CHRONOBIOLOGICAL STUDY OF ANTIMUTAGENIC ACTIVITY OF METHYLURACIL IN CYTOGENETIC LESIONS INDUCED IN MICE BY A VACCINE STRAIN OF POLIOMYELITIS VIRUS

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Many vaccine strains of viruses widely used for the prevention of widespread infectious diseases induce considerable cytogenetic disturbances in man and animals [3, 4, 9, 10]. Considering that every year nearly 170 million persons are vaccinated in the USSR [2] and that in the foreseeable future it will be virtually impossible to refuse preventive vaccinations, it will be evident that the search for substances weakening the mutagenic effects of the vaccines used is essential. Previous investigations have shown that living Sabin poliomyelitis virus induces cytogenetic disturbances in mouse bone marrow [5], and that preventive injection of methyluracil has an antimutagenic action [6]. The effectiveness of drugs is known to differ considerably depending on the time of day when they are given [12], evidently because of differences in the pattern of function of cells, so that the body as a whole responds most effectively to a drug when administered at a particular moment of time.

This paper describes a study of the antimutagenic action of methyluracil depending on the time of its administration to mice infected with a vaccine strain of poliomyelitis virus.

EXPERIMENTAL METHOD

Depending on the time of the experiment, the chromosome set in bone marrow cells of mature male mice was studied by the method described in [13] in four groups of animals. All the experiments with mice of group 1 were carried out at 3 a.m., those of group 2 at 9 a.m., of group 3 at 3 p.m., and of group 4 at 9 p.m. Methyluracil was given perorally in a dose of 200 mg/kg body weight once daily for 5 days, after which half of the mice of each group received an intraperitoneal injection of living Sabin triple poliomyelitis vaccine (LSV) in a dose of 0.2 ml of the standard liquid vaccine. The mice were decapitated 3 days after infection and uninfected mice ("treated" with methyluracil) and infected mice (not "treated" with methyluracil) were killed at corresponding times. The last two groups of mice served as the "experimental" control. At each time of the experiment, the chromosome set also was studied in intact mice. Altogether, the chromosome set was studied in 96 mice, consisting of six animals in each control and experimental group. The results were subjected to statistical analysis by the usual methods [10].

EXPERIMENTAL RESULTS

As Table 1 shows, methyluracil acted most effectively when given at 9 p.m., when the mutagenic effect of the poliomyelitis virus was completely abolished. Whereas in the "experimental" control with methyluracil the frequency of cells with cytogenetic disturbances was 6.3 ± 1.6%, and in the infected mice it was 12.7 ± 2.6%, in animals receiving vaccination and methyluracil it was 6.2 ± 1.4%. Meanwhile, it must be pointed out that injection of the poliomyelitis virus at this time of day caused the least mutagenic effect (Table 1). Methyluracil had the least antimutagenic action when injected at 9 a.m., when only a small decrease was observed in the number of cells with a hypoploid karyotype: from 17.3 ± 2.2 to 11.0 ± 0.9% ($P < 0.01$) compared with 2.5 ± 0.6% in uninfected mice receiving methyluracil. In these mice (9 a.m.) no decrease was observed either in the number of cells with structural disturbances of their chromosomes or in the number of hyper- and polyploid cells. At other times of the experiment (3 a.m. and 3 p.m.) methyluracil also exhibited its antimutagenic properties. Administration of methyluracil at 3 a.m. prevented the increase in the number of cells with structural disturbances of their

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TABLE 1. Chromosomal Disturbances in Enterocytes of Mice Infected with Poliomyelitis Virus and Treated with Methyluracil at Different Times of the 24-h Period (in %)

Clock time	Experimental conditions	Number of cells with chromosomal disturbances	Frequency of chromosomes with disturbances				
			total	chromosomal breaks	chromatid breaks	exchanges	other
3 a.m.	C	2,3±0,5	3,0±0,7	0,7±0,4	1,5±0,5	0,5±0,4	0,3±0,2
	M	3,5±0,7	4,2±1,0	0,8±0,5	2,5±0,6	0,3±0,2	0,5±0,4
	I	10,0±0,9*	11,2±1,0*	1,8±0,6	7,8±0,6*	1,0±0,5	0,5±0,4
	M+I	4,5±0,8***	4,8±1,0***	0,7±0,5	3,3±0,8***	0,5±0,4	0,3±0,2
9 a.m.	C	2,7±1,1	3,0±1,3	0,5±0,4	1,7±0,7	0,5±0,4	0,3±0,2
	M	5,3±1,4	6,7±1,3	1,7±0,9	4,0±0,9	0,3±0,2	0,7±0,4
	I	12,0±1,6*	13,7±1,6*	2,2±0,5	2,3±1,1	1,2±0,6	1,0±0,4
	M+I	11,2±2,5**	12,7±1,4 *,**	1,8±0,5	8,7±1,5*,**	1,7±0,5**	0,5±0,4
3 p.m.	C	1,7±0,4	1,8±0,3	0,1±0,1	1,2±0,2	—	0,5±0,2
	M	3,1±0,8	3,3±0,7	0,4±0,2	2,1±0,4	0,3±0,2	0,5±0,2
	I	8,2±1,1*	9,2±1,4*	1,8±0,7*	5,5±0,8*	0,4±0,3	1,5±0,4
	M+I	3,3±0,8***	3,7±0,9***	0,7±0,4	2,2±0,5***	0,3±0,2	0,5±0,2***
9 p.m.	K	2,2±0,7	3,2±1,0	1,7±0,5	0,8±0,5	0,3±0,2	0,3±0,2
	M	3,8±0,6	4,6±0,8	1,2±0,3	2,8±0,5*	0,5±0,4	0,3±0,2
	C	5,5±1,2	5,8±1,4	1,2±0,5	3,7±0,5*	0,3±0,2	0,7±0,4
	M+I	3,2±0,8	4,0±1,1	0,7±0,4	2,5±1,0	0,5±0,4	0,3±0,2

Clock time	Experimental conditions	Frequency of cells with an abnormal number of chromosomes				Total number of cells with cytogenetic disturbances
		total	hypoploid	hyperploid	polyploid	
3 a.m.	C	3,3±0,6	1,8±0,6	0,7±0,4	0,3±0,2	4,0±0,7
	M	3,2±1,0	2,3±0,7	0,5±0,4	0,3±0,2	5,7±0,9
	I	30,0±4,5*	20,7±2,7*	4,3±0,4*	2,2±0,4	37,7±7,9*
	M+I	10,7±2,0*, **, ***	7,3±1,4*, **, ***	2,3±0,4*, **, ***	1,0±0,5	12,8±1,7*, **, ***
9	C	4,5±1,4	2,2±0,5	1,0±0,4	0,5±0,4	6,0±1,4
	M	4,8±1,0	2,5±0,6	1,7±0,4	0,7±0,4	8,5±1,4
	I	21,0±2,4*	17,3±2,2*	3,0±0,7*	1,8±0,5	25,8±2,1*
	M+I	15,0±1,1*, **, ***	11,0±0,9*, **, ***	2,3±0,8	1,7±1,1	23,2±3,0*, **, ***
3 p.m.	C	2,6±0,4	2,0±0,3	0,4±0,2	0,2±0,1	3,6±0,4
	M	4,1±0,7	3,2±0,6	0,5±0,2	0,4±0,2	6,0±0,8
	I	12,6±1,8*	8,3±1,6*	3,6±0,9*	0,7±0,3	18,2±2,4*
	M+I	10,8±1,2*, **	8,7±1,4 *'***	1,4±0,5***	0,7±0,4	11,8±0,9*, **, ***
9 p.m.	C	3,8±0,9	2,3±0,4	0,7±0,2	0,2±0,1	4,8±1,3
	M	3,5±1,0	2,8±1,0	0,3±0,2	0,3±0,2	6,3±1,6
	I	9,3±2,3*	6,0±1,1*	1,7±0,4	1,7±0,5*	12,7±2,6*
	M+I	4,7±1,1***	3,5±0,6***	0,7±0,4	0,5±0,4	6,2±1,4***

Legend. M) Mice receiving methyluracil; I) infected mice; M+I) combination of methyluracil and infection; C) intact mice. *) Significance of differences from C, **) the same between M+I and M; ***) the same between M+I and I ($P \leq 0.05$).

chromosomes; the number of cells with an altered number of chromosomes, moreover, was reduced by almost two-thirds. A similar pattern was observed when methyluracil was given at 3 p.m., although it was less effective.

The results thus show that administration of methyluracil at different times of day differed in its antimutagenic effect in mice with cytogenetic lesions induced by LSV. Methyluracil is known to stimulate immunogenesis [8], whereas the immunoreactivity of an individual is subject to considerable fluctuations due to both seasonal and circadian biorhythm [11, 14]. The state of the immune system can be considerably modified by the action of mutagenic factors, including infective, on the body [7]. The antimutagenic effectiveness of methyluracil may also be connected not only with the immunoreactivity of the individual, but also with the effect of this compound on nucleic acid and protein synthesis [8, 10]. Since most cytogenetic aberrations in virus infections occur in the stage of DNA synthesis [3, 4], and since the mitotic cycle of most cells in the body obeys a circadian rhythm [11], it can evidently be postulated that the chronobiological features of the antimutagenic effectiveness of methyluracil may be connected with the effect of this compound on the system responsible for synthesis and, in particular, for repair, not to mention its effect on protection against infection and, possibly, on the mechanisms of immunologic surveillance of the genetic constancy of the organism which, like most systems of the body which have been studied, function according to biorhythmologic principles.

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MAKING ALLOGENEIC BONE MARROW TRANSPLANTATION MORE EFFECTIVE

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During allogeneic bone marrow transplantation, there is the risk of development of immunologic conflicts: recipient versus graft and graft versus host (DVHR) reactions. If, however, a mixture of genetically different cells from two or more donors is transplanted, immunologic conflict arises between the donors' cells: a graft versus graft reaction [2, 7, 9]. This gives rise to serious adverse effects, for inhibition or complete blocking of proliferation of the stem cells of one donor under the influence of nonsyngeneic lymphocytes can sharply reduce the therapeutic effect of a mixed graft [5].

In clinical practice in the treatment of depressions of hematopoiesis, it is often necessary to use large doses of bone marrow from several donors, and for that reason the development of an effective method of conservation of allogeneic bone marrow, enabling the activity of the immunocompetent cells of the graft to be reduced, would be promising.

The investigation described below was devoted to a search for ways of making allogeneic bone marrow grafting more effective by measures aimed at particular populations of lymphocytes (T lymphocytes) of the graft.

EXPERIMENTAL METHOD

The immunologic activity of lymphocytes located in bone marrow and lymph nodes was determined by methods of estimating inactivation of nonsyngeneic stem cells and abolition of endogenous colony formation.

Mice of strains CBA and C57BL/6 were used as donors, (CBA × C57BL/6)F_1 hybrids as recipients. A cell suspension was prepared in TsOLIPK-3* conserving solution. The recipient mice were irradiated in a lethal dose (880 R). Lymph nodes or bone marrow cells from CBA mice were

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