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Repeated daunomycin administration in rats

Pharmacokinetics and bone marrow toxicity

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Summary. *In the experiments described here, rats received three IV bolus injections (7.5 mg/kg) of daunomycin. The plasma data obtained after a single IV injection could be described by a two-compartment open model with $t_{1/2}\alpha$ and $t_{1/2}\beta$ values of 18.4 and 472.1 min. Of the tissues, the lungs contained the most daunomycin per gram of tissue, followed by the kidneys, liver, heart, and spleen. Daunomycinol was the main metabolic product and no substantial differences were found in daunomycinol content among the different organs. For all tissues and plasma, higher drug concentration values than would be expected on the basis of accumulation alone were observed after the second but not after the third injection. The cumulative urine excretion of daunomycin and daunomycinol remained essentially unchanged after one, two, and three daunomycin injections. However, the cumulative bile excretion increased after repeated daunomycin administration. The experiments in which the myelotoxicity was assessed by CFU-S survival after daunomycin treatment showed that three successive daunomycin administrations lead to a proportional reduction in stem cells.*

Introduction

The anthracycline antibiotics adriamycin and daunomycin have long been major components of cancer treatment regimens [3]. The most serious toxic side-effects of anthracycline administration are dose-related cardiotoxicity [2] and myelosuppression [6]. In many cancer treatment protocols in which these agents are used the drug is administered in repeated dosages [4]. For example, in the clinical trial protocol (LAM 6) developed for the treatment of adult acute nonlymphocytic leukemia by the EORTC* Leukemias and Hematosarcomas Cooperative Group, daunomycin is administered on three successive days as an IV rapid bolus dose. The study reported here was designed to evaluate the effects of three repeated daunomycin administrations on drug metabolism and elimination and bone marrow toxicity.

Materials and methods

Experimental procedures. Daunomycin (7.5 mg/kg body weight) was administered IV as a bolus injection into the tail vein of 12-week-old female Brown Norway (BN) rats weighing 140–160 g and under light ether anesthesia. The drug was dissolved in 0.5 ml physiological saline. At specific time intervals, the animals were sacrificed by exsanguination under ether anesthesia.

Plasma was obtained from aortic blood samples, which were prevented from coagulation by the addition of EDTA. Organs of interest were removed and rapidly cooled in liquid nitrogen and then stored at −20° C until further processing. Urine and bile were collected at 1-h intervals and stored at −20° C. Bile was obtained by cannulation of the bile duct.

Drug determination. Daunomycin, daunomycinol and adriamycin were kindly supplied by Farmitalia (Milan, Italy).

Daunomycin and daunomycinol concentrations were determined by straight-phase high-performance liquid chromatography as described previously [1]. For drug quantification, adriamycin was used as an internal standard. Urine and bile samples were extracted in the same way as was plasma [1]. Tissues were extracted as 10% homogenates in phosphate buffer (0.05 *M*; pH 8.3) with a chloroform/methanol (4 : 1) mixture. Each point in the plasma, urine, bile, and tissue concentration/time courses represents the mean of values recorded in six to eight animals.

Hematopoietic stem cell assay. The number of pluripotent hematopoietic stem cells in the BN rat can be determined by means of the modified colony forming unit-spleen (CFU-S) assay [5].

Pharmacokinetic calculations. For the pharmacokinetic modeling of the drug, the equations describing an open two-compartment model with excretion from the central compartment only were used [7]. The coefficients, exponents and compartmental volumes in the integrated equations were estimated by fitting the biexponential function $A.e^{-\alpha.t} + B.e^{-\beta.t}$ to the observed plasma concentrations. The concentration/time curves were generated by iterative numerical analysis.

Results

Plasma

After a single IV bolus injection of 7.5 mg/kg body weight into BN rats, there is a biphasic elimination of daunomycin from the plasma (Fig. 1), with a relatively rapid α-distribution phase (18.4 min) and a slower β-elimination phase (472.1 min). In all

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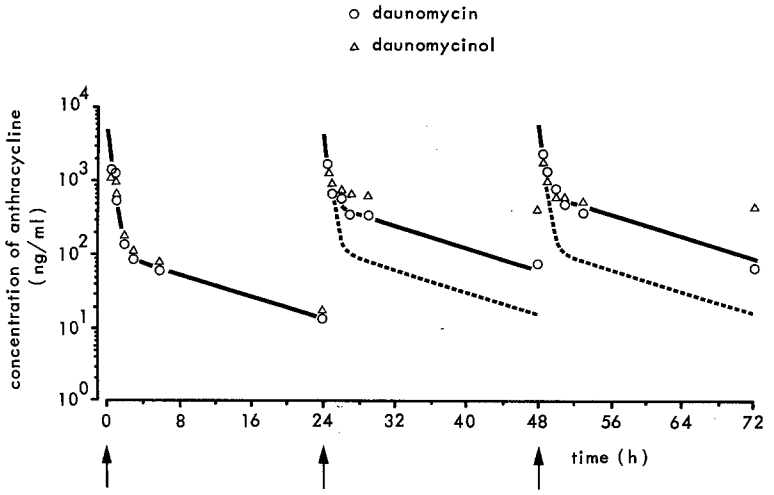

Fig. 1. Plasma disappearance curves for daunomycin and daunomycinol after three successive IV bolus injections of daunomycin. The time points of drug administration are indicated by the *arrows*. The symbols represent the concentrations actually detected; the *solid* and *broken lines* represent the calculated disappearance of daunomycin

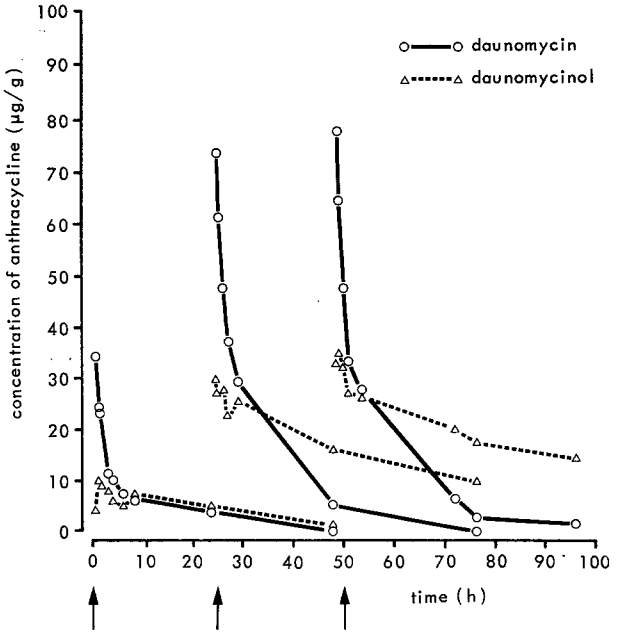

Fig. 2. Kidney tissue concentration/time course of daunomycin and daunomycinol after three successive IV bolus injections of daunomycin. The time points of drug administration are indicated by the *arrows*

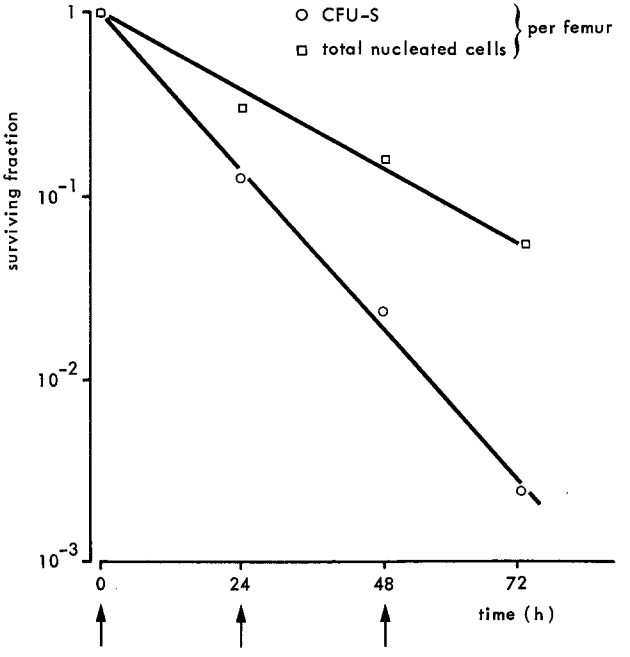

Fig. 3. Cytoreductive effect of three successive daunomycin (7.5 mg/kg) injections on the femoral marrow in rats. Drug administration is indicated by the *arrows*

Table 1. Cumulative 24-, 48-, and 72-h urine and bile excretion after three successive daunomycin (7.5 mg/kg) IV bolus injections

	Number of drug injections	Excretion[a]	
		Daunomycin	Daunomycinol
Urine	I	4	5
	II	4	6
	III	4	6
Bile	I	13	13
	II	20	15
	III	20	25

[a] Expressed as a percentage of the total dose administered

cases, daunomycinol was the major fluorescent metabolic product. Only a minute amount of aglycon was detected in the plasma. The model parameters α, β, V1, K12, K21, and Kel (see [7]) were used to calculate plasma disappearance curves. Two types of calculations were made. In one, the model parameters obtained after the first injection were kept constant for the second and third injections (the broken lines in Fig. 1). In the other, only the rate constants α and β were kept constant (the solid lines in Fig. 1).

Organs

Of the tissues examined, the lungs and kidneys showed the highest uptake of the drug (at $t = 0.5$ h, 45 and 35 μg/g wet tissue, respectively). The liver, heart, and spleen showed

intermediate levels (at t = 0.5 h, 24, 20, and 18 μg/g, respectively), followed by skeletal muscle, which had low concentrations of drug (at t = 0.5 h, 3 μg/g). The main fluorescent metabolite in all organs was daunomycinol, and the highest levels were found in the kidneys (maximally, 10 μg/g). In all tissues examined a second daunomycin injection led to higher drug concentrations. However, a third drug administration had only a marginal effect on the amount of daunomycin per gram of tissue. Figure 2 shows an example of daunomycin and daunomycinol disappearance for the kidneys after one, two, and three successive IV bolus injections of daunomycin. The elimination pattern is typical for well-perfused organs such as the liver, kidneys, lungs, and heart. Due to the low amount of drug in the plasma compared with the tissues, the tissue drug levels are only minimally influenced by daunomycin and daunomycinol in the blood present in the organs.

Drug excretion

Table 1 shows the cumulative 24-, 48-, and 72-h urine and bile excretions of daunomycin and daunomycinol, expressed as percentages of the total dose administered after three successive drug injections.

Bone marrow toxicity

The decrease in femoral total nucleated cells (TNC) and CFU-S after three successive drug administrations is shown in Fig. 3. After the third daunomycin injection the CFU-S are reduced by a factor of about 500, while the TNC are reduced by a factor of only about 20.

Discussion

Rats received three daunomycin doses of 7.5 mg/kg body weight at 24-h intervals. The plasma data obtained after a single IV bolus injection appeared to be suitable for description by a two-compartment open model for drug disposition. When these half-time values were kept constant for the second and third drug injections, good fits were also obtained between the actually detected values and the calculated ones. Apparently the plasma half-life times of daunomycin do not change after repeated drug injections. However, calculations on plasma data with all model parameters (α, β, V1, K12, K21, and Kel) obtained after the first injection kept constant leads to a discrepancy; for the second and third injections the actually detected values are much higher than the calculated ones. It is remarkable that this discrepancy is found only after the first injection and not after the second one.

The increase in tissue concentration after IV bolus injections is a rapid phenomenon which is in agreement with the decrease in plasma concentration. For all tissues examined, a second daunomycin injection led to higher drug concentrations. However, a third drug administration had hardly any effect on the amount of daunomycin per gram of tissue. After one drug injection at t = 0.5 h, about 40% of the total administered dose of daunomycin was found in heart, lungs, liver, kidneys, skeletal muscle, and spleen. After a second injection, this percentage was increased to 60%, but a subsequent daunomycin injection produced no further change.

The phenomenon discussed above, which occurs simultaneously in plasma and tissues, cannot be explained by decreased urine and bile excretion. On the contrary, the cumulative urine and bile excretions tend to increase after the second and third drug administrations. Apparently the distribution volume of the drug is drastically decreased after the second injection, leading to higher tissue levels. Theoretically, one could think in terms of a yet unknown drug depot, which is filled after the first injection and which releases the drug very slowly, leading to a jump in drug concentration after a second injection. However, we have no experimental evidence so far for such a drug depot.

The experiments in which the myelotoxicity was assessed by CFU-S survival after daunomycin treatment showed a differential effect on TNC and CFU-S. This apparent selective kill of stem cells might indicate a preferential killing of proliferating cells, since it has been shown for the rat that the majority of bone marrow CFU-S is in cell cycle [5]. Although we have shown that the increase in tissue content is much more pronounced after the second injection than after the third, a significant additional effect of a third drug injection on the stem cell kill is still observed. After each dose, a constant fraction of CFU-S is killed. However, besides a cytoreductive effect on normal stem cells, the therapeutic effects of anthracycline treatments are of importance. Thus, the effects of repeated daunomycin injections on specific targets, such as leukemic cells, remain to be clarified.

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