CYTOGENETIC ACTION OF 9-(2-HYDROXYETHOXYMETHYL)GUANIDINE

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Therapeutic preparations constitute a group of substances for which the testing of potential genetic risk is of the greatest importance. The new and highly effective antiherpetic agent 9-(2-hydroxyethoxymethyl)quanidine (acyclovir) belongs to the class of abnormal nucleosides [7, 11], many of which exhibit marked mutagenic activity [4, 5, 9, 15]. It was therefore interesting to test this antiviral chemotherapeutic agent for remote genetic aftereffects in mammals.

EXPERIMENTAL METHOD

To test acyclovir, the therapeutic form of the compound known as Zovirax I. V. (Wellcome, Great Britain), was used, with cyclophosphamide (Jenaphram, East Germany) as the positive control. The substances were injected into random-bred albino mice weighing 20-22 g (6-8 min in a group) intraperitoneally or intravenously. To assess the cytogenetic action, the method of counting micronuclei in bone marrow reticulocytes [1, 13] was chosen. The predictive value of the micronuclear test with regard to carcinogenic effect exceeds 90% [8] and it has been recommended as one of the principal methods of detection of the mutagenic activity of chemical substances in mammals [8, 10]. Bone marrow films were prepared by the rapid method [2], the cells were isolated in mouse blood serum, and stained by Pappenheim's method (Fig. 1); they were numbered before examination. When reticulocytes with micronuclei were counted, 1000 reticulocytes from each mouse were analyzed. The toxic action on erythropoiesis was assessed by the ratio of the number of nucleated cells, of mature erythrocytes, and of reticulocytes [14]. The results were subjected to statistical analysis by the chi-square and Student's tests. The mean lethal dose (LD_{50}) was calculated by Kärber's method.

EXPERIMENTAL RESULTS

Preliminary experiments showed that LD_{50} for acyclovir for a single intravenous injection was 1250 ± 110 mg/kg, and for intraperitoneal injection 510 ± 70 mg/kg. Administration in three fractional doses at intervals of 1 h reduced the toxicity of intraperitoneal injection to the level observed with intravenous injection. In the cytogenetic investigations

TABLE 1. Frequency of Detection of Reticulocytes with Micronuclei in Mouse Bone Marrow 24 h after Injection of Acyclovir (M ± m)

Dose, mg/kg	Number of reticulocytes with micronuclei per 1000 reticulocytes	
	single intravenous injection	intraperitoneal injection of fractional doses
0	1,7±0,4	2,3±0,5
5	2,7±0,7	2,0±0,5
25	2,3±0,6	3,5±0,7
75	5,5±1,0	4,0±0,8
150	6,7±0,7	4,4±0,4
300	14,0±1,0	8,9±1,0
600	12,4±1,2	31,4±1,9
750	11,4±1,2	20,8±1,6

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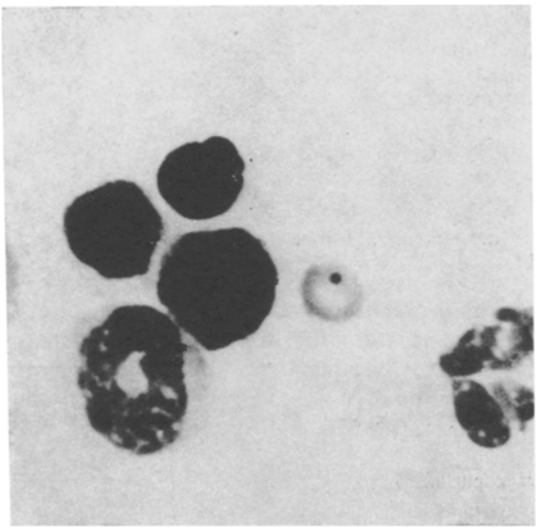

Fig. 1

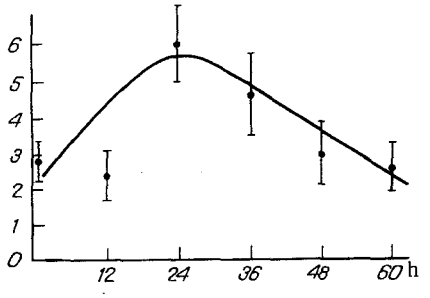

Fig. 2

Fig. 1. Film of mouse bone marrow cells isolated in mouse blood serum. 900 ×. Pappenheim's strain.

Fig. 2. Time course of frequency of reticulocytes with micronuclei in mouse bone marrow after injection of acyclovir. Acyclovir injected intraperitoneally in a dose of 150 mg/kg (three injections, each of 50 mg/kg, at intervals of 1 h). Abscissa, time (in h); ordinate, number of reticulocytes with micronuclei per 1000 reticulocytes.

acyclovir was given to the mice either as a single intravenous injection or intraperitoneally in fractional doses. The cytogenetic effect of cyclophosphamide in a dose of 5 mg/kg was 4.7 ± 1.2‰ reticulocytes with micronuclei, compared with 1.4 ± 0.7‰ (χ^2 = 10.8) in the control, evidence of the standard sensitivity of the method [1, 3]; the maximal effect (18.8 ± 2.1‰) was observed with a dose of 40 mg/kg.

To determine the optimal time of preparation of bone marrow films after injection of acyclovir the frequency of reticulocytes with micronuclei was analyzed after 12, 24, 36, 48, and 60 h (Fig. 2). The maximal cytogenetic effect (6.7 ± 0.7‰) was observed 24 h after injection, so that when dose dependence was studied this time was chosen to prepare the bone marrow films. The results are given in Table 1. In a dose of 75 mg/kg acyclovir caused a statistically significant increase in frequency of reticulocytes with this disturbance (χ^2 = 9.0). With an increase in the dose up to the sublethal level, the intensity of the mutagenic effect increased by both methods of injection. The higher mutagenic activity of acyclovir than of cyclophosphamide was noted (the maximal genetic effect of the first was 31.4‰, and that of the second 18.8‰.

TABLE 2. Effect of Acyclovir in Erythropoiesis in Mice (M ± m)

Mode of injection	Dose, mg/kg	Ratio of types of cells		
		nucleated, %	reticulocytes, %	erythrocytes, %
	0	72,7±2,4	10,7±0,9	16,6±1,9
Single intravenous injection	150	48,5±5,5 (4,0)	10,8±1,6 (3,9)	40,6±5,7 (4,0)
	300	39,3±1,4 (12,0)	14,0±2,5 (1,2)	41,3±4,2 (5,4)
	600	47,7±4,2 (5,2)	9,9±1,7 (0,4)	44,8±4,4 (5,9)
Intraperitoneal injection of fractional doses	150	36,7±2,6 (10,2)	14,2±4,1 (0,8)	49,2±3,1 (9,0)
	300	29,8±5,0 (7,7)	12,0±1,1 (0,9)	58,2±5,8 (6,8)
	600	25,1±5,3 (8,1)	12,0±3,7 (0,3)	62,8±4,9 (8,8)

Legend. Altogether 1000 cells were counted to estimate the ratio between nucleated and non-nucleated cells, and 1000 non-nucleated cells were counted to estimate the ratio between reticulocytes and erythrocytes. Values of Student's t relative to the control given in parentheses.

Table 2 gives the results of the study of the cytotoxic action of acyclovir on erythropoiesis in mice. In a dose of 150 mg/kg inhibition of the bone marrow was observed, in the form of a decrease in the fraction of nucleated cells and filling of the bone marrow with peripheral blood. With an increase in dose the intensity of these changes increased only in series in which fractional doses were injected.

Acyclovir belongs to the group of antiherpetic chemotherapeutic agents with a narrowly targeted mechanism of inhibition. Initiation of the antiviral activity of acyclovir is effected by phosphorylation by virus-induced thymidine kinase [12], and for that reason its toxic concentrations for normal cells in tissue culture are approximately 3000 times higher than concentrations inhibiting the reproduction of herpes virus [6]. All these properties make acyclovir the "ideal" antiviral preparation. It is interesting to note that it did not induce sister chromatid exchanges of chromosomes in cultures of human lymphocytes and fibroblasts [5], but induction of events of this type cannot be regarded as unconditional evidence that the substance possesses mutagenic activity. By using the micronuclear test on mice we were able to show the dose-dependent cytogenetic activity of acyclovir. Depending on the character of its toxic action on hematopoiesis, when given in a single dose or fractionally, acyclovir must be regarded as an antimetabolite of nucleic acid synthesis, and not as a substance of cytostatic type (division spindle blockers, alkylating compounds) [15].

The results of this investigation can be used to assess the degree of potential genetic risk of acyclovir, in accordance with the classification of risk of therapeutic forms based on the results of cytogenetic experiments on mammalian bone marrow cells, adopted by the pharmacological committee of the Ministry of Health of the USSR. A fivefold increase in the background frequency of cells with disturbances was observed after injection of acyclovir in a dose of 75 mg/kg, which is equivalent to 15 therapeutic doses (recommended for a single injection). When doses exceeding the therapeutic level by more than 15 times were given, the background level was exceeded by 15 times. By both criteria acyclovir can be placed in risk class B, and this must be taken into account when it is used therapeutically.

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