EFFECT OF DEOXYCYTIDINE ON THE EFFICACY OF CYTOSINE ARABINOSIDE AGAINST RAUSCHER ERYTHROBLASTIC LEUKEMIA IN BALB/c MICE

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The search for combinations of drugs capable of selectively inhibiting tumor growth is an urgent task in oncology. The possibility of enhancing the therapeutic effect and widening the spectrum of sensitive tumors through administration of large doses of methotrexate under folic acid protection orin combination with thymidine, has proved a stimulus for the study of the efficacy of pairs of compounds of the metabolite–antimetabolite type [3, 4]. For instance, it has been shown in the writers' laboratory that deoxycytidine (dC) in normal mice protects the tissues of the gastrointestinal tract and the myeloid and erythroid branches of hematopoiesis against the toxic effect of cytosine arabinoside (araC). Meanwhile depression of the lymphoid branch, which is especially marked in the thymus and to a lesser degree in the bone marrow, is completely preserved. Administration of araC to mice in lethal doses under dC protection enabled manifestation of a powerful antitumor effect against developed lymphoblastic leukemia L1210, but not against Graffi myeloid leukemia [1, 2]. The absence of antitumor effect in the latter case was evidently due to protection by dC of the malignant myeloid cells against the toxic effect of araC.

In the investigation described below the effect of dC on the antitumor effect of araC was studied in Rauscher erythroblastic leukemia.

EXPERIMENTAL METHOD

Female BALB/c mice weighing 20-22 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. Rauscher leukemia was induced by intraperitoneal inoculation of $2 \cdot 10^7$ leukemic spleen cells. The day of injection of the leukemic cells was counted as 0. The araC was used in the form of "Cytoza" from Upjohn (USA) and dC in the form of 2-deoxycytidine hydrochloride, from Reanal (Hungary). Both substances were dissolved in physiological saline and administered simultaneously in a dose of 0.2 ml per mouse: dC by mouth, araC intraperitoneally four times a day, every day. The daily dose of dC was 480 mg/kg body weight, and of araC 160 mg/kg. The course began on the 15th or 16th day after inoculation of leukemic cells. The antitumor effect was estimated either from the degree of splenomegaly or from the mean lifespan (MLS). The lengthening of the lifespan, in percent, was calculated by the formula:

$$\frac{MLS_{expt.} - MLS_{control}}{MLS_{control}} \times 100.$$

The cell composition of the spleen and peripheral blood was investigated in blood films stained by the Romanovsky–Giemsa method and in squash preparations of the spleen, in which 200 and 1000 cells, respectively, were identified. The proportions of cells on squash preparations of the spleen were compared by the chi-square test and the remaining mean values were compared by Student's t-test. Differences for which $P < 0.05$ were taken to be significant.

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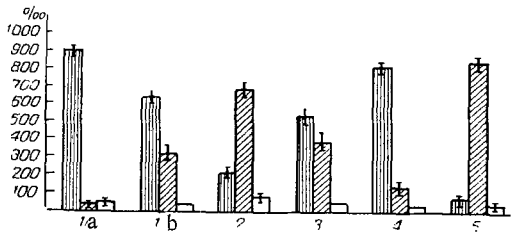

Fig. 1. Effect of araC—dC combinations on cell composition of spleen of BALB/c mice with Rauscher leukemia. Ordinate, number of cells per 1000 spleen cells (in ‰). Preparations administered on 16th-17th day of leukemia. Data for all groups obtained on 20th day of leukemia or on 3rd day after end of course of treatments for group 1a on 16th day of leukemia. 1a) Rauscher leukemia; 1b) Rauscher leukemia; 2) Rauscher leukemia + araC; 3) Rauscher leukemia + araC + dC; 4) Rauscher leukemia + dC; 5) Intact control. Columns with vertical shading cells of erythroid series; columns with oblique shading, cells of lymphoid series; unshaded columns, cells of myeloid series.

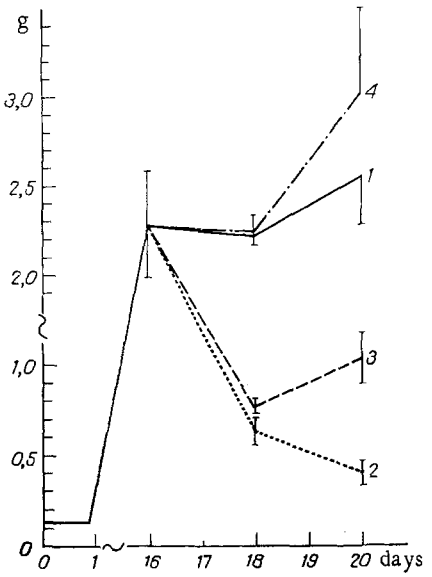

Fig. 2. Effect of araC—dC combinations on weight of spleen of mice with Rauscher leukemia. Compounds administered on 16th and 17th days of leukemia. 1) Rauscher leukemia; 2) Rauscher leukemia + araC; 3) Rauscher leukemia + araC + dC; 4) Rauscher leukemia + dC. Abscissa — time of investigation (in days); ordinate — weight of spleen (in g).

EXPERIMENTAL RESULTS

At the time when treatment of the mice began a well-marked picture of leukemia was observed: The weight of the spleen was increased by 15-20 times and the lymphoid tissue was replaced by young cells of the erythroid series. The number of myeloid and lymphoid cells also increased until the 20th day (Fig. 1). The number of nucleated cells in the peripheral part increased to 189,000/μl on account of all types of leukocytes and young cells of the erythroid series, followed by the development of increasing anemia, with signs of anisocytosis and poikilocytosis. Untreated mice died on average on the 26th day (26 ± 4 days, n = 7). Administration of araC in a dose of 20 mg/kg three times a day from the 15th through the 17th days led to a small but significant lengthening of the life span of the mice by 24%. However, administration of araC in a higher dose (160 mg/kg daily), either alone or in combination with dC (480 mg/kg daily), in one two-day course or as three such courses with intervals of three days between them, caused no increase in MLS of the mice compared with its duration in

the untreated control animals. Although MLS of the leukemic mice treated with araC alone or with dC protection was the same, it can be postulated that the main cause of death of the animals in these groups was different. In the first case their death was most probably due to the development of lethal toxicosis, in the second case to progression of the leukemia as a result of weakening of the antitumor effect. To test this hypothesis experiments were carried out to study the effect of dC on the toxic and lethal effects of araC in healthy mice, and also on the weight and cell composition of the spleen of the leukemic mice. In normal mice, after administration of araC for two days a toxic effect was exhibited from the 4th through the 7th days, but all the mice survived. Two or three 2-day courses of araC with intervals of three days between them caused death of most mice: MLS was 10 ± 0 and 8 ± 1 days, respectively, for 2 and 3 courses. dCprevented the toxic and lethal effects of araC. In leukemic mice administration of araC caused a rapid and sudden fall in weight of the spleen, and a tendency toward normalization of the cell composition of the spleen also was observed (Figs. 1 and 2). In mice treated with araC under dC protection, the weight of the spleen 3 days after the end of the course was significantly greater and the number of cells of the erythroid series was six times greater than in the spleen of mice treated with araC alone. Meanwhile the number of lymphoid and myeloid cells was approximately the same in the mice of both groups. The results of this series of experiments thus confirmed the earlier views expressed on the causes of death of the mice.

Analysis of the cell composition of the peripheral blood of the mice showed that by the 3rd day after the beginning of a two-day course of treatment, whether with araC alone or under dC protection also, the number of nucleated cells per microliter fell sharply. The protective effect of dC was exerted only on postmitotic granulocytes on the 5th day after the beginning of treatment. It was impossible to analyze the peripheral blood picture later during the investigation because of death of the mice.

It can thus be concluded that, in contrast to lymphoblastic leukemia L1210, administration of araC in lethal doses under dC protection to mice with Rauscher erythroblastic leukemia causesa decrease in the antitumor and toxic effects. Meanwhile dC does not weaken the inhibition of excessive proliferation of lymphocytes by araC in the spleen. This indicates that an oriented action on proliferation of lymphoid tissue is possible in such nonlymphoid forms of leukemia by means of the metabolite–antimetabolite combination studied.

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