BIOCHEMICAL BASIS OF THE FTORAFUR-POTENTIATING EFFECTS OF THE CYTOCHROME P-450-DEPENDENT MICROSOMAL MONOOXYGENASE INDUCER PERFLUORODECALIN

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Ftorafur (FT) is one of the few agents which can be used in clinical practice to treat solid tumors [7]. It is the depot form of 5-fluorouracil and, for its pharmacologic activity to be manifested, it requires preliminary biotransformation in vivo by the cytochrome P-450-dependent liver monooxygenases [1, 2, 6, 7]. When malignant tumors are treated by ftorafur, microsomal enzymes must be constantly stimulated, since FT itself and the development of the tumor considerably inhibit their activity [2, 6, 7].

The aim of this investigation was to study the biochemical basis of resistance of experimental tumors (Lewis lung carcinoma and hepatoma H-2-73) to ftorafur and the possibility of overcoming it by combining the ftorafur with perfluorodecalin, an effective liver cytochrome P-450 inducer [8].

EXPERIMENTAL METHOD

Male inbred C57BL/6 and (CBA × C57BL/6)F_1 hybrid mice aged 2-3 months and weighing 20-25 g were used. Strains of a transplantable Lewis lung carcinoma (LLC) were obtained from the Laboratory of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and strains of hepatoma H-2-73 from the Laboratory of Pharmacology and Toxicology, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Tumors in the experiment were implanted subcutaneously in the region of the axilla in a dose of 0.2 ml of a 30% suspension; the day of implantation was taken as day 0.

Perfluorodecalin (PFD, $C_{10}F_{18}$, mol. wt. 462.11 daltons, bp 142-143°C, density 1.93 g/ml) was injected intraperitoneally in a dose of 0.5 ml per mouse. FT (4% solution in ampuls, from "Olainefarm") was injected intravenously into the retro-orbital plexus of the mice. Treatment with FT began on the 5th-6th day after implantation, when the calculated mass of the tumor was 0.5-1% of the animal's body weight. The mice were killed on the 6th day after injection of FT.

Isolation of microsomal fraction from the liver homogenate, and measurement of the concentrations of cytochromes P-450 and b_5, and of the rate of N-demethylation of aminopyrine and of p-hydroxylation of aniline were carried out as described in [4]; the microsomal protein concentration was measured by a modified Lowry's method [10]. The results were analyzed by Student's t test.

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TABLE 1. Effect of Tumor (LLC) Growth on State of Cytochrome P-450 System in Liver of C57BL/6 Mice (M ± m, n = 15)

Time after inoculation of tumor, days	Group	Cytochrome, nmoles/mg protein		Enzyme, nmoles substrate/min/mg protein	
		P-450	b_5		
5	Intact animals	0,64±0,03	0,61±0,04	6,8±0,13	1,2±0,09
	Tumor bearers	0,53±0,02*	0,50±0,02*	6,5±0,49	1,05±0,04
8	Intact animals	0,56±0,04	0,58±0,02	5,91±0,04	0,96±0,10
	Tumor bearers	0,43±0,03*	0,57±0,03	5,20±0,12*	0,79±0,04*
14	Intact animals	0,54±0,04	0,55±0,02	5,02±0,27	1,05±0,08
	Tumor bearers	0,41±0,02*	0,46±0,03*	4,15±0,33*	0,78±0,15*

Legend. Here and in Tables 2 and 3: *$p < 0.05$.

TABLE 2. Effect of Single Injection of FT on Activity of Cytochrome P-450 System in Liver of Intact F_1 Mice (M ± m, n = 15)

Group	Cytochrome, nmoles/mg protein		Enzyme, nmoles substrate/min/mg protein	
	P-450	b_5	aminopyrine demethylase	aniline hydroxylase
Intact control	0,69±0,05	0,54±0,03	7,95±1,09	0,99±0,08
Time after injection of FT in dose of 100 mg/kg, h				
1	0,75±0,09	0,62±0,04	7,01±0,21	0,91±0,14
4	0,56±0,03*	0,53±0,04	7,12±1,09	0,83±0,05*
24	0,54±0,02*	0,44±0,03*	7,62±0,35	1,00±0,12

EXPERIMENTAL RESULTS

Table 1 gives data showing the effect of tumor growth on the liver monooxygenases of mice with LLC. The concentrations of cytochromes P-450 and b_5 and microsomal monooxygenase activity, using substrates of types I and II, were determined during the latent period of tumor development (5th day), at the time of appearance of a palpable tumor (8th day), and at the height of manifest growth of LLC (11th day after inoculation). As Table 1 shows, tumor development leads to lowering of the levels of cytochromes P-450 and b_5 and also of activity of the monooxygenase reactions responsible for biotransformation of drugs of the FT type. Similar results showing inhibition of activity of liver monooxygenases by tumor development were obtained in our experiments on mice with hepatoma H-2-73. Levels of cytochromes P-450 and b_5 and N-demethylase and p-hydroxylase activity on the 11th day after inoculation of the tumor (mass of tumor 1657 ± 232 mg) were 67, 93, 72, and 98% respectively of the level of these parameters in intact mice of the same batch.

Injection of FT, in turn, causes inhibition of monooxygenase function of the liver. The time course of parameters of the state of the biotransformation function of the liver in native mice 1, 4, and 24 h after receiving a single injection of FT in a dose of 400 mg/kg is illustrated in Table 2. A single injection of FT lowered the cytochrome P-450 level after 4 h significantly ($p < 0.05$), and the effect remained 1 day after injection ($p < 0.01$). The cytochrome b_5 level was significantly lowered only when 1 day had elapsed after the injection of FT ($p < 0.01$), and aniline phydroxylase activity was significantly inhibited after 4 h ($p < 0.05$) and returned to normal 24 h after injection of FT. Activity of aminopyrine N-demethylase also showed a tendency to fall, which was most marked 1-4 h after injection of the cytostatic.

Inhibition of function of the FT-transforming enzymes of the liver in tumor-bearing mice, induced by tumor growth, is evidently the cause of resistance of most experimental tumors to FT [3]. To overcome the biochemical resistance of tumors due to inhibition of FT biotransformation, we used a combination of FT with PFD — an effective inducer of liver microsomal monooxygenases [8]. Table 3 gives data on the effect of PFD on somatic parameters, namely tumor development and activity of the cytochrome P-450-dependent liver monooxygenases of native and inoculated C57BL/6 mice. As Table 3 shows, PFD did not affect the leukocyte count, body weight, or weight of the spleen and thymus of native and tumor-bearing animals and had no effect on development of the leukemoid reaction induced by growth of LLC [11]. Injection of PFD led to an increase in mass of the liver (on average by 37% in native and 20% in tumor-bearing mice) and completely prevented the suppression of function of cytochrome P-450-dependent monooxygenases induced by tumor development. Comparison of levels of biotransforming enzymes in native and tumor-bearing animals, induced under the influ-

TABLE 3. Effect of PFD on Somatic Parameters, Tumor Development, and Activity of Liver Cytochrome P-450-Dependent Monooxygenases in C57BL/6 Mice (M ± m, n = 15)

Parameter studied	Native animals		Animals with LLC tumors, 14th day	
	without PFD	with PFD	without PFD	with PFD
Morphometric index:				
body weight, g	28,48±1,7	30,14±1,76*	27,39±2,3	28,54±2,7
weight of: liver, g	1,26±0,2	1,73±0,1*	1,54±0,2	1,82±0,2*
spleen, mg	88,2±22,7	99,8±15,2	260±55	301±69
thymus, mg	36,4±13,9	34,2±9,63	38,2±8,8	39,3±5,8
tumor, mg	—	—	1665±140	1625±209
Blood leukocyte count, thousands/mm^3	9,1±0,9	8,4±0,9	13,4±3,7	13,0±4,1
Cytochrome, nmoles/mg protein:				
P-450	0,58±0,03	1,04±0,05*	0,46±0,04	0,87±0,01*
b_5	0,58±0,01	0,77±0,02*	0,51±0,03	0,64±0,03*
Enzyme, nmoles substrate/min/mg protein:				
aminopyrine N-demethylase	6,57±0,84	20,99±2,34*	5,98±0,95	18,31±0,06*
aniline p-hydroxylase	1,07±0,07	2,49±0,11*	0,87±0,09	2,35±0,10*

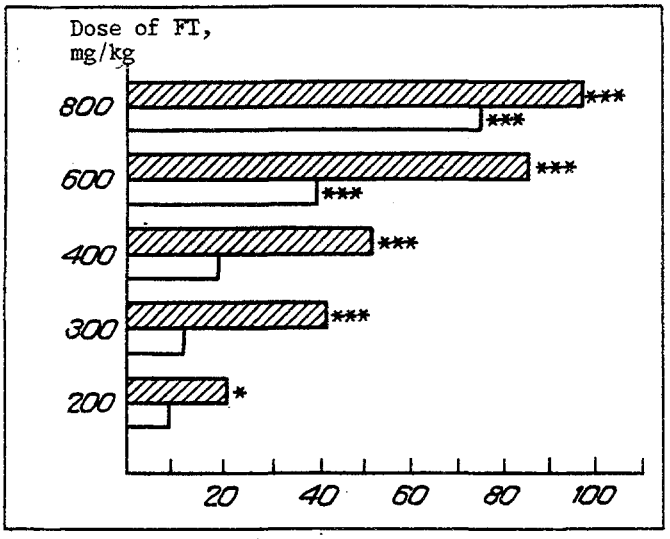

Fig. 1. Potentiation by PFD of dose-dependent antitumor efficacy of FT. Evaluation of effect on 6th day after injection of FT and on 11th day after inoculation of F_1 mice with hepatoma H-2-73. PFD in a dose of 0.5 ml/mouse injected 5 days before inoculation of tumor. Unshaded columns — FT, shaded — FT + PFD. *p < 0.05. Abscissa, inhibition of tumor growth (in % of control); ordinate, dose of FT (in mg/kg).

ence of PFD, indicates that by the use of PFD it is possible to overcome the resistance of the monooxygenase systems of the liver, formed during tumor development, to the action of classical inducers of phenobarbital type [5].

Evaluation of the cytotoxic effects of FT using two test systems, namely mass of the tumor and blood leukocyte count, showed that on the 6th day after injection of FT in a dose of 400 mg/kg (11th day after inoculation of LLC) the leukocyte count fell from 14.0 ± 5.6 to 10.5 ± 5.6 thousands/mm^3, whereas the dimensions of the tumor were virtually unchanged: 1470 ± 792 mg in the control and 1371 ± 961 mg after FT. Against the background of PFD the same dose of cytostatic caused a fall of the leukocyte count from 15.0 ± 5.2 to 9.4 ± 3.4 thousands/mm^3 and reduction of the mass of the tumor to 562 ± 318 mg ($p < 0.05$). In view of data in the literature on the resistance of LLC to 5-fluorouracil, the principal antitumor metabolite of FT [3], it can be tentatively suggested that PFD activates other pathways of FT biotransformation, resulting in the formation of other FT metabolites than 5-fluorouracil, which possess marked antitumor activity [9].

Data on the action of PFD on the dose-dependent effect of FT, obtained on a model of hepatoma H-2-73, are given in Fig. 1. Clearly a significant antitumor effect of FT was recorded in a dose of 600 mg/kg and higher, whereas in combination with PFD significant inhibition of tumor growth was observed with a dose as low as 300 mg/kg. Reduction of the therapeutic dose of FT without loss of its antitumor efficacy is of considerable importance in the clinical treatment of tumors, for FT in high doses exhibits a marked neurotoxic effect [1, 6, 7].

The results thus indicate that it is possible to modify the antitumor efficacy of FT in a particular direction by means of PFD, an inducer of the liver cytochrome P-450 system. A combination of FT with PFD potentiates the action of FT on tumors resistant to it (LLC, hepatoma H-2-73) on average by a factor of 2.5. The possibility of significantly reducing the dose of FT, which exhibits marked neurotoxicity, without any loss of antitumor efficacy is evidence that the use of such combinations in clinical practice is promising.

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SOME MOLECULAR MECHANISMS OF THE ANTIOXIDATIVE ACTION OF DALARGIN ON THE LIVER IN EXPERIMENTAL CHOLESTASIS

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Dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a synthetic analog of the opioid Leu-enkephalin, synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Professor M. I. Titov, and which possesses antistressor activity and exerts a protective effect on organs (including the liver), has begun to be used in recent years as a protective agent in anesthesiology. In such cases dalargin has been observed not to have a direct membrane-stabilizing effect on the myocardium [4], and the protective action of dalargin (in myocardial infarction, after wounding) has been shown to be realized in opioidergic receptor processes, for it is completely abolished by simultaneous administration of the structural morphine analog naloxone, a universal opioid antagonist [1, 7].

Meanwhile, the molecular mechanisms of the protective action of dalargin on the liver have not been adequately studied. There is no information in the literature on the action of the dalargin antagonist, naloxone, on liver function.

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