EFFECT OF FINOPTIN ON METABOLISM AND PHARMACOLOGIC ACTION OF CYCLOPHOSPHAMIDE IN VIVO AND IN VITRO

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Interest in the use of Ca^{2+} antagonists in oncology has recently increased, because of the ability of these compounds to overcome the resistance of tumor cells to natural cytostatics. Although the mechanism of action of Ca^{2+} antagonists on resistant tumor cells is still being studied, experiments are being conducted in vitro and in vivo to study their use in order to overcome multiple drug resistance [6, 13]. Clinical trials of these substances in oncologic practice also have been reported [11], and the possible effects of these modifiers on the pharmacological action of cytostatics also is being investigated. It has been shown, for instance that verapimil modifies the pharmacokinetics of doxorubicin in mouse blood plasma and may affect the distribution of the cytostatics in tissues of the body [5]. The development of this trend in the study of the effects of Ca^{2+}-channel blockers on the pharmacologic action of cytostatics is logical. We know that Ca^{2+}-channel blockers are actively metabolized in the liver, where they undergo O- and N-demethylation on cytochrome P-450, the key enzyme of metabolism of many cytostatics [9]. Since polychemotherapy is used in current oncologic practice, the use of Ca^{2+}-channel blockers to overcome the resistance of tumor cells to certain cytostatics may have some influence on the metabolism of other antitumor agents, and may thus change the efficacy of treatment.

This paper describes a study of modification of the pharmacologic action of cytophosphamide (CP) under the influence of finoptin (FO). CP is known to be actively metabolized in the liver and, consequently, changes in the rate of its metabolism through the use of a Ca^{2+}-channel blocker may be expected.

EXPERIMENTAL METHOD

Finoptin was obtained from "Orion," Finland and cyclophosphamide from Saransk Medical Preparations Factory, USSR. The animals used were male (CBA × C_{57}BL/6)F_1 and male C_{57}BL/6 mice.

The therapeutic action of CP was assessed by the increase in length of survival (ILS) and the number of animals with hemocytoblastosis La cured (surviving more than 60 days after transplantation of the tumor). Hemocytoblastosis La cells were injected in a dose of $1 \cdot 10^6$ per mouse intraperitoneally in 0.2 ml of medium 199. Treatment was carried out 24 h after implantation of the tumor cells. CP was dissolved in physiological saline and injected as a single dose intraperitoneally 15 min after injection of FO. FO was injected in a dose of 20 mg/kg 15 min before injection of CP and 20 min after the injection of CP. The dose of FO corresponded to that described previously, used to overcome multiple drug resistance [2].

The concentration of reactive metabolites of CP (NBP-products) was determined in the blood plasma of the F_1 mice weighing 29-26 g by the method described previously [1, 4]. A calibration curve was plotted by the aid of methyl-*bis*-(β-chloroethyl)amine hydrochloride. Acrolein was determined in the blood plasma of the mice and the rate of microsomal oxidative hydroxylation of CP in vitro was determined by a spectrofluorometric method [3] ($\lambda_{excitation}$ = 360 nm, $\lambda_{emission}$ = 510 nm). The

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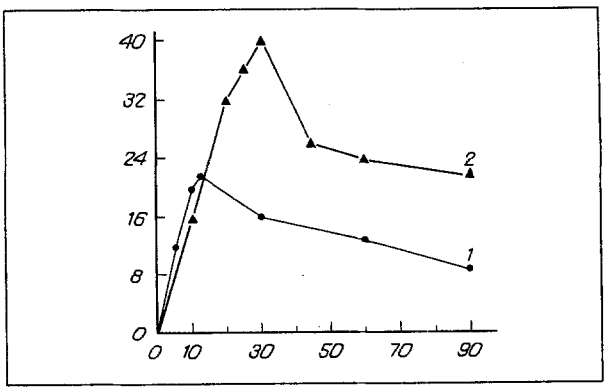

Fig. 1. Effect of finoptin on concentration of NBP-product of cyclophosphamide in mouse blood plasma. Abscissa, time after injection of CP (in min); ordinate, concentration of NBP-product of CP (in μM). 1) CP, 2) FO + CP.

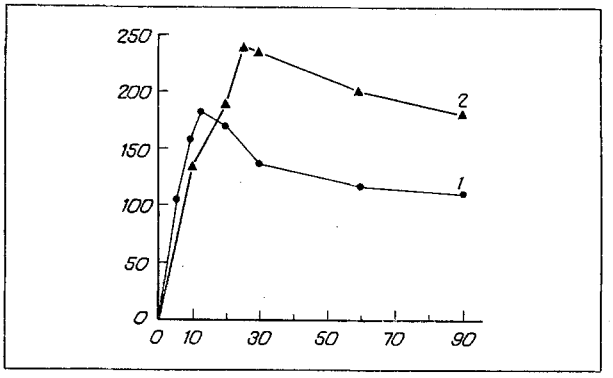

Fig. 2. Effect of finoptin on acrolein concentration in mouse blood plasma. Ordinate, acrolein concentration (in μM). Remainder of legend the same as to Fig. 1.

instrument was calibrated against a standard emission solution of 7-ethoxycoumarin, at the same wavelengths of excitation and emission.

The microsomal fraction of the liver was isolated and the cytochrome P-450 assayed by methods described previously [10]. Protein was determined by Lowry's method [7].

LD_{50} of the cytostatic was calculated by Berings' method.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Recording spectra of binding of cytochrome P-450 and FO showed that FO is a type I substrate with maximum of absorbance at 380 nm and minimum at 414 nm, and the binding constant of FO with cytochrome P-450 is 15.0 μM. CP is also a type I substrate, but its binding constant with cytochrome P-450 is 2150 μM, i.e., 193 times higher than the corresponding value for FO. The spectral type of binding indicates that both CP and FO may be substrates for cytochrome P-450, but CP has significantly lower affinity for the hemoprotein and, consequently, its metabolism on cytochrome P-450 ought to be inhibited by FO. We found that FO, in a concentration of 100 μM inhibits the reaction of cytochrome P-450-dependent oxidative hydroxylation of CP (1 mM) by 88% relative to the control level, and in a concentration of 400 μM, it blocks it completely. These data are evidence that the value of the inhibition constant for the reaction of cytochrome P-450-dependent hydroxylation of CP by FO is 40 μM.

TABLE 1. Effect of Finoptin on Therapeutic Action of Cyclophosphamide in Mice with Hemocytoblastosis La (M ± m)

Dose of CP, mg/kg	Modifier	Mean length of survival of animals which died, days	ILS, percent of control	Number of animals cured, percent
Control		8,3±0,2		0
—	FO	8,5±0,2	2	0
100	—	24,6±1,1	196	0
100	FO	27,0±0,9	225	10
200	—	25,2±3,3	204	0
200	FO	31,5±3,8	280	60
300	—	16,7±2,7	101	60
300	FO	13,8±6,1	66	30
350	—	10,6±2,4	28	0
350	FO	4,4±0,5	—47	0
400	—	10,0±2,4	20	0
400	FO	5,6±0,9	—33	0

The pharmacokinetics of the NBP-reactive metabolite of CP in mice receiving the cytostatic alone or in combination with FO is shown in Fig. 1. Use of the modifier causes a shift of the maximum of the concentration of the NBP-product from 10 to 30 min. The maximal concentration of reactive metabolites in the blood plasma of the mice under these circumstances was almost doubled. Similar changes took place in the pharmacokinetics of acrolein in the animals' blood plasma against the background of FO (Fig. 2). The maximum of the acrolein concentration shifted from the 10th to the 20th-30th minute and the maximal acrolein concentration in the blood plasma of the mice increased from 180 to 240 μM. Reactive metabolites of CP are known to mediate its therapeutic and toxic action, whereas acrolein, another metabolite of CP, mediates only its toxic action. On the whole, changes in the pharmacokinetics of CP against the background of FO are characteristic of inhibition of liver monooxygenase activity. It follows from the pharmacokinetic data for the effect of FO on CP metabolism that FO may enhance both the therapeutic and the toxic action of the cytostatic. Toxicologic investigations showed that FO significantly depresses LD_{50} of CP from 388.0 ± 13.9 to 342.8 ± 16.0 mg/kg body weight. This confirms the hypothesis that FO increases the toxicity of CP.

The effect of FO on the therapeutic action of CP also was studied in mice with hemocytoblastosis La (Table 1). It was shown that FO enhances the therapeutic action of CP within the dose range from 100 to 200 mg/kg. ILS of animals receiving CP in a dose of 100 mg/kg was 196%, whereas against the background of the modifier, this parameter was increased to 225%, somewhat higher than ILS of animals receiving CP alone, in a dose of 200 mg/kg. Incidentally, the use of the modifier caused an increase in the number of animals cured up to 60%, whereas in groups of animals receiving the cytostatic alone, no cures were observed. However, with a further increase in the dose of CP from 300 to 400 mg/kg the efficacy of the combination was less than that of the cytostatic alone. If CP was injected in a dose of 300 mg/kg 60% of the animals were found to be cured, but when the modifier was used, this figure was reduced to 30%. The value of ILS also was reduced in groups of animals receiving a combination of FO and CP. This kind of effect of FO on ILS and the percentage of cured animals can probably be explained by an increase in toxicity of the combination with doses of the cytostatic of 300 mg/kg or higher, in agreement with the results of a study of the effect of FO on the toxicity of CP, obtained previously.

The use of modifiers to overcome multiple drug resistance thus leads to confrontation with the following problems. First, the modifier must be used at the time of action of the cytostatic, when it may have an unpredictable effect on the therapeutic and toxic action of antitumor preparations, used in the schedule of polychemotherapy. Second, the modifiers must have definite polycyclic structures, which permit interaction of the molecule with the P-glycoprotein pharmacophore, as well as high affinity for cytochrome P-450 [8,12]. This kind of interaction of FO both with the P-glycoprotein molecule and with cytochrome P-450, explains the change in pharmacologic properties of CP when FO is used. This change in the pharmacologic action of the cytostatic must be taken into account when chemotherapy for malignant neoplasms is undertaken.

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