EFFECT OF FINOPTIN ON DOXORUBICIN ACCUMULATION IN LEUKEMIA P388 CELLS WITH INDUCED ANTIBIOTIC RESISTANCE

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The development of resistance of tumor cells to cytostatics essentially restricts the scope for tumor chemotherapy. Many authorities consider that the development of resistance to anthracycline antibiotics and other cytostatics of natural origin is connected with acceleration of energy-dependent transport of antitumor preparations from the cells [2, 7]. One way of overcoming the resistance of tumor cells to cytostatics of natural origin in a system in vitro is to add calcium channel blockers or calmodulin inhibitors to the incubation medium of the cells [4, 6]. Meanwhile there are no data in the literature relating to the study of the effect of these preparations on accumulation of cytostatics in tumor cells with induced resistance in an in vivo system. The importance of such research is quite obvious, for we know that it is virtually impossible in an in vivo system to achieve concentrations of modifiers that are used in an in vitro system.

We have studied the effect of finoptin (FP), a calcium channel blocker, on accumulation of doxorubicin (DX) in leukemia P388 cells in mice with a tumor with induced resistance to the antibiotic.

EXPERIMENTAL METHOD

Experiments were carried out on BDF_1 hybrid male mice aged 2-3 months. Leukemia P388 cells sensitive to DX (P388/0, tumor strain bank, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) and with induced resistance to the antibiotic (P388/DX) were transplanted intraperitoneally in a dose of 1×10^6 cells in 0.2 ml of medium 199. DX and FP (pharmacopeial preparations) were injected intraperitoneally in the doses and under the conditions specified in the captions to the figures and in Table 1.

Accumulation of DX in leukemia P388/0 and P388/DX cells was determined spectrofluorometrically by the method described previously [1]. Ascites fluid was taken from the peritoneal cavity in a volume of 3 ml on the 7th day after transplantation of the tumor, 30, 60, 120, 180, and 240 min after injection of the antibiotic, and washed by centrifugation in 5 ml of Earle's solution at 1000g for 8 min. The cells were counted in a Goryaev counting chamber.

The pharmacokinetics of DX was determined as the concentration of DX in mouse blood plasma 10, 20, 40, 60, and 90 min after injection. Four mice were used at each point. The animals were killed by decapitation and blood was collected in glass test tubes with 50 μg of heparin, and centrifuged at 1000g for 5 min; 1 ml of plasma was transferred to clean test tubes, 1 ml each of ethyl and butyl alcohol was added to the tubes, which were again centrifuged at 1000g for 20 min. The DX concentration was determined as the intensity of fluorescence of the compound in the supernatant.

The therapeutic action of DX in mice with leukemia P388/DX was assessed on the basis of the increase in the mean life span of the animals which died. Ten mice were used in each group.

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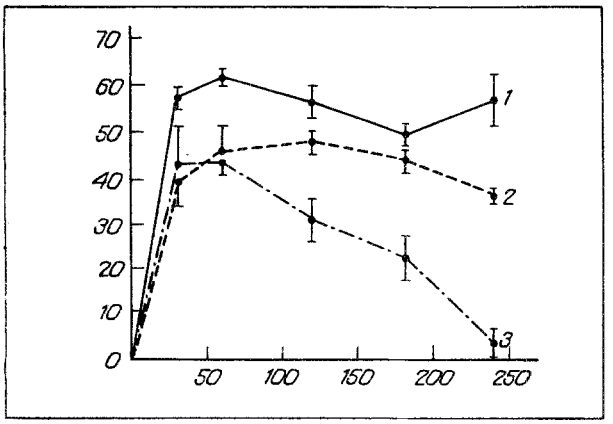

Fig. 1. Effect of FP on DX accumulation in leukemia P388/DX cells. Abscissa, time after injection of DX (in min); ordinate, DX concentration (in pg/l × 10^6 cells). 1) P388/0 + DX, 2) P388/DX + FP + DX, 3) P388/DX + DX. Here and in Fig. 2: DX in a dose of 15 mg/kg was injected 15 min after injection of FP in a dose of 20 mg/kg.

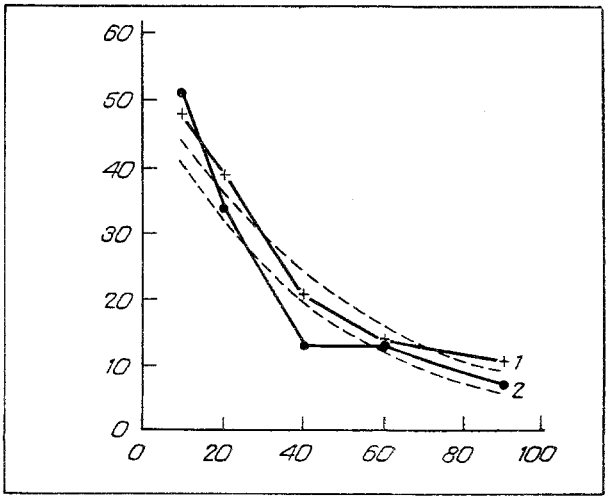

Fig. 2. Effect of FP on pharmacokinetics of DX. Abscissa, time (t) after injection of DX (in min); ordinate, concentration (C) of DX (in pg/ml plasma). 1) DX, 2) FP + DX; broken lines indicate exponential approximation of experimental dependence of C on (t).

The numerical results were subjected to statistical analysis by the Fisher–Student method. The difference was considered significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

The results of the study of the effect of FP on DX accumulation in leukemia P388/DX cells are given in Fig. 1. They show that the DX concentration in leukemia P388/DX cells was the same during 60 min of observation as in leukemia P388/0 cells, namely about 50 pg/l × 10^6 cells. However, the DX concentration in leukemia P388/DX cells then fell sharply and after 240 min of observation, it was less than 10 pg/l × 10^6 cells. Meanwhile the DX concentration in leukemia P388/0 cells remained virtually unchanged during 4 h of observation.

Administration of FP disturbs elimination of DX from leukemia P388/DX cells. For instance, the DX concentration in leukemia P388/DX cells during the first 60 min of observation

TABLE 1. Effect of FP on Therapeutic Action of DX in Mice with Leukemia P388/DX

No. of group of mice	Conditions	Mean life span of animals which died, days (M ± m)	p
1	P388/DX	10,0±0,5	
2	FP	10,7±0,2	
3	P388/DX + DX (4 mg/kg)	10,8±0,1	
4	P388/DX + FP + DX (4 mg/kg)	13,4±0,5	<0,05
5	P388/DX + DX (8 mg/kg)	10,5±0,4	
6	P388/Dx + FP + DX (8 mg/kg)	13,5±0,6	<0,05

Legend. p) Significance of differences between group of animals treated with DX and group treated with DX + FP. DX in doses of 4 and 8 mg/kg was injected 15 min after injection of FP in a dose of 20 mg/kg.

after injection of FP, did not differ significantly from the DX concentration in leukemia P388/DX cells isolated from animals not receiving the modifier. At a later time of observation (120-240 min), however, the DX concentration in leukemia P388/DX cells after injection of FP was considerably higher than in leukemia P388/DX cells isolated from animals not receiving FP, although it was lower in absolute terms than the DX concentration in leukemia P388/0 cells.

The next stage of the investigation was to study the effect of FP on the pharmacokinetics of DX. As will be clear form Fig. 2, FP did not increase the circulating time of DX in the blood plasma of the mice ($T_1/_2$ of DX = 72 min; T_{1-2} of DX + FP = 62 min).

The results suggested that administration of DX against the background of FP can overcome resistance of mice with transplanted leukemia P388/DX to the antibiotic.

As Table 1 shows, FP facilitates manifestation of the therapeutic action of DX in mice with leukemia P388/DX. Injection of DX in doses of 4 and 8 mg/kg had no therapeutic action on animals with leukemia P388/DX. The average life span of the mice in these groups was the same as in the control group. The mean life span of the animals with leukemia P388/DX, treated with DX + FP, was longer than that of animals treated with DX alone.

The results can be summed up in the statement that FP can overcome the resistance of tumor cells of leukemia P388/DX not only in an in vitro system [4, 7], but also in vivo. An essential difference of the experiments in vivo, in our opinion, is the prolonged presence (over 4 h) of the cytostatic in the tumor cells, whereas in the in vitro system, investigators usually restricted their observations to 30-60 min [3]. It has to be pointed out that injection of FP causes no change in the pharmacokinetics of DX. The effect of FP we have described is evidently due to the influence of the modifier on disturbance of elimination of the cytostatic from cells with induced resistance.

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