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Short communication

Effects of verapamil on the pharmacokinetics of daunomycin in the rat

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Summary. We compared the pharmacokinetics of daunomycin in two groups of rats: one group was treated with daunomycin (7.5 mg/kg) alone and the other group was treated with daunomycin (7.5 mg/kg) plus the calcium antagonist verapamil (2 × 50 mg/kg i. p.). Due to a much slower decrease in plasma concentrations the daunomycin $AUC_{0\rightarrow\infty}$ was dramatically increased (8 times) in the animals treated with anthracycline plus verapamil. The daunomycin plasma clearance was found to be decreased about 9 times in the verapamil-treated group. Verapamil had a differential effect on the tissue distribution of daunomycin. Of the organs examined the heart, liver, and lungs showed an increased (about 2–3 times) AUC of daunomycin. In the kidneys and spleen the AUCs of daunomycin were about equal in both groups of rats, while in the femoral bone marrow the daunomycin AUC was significantly reduced by the simultaneous administration of verapamil. Our data suggest that an increased risk for anthracycline-induced cardiotoxicity can be anticipated by the combined treatment of anthracycline drugs with calcium antagonists.

Introduction

A major problem in cancer chemotherapy is acquired drug resistance. A variety of agents, among which are calcium antagonists such as verapamil, have been shown to overcome resistance to anthracyclines in both murine and human multidrug-resistant tumor cells in vitro, most likely by increasing the intracellular anthracycline concentrations [7]. The agents capable of reversing pleiotropic drug resistance have no effect on the cellular accumulation of anthracyclines in drug-sensitive cells in vitro. These results suggest a potential for therapeutic gain through the use of, e. g., calcium antagonists in combination with classic chemotherapeutic agents. Clinical trials of verapamil plus anthracyclines have indeed been started [5, 6].

In the present study, we compared the tissue distribution of the anthracycline antibiotic daunomycin in rats with or without simultaneous treatment with verapamil.

Materials and methods

Two groups of experimental rats were used. One group received daunomycin alone and the other group daunomycin plus verapamil. Daunomycin (7.5 mg/kg body weight) was administered intravenously (i. v.) 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. This anthracycline dosage in rats is comparable to a clinical dose of 40 mg/m^2 [1]. Verapamil (50 mg/kg body weight) was administered intraperitoneally (i. p.) 2 h prior to (as a loading dose) and simultaneously with the daunomycin injection. The control group was treated with physiological saline alone. At specific time intervals after drug injection the animals were killed by exsanguination under ether anesthesia. Plasma was obtained by anticoagulation of aortic blood samples by ethylenediaminetetraacetic acid (EDTA). Organs of interest were removed and rapidly cooled in liquid nitrogen and then stored at −20° C until further processing.

Daunomycin and daunomycinol concentrations were determined by straight-phase high-performance liquid chromatography as described previously [4]. Verapamil did not interfere with the chromatographic quantification of daunomycin and daunomycinol. Each point in the plasma and tissue concentration – time curves represents the mean ±SD of 6–8 animals. The results are expressed as ng/ml for plasma, as μg/g wet weight for the tissues, and as μg/10^9 nucleated bone marrow cells. For the pharmacokinetic modeling of daunomycin, the equation describing an open three-compartment model with excretion from the central compartment alone was used [8]. The coefficients, exponents, and compartmental volumes in the integrated equations were estimated by fitting the triexponential function, $A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} + C \cdot e^{-\gamma \cdot t}$, to the observed plasma concentrations. The concentration-time curves were generated by iterative numerical analysis. The half-life of plasma drug concentrations during the α-, β- and γ-phases ($t_{\frac{1}{2}} = ln2/\alpha$, β, or γ). The AUC $({}_0\int^\infty C(t)dt)$, and the plasma clearance (dose/AUC) were also calculated.

Results and Discussion

After a single i. v. bolus injection of daunomycin (7.5 mg/kg), the plasma concentration – time relationship could be very well described by a triexponential equation for animals injected either with anthracycline alone or with anthracycline plus verapamil (Fig. 1; Table 1). The major fluorescent metabolite was daunomycinol. The half-times of all three phases of the plasma decay curve of daunomycin were substantially increased in the rats treated with dau-

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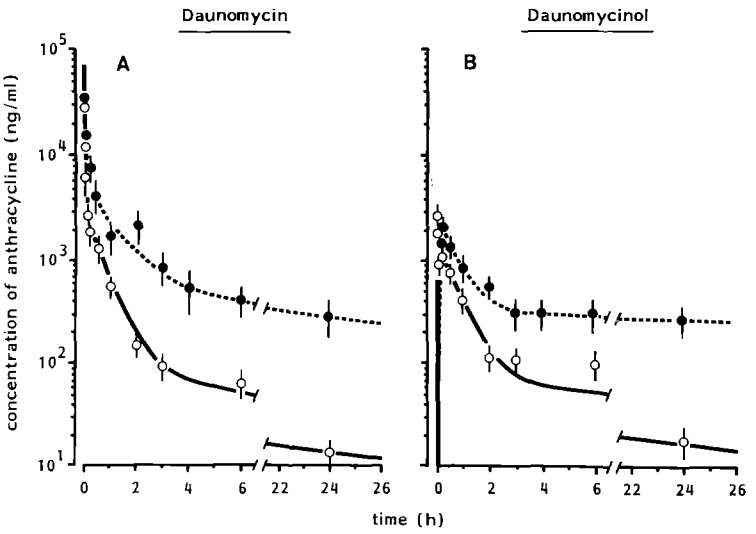

Fig. 1 A, B. Plasma disappearance curves in rats treated with daunomycin (7.5 mg/kg) as an i. v. bolus dose (○———○) or with daunomycin (7.5 mg/kg) plus verapamil (50 mg/kg i. p.) (●———●). **A**, Daunomycin; **B**, daunomycinol. *Bars*, SD

Table 1. Pharmacokinetic parameters of daunomycin (D) ± verapamil (V) in rats

	D	D + V
$t_{1/2}\,\alpha$	0.8 min	5.4 min
β	29.6 min	3.2 h
γ	9.9 h	32.9 h
$AUC_{0\to\infty}$	3.73 μg · ml^{-1} · h	30.2 μg · ml^{-1} · h
Cl_p	330 ml · h^{-1}	36 ml · h^{-1}

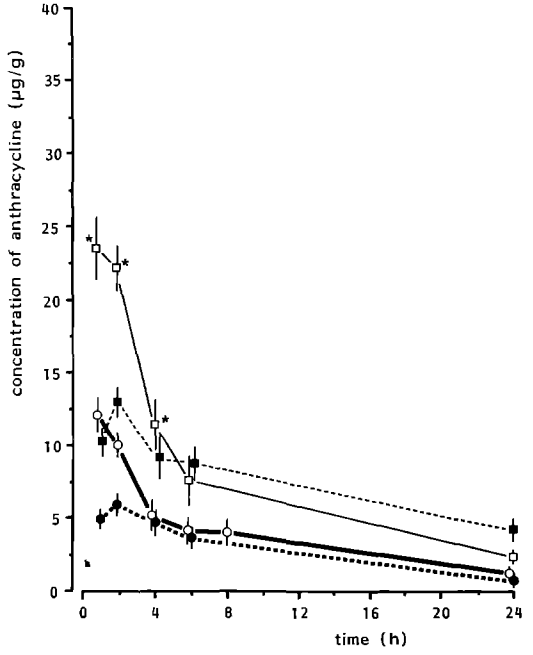

Fig. 2. Time course of concentrations of anthracyclines in heart tissue of rats treated with daunomycin (7.5 mg/kg i. v.): ○———○ daunomycin; ●———● daunomycinol, or treated with daunomycin (7.5 mg/kg i. v.) plus verapamil (50 mg/kg i. p.): □———□ daunomycin; ■———■ daunomycinol. *Bars*, SD. Daunomycin concentrations (indicated by an *asterix*) differ significantly from the controls (Wilcoxon's signed-rank test, $\alpha = 0.05$)

nomycin plus verapamil, as compared to the animals treated with daunomycin alone (Table 1). Due to the increased plasma concentrations, the $AUC_{0\to\infty}$ was increased from 3.73 μg·ml^{-1}·h to 30.2 μg·ml^{-1}·h in the animals treated with verapamil. Since plasma clearance (CL_p) is defined by dose / AUC, Cl_p is decreased about 9 times in the verapamil-treated rats. This decreased Cl_p could theoretically be due to reduced renal and/or hepatic clearances induced by verapamil.

Recent clinical data indicate that verapamil can also have a significant effect on the plasma pharmacokinetics of adriamycin [2]. Adriamycin peak levels, AUC, and terminal half-life were elevated, while plasma clearance was found to be lower in patients treated with adriamycin and concomitantly with verpamil.

Differences were also found in the amount of drug taken up by the tissues between rats treated with daunomycin alone and rats treated with daunomycin plus verapamil. For example, daunomycin concentrations in heart tissue were significantly (Wilcoxon's signed-rank test, $\alpha = 0.05$) increased (about two times) by the simultaneous use of verapamil (Fig. 2). On the other hand, concentrations of daunomycin in the femoral bone marrow of rats treated with daunomycin plus verapamil were about twice as low as those found in the control rats treated with daunomycin alone (Fig. 3). In Table 2, the tissue exposure (expressed as $AUC_{0\to 24\,h}$) to daunomycin and daunomycinol in the different organs is listed. An increased AUC of daunomycin was found in the heart (about 2 times), in the liver (about 3 times), and in the lungs (about 2 times) as a result of the verapamil administration (Table 2). In other organs such as kidneys and spleen, about equal daunomycin AUCs were found in both groups of rats. In liver and kidneys the daunomycinol AUC was increased out of proportion compared to the AUC of daunomycin, a phenomenon that may be related to the sites of metabolism of the parent drug.

These results suggest a differential effect of verapamil on the tissue distribution of anthracycline drugs. In this respect two organs are of special interest: the bone marrow as a target organ for the drug in the treatment of leukemias, for example, and the heart as a major organ by the anthracycline-induced toxicity. When we assume that in the case of daunomycin treatment, tumor cell kill is the result

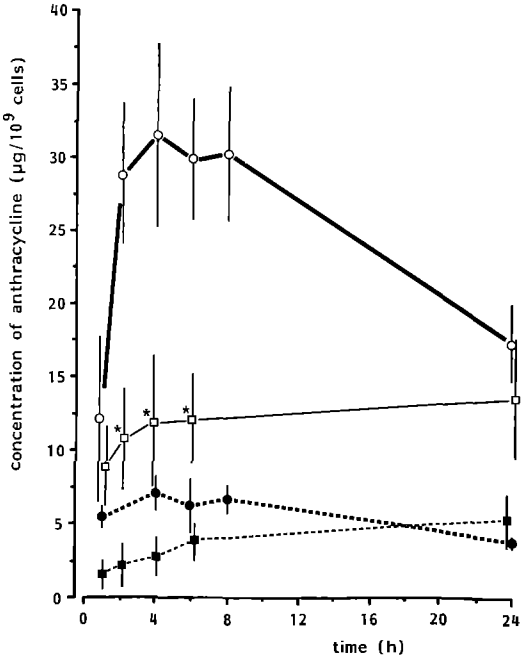

Fig. 3. Time course of concentrations of anthracyclines in the femoral bone marrow of rats treated with daunomycin (7.5 mg/kg i. v.): ○———○ daunomycin; ●———● daunomycinol, or treated with daunomycin (7.5 mg/kg i. v.) plus verapamil (50 mg/kg i. p.): □———□ daunomycin; ■———■ daunomycinol. *Bars*, SD. Daunomycin concentrations (indicated by an *asterix*) differ significantly from the controls (Wilcoxon's signed-rank test, $\alpha = 0.05$)

Table 2. Daunomycin (D) and daunomycinol (DNOL) tissue exposure (AUC)[a] in organs of rats treated with daunomycin alone or with daunomycin plus verapamil

	$AUC_{0 \to 24\,h}$ in rats treated with			
	daunomycin[b]		daunomycin plus verapamil[b]	
	D	DNOL	D	DNOL
Heart	90.1± 6.1	67.2± 8.7	172.7±17.1[c]	136.4±12.4[c]
Liver	115.8± 9.4	41.3± 8.6	365.8±31.3[c]	231.6±25.9[c]
Lungs	305.5±23.5	109.5±13.6	612.0±34.2[c]	163.6±17.0[c]
Kidneys	224.1±36.9	161.7±16.5	249.0±21.1	351.9±44.9[c]
Spleen	585.8±61.9	174.8±23.4	614.5±48.4	250.9±30.6
Bone marrow	589.4±43.5	128.9± 8.5	290.1±47.0[c]	98.1±17.5

[a] Mean ± SD, expressed as microgram per gram per hour for the tissues and as microgram per 10^9 cells per hour for the bone marrow. The AUC was estimated by means of the trapezoidal rule

[b] Rats were treated with daunomycin (7.5 mg/kg) as an i.v. bolus dose or were pretreated with an i.p. loading dose of verapamil (50 mg/kg) 2 h prior to a simultaneous administration of dauno mycin (7.5 mg/kg i.v.) and verapamil (50 mg/kg i.p.)

[c] Anthracycline concentrations obtained in rats treated with daunomycin plus verapamil indicated a difference of more than 3 SDs from those obtained in rats treated with daunomycin alone

of the total tissue exposure to the drug (C × t), then we expect less kill in the femoral marrow when daunomycin is used in combination with verapamil. On the other hand, a milder suppression of normal hemopoiesis, a frequently observed toxic effect accompanying the clinical use of anthracyclines, could be a positive effect of the combined use of daunomycin and verapamil. A dose-limiting toxicity of anthracycline drugs is congestive heart failure. This toxicity has been found to be related to the height of the peak plasma concentration [3] and most likely also to the peak concentration in the heart. In a series of patient studies it appeared that reduction in anthracycline peak concentration, by changing from rapid bolus administration to continuous infusion, the total cumulative anthracycline dose could be increased with about 30% [3]. In our experiment the daunomycin peak concentration and the AUC in heart tissue were increased by about a factor of two in the rats treated with daunomycin plus verapamil, as compared to rats treated with daunomycin alone. It can thus be expected that anthracyclines are more cardiotoxic when administered simultaneously with verapamil.

It must be emphasized that the verapamil dose given to our rats (2 × 50 mg/kg i. p.) is far beyond the range routinely used in man (0.1–0.05 mg/kg i. v.). However, it can be anticipated that any clinical dose of verapamil that leads to increased anthracycline concentrations in resistant tumor cells in vivo (which is the ultimate goal of the treatment) will also give rise to altered tissue distribution of anthracyclines' and to increased risk of anthracycline-induced cardiotoxicity.

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