SELECTIVE DEPRESSION OF THE PRIMARY HUMORAL IMMUNE RESPONSE IN MICE BY A COMBINATION OF DEOXYCYTIDINE WITH CYTOSAR

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Injection of cytosine-arabinoside in a lethal dose into female C57BL/6j mice immunized with sheep's red blood cells, under protection of deoxycytidine, leads to a decrease in the serum hemagglutinin level on the 5th day without the development of toxicosis. Simultaneous injections of the metabolite and antimetabolite is optimal.

KEY WORDS: deoxycytidine; Cytosar; selective immunodepression.

The absence of selectivity of the therapeutic effect is one of the most important disadvantages of drugs with a cytostatic mechanism of action, such as are widely used for tumor chemotherapy, suppression of the immunologic reaction during organ allografting, and the treatment of autoimmune processes. This disadvantage leads to the development of serious complications which interfere with planned treatment. The development of methods of selective cytostatic therapy is thus extremely important. The writers recently found that injection of lethal doses of cytosine arabinoside (araC) into mice under protection of deoxycytidine (dC) leads to selective inhibition of medullary lymphopoiesis without the development of toxicosis [1]. By means of this combination of antimetabolite with metabolite, a powerful and selective therapeutic effect was found to be obtained against L1210 lymphatic leukemia, but not against myeloid leukemia of mice [2].

The possibility of selective depression of the primary humoral response by means of this combination in mice immunized with sheep's red cells was studied in the investigation described below.

EXPERIMENTAL METHOD

Female C57BL/6j mice weighing from 20 to 30 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, were used. The compounds – 2'-deoxycytidine hydrochloride (from Reanal, Hungary) and cytosine arabinoside (Cytosar, from Upjohn, USA) – were dissolved in physiological saline and diluted with it to the necessary concentration, and injected in a dose of 0.2 ml per mouse. Peroral administration was carried out with a curved metal tube. The mice were immunized (intraperitoneally) with sheep's red cells in a dose of 0.2 ml of a 10% suspension per mouse (day 0), and the compounds were administered from the 1st to the 4th day inclusive; the daily dose was given at three injections at intervals of 2 h. Blood for determination of the serum antibody level was taken from the retro-orbital plexus from each animal separately. The hemagglutinin titer was determined by means of a microtitrator of the Takachi system and expressed in $\log_2$ units. The development of toxicosis was deduced from three parameters: mortality, changes in body weight, and leukocyte count in 1 μl of peripheral blood. A fall in the leukocyte count below 5000/1 μl blood was taken as leukopenia. Since injection of dC alone, according to our own observations and data in the literature, is not accompanied by development of a toxic or immunodepressive effect, in most experiments whose results are given below no control group of mice receiving the metabolite alone was used. The results were analyzed by the methods of least squares and by Student's t-test.

EXPERIMENTAL RESULTS

Optimal dose–time relationships were first established for dC and araC to depress hemagglutinin production without the development of toxicosis. This problem was solved by mathematical planning methods. A three-level plan of nine experiments, whereby the effects of each factor could be assessed independently and

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equations connecting their values with the values of the output indices could be obtained, was chosen as the experimental plan. The conditions and results of the experiments are given in Table 1. From the results of the experiments coefficients of influence of the various factors on the output indices were estimated by the method of least squares:

$$Y_1=2.58;$$

$$Y_2=3.7017-0.2705X_1+0.1773X_1^2-0.2492X_2+0.9639X_3-0.4464X_3^2;$$

$$Y_3=59.42+17.505X_2-118.44X_3+59.22X_3^2;$$

$$Y_4=34.82-10.97X_1+25.57X_2-98.82X_3+49.41X_3^2;$$

$$Y_5=41.17-17.32X_1+25.57X_2-81.12X_3+40.56X_3^2;$$

$$Y_6=-2.15-0.97X_1+0.77X_1^2-0.7X_2+3.86X_3-1.93X_3^2.$$

The significance of the coefficients was tested by using the experimental error, calculated from the results of duplicated experiments:

$$=S^2Y_1=1.7;\ S^2Y_2=0.03;\ S^2Y_3=S^2Y_4=S^2Y_5=168;\ S^2Y_6=0.2.$$

Testing models by application of the F-criterion showed that they adquately describe the experimental results. Analysis of the equations showed the following. 1) The antibody titer is independent of the doses of the two compounds and the interval between them. 2) The toxic effect is minimal with simultaneous administration of the compounds and rises as a linear function with an increase in the dose of araC and falls with an increase in the dose of dC. 3) Selectivity of the immunodepressive effect without the development of toxicosis within the chosen ranges of araC doses can be achieved by simultaneous administration of araC in a dose of 30-120 mg/kg·day and dC in a dose of 120 mg/kg·day. Vertificatory experiments (Table 2) confirmed this conclusion: Administration of dC in a dose of 120 mg/kg·day prevents the development of lethal toxicosis while retaining the immunodepressive effect.

However, at later times of blood sampling (7th day) the selective effect remained only when a lethal dose of araC (120 mg/kg·day) was given. The immunodepressive effect of a smaller dose of araC (30 mg/kg·day) was weakened. The experiments thus confirm the view that selective depression of antibody formation by injection of lethal doses of araC is possible if accompanied by simultaneous protection with dC. Administration of dC 1 h before or 1 h after araC is much less effective. It is interesting to note that administration of araC alone in lower, nontoxic doses (10 mg/kg per injection, 3 times a day, from the 1st through the 4th day) also leads to depression to antibody formation, as shown by the virtual absence of hemagglutinins in the serum on the 5th day after immunization of the mice with sheep's red cells. These results agree with those in the literature. It can be postulated on the basis of analysis of medullary hematopoiesis [1] and of the data in this paper that there is a subpopulation of lymphocytes which is vulnerable to araC even if optimal doses of dC are administered. If smaller doses are used, the increased sensitivity of these cells to the depressant action of araC may be connected with the inability of dC synthesized in vivo to protect them.

LITERATURE CITED

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TABLE 1. Determination of Optimal Proportions of dC and araC To Give a Selective Immunodepressive Effect (conditions, planning matrix, and results of experiment; data for untreated control given in Table 2, experiment 1, group 1)

Levels of variation	Factors			Indices					
	X_1, dose of dC, mg/kg·day	X_2, dose of araC, mg/kg·day	X_3, interval relative to araC, hr	Y_1, mean antibody titer in group, $\log_2$	Y_2, mean leukocyte count in 1 μl blood in group, log	Y_3, fraction of animals with a toxic effect*	Y_4, fraction of animals with leukopenia*	Y_5, fraction of animals with lethal outcome*	Y_6, change in body weight, g
0	30	30	−1						
1	60	60	0						
2	120	120	+1						
Expt. No.	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
1	0	0	0	3	3,7669	50,77	26,57	26,57	−2
2	0	1	1	2,6	3,9378	26,57	0	26,57	−0,6
3	0	2	2	1,8	3,3127	90	90	90	−4
4	1	0	1	3,2	4,0930	0	0	0	−1
5	1	1	2	2,6	3,6318	90	39,25	50,77	−2,4
6	1	2	0	2,7	3,0127	90	90	90	−3,8
7	2	0	2	3,2	3,9148	50,77	0	0	−0,8
8	2	1	0	2	3,6530	90	50,77	39,23	−2,22
9	2	2	1	2,8	3,9543	26,57	0	0	+1,2

*Transformed percentages of animals obtained after normalizing transformations Y = arc sin $\sqrt{p}$ given in this table.
†Time of administration of araC taken as 0.

TABLE 2. Level of Serum Hemagglutinins and Degree of Toxicosis in Mice Receiving Lethal Doses of araC under Protection of dC (M ± m)

Expt. No.	dC, mg/kg day	araC, mg/kg day	l/n	Change in body weight, g	Leukocytes, log/μl	Serum hemagglutinin titer, $\log_2$ 5th day	Serum hemagglutinin titer, $\log_2$ 7th day
1	0	0	0/8	+1,0±0,2	4,32±0,02	5,9±0,2*	ND
	0	30	1/5	−0,8±0,4	4,13±0,04	2,4±0,4	ND
	0	60	1/5	−1,8±0,2	3,86±0,10	3,4±0,4	ND
	0	120	5/5	−2,8±0,6	3,28±0,13	2,2±0,9	ND
2	0	0	0/30	−0,1±0,2	4,20±0,02	ND	4,4±0,2*
	120	30	0/30	+0,3±0,2	4,15±0,02	ND	3,8±0,1*
	0	30	2/30	0±0,2	4,20±0,01	ND	1,5±0,3
	120	120	0/30	−0,1±0,2	4,23±0,02	ND	2,4±0,4
3	0	0	0/10	+0,4±0,3	ND	3,9±0,6*	HO
	0	30	0/10	−0,1±0,2	ND	1,2±0,2	HO
	120	30	0/10	+0,4±0,3	ND	1,6±0,5	HO
4	0	0	0/15	ND	ND	5,6±0,6*	5,5±0,5*
	0	60	15/15	ND	ND	1,8±0,3	— †
	120	60	0/10	ND	ND	1,6±0,3	2,6±0,9
	180	60	0/10	ND	ND	1,9±0,3	1,8±0,8
	120	0	0/10	ND	ND	3,4±0,6*	4,2±1,4*
	180	0	0/10	ND	ND	4,5±0,6*	5,3±0,6*

Legend. 1) In experiments 1, 2, and 3, dC was injected intraperitoneally, in experiment 4 it was given by the peroral route; araC was injected intraperitoneally. 2) ND: not determined; *l*) number of mice dying; n) total number of mice. 3) (*): Difference significant ($P < 0.05$–0.001) compared with mean for group of mice receiving araC alone; (†) all mice died.