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Changes in Toxic and Antitumor Properties of Ftorafur by Induction or Inhibition of the Microsomal Enzymes Activity*

G. A. Belitsky, V. M. Bukhman, and I. A. Konopleva

Laboratory of Chemical Carcinogenesis, Cancer Research Center of the USSR AMS, Kashirskoye Shosse 6, Moscow, 115478, USSR

Summary. *The inducers of microsomal drug-metabolizing enzymes phenobarbital (PB) and 20-methylcholanthrene (MC) inhibited the lethargic effect of high doses of ftorafur in C57BL/6j mice, but stimulated the animal mortality at days 4–8 after the drug administration. The opposite effect has been obtained by the combination of ftorafur with the inhibitor of the microsomal enzymes SKF 525A. Animal pretreatment with PB or with PB + MC markedly enhanced the antineoplastic activity of ftorafur in Rauscher leukemia-, leukemia La-, or hemangiopericytoma-bearing mice but seemed unlikely to affort any therapeutic advantage over this drug because the lethal toxicity of ftorafur was increased.*

Introduction

Ftorafur has become a commonly used antineoplastic drug because it causes milder bone marrow depression and milder gastrointestinal toxicity than 5-fluorouracil [5, 16, 17].

These properties of ftorafur are attributed to its slow metabolic activation, i.e., conversion to 5-fluorouracil and some other suspected cytotoxic metabolites [2, 3, 9, 11, 15, 25].

The slow release of the active drug resulted not only in the low cytotoxicity of ftorafur, but also in its lower effectiveness against some experimental tumors in contrast to 5-fluorouracil [10, 29].

Attempts have been made to increase the antitumor activity of ftorafur by using high doses of the drug, but it produces extensive toxicity of the central nervous system [14]. The clinical use of ftorafur in high single intermittent doses was also limited by unacceptable manifestations of central nervous system toxicity, including lethargy, confusion, ataxia, and dizziness [13].

We supposed that the neurotoxic effects produced by ftorafur might be affected by influencing the enzymes involved in ftorafur metabolism.

Studies performed in tissue homogenates or isolated liver microsomes indicated that microsomal drug-metabolizing enzymes such as cytochrome P-450 may participate in transformation of ftorafur to 5-fluorouracil [9, 20–22]. Attempts were also made to enhance the conversion of ftorafur to 5-fluorouracil by the induction of drug-metabolizing enzymes activity [8, 21, 22].

This gave us the basis for studying the effect of inducers and an inhibitor of the given enzymatic system on toxic effects produced by ftorafur and its antitumor activity.

Materials and Methods

Animals and Tumors. The animals used were 2- to 3-month-old C57BL/6j (B6), BALB/C (C), and (CBA × C57BL/6j) F_1 ($CBAB6F_1$) female mice obtained from the Stolbovaya breeding farm of the USSR Academy of Medical Sciences.

Rauscher leukemia [6], leukemia La [24] and the solid tumor hemangiopericytoma [23] were maintained in C, B6, and $CBAB6F_1$ mice, respectively.

Drugs. Ftorafur in 4% conventional aqueous solution was obtained in ampules from the Himpharm Factory no. 6 (USSR). The stock solution was diluted with water.

Sodium phenobarbital (Merk) and SKF 525A (Smith Kline & French Labs. Ltd) were dissolved in water, while 20-methylcholanthrene (Fluka A.G.) was dissolved in sunflower oil. All the concentrations were prepared immediately before use so that 0.1 ml/10 g body weight provided the doses desired for mice.

Administration Schedule for the Agents. Microsomal enzyme activities were induced by administration of three successive IP injections of sodium PB (60 mg/kg) or one IP injection of MC

Reprint requests should be addressed to G. A. Belitsky

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(15 mg/kg). When both inducers were combined MC was injected on the same day as the third PB injection but 4 h later. A single IP injection of ftorafur was then inoculated 24 h after the last dose of the inducer. A single IP injection of SKF 525A (50 mg/kg) was given 1 h before ftorafur administration.

Study of Ftorafur Lethargy Effect. Normal non-tumor-bearing B6 mice pretreated with inducers of the microsomal enzymes or SKF 525A and untreated controls received ftorafur IP at a dose of 300 mg/kg. The duration of lethargy was estimated by the time period during which the mice remained in a certain position on their side as a result of adynamia and muscular relaxation.

Study of Antitumor Effects. In our experiments $1 \cdot 10^6$ spleen nucleated cells from Rauscher leukemia-bearing mice were inoculated IP on day 0. Ftorafur was injected on day 15 and the inducers or SKF 525A earlier. Three days after ftorafur administration the animals were killed by cervical dislocation and the spleen weight was determined.

For transplantation of leukemia La each mouse received $1 \cdot 10^6$ tumor cells by IP injection on day 0. Phenobarbital was administered on days −2, −1, 0, and/or MC on day 0. The injections of inducers on day 0 were performed 4 h after tumor cell transplantation. Ftorafur was administered in day 1. The antitumor effect was evaluated from the mean survival time and the percentage increase in lifespan (ILS) was calculated. All dead mice were autopsied to determine macroscopic manifestations of leukemia. The data obtained showed whether the mice concerned died of leukemia or drug-related toxicity.

The solid tumor hemangiopericytoma was transplanted by SC injections of $1 \cdot 10^6$ tumor cells into the right axilla on day 0. The treatment of the animals with PB and ftorafur was performed according to the same schedule as in leukemia La. On day 8 mice were sacrificed and tumors were weighed.

Results

In our experiments a single ftorafur administration at a dose of 300 mg/kg caused lethargy lasting 5–20 h in B6 mice. Pretreatment of the animals with inducers of microsomal enzymes diminished this complication, the highest antilethargic effect being registered in the case of concurrent PB and MC injections (Fig. 1). In this case the duration of animals lying motionless was reduced almost ten times.

In contrast, administration of an inhibitor of drug-metabolizing enzymes increased the duration of the neurotoxic reaction three or more times, some mice dying during the first day. At the dose administered SKF 525A inhibited the antilethargic action of inducers, its action being least marked against the PB plus MC combination.

The inducers used had precisely the opposite effect on the delayed toxic action of ftorafur (Tables 1 and 2). Here the 100% death rate of B6 mice pre-inoculated with PB and MC was observed with a ftorafur dose of as little as 200 mg/kg. At a dose of 300 mg ftorafur/kg all the mice pretreated with at least one of the inducers died.

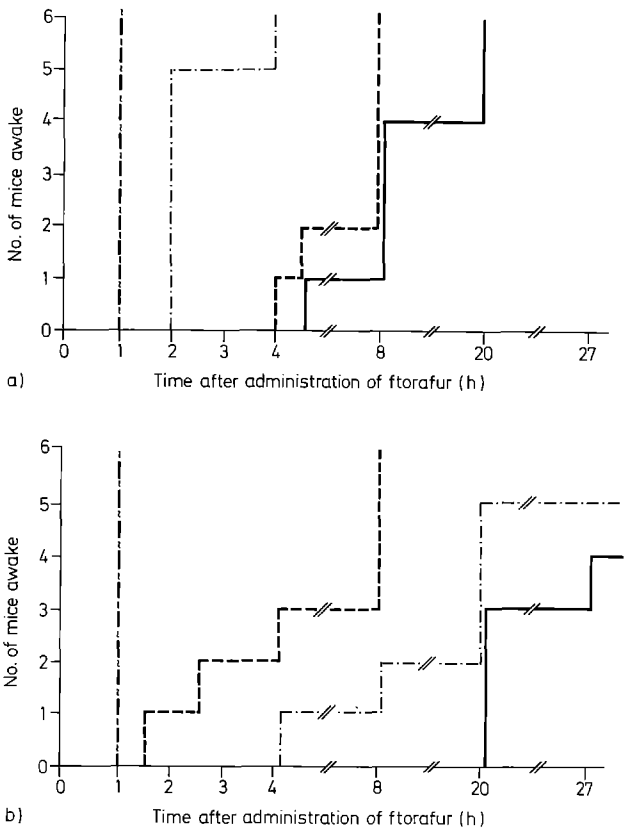

Fig. 1A and B. Effect of induction and inhibition of the microsomal enzymes on duration of lethargy induced by ftorafur in mice. B6 females received PB 60 mg/kg IP on 3 consecutive days (days 0–2). MC (15 mg/kg) was injected in a single IP dose on day 2 and SKF 525A, on day 3. Ftorafur was administered in a single IP dose of 300 mg/kg on day 3, i.e., 24 h after the last injection of the inducer and 30 min after administration of SKF 525A. Each experimental group contained six mice. **A** (———) ftorafur (FT); (– – – –) MC + FT; (– · – · – · –) PB + FT; (–~–~–~–) PB+MC+FT; **B** (———) SKF 525A + FT; (– – – –) MC + SKF 525A + FT; (– · – · – · –) PB + SKF 525A + FT; (–~–~–~–) PB + MC + SKF 525A + FT

SKF 525A at a dose of 50 mg/kg inhibited the delayed toxic effect of the PB plus ftorafur combination, though it showed no inhibiting action in the case of ftorafur combined with MC or with both inducers.

Further, we studied the effect of ftorafur on a series of experimental tumors under the conditions of changed activity of microsomal enzymes. We demonstrated that pretreatment of mice with PB and MC intensifies the effect of ftorafur on Rauscher leukemia cells. The spleen weight of infected mice C was diminished most markedly when ftorafur was combined with both inducers (Fig. 2). For example, if given at a dose of 200 mg/kg ftorafur alone did not affect spleen weight, while in combination with two inducers it diminished spleen weight almost five

Table 1. Changes in the mode of ftorafur toxicity at action on the microsomal enzymes

Pretreatment[a]	Mortality[b]: dead animals/total					
	Ftorafur dose (mg/kg)					
	100		200		300	
	Early	Late	Early	Late	Early	Late
None	0/12	0/12	0/12	1/12	6/24	1/18
PB + MC	0/6	0/6	0/6	6/6	0/18	18/18
PB	–	–	–	–	0/6	6/6
MC	–	–	–	–	0/6	6/6
SKF 525A	–	–	1/6	0/5	1/6	0/5
PB+MC+SKF 525A	–	–	–	–	0/6	6/6
PB + SKF 525A	–	–	–	–	1/6	2/5
MC + SKF 525A	–	–	–	–	1/6	5/5

[a] B6 females received PB 60 mg/kg IP on 3 consecutive says (days 0–2). MC (15 mg/kg) was injected in a single IP dose on day 2; SKF 525A on day 3. Ftorafur was administered in a single IP dose on day 3, i.e., 24 h after the last injection of the inducer and 30 min after administration of SKF 525A

[b] Early mortality, death at 20–24 h (in a lethargic state); late mortality, death on days 4–7 after ftorafur administration

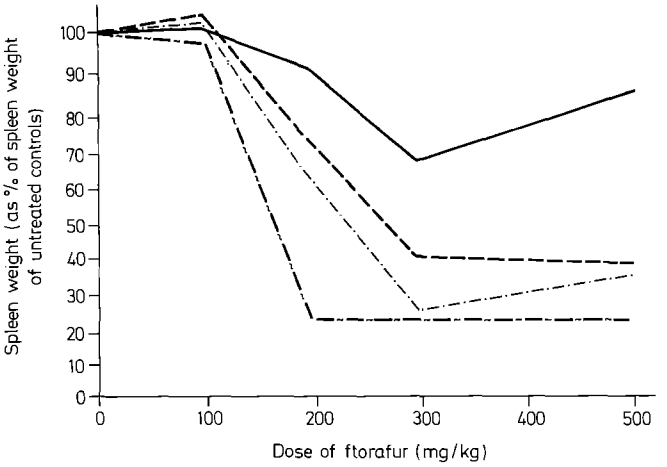

Fig. 2. Spleen growth inhibition in Rauscher leukemia-bearing C mice treated with ftorafur in combination with inducers of the drug-metabolizing enzymes. C females were inoculated with Rauscher leukemia cells IP on day 0. On days 12–14 the animals in groups 3 and 4 received PB 60 mg/kg IP. The mice in groups 2 and 4 received a single IP injection of MC (15 mg/kg) on day 14; ftorafur was administered in a single IP dose on day 15. On day 18 the spleen weight was estimated. Each experimental group contained seven or eight animals. Standard error did not exceed ± 10%. 1. (———) FT; 2. (– – – –), MC + FT; 3. (– ·– · – · –) PB + FT; 4. (–~–~–~–) PB + MC + FT

Table 2. Effect of PB or MC pretreatment followed by injection with ftorafur on the drug-related death of B6 mice bearing La leukemia

Pretreatment	Mortality: dead animals/total			
	Ftorafur (mg/kg)			
	50	100	150	200
0	–	0/6	–	0/6
PB + MC	1/6	2/5	6/6	5/5
PB	0/6	0/6	3/6	4/6
MC	0/6	0/6	1/6	3/5

Groups of five to six mice received IP injection of $1 \cdot 10^6$ tumor cells on day 0. PB (60 mg/kg) was administered as a single daily IP injection on days −2, −1, and 0. A single IP injection of MC (15 mg/kg) was administered on day 0, 6 h after tumor inoculation and 4 h after PB administration. Ftorafur was given as a single IP injection on day 1

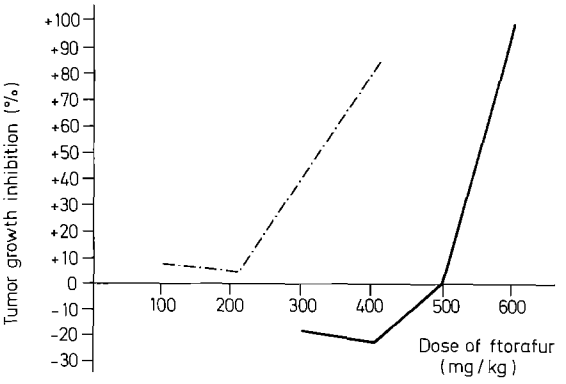

Fig. 3. Phenobarbital effect on ftorafur antitumor activity in hemangiopericytoma-bearing mice. $CBAB6F_1$ female mice pretreated with PB 60 mg/kg IP on days −2, −1, and 0 were inoculated SC with tumor cells on day 0. Ftorafur was administered in a single dose on day 1 and subcutaneous tumors were weighed on day 8. Each experimental group contained six or seven animals. (———) FT; (– · – · – · –) PB + FT

times. The achieved effect turned out to be greatest on the third day after ftorafur injection, since it did not increase when the dose of ftorafur was augmented to 500 mg/kg. A 'superhigh' dose of ftorafur (500 mg/kg) was tolerated only by the mice that were treated with PB or with both inducers together (they survived till the third day). The mice which were not treated in the above way died in a lethargic state 20–24 h after administration of the drug. MC partially decreased the neurotoxic effect of this ftorafur dose and intensified its influence on tumor cells, though its action was weaker than that of PB. It should be noted that PB and MC without ftorafur had no effect on the growth and development of tumors used in our experiments.

$CBAB6F_1$ mice bearing hemangiopericytoma were more tolerant of ftorafur and could tolerate high doses of this drug. Without additional effects ftorafur

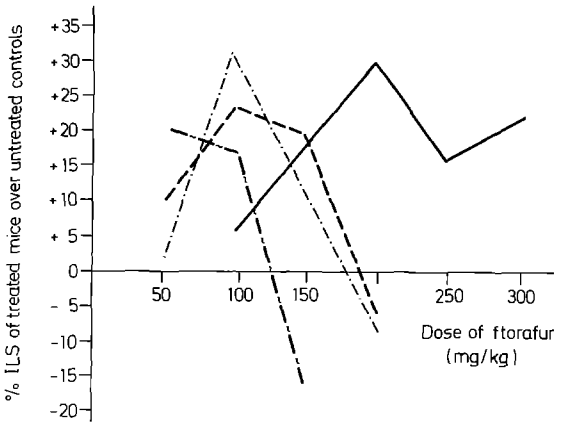

Fig. 4. Treatment of leukemia La with ftorafur in combination with PB and (or) MC. B6 females pretreated with PB 60 mg/kg IP on days −2, −1, and 0 and (or) with MC 15 mg/kg IP on day 0 were inoculated with tumor cells IP on day 0. Ftorafur was administered in a single dose on day 1. Each experimental group contained five or six animals. (———) FT; (– – – –) MC + FT; (– · – · – · –) PB + FT; (–~–~–~–) PB + MC + FT

inhibited the growth of hemangiopericytoma at a dose of 600 mg/kg. This dose appeared to be toxic.

It produced pronounced lethargy and manifestations of delayed toxicity, in particular loss of over 20% of body weight by the fifth day after ftorafur administration. One of six mice in this group died as a result of drug-related toxicity. Doses of 400–500 mg/kg inducing manifestations of lethargy were ineffective against the tumor (Fig. 3).

After PB pretreatment considerable inhibition of tumor growth was observed with as little as 300 mg ftorafur/kg, and the antitumor and delayed toxic effects of 400 mg/kg were equal to those of 600 mg/kg without enzyme induction.

Leukemia La was also markedly resistant to ftorafur. With a single administration of this drug the duration of survival was only increased by 30%. This maximum effect was achieved at a ftorafur dose of 200 mg/kg without additional treatment and of 100 mg/kg after PB pretreatment (Fig. 4).

The combination of PB, MC, and ftorafur exhibited marked antitumor activity even at 50 mg ftorafur/kg, though this combination was very toxic (Table 2).

SKF 525A did not stimulate ftorafur's antitumor action at low doses. Its application with high doses of ftorafur was impossible due to neurotoxic complications which it intensities.

Discussion

Ftorafur in high experimental doses is known to cause two types of acute toxic complications, which can be designated as direct and delayed. The former develop about 30 min after drug administration and involve various types of neurotoxic shock [4, 14]. Lethality as a result of delayed toxic effect is observed from the fourth day after drug administration [16, 17].

It is possible that the lethargic effect of ftorafur is induced by the unchanged drug, but its delayed toxicity and antitumor activity are due to metabolites formed under the influence of microsomal enzymes [13, 18, 28].

Our data indicate that pretreatment of mice with the inducers of microsomal enzymes caused a marked inhibition of the lethargic effect and pronounced augmentation of ftorafur delayed toxicity and antitumor action.

The lethargic effect of ftorafur is connected with its ability to cross the blood-brain barrier and concentrate in cerebrospinal fluid [7, 13, 19]. The inducers presumably intensify the conversion of ftorafur to less hydrophobic derivatives which do not so easily penetrate the blood-brain barrier and are accumulated to a lesser degree in the brain tissue.

This assumption is in keeping with data published by the present authors [8, 22], who showed that phenobarbital pretreatment increased the 5-fluorouracil concentration in the blood of patients or animals treated with ftorafur. This was followed by an increase in the ftorafur antitumor activity [8, 21, 22].

Differences between PB and MC in their ability to prevent the lethargie action of ftorafur and to stimulate its antitumor action are connected, evidently, with the fact that in microsomal mono-oxygenases these compounds induce a synthesis of various final links [1, 27]. PB stimulates the formation of cytochrome P-450, which is known to be complexed with ftorafur and to oxidize it, whereas MC induces the synthesis of cytochrome P-448, which predominantly catalyses oxidation of polycyclic aromatic hydrocarbons. Combination of PB + MC may result in a synergistic increase of the enzyme activity over that induced by PB or MC alone [12].

Inhibition by SKF 525A of the activity of enzymes metabolizing ftorafur contributes to longer circulation of unchanged drug in blood. This is reflected in the observed prolongation of lethargy. It seems likely that SKF 525A inhibited the background and PB-induced enzymes involved in ftorafur metabolism much more than that induced by MC. At the dose used SKF 525A does not alter the antilethargic or toxic effect produced by the PB + MC combination. This might be a result of high level enzyme formation or generation of an enzyme profile similar to that induced by MC.

Stimulation of the antineoplastic activity of ftorafur in our experiments was not likely to afford any therapeutic advantage over this drug. This was because of an increase in the ftorafur antitumor effects due to the induction pretreatment, which also increased the drug's lethal toxicity. We were able to detect a certain therapeutic advantage only when Rauscher leukemia was treated with the combination of PB or PB + MC and ftorafur. It was not possible to reach the effect obtained in this case with high doses of ftorafur only, because of the animals' death in a lethargic state.

In the other cases the maximum therapeutic effects of ftorafur in animals pretreated with inducers were the same as those in mice receiving a high dose of ftorafur alone. It should be noted that in the last case all animals had pronounced manifestations of central nervous system toxicity.

Stimulation of ftorafur-activating enzymes in cancer patients with low activity of microsomal hydroxylases [26] seems to be of practical value because it may lead both to a decrease in neurotoxic manifestations following ftorafur and to an increase in its antitumor action.

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