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MODIFICATION OF THE TOXIC AND ANTITUMOR PROPERTIES OF FTORAFUR BY ACTION AIMED AT NONSPECIFIC MICROSOMAL OXIDASES

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Phenobarbital and methylcholanthrene, inducers of nonspecific microsomal oxidases, inhibit the development of neurotoxic shock produced in mice by large doses of ftorafur, but they increase the mortality among the animals on the 4th-8th days after administration of the compound. Inhibitor SKF525 A has the opposite action on both types of toxic manifestations. The antitumor effect of ftorafur, determined on the basis of the decrease in weight of the spleen in mice with Rauscher leukemia, is greatly enhanced by preliminary administration of phenobarbital, alone or together with methylcholanthrene, to mice.

KEY WORDS: ftorafur; nonspecific microsomal oxidases.

The antitumor agent ftorafur has achieved widespread popularity because of its lower toxicity, compared with other fluoropyrimidines, toward the epithelium of the small intestine and hematopoietic organs [1, 2, 8].

It is considered that this property of ftorafur is most probably due to its slow breakdown and conversion into the active principle, 5-fluorouracil [5, 6].

This character of its metabolism, ensuring the long circulation of low concentrations of 5-fluorouracil in the body, also gives ftorafur lower antitumor activity than 5-fluorouracil in relation to several experimental tumors [3, 7]. Attempts to enhance its antitumor activity by the use of massive doses of the compound have proved it possible, for in large doses ftorafur gives rise to neurotoxic complications, the severity of which is proportional to the sessional dose of the drug [4].

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The results of investigations in a cell-free system have shown the metabolic conversion of ftorafur into 5-fluorouracil is effected by nonspecific microsomal oxidases [6, 9]. It was accordingly decided to study the effect of inducers and an inhibitor of this enzyme system on the toxic action of ftorafur and its antitumor activity.

EXPERIMENTAL MATERIAL AND METHOD

Mice of two lines, sensitive to induction of microsomal enzymes, were chosen for the experiments: C57BL/6J (B) and BALB/c (C). In each experimental group there were 6 or 7 female mice weighing 19-21 g.

Phenobarbital (PH) (from "Pharmakhim") was used as the type I inducer, 20-methylcholanthrene (MCh) (from Fluka) as type II inducer, and SKF525 A (from Smith-Kline and French) as inhibitor. These compounds are most widely used to modify the activity of microsomal enzymes [11, 13].

All the compounds were injected intraperitoneally: PH in a dose of 60 mg/kg once daily for 3 consecutive days, MCh in a single dose of 15 mg/kg, and SKF525 A also as a single dose of 50 mg/kg. Ftorafur (from the No. 6 Pharmaceutical Chemical Factory, Riga) also was injected as a single dose 24 h after the last injection of the inducer or combination of inducers or 1 h after injection of the inhibitor. The volume of solvent (sunflower oil or MCh, distilled water for the other compounds) was 0.1 ml/10 g body weight.

The duration of neurotoxic shock was determined as the time during which the mice remained in whatever position they were put, in the lateral position, for example, as a result of adynamia and muscular relaxation.

Rauscher leukemia [10] was induced in C mice by intraperitoneal injection of a suspension of spleen cells in a dose of $1 \cdot 10^6$ cells per mouse in 0.25 ml medium No. 199. Administration of ftorafur began 15 days after injection of the cell suspension, and of the inducers appropriately earlier. The mice were killed 3 days after injection of ftorafur and the weight of the spleen and their body weight were measured. The animals also were weighed on the day of starting treatment.

EXPERIMENTAL RESULTS AND DISCUSSION

Large doses of ftorafur caused two types of acute complications, which can be described as immediate and delayed. The first type developed 30-60 min after injection of the compound and was a variety of neurotoxic shock. Its principal features were adynamia, dyspnea, hypothermia, and lowering of muscle tone, amounting sometimes to complete flaccidity of the animal [4]. After a particularly large dose the animals died without recovering from this state.

Death as a result of delayed toxic action was observed with effect from the 4th day after injection of the compound. At that time, just as during the action of the other fluoropyrimidines, necrosis of the epithelium of the small intestine and inhibition of hematopoiesis are observed and immunodepression develops [2, 8].

In the present experiments a single injection of ftorafur in a dose of 300 mg/kg induced neurotoxic shock lasting from 8 to 12 h in B mice. Preliminary injection of inducers of nonspecific oxidases into the mice weakened this effect, and the strongest antishock action was observed after the use of a combination of PH and MCh (Fig. 1). In this case the duration of flaccidity of the animals was reduced to almost one-tenth.

Conversely, injection of the inhibitor of nonspecific oxidases prolonged the neurotoxic reaction by three times or more. Under these circumstances some mice died during the first day. In the dose used, SKF525 A inhibited the antishock action of the inducers, and its action in this direction was weakest against the background of a combination of PH + MCh (Fig. 1).

The inducers used in these experiments had the directly opposite action on the delayed toxic action of ftorafur (Table 1). Death of 100% of B mice, receiving PH and MCh beforehand, was observed after ftorafur in a dose of only 200 mg/kg. If ftorafur was given in a dose of 300 mg/kg, all the mice receiving even one of the inducers beforehand died.

SKF525 A, in a dose of 50 mg/kg, inhibited the delayed toxic effect of a combination of PH + ftorafur, but no inhibitory action was manifested by a combination of ftorafur with MCh or with both inducers (Table 1).

The results can be explained in terms of the known data on the properties and metabolism of ftorafur [6, 9, 15]. Neurotoxic shock is evidently caused by the action of ftorafur itself, which passes through blood–brain barrier and is concentrated in the brain tissue because of its hydropholic properties. The presence of the fluorine atom in the ftorafur molecule is not the cause of the neurotoxic effect, for in preliminary experiments a similar reaction of the mice was observed to administration of tetrahydrofurylthymine.

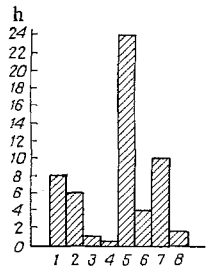

Fig. 1. Effect of inducers and inhibitor of nonspecific microsomal oxidases on duration of neurotoxic shock induced in B mice by ftorafur. Abscissa, groups of animals: 1) ftorafur (FF) 300 mg/kg, 2) MCh + FF, 3) PH + FF, 4) PH + MCh + FF, 5) SKF525 A + FF, 6) MCh + SKF525 A + FF, 7) PH + SKF525 A + FF, 8) PH + MCh + SKF525 A + FF; ordinate, minimal duration of neurotoxic shock (time taken for half of the animals of a given group to recover their motor activity).

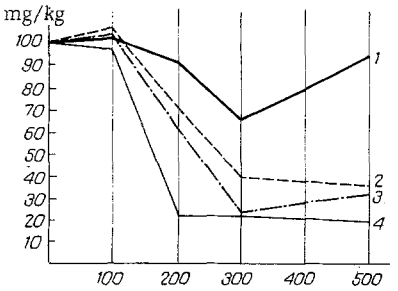

Fig. 2. Change in weight of spleen in C mice during treatment of Rauscher leukemia with ftorafur. Abscissa, mean weight of spleen in per cent of control (untreated animal); in all cases error of mean did not exceed 10%; ordinate, dose of ftorafur. Experimental groups: 1) ftorafur (FF), 2) MCh + FF, 3) PH + FF, 4) PH + MCh + FF.

Inducers of nonspecific oxidases evidently sharply potentiate the activity of enzymes converting ftorafur into less hydropholic derivatives, which pass less readily through the blood–brain barrier and which accumulate to a lesser degree in the brain tissue. These metabolites, the principal of which is 5-fluorouracil, possess higher cytotoxicity, and this explains the enhancement of the delayed toxic action of ftorafur against the background of increased activity of metabolizing enzymes.

Differences in the ability of PH and MCh to prevent the neurotoxic action of ftorafur are evidently connected with the fact that these agents induce synthesis of different terminal components of the nonspecific oxidase

TABLE 1. Change in Character of Toxicity of Ftorafur during Induction and Inhibition of Nonspecific Oxidase Activity in B Mice

Preliminary treatment	ITE	DTE	ITE	DTE	ITE	DTE
	dose of ftorafur, mg/kg					
	100		200		300	
Control	0/12	0/12	0/12	1/12	6/24	1/18
PH+MCh	0/6	0/6	0/6	6/6	0/18	18/18
PH	—	—	—	—	0/6	6/6
MCh	—	—	—	—	0/6	6/6
SKF525-A	—	—	1/6	0/5	1/6	0/5
PH+MCh+ SKF525 A	—	—	—	—	0/6	6/6
PH+SKF525-A	—	—	—	—	1/6	2/5
MC+SKF525-A	—	—	—	—	1/6	5/6

* This group contained, besides the pure control, mice receiving the solvent of MCh (sunflower oil) and the solvent of PH (distilled water).

Legend. Numerator shows number of animals which died, denominator number of animals used in experiments. ITE) Immediate toxic effect – death during 1st day; DTE) delayed toxic effect – death during 4-7th days.

system in microsomes. Phenobarbital stimulates the formation of cytochrome P-450, which is known to form a complex with ftorafur and to oxidize it [6, 9]. Methylcholanthrene induces the synthesis of cytochrome P-448 in the microsomes, and this substance catalyzes predominantly the oxidation of polycyclic aromatic hydrocarbons, which it intensifies about tenfold [11, 12], but which is evidently less active against ftorafur.

SKF525A, which inhibits the activity of enzymes catalyzing rupture of the pseudoglucoside C-N bond in the ftorafur molecule between the tetrahydrofuran ring and 5-fluorouracil, ought to lead to the longer circulation of unchanged ftorafur in the blood stream, and this explains the effects it produces.

The action of ftorafur on Rauscher leukemia was next investigated after modification of the activity of the microsomal enzymes.

The results of these experiments showed that preliminary treatment of the mice with PH or MCh potentiates the action of ftorafur on Rauscher leukemia cells. The weight of the spleen of the affected animals fell particularly sharply after a combination of ftorafur with both inducers (Fig. 2). Whereas in a dose of 200 mg/kg ftorafur alone had no effect on the weight of the spleen, when combined with the two inducers it reduced it by almost four-fifths. This effect was evidently maximal on the 3rd day after injection of ftorafur, for it was not increased by increasing the dose of ftorafur to 500 mg/kg or by injecting large doses of 5-fluorouracil into the mice. A "very high" dose of ftorafur (500 mg/kg) could be tolerated and survived up to 3 days only by mice treated with PH or with both inducers together. Mice not so treated died 20-24 h after administration of ftorafur from neurotoxic shock. Some weakening of the neurotoxic action of this dose of ftorafur was produced by MCh, as a result of which four of the seven experimental mice survived until the 3rd day. This inducer also potentiated the action of ftorafur on the tumor cells, although less strongly than PH. Incidentally, PH and MCh without ftorafur did not inhibit the increase in weight of the spleen of mice infected with Rauscher leukemia: during the period of administration of the inducers the weight increased, just as in the control, by 40%.

SKF525 A did not stimulate the antitumor action of ftorafur in a dose of 100-200 mg/kg. Its administration with higher doses of ftorafur was impossible because of the neurotoxic complications, which are potentiated by this agent.

By means of inducers or inhibitors of microsomal enzymes it is thus evidently possible to regulate the metabolism of ftorafur. Besides simple acceleration or retardation of the liberation of active metabolites, inducers of nonspecific oxidases also enable the character of the metabolites thus obtained to be changed [14]. This opens up new prospects for the use of ftorafur and calls for further research on different experimental objects.

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CELLS WITH FRAGMENTED NUCLEI IN ASCITES HEPATOMA 22A AND THEIR ROLE IN PROLIFERATION

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During aging of an ascites hepatoma 22A (AH22A) the number of cells with fragmented nuclei (especially multilobate) increases: in an AH22A aged 6 days they numbered $15 \pm 9.3^0/_{000}$, in a tumor aged 14 days $196 \pm 53^0/_{00}$, and in a delayed tumor aged 18 days $453 \pm 51^0/_{00}$. The main method of formation of fragmented nuclei is by amitosis. Approximately 150 and $170^0/_{00}$ of cells with fragmented nuclei in a 14- and 18-day old AH22A were in the reversible resting R_1 stage (or in a very protracted G_1-period, extending over 4 days), whereas the remaining 50 and $230^0/_{00}$ of cells respectively had left the mitotic cycle irreversibly and were evidently undergoing involution, which takes place more rapidly during passage-stimulated division.

KEY WORDS: ascites hepatoma; fragmented nuclei; mitotic cycle.

As a result of a few investigations, evidence has been obtained that during growth of certain ascites tumors the number of cells with fragmented nuclei (FN) in them increases, [2, 3]. Two types of fragmentation have been observed: multinuclear and multilobate (nuclei with deep invaginations). Many of these cells are viable: during stimulation of division by passage, DNA synthesis takes place in the FN [2] and these cells pass through normal mitosis [3]. The authors cited above suggested that FN are formed in ascites tumor cells as a result of disturbances of mitosis. During a long study of ascites hepatoma 22A (AH22A) the present writers

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