-\beta.t}$ (infusion). For the bolus injection, all plasma data and in the case of infusion only the postinfusion data were used. From the data of the distribution (α), intermediate (β) and elimination (γ) phases, linear regression lines of log C vs t leading to values for A, B, C, α, β and γ were calculated. These rough estimates of A, B, C, α, β and γ were subsequently used for nonlinear least square computer analysis, resulting in more precise values for A, B, C, α, β and γ.

Hemopoietic stem cell assay

The number of pluripotent hemopoietic stem cells in the BN rat can be determined by means of the modified colony forming unit-spleen (CFU-S) assay [18]. After a single daunomycin bolus injection as well as after a 3-hr infusion the bone marrow CFU-S survival was determined by removing the femoral bone marrow 24 hr after drug administration and subsequent inoculation into lethally irradiated mice. Nine days later spleen colonies can be counted. One spleen colony equals about 140 pluripotent hemopoietic stem cells. By doing repeated CFU-S assays at different time intervals after daunomycin administration, we have found that 24 hr is the best interval for determination of the daunomycin-induced CFU-S kill. Twenty-four hours after daunomycin administration there is a gradual increase of CFU-S and at day 6 after drug administration the femoral CFU-S counts are at the control level again.

RESULTS

Distribution and elimination of daunomycin after i.v. bolus injection

After i.v. bolus administration of 7.5 mg/kg to BN rats, the plasma concentration/time relationship could be fitted by an equation describing an open linear three compartmental distribution with drug elimination from the central compartment only (correlation coefficient of 0.99599) (Fig. 1). Daunomycinol was the major fluorescent metabolic product. Only a minute amount of aglycon was detected in the plasma. The alpha (distribution) phase for daunomycin was very short ($t½\alpha$ = 0.8 min) (Table 1). This phase was followed by a longer lasting intermediate (β) phase ($t½\beta$ = 29.6 min) and a relatively slow elimination (γ) phase ($t½\gamma$ = 9.9 hr). Some other relevant pharmacokinetic parameters (area under the total plasma concentration/time curve — AUC — and drug plasma clearance — Cl p) for daunomycin

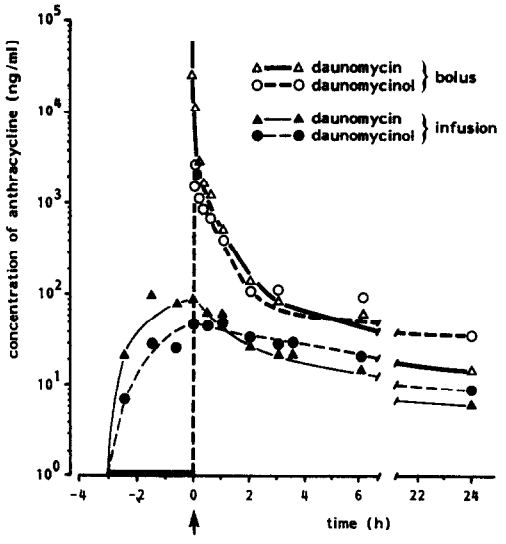

Fig. 1. Plasma disappearance curves in rats treated with daunomycin (7.5 mg/kg) as an i.v. bolus injection or as a 3-hr infusion. The plasma data were fitted by equations describing an open linear three compartimental distribution for the bolus administration and a two compartimental distribution for the infusion. The lines were generated by iterative numerical analysis. The solid bar on the ordinate represents the infusion period. The arrow indicates the time point of bolus injection.

Table 1. Pharmacokinetic parameters of daunomycin in the rat after a rapid i.v. bolus injection or a 3-hr i.v. infusion

		Bolus	Infusion
$t½\alpha$	(min)	0.8	38.9
$t½\beta$	(min)	29.6	589.1
$t½\gamma$	(hr)	9.9	—
AUC	($ng.ml^{-1}.hr$)	3739.8	710.4
Cl p	($ml.hr^{-1}$)	304.1	1875.1

$t½$; half life of elimination ($t½ = \frac{\ell n2}{\alpha, \beta \text{ or } \gamma}$)

AUC; area under the plasma concentration/time curve ($_0\int^{\infty} C(t)dt$).

Cl p; plasma clearance ($\frac{\text{dose}}{\text{AUC}}$)

(7.5 mg/kg) in the rat after a rapid i.v. bolus injection are given in Table 1.

Tissue uptake of daunomycin by the well-perfused organs appeared to be very rapid after a single i.v. bolus injection. Of the tissues examined, the lungs and kidneys showed the highest uptake of daunomycin (at t = 0.5 hr, 45 and 35 μg/g wet wt, respectively). The liver, heart and spleen showed intermediate levels (at t = 0.5 hr, 24, 22 and 18 μg/g wet wt, respectively) followed by skeletal muscle which had low amounts of drug (at t = 0.5 hr, 3 μg/g wet wt). Brain tissue contained no detectable levels of daunomycin or daunomycinol, suggesting that the drug does not pass the blood brain barrier in significant amounts. The main fluorescent metabolite in all organs was daunomycinol and the highest levels were found in the kidneys (maximally, 10 μg/g wet wt).

Examples of a graphical representation of a tissue concentration/time relationship are shown in Fig. 2. The initial rapid uptake of the drug by the heart tissue is followed by a slower elimination phase. Other organs such as the lungs, liver and kidneys showed essentially identical disappearance patterns (figures not shown). However, a different kind of concentration time relationship is found in spleen tissue (Fig. 3). Here, there is no clear peak

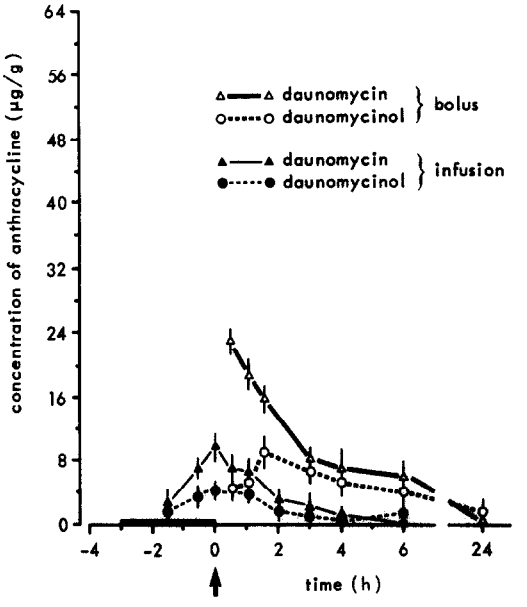

Fig. 2. Time course of concentrations (mean ± S.D.) of daunomycin and daunomycinol in heart tissue of rats treated with daunomycin (7.5 mg/kg) either as an i.v. bolus injection or as a 3-hr infusion. The solid bar on the ordinate represents the infusion period. The arrow indicates the time point of bolus injection.

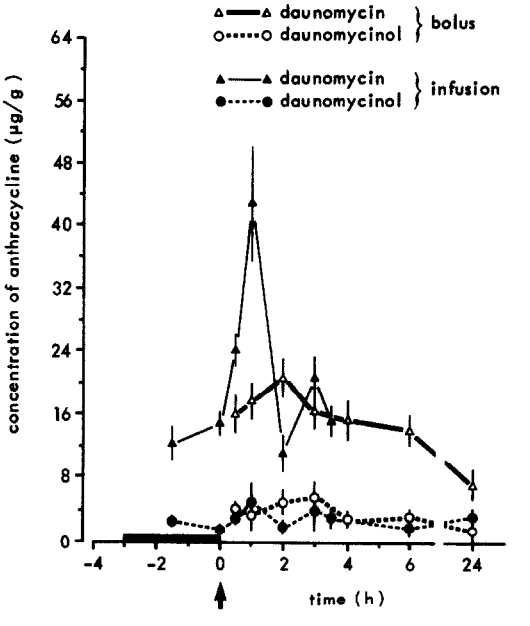

Fig. 3. Time course of concentrations (mean ± S.D.) of daunomycin and daunomycinol in spleen tissue of rats treated with daunomycin (7.5 mg/kg) either as a bolus injection or as a 3-hr infusion.

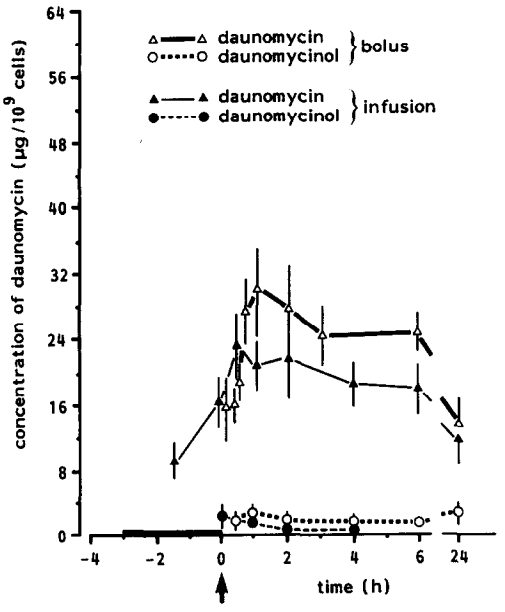

Fig. 4. Time course of concentration of daunomycin (mean ± S.D.) in nucleated bone marrow cells of rats treated with daunomycin (7.5 mg/kg) *either as a bolus injection or a a* 3-hr *infusion.*

value and a certain level of daunomycin is maintained over a relatively long time period (about 8 hr). Figure 4 shows the time course of daunomycin concentration in nucleated bone marrow cells after a bolus injection of daunomycin.

The cumulative 24-hr urine and bile excretion of daunomycin and daunomycinol are given in Table 2.

Distribution and elimination of daunomycin after a 3-hr *i.v. infusion*

During a 3-hr i.v. infusion the drug concentrations increase gradually. However, the highest plasma concentration is only about one tenth of the one obtained after a bolus injection (Fig. 1). After the end of the infusion period the plasma disappearance is biphasic with $t½\alpha$ = 38.9 min and $t½\beta$ = 589.1 min. The pharmacokinetic parameters after a rapid bolus injection are compared with those of a 3-hr infusion in Table 1.

In heart, liver and kidney tissues, considerable lower amounts of daunomycin were detected after infusion of the drug than after bolus injection. For example, heart tissue (Fig. 2) showed a maximum concentration of 10 μg/g wet wt after infusion as compared with 22 μg/g tissue for a bolus injection. After the end of the infusion period, the drug concentrations gradually decreased. However, in spleen tissue (Fig. 3), the disappearance pattern showed an unexpected phenomenon. After stopping the infusion, the daunomycin concentrations increased by a factor of 2 or 3, with peak values at 4 hr after the start of the infusion. Comparable results were obtained for bone marrow (Fig. 4).

In Table 2 are compared the cumulative urine and bile excretion of daunomycin and daunomycinol after daunomycin treatment either as a rapid bolus injection or as a 3-hr infusion.

Table 2. Cumulative 24-hr *urine and bile excretion* of daunomycin and daunomycinol*

	Daunomycin administered as Bolus		Infusion	
	DAU†	DAUNOL‡	DAU†	DAUNOL‡
Urine	4.5	5.0	1.0	2.0
Bile	13.0	15.0	8.5	7.0

* Expressed as a percentage of the total administered dose.
† Daunomycin.
‡ Daunomycinol.

Bone marrow toxicity

The toxicity of daunomycin treatment for the bone marrow *in vivo* was assessed by CFU-S survival at 24 hr after the drug administration. When daunomycin (7.5 mg/kg) was administered by infusion, about an equal number of CFU-S survived the treatment (mean ± S.D.: 13.5 ± 2.4 of control) as compared with a bolus injection (mean ± S.D.: 11.8 ± 5.3 of control) (Table 3).

Table 3. Cytoreductive effect of daunomycin* (7.5 mg/kg) *on the femoral bone marrow in rats: comparison of an* i.v. *bolus injection with a* 3-hr *infusion*

	CFU-S survival†
i.v. bolus injection	11.8 ± 5.3 (n = 4)
3-hr infusion	13.5 ± 2.4 (n = 4)

* CFU-S were determined 24 hr after daunomycin treatment.
† expressed as percentage survival ± S.D. of the control.

DISCUSSION

Adriamycin and daunomycin are usually administered in man as a rapid intravenous (i.v.) bolus dose over a few minutes. With this regimen, several pharmacokinetic studies in animals and man have yielded data on anthracycline plasma and tissue distribution and urine and bile elimination [1, 19, 20]. In the study reported here, we compared the distribution and elimination and bone marrow cytotoxicity of daunomycin injected i.v. or infused in rats. The animals received a dose of 7.5 mg/kg body wt, which is comparable with a clinical dose in man [21].

The increase in tissue concentration after an i.v. bolus injection is a rapid phenomenon which is in agreement with the decrease in plasma concentration. The lungs contained most daunomycin per gram of tissue, followed by the kidneys, liver, bone marrow, heart and spleen. Daunomycinol appeared to be the main metabolic product and no

substantial differences were found in daunomycinol content per gram of tissue among the different organs. The organs exhibited two types of concentration/time curves. One type was found in the lungs, liver, kidneys and heart which showed an initial high drug concentration followed by a relatively slow elimination phase. The other type was shown by hemopoietic tissues such as the spleen and bone marrow: the drug uptake phase lasted longer (at least 1.5 hr) and the drug elimination phase was much slower.

Remarkable differences were found in plasma profiles and tissue distribution when daunomycin was administered by infusion. The high peak plasma value as found for an i.v. bolus injection was not evident after infusion and the area under the plasma concentration vs. time curve for daunomycin infusion was about 5 times smaller. Consequently, the plasma clearance is increased. In another experimental study with daunomycin in rabbits the absence of peak values in plasma and tissues after drug infusion was also shown [10]. However, in our study plasma kinetics and urine and bile excretions were studied as well, thus providing a complete overview of the fate of administered daunomycin both after bolus injection and continuous infusion. The increased plasma AUC after daunomycin bolus injection as compared with infusion could theoretically be due to saturation of the enzyme system responsible for daunomycin metabolism. However, the ratio of excreted daunomycin and daunomycinol either via the urine or via the bile is not affected by the route of daunomycin administration. Thus, there is no experimental evidence for such a saturation. Since not only the plasma AUC is smaller after daunomycin infusion but also the daunomycin and daunomycinol urine and bile excretions are decreased the total tissue exposure must be larger. After daunomycin infusion again the hemopoietic organs behaved in a different way as compared to e.g. heart. For heart the total AUC seems to be smaller after daunomycin infusion. However, in spleen and bone marrow the total drug exposure seems to be larger or equal than those obtained after bolus injection. Spleen and to a lesser extent bone marrow showed a tendency for an increase in drug content after the end of the infusion. For example, in spleen a 2- to 3-fold increase in daunomycin content per gram of tissue was found after stopping the infusion. Spleen and bone marrow also showed this phenomenon after a bolus injection. An explanation is lacking at the moment. The final drug concentration in tissues after *in vivo* exposure is the result of physiological processes (e.g. blood flow) in combination with anatomical (e.g., vascularization) and cell characteristics (e.g. drug influx/efflux ratio, DNA content per gram wet weight). The observed differences in pharmacokinetic behaviour between hemopoietic tissues (spleen and bone marrow) on the one hand and, e.g., heart tissue on the other clearly reflects differences in the above-mentioned factors.

It has been demonstrated that the cardiotoxicity of anthracyclines can be reduced changing from rapid bolus administration to continuous infusion [13–15]. In experimental [10] and clinical studies [14] in which plasma levels of anthracyclines were monitored it was generally concluded that a reduction in peak plasma levels might be responsible for the reduction in cardiotoxicity. Several mechanisms of action have been postulated for the cytostatic activity of anthracycline drugs among which are intercalation between base pairs in the DNA [1], generation of free radicals [22] and interactions with the cell plasma membrane [23, 24]. Most likely the anthracycline-induced cardiotoxicity is also based on one (or more) of these molecular interactions.

After a bolus injection, 28% of the total administered dose is excreted via the bile route within 24 hr and 9.5% via the urine. In the case of infusion, these figures are quite different: 16.5% for the bile and 3% for the urine excretion. These reduced bile and urine excretions are in agreement with the lower plasma levels found after daunomycin infusion.

The experiments in which the myelotoxicity was assessed by CFU-S survival after daunomycin treatment showed that the two methods of administration, bolus and infusion, had an equal effect on the CFU-S per femur. Thus, infusion of daunomycin, which gives rise to almost equal drug concentrations in the bone marrow compartment as compared with bolus injection, leads to an equal kill of hemopoietic stem cells in the bone marrow. In a clinical study it was found that after anthracycline infusion the myelosuppression as estimated by peripheral blood cell counts appeared to be similar to that observed after bolus injection [14]. This observation is in agreement with our data.

Studies of the relative cytotoxicity of adriamycin and adriamycinol have shown that adriamycinol can have a cytotoxic effect, although to a lesser degree than adriamycin [25]. It thus can be expected that daunomycinol will also be cytotoxic. However, since daunomycinol reached only low levels in the femoral bone marrow either after a push injection or a continuous infusion of daunomycin this metabolite does not seem to play a role in the reduction of femoral CFU-S after daunomycin administration in the rat.

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