3. E. A. Komarova, "Expression of antigens of mouse mammary carcinoma virus in the course of their individual development and of carcinogenesis, and also of related antigens in human breast carcinomas," Author's Abstract of Candidate's Disseration, Moscow (1981).
4. I. N. Kryukova, S. L. Kolobkov, M. M. Beriashvili, et al., Neoplasma, 29, 19 (1982).
5. I. N. Kryukova, in: Immunoluminescence in Medicine [in Russian], Moscow (1977), pp. 184-226.
6. S. Avrameas and T. Ternynck, Immunochemistry, 6, 53 (1969).
7. R. Dulbecco, M. Bologna, and M. Unger, Proc. Natl. Acad. Sci. USA, 76, 1848 (1979).
8. A. Farri and P. Nakane, J. Immunol. Methods., 47, 129 (1981).
9. H. Hosick and S. Nandi, J. Natl. Cancer Inst., 52, 897 (1974).
10. J. Ledbetter and L. Herzenberg, Immunol. Rev., 47, 63 (1979).
11. V. Lennon, M. Unger, and R. Dulbecco, Proc. Natl. Acad. Sci. USA, 75, 6093 (1978).
12. A. Reif and J. Allen, J. Exp. Med., 120, 413 (1964).
13. J. Roubinian and P. Blair, J. Natl. Cancer Inst., 58, 727 (1977).
14. F. Squartini, Israel J. Med. Sci., 7, 26 (1971).
15. K. Thompson, J. Natl. Cancer Inst., 67, 143 (1981).
16. R. Turkington and M. Riddle, Cancer Res., 30, 127 (1970).
17. H. Young, E. Scolnick, and W. Parks, J. Biol. Chem., 250, 3337 (1975).

PROTECTION OF MICE WITH HEMOBLASTOSIS BY ALLOPURINOL DURING CHEMOTHERAPY WITH 5-FLUOROURACIL

V. M. Bukhman and F. F. Mamedov

UDC 616.155.392-085.277.3.015.25:615.254.1:547.828

KEY WORDS: hemoblastosis; allopurinol.

Frequent involvement of normal tissues is one of the most important obstacles to the planned treatment of cancer patients with 5-fluorouracil (FU). For instance, because of the rapid development of toxic complications affecting the gastrointestinal tract, FU is virtually never used in leukemias [4]. It has recently been demonstrated that normal tissues can be protected against the harmful action of FU by means of allopurinol (AP) [5, 6, 8, 9]. Under these circumstances the antitumor effect against certain tumors is maintained [9]. Whereas in an *in vitro* system the cytotoxic action of FU on cells of mouse lymphoblastic leukemias is weakened by AP [10], damage to bone marrow cells still remains [9].

It might be expected that during protection of the animal by AP against the toxic action of FU, damage to malignant cells of nonlymphoid leukemias would be preserved. We give below the results of experiments carried out on a model of hemoblastosis in mice undertaken to test this hypothesis.

EXPERIMENTAL METHOD

Male C57Bl/Gj mice weighting 20-26 g, reared at the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used (the line will hereafter be abbreviated to B6). A strain of syngeneic hemoblastosis La [2], obtained from the Laboratory of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, was maintained by weekly intraperitoneal passages. In the experiment 10^5 splenic tumor cells were injected intraperitoneally into mice on day 0. AP powder (Hungary) was diluted with physiological saline to a concentration of 1-3 mg/ml and kept in a refrigerator. Before use the suspension was dissolved by heating on a waterbath at 40°C and administered in the necessary volume per os through a gastric tube. A 5% ampul solution of FU (USSR) was diluted immediately before

Department of Molecular Pathology, Research Institute for Biological Trials of Chemical Compounds, Ministry of the Medical Industry of the USSR, Staraya Kupavna, Moscow Region.
(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Buylleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 84-86, August, 1983. Original article submitted October 11, 1982.

TABLE 1. Effect of AP on Toxic and Antitumor Effects of FU

Expt. No.	Group	Preparation, mg/kg/day		MLS, days	(Range)	LLS, %	l/n	%
		AP	FU					
1	1	0	0	12,0***	(12—12)	—	0/9	(0)
	2	30	0	12,0***	(12—12)	0	0/9	(0)
	3	0	60	14,7	(14—15)	22	0/9	(0)
	4	30	60	14,6	(14—16)	22	0/9	(0)
2	1	0	0	9,0***	(8—10)	—	0/8 c	(0)
	2	25	0	8,7***	(8—9)	3	0/9 c	(0)
	3	0	30	14,0	(8—22)	56	3/15c	(20)
	4	0	50	14,2	(8—22)	58	7/15 a	(47)
	5	0	60	11,7*	(10—18)	30	13/15	(87)
	6	25	60	14,7	(8—20)	63	4/15 c	(27)
3	1	0	0	10,0**	(10—10)	—	0/6	(0)
	2	25	0	10,0**	(10—10)	0	0/6	(0)
	3	0	30	14,7	(10—17)	47	0/6	(0)
	4	0	60	18,1*	(15—23)	81	3/6	(50)
	5	25	60	26,0**	(17—47)	160	0/6	(0)
4	1	0	0	9,9***	(8—11)	—	0/9 b	(0)
	2	20	0	9,2**	(8—11)	7	0/6 b	(0)
	3	0	30	13,5	(11—16)	36	0/6 b	(0)
	4	0	60	10,7*	(8—16)	8	5/6	(83)
	5	20	60	11,8	(9—14)	19	3/6	(50)
5	1	0	0	9,5	(9—10)	—	0/4 b	(0)
	2	0	30—60	9,4	(7—17)	1	12/15	(80)
	3	25	60	7,8*	(5—15)	18	4/5	(80)
	4	50	60	8,2	(7—9)	14	4/4	(100)
	5	75	60	8,8	(3—15)	7	4/5	(80)
	6	100	60	8,6	(7—11)	9	4/5	(80)
	7	150	60	5,0***	(3—8)	47	6/6	(100)
	8	200	60	5,7**	(3—13)	40	5/6 c	(83)
	9	25×2—3	60	13,7***	(9—15)	44	1/10	(10)

Legend. In experiment No. 1 the preparations were given on the 1st day, in experiments Nos. 2, 4, and 5, on the 1st and 5th day, in experiment No. 3 on the 1st, 6th, and 11th days, and in experiment No. 4 on the 1st, 5th, 9th, and 13th days. In experiment No. 5, because of equal effectiveness, mice receiving FU in doses of 30, 50, or 60 mg/kg daily were combined in group 2, and those receiving AP in two or three fractional doses daily were combined in group 9. In experiments Nos. 1-4 the preparations were given in a single dose, in experiment No. 5 the last dose of AP was given 30 min before FU. *$P \leq 0.05$ compared with group of mice receiving FU in a dose of 30 mg/kg/day; **$P \leq 0.01$, and ***$P \leq 0.001$ respectively; a, b, c: $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively compared with group of mice receiving FU in a daily dose of 60 mg/kg; l/n) ratio of number of animals dying from toxicosis to total number of mice.

the experiment to the required concentration with sterile physiological saline and injected intraperitoneally in a volume of 0.1 ml/10 g body weight. The toxic effects were judged from the frequency of death of the animals due to the action of FU, changes in body weight, and the general condition of the mice. The antitumor effect was judged on the basis of the survival rate [2]. Lengthening of the life span (LLS) was calculated by the equation

$$\text{LLS } (\%) = \frac{\text{MLS}_e - \text{MLS}_c}{\text{MLS}_c} \times 100,$$

where MLS_e and MLS_c denote the mean life span of mice of the experimental and control (untreated animals) groups respectively.

All mice which died were autopsied and the spleen was removed and weighed. At the moment of death as a result of the development of leukemia La the weight of the spleen was increased

by 5-10 times, whereas in the case of death from the toxic action of FU, besides considerable loss of body weight and damage to the intestine, inhibition of the splenic lymphoid tissue also was observed, and this was reflected in the small weight of the organ (usually less than the weight of the spleen in normal mice). The weight of the spleen thus provides a convenient indicator by means of which the main cause of death of the animal can be identified with a high degree of probability. The significance of differences in the survival rates of the mice were judged by Wilcoxon's U test [1], and the frequency of toxic death by the chi-square test [3]. Differences were taken to be significant at the $P \leq 0.05$ level.

EXPERIMENTAL RESULTS

The object of this series of experiments was to determine, first, whether AP can reduce the toxic damage to mice inoculated with hemoblastosis La cells and treated with FU, and whether antitumor activity of FU is exhibited under these circumstances, and second, to determine which treatment is more effective: with FU alone in optimal therapeutic doses, or with lethal doses of FU under protection by AP. A continuous scheme of administration of the preparations was used from the beginning of treatment on the day after inoculation of the tumor cells. AP in the first four experiments was given once a day, simultaneously with FU. In the last experiment single and fractional administration of AP were compared; the total dose or its last part was given 30 min before FU (Table 1). In the first experiment the preparations were given only once. Simultaneous injection of AP did not affect the weak but significant antitumor effect of FU. In the remaining experiments, in which FU was given 2-4 times at intervals of 4-5 days, the toxic effect of the antimetabolite varied. For instance, in experiments Nos. 3 and 4 the mice tolerated four injections of FU in a daily dose of 30 mg/kg without any signs of toxicosis, whereas in experiments Nos. 2 and 5, in which the treatment was given twice — on the 1st and 5th days, this same dose of FU caused toxic death of 20 and 60% of mice respectively. In most cases the effect of AP on the toxic and antitumor effect of FU was determined in a daily sessional dose of 60 mg/kg (when FU alone was given in this dose from 50 to 90% of the mice died from lethal poisoning). AP always weakened the toxic action of FU to some degree or other, reducing the frequency of development of a lethal effect or completely preventing it. The antitumor action was potentiated in experiment No. 3 (group 5), and in experiment No. 5, abolition of the high toxicity of FU by AP was observed only when AP was given in fractional doses of two or three injections and this was accompanied by a marked antitumor effect (group 9). In experiments Nos. 2 and 4 considerable but incomplete reduction of the toxicity of FU was observed; the antitumor effect in groups of mice receiving a combination of AP and FU did not differ significantly from that in comparable groups of mice receiving FU only.

The results indicate that effective treatment of nonlymphoid leukemias by FU is possible under protection of the organism by AP. It must be pointed out that recently Kroener et al. [8] reported the occurrence of a partial remission in a patient with histiocytic lymphoma as a result of treatment with high doses of FU under AP protection. AP itself is known to have a weak antitumor action experimentally [7]. In our experiments AP in doses from 20 to 200 mg/kg daily caused neither toxic nor antitumor effect. It must be recalled, however, that in experiment No. 5 AP in doses of 150 and 200 mg/kg daily, when given together with FU in a dose of 60 mg/kg daily, led to the development of a serious condition in the mice with evidence of CNS damage and with early death of the mice during the 72 h after administration of the compounds. A lesion of the CNS when a combination of AP and FU was used also has been reported clinically [8]. Our own data show that these phenomena are observed only when fairly high doses of AP are given.

According to recent data the protective effect of AP during treatment with FU is connected with competition at the molecular level [8, 9]. The leading factor is evidently accumulation of orotic acid as a result of interaction of AP metabolites with orotidylate decarboxylase or hypoxanthine as a result of inhibition of xanthine oxidase. This leads to inhibition of conversion of FU into FU monophosphate, which takes place with the aid of orotate phosphoribosyltransferase and which utilizes 5-phosphoribosyl-1-pyrophosphate. Those cells in which this pathway of biotransformation of FU is the principal one are protected by AP against damage by FU. Cells in which other pathways of FU metabolism predominate or in which conversion of AP into hydroxypurinol does not take place remain affected.

The model of successful treatment of hemoblastosis La with lethal doses of FU under AP protection, described above, is convenient for the further study of mechanisms of competition between these two preparations.

LITERATURE CITED

1. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973), pp. 21-31.
2. Z. P. Sof'ina, A. B. Syrkina, A. Goldin, et al. (editors), Experimental Evaluation of Antitumor Preparations in the USSR and USA [in Russian], Moscow (1980), pp. 74-75.
3. V. Yu. Urbakh, Statistical Analysis in Biological and Medical Research [in Russian], Moscow (1975), pp. 102-110.
4. V. A. Chernov (editor), Therapeutic and Diagnostic Substances Used in Oncologic Practice [in Russian], Moscow (1982), pp. 53-54.
5. R. M. Fox et al., Cancer Treat. Rev., 6, Suppl., 143 (1979).
6. S. B. Howell et al., Cancer (Philadelphia), 48, 1281 (1981).
7. A. Kinz, Gann, 67, 903 (1976).
8. J. F. Kroener et al., Cancer Treat. Rep., 66, 1133 (1982).
9. P. M. Schwartz et al., Cancer Res., 40, 1885 (1980).
10. P. M. Schwartz and R. E. Handschumacher, Cancer Res., 39, 3095 (1979).