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EFFECT OF FINOPTIN ON DOXORUBICIN ACCUMULATION IN P-388 LEUKEMIA CELLS WITH INDUCED RESISTANCE TO A COMBINATION OF FINOPTIN AND DOXORUBICIN

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It has recently been shown that the phenomenon of multiple drug resistance (MDR) is connected with active transport of antitumor preparations from cells with the participation of glycoprotein P, which leads to reduction of cytostatic accumulation inside tumor cells. One way of overcoming MDR, in the opinion of many authorities, is to use calcium antagonists and calmodulin inhibitors, blocking glycoprotein P, and thereby causing retention of antitumor agents in the cells and potentiating their cytotoxicity [2, 4, 6]. Despite extensive discussion of this approach to the suppression of MDR, the problem of induction of resistance of tumors to a combination of cytostatics with cell calcium channel blockers and calmodulin inhibitors remains completely unstudied.

The aim of the present investigation was to study induction of resistance of tumor cells of leukemia P-388 to a combination of doxorubicin (Dx) and finoptin (Fp) and also the effect of Fp on Dx accumulation in leukemia P-388 cells sensitive to the antibiotic, leukemia P-388 with induced resistance to Dx, and leukemia P-388 with induced resistance to a combination of Fp + Dx.

EXPERIMENTAL METHOD

Experiments were carried out on male BDF_1(C57BL/6j × DBA) mice aged 2-3 months. Leukemia P-388 cells with induced resistance to Dx (P-388/Dx) were obtained by selection from leukemia P-388 cells (P-388/O, original strain, tumor strain bank, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) during treatment of animals with small doses of the antibiotic. Altogether 35 passages were needed to induce resistance. Leukemia P-388 cells resistant to a combination of Fp + Dx (P-388/Fp + Dx) were obtained from the P-388/Dx strain by selection of cells resistant to this combination. Altogether six passages were needed for leukemia P-388/Dx cells to develop resistance to the combination Fp + Dx. Tumor cells

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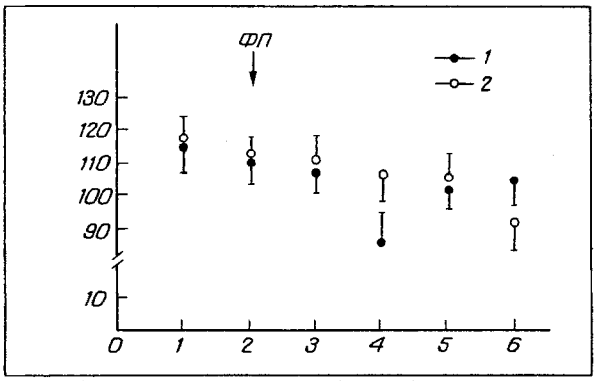

Fig. 1. Effect of Fp on Dx accumulation in leukemia P-388 cells sensitive to the antibiotic. Abscissa, time after injection of Dx (in h); ordinate, concentration of Dx (in ng/10^6 cells). 1) Mice receiving Dx alone, 2) mice receiving Fp + Dx.

TABLE 1. Evaluation of Therapeutic Action of Dx and the Combination Fp + Dx in Mice with Leukemia P-388, Sensitive to Dx, and with Induced Resistance to Dx and to the Combination Fp + Dx

Group	Treatment	Mean survival time of dying animals (M ± m)	No. of animals cured
P-388/0	—	10,0±0,0	0
P-388/0	Dx	22,7±1,2*	0
P-388/0	Fp + Dx	26,7±2,0*	70
P-388/ Dx	—	11,3±0,1	0
P-388/ Dx	Dx	11,3±0,2	0
P-388/ Dx	Fp + Dx	13,0±0,5**	0
P-388/Fp + Dx	—	10,0±0,0	0
P-388/ Fp + dx	Dx	10,1±0,1	0
P-388/ Fp + Dx	Fp + Dx	10,1±0,1	0

Legend. *) Significance of differences between mean survival time of treated and untreated animals: $*p < 0.001$, $**p < 0.05$.

were transplanted intraperitoneally in a dose of 10^6 cells in 0.2 ml of medium 199. Fp (pharmacopoeial preparation) was injected in a dose of 30 mg/kg 15 min and 2 h after injection of 15 mg/kg of Dx (pharmacopoeial preparation).

Accumulation of Dx in the tumor cells was determined spectrofluorometrically, by the method described previously [1]. Ascites fluid was withdrawn from the peritoneal cavity in a volume of 3 ml on the 7th day after transplantation of the tumor and 1, 2, 3, 4, 5, and 6 h after injection of the antibiotic, and it was washed by centrifugation in 5 ml phosphate buffer (pH 7.4) for 8 min (4°C). The cells were counted in a Goryaev's chamber.

The therapeutic action of the Fp + Dx combination was evaluated by observing the duration of survival of the animals which died and the number of cured animals during observation for 60 days. Fp in a dose of 30 mg/kg was injected 15 min and 2 h after injection of Dx in a dose of 4 mg/kg. Ten animals were used in each group.

The data were subjected to statistical analysis by the Fisher–Student method. Differences were considered significant at the $p \leq 0.05$ level.

EXPERIMENTAL RESULTS

The first stage of the investigation was to compare the therapeutic action of the Fp + Dx combination in mice with leukemia P-388/O, P-388/Dx, and P-388/Fp + Dx (Table 1). Injection of Fp was shown to potentiate the therapeutic action of Dx

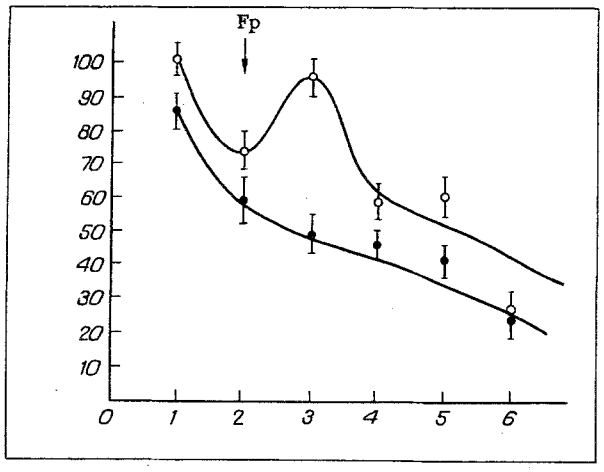

Fig. 2. Effect of Fp on Dx accumulation in leukemia P-388 cells resistant to the antibiotic. Legend as to Fig. 1.

in mice with leukemia P-388/O. The mean length of survival of animals treated with the Fp + Dx combination was greater than in animals treated with Dx alone. Furthermore, a cure rate of 70% was observed in the group of animals treated with Fp + Dx.

Administration of Dx had no therapeutic action in mice with leukemia P-388/Dx and P-388/Fp + Dx. The mean length of survival of the mice in these groups was the same as in the control. Injection of FP enhanced the therapeutic action of Dx in mice with leukemia P-388/Dx, and significantly increased the mean length of survival of animals treated with the Fp + Dx combination compared with those treated with Dx alone. The use of the Fp + Dx combination was ineffective in mice with leukemia P-388/Fp + Dx. The mean length of survival of the group of animals treated with Fp + Dx was the same as in untreated mice.

The results are evidence of resistance of leukemia of strain P-388/FP + Dx to treatment by the Fp + Dx combination.

The next stage of these investigations was to study the effect of Fp on accumulation and elimination of Dx in cells of leukemia P-388/O, P-388/Dx, and P-388/Fp + Dx.

Injection of Fp did not change the Dx concentration in leukemia P-338/O cells (Fig. 1). The Dx concentration in tumor cells of mice receiving Fp + Dx and those receiving Dx alone, 1 h after injection of the antibiotic, was virtually the same, namely 115-118 ng/10^6 cells. The Dx level recorded in animals of both groups lasted for 6 h of observation.

The results of a study of the effect of Fp on accumulation and elimination of Dx in leukemia P-388/Dx cells are given in Fig. 2. Injection of Fp caused a significant increase in the concentration of the antibiotic during 5 h of observation. For instance, 1 h after injection of Dx the concentration of the antibiotic in tumor cells in mice receiving Fp + Dx was 101 ng/10^6 cells, whereas in the group of animals receiving Dx alone it was only 86 ng/10^6 cells. The increase in accumulation of Dx in the group of animals receiving Fp + Dx, 3 h after injection of the antibiotic, was caused by a repeated injection of Fp. After observation for 6 h the Dx concentration in both groups of animals was virtually identical, namely about 25 ng/10^6 cells.

The data in Fig. 3 show that Fp does not affect accumulation or elimination of Dx in leukemia P-388/Fp + Dx cells. For instance, 1 h after injection of Dx the concentration of the antibiotic in groups of animals receiving Fp + Dx and Dx alone was virtually identical, namely about 75 ng/10^6 cells. After 6 h of observation the Dx concentration in the two groups fell to 30-40 ng/10^6 cells.

The following conclusions can thus be drawn from the results described above.

1. Administration of Fp does not change the accumulation and elimination of Dx in leukemia P-388 cells sensitive to the antibiotic.

2. Fp enhances accumulation but does not change elimination of Dx in leukemia P-388 cells with induced resistance to the antibiotic.

3. Fp has no effect on accumulation and elimination of Dx in leukemia P-388 cells with induced resistance to the Fp + Dx combination.

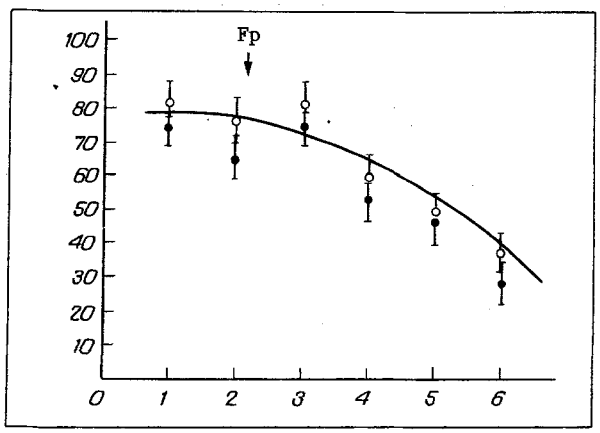

Fig. 3. Effect of Fp on Dx accumulation in leukemia P-388 cells resistant to the Fp + Dx combination. Legend as to Fig. 1.

It can be concluded from an analysis of these results that the phenomenon of suppression of MDR observed by many investigators in relation to antitumor agents of natural origin and, in particular, to Dx is evidently due to an increase in accumulation of the antibiotic in tumor cells under the influence of a modifier. On the other hand, it has been shown that the use of Fp to overcome the resistance of tumors to Dx even for quite a short time (six passages under experimental conditions for leukemia P-388) leads to the selection of cells resistant to the Fp + Dx combination. In our opinion this is evidence that there is no future for the use of Fp to overcome the phenomena of MDR. The results described above correlate with previous data on the absence of effect of Fp on Dx accumulation in tumor cells with resistance to the antibiotic induced in an in vitro system [3].

The fact must be emphasized that Fp enhances the therapeutic action of Dx in mice with leukemia P-388 sensitive to the antibiotic, and this is probably connected with the influence of the modifier on Dx distribution within the tumor cell [5].

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