POTENTIATION BY BLASTOLYSIN OF THE ANTIRELAPSE EFFECT OF CYCLOPHOSPHAMIDE IN MICE WITH T CELL LEUKEMIA

A. I. Chertkova, V. M. Bukhman, and A. B. Syrkin

UDC 616.155.392.2-036.87-085.277.3.015.21:615.331:579.864.1]-039.71-092.9

KEY WORDS: T cell leukemia, blastolysin, cyclophosphamide.

The development of ways of preventing relapses in patients in whom a remission has been obtained is an urgent problem in the treatment of hemoblastoses at the present time. Analysis of experimental and clinical data on the state of immunoreactivity during remission suggests that one of the main causes of relapse is a disturbance of immunologic control due both to the disease itself and to cytostatic treatment [2]. Attempts to use certain chemical and biological agents capable of restoring or correcting antitumor immunity are consequently promising.

Intensive treatment with cytosar (cytosine arabinoside, araC) under deoxycytidine (dC) protection induces remission of T cell leukemia EL-4 in C57BL/6j (B6) mice in virtually 100% of cases [1]. However, in most mice relapse occurs after a longer or shorter period.

This paper describes an attempt to lengthen the period of remission by means of agents with an immunomodulating action, namely cyclophosphamide and blastolysin [5, 14].

EXPERIMENTAL METHOD

Male B6 mice aged 10-16 weeks, bred at the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. The EL-4 T cell lymphoma, induced with benzopyrene in B6 mice [8], was maintained in the ascites form by passage through syngeneic mice. In the experiments, 10^5 ascites cells were injected subcutaneously into the right flank of each mouse. Each experimental group consisted of 15-20 mice.

To induce remission of leukemia, araC (Upjohn, USA) and dC (Reanal, Hungary) were injected simultaneously, intraperitoneally three times a day in doses of 20 and 5 mg/kg, respectively, per injection, for 4 days, in three courses with intervals of 3 days between them. Treatment began on the 11th day after inoculation of the tumor cells when the mean diameter of the lymphoma was 10-12 mm. Toward the end of the second course of treatment subcutaneous lymphomas were absorbed in 100% of mice. All the untreated mice died by the 25th-26th day. Cyclophosphamide (CP; USSR product) and blastolysin (BL) [3] were injected into mice with an induced remission once a week, intraperitoneally, in doses of 100 and 25 mg/kg, respectively.

The mean duration of survival and its increase (MDS, IDS) and the mean duration of remission (MDR) were calculated. The difference between survival and the duration of remission was analyzed by Wilcoxon's nonparametric U test. The difference in the frequency of relapse was assessed by the chi-square test or by Fisher's modification of it.

EXPERIMENTAL RESULTS

Data on the antitumor activity of BL [3] and its ability to potentiate the antitumor action of irradiation [4] served as the basis for testing it to maintain remission in mice with EL-4 lymphoma. BL is known to possess adjuvant properties: It nonspecifically stimulates immunogenesis and activates cells of the mononuclear phagocyte system [5], whose functions are disturbed in mice with EL-4 lymphoma [12].

On a model of LSTRA mouse lymphatic leukemia induced by Moloney virus, Chirigos et al. [6] demonstrated that the length of survival of the mice could be prolonged by the use of im-

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Research Institute for Biological Testing of Chemical Compounds, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 7, pp. 57-59, July, 1984. Original article submitted July 22, 1983.

0007-4888/84/9807-0919$08.50

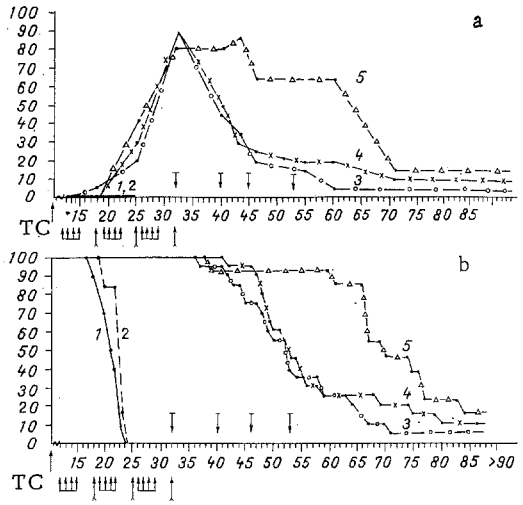

Fig. 1

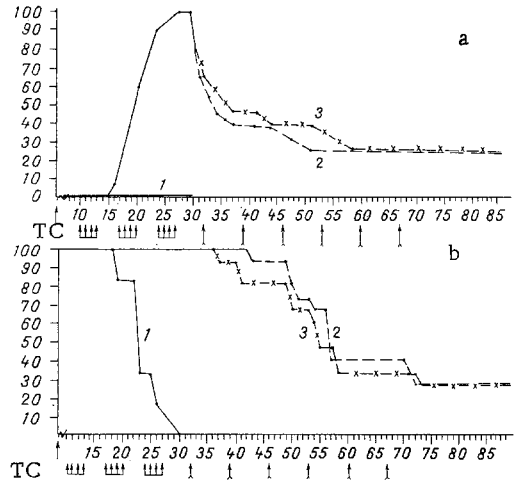

Fig. 2

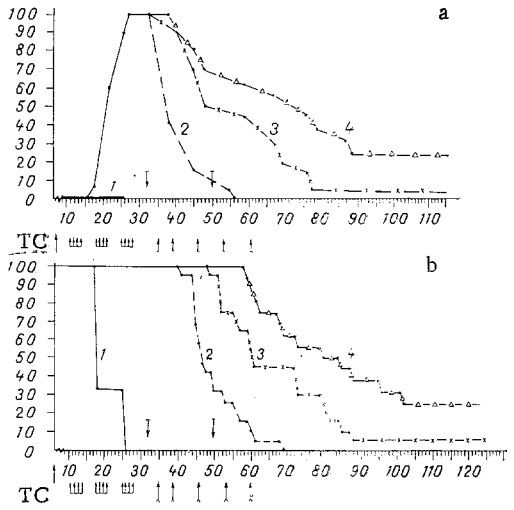

Fig. 3

Fig. 1. Effect of CP and BL on frequency of remission induced by araC + dC combination and on survival rate of mice with EL-4 lymphoma. Each mouse received 10^5 EL-4 ascites tumor cells (TC) subcutaneously on day 0. 1) Untreated control; 2) BL; 3) araC + dC + BL; 4) araC + dC; 5) araC + dC + CP. Arrows indicate days of injection of preparations: ↑↑↑↑ araC + dC, ↑ BL, ↓ CP. Here and in Figs. 2 and 3: abscissa, days of observation; ordinate: a) % of mice with remission, b) % of mice in group still alive.

Fig. 2. Effect of BL on antileukemic activity of araC + dC. 1) Untreated control; 2) araC + dC; 3) araC + dC + BL. Remainder of legend as to Fig. 1.

Fig. 3. Effect of combined administration of CP and BL on frequency of remission and survival of mice with lymphoma EL-4. 1) Untreated control; 2) araC + dC; 3) araC + dC + CP; 4) araC + dC + CP + BL. Remainder of legend as to Fig. 1.

munoadjuvants levamisole and tetramisole in a period of remission induced by the chemotherapeutic agent.

We were unable to find any antitumor activity of BL against a developed EL-4 lymphoma (data not described). Injection of BL in periods between courses of araC and dC or in the period of maintenance of remission likewise proved ineffective (Figs. 1 and 2).

During the development of EL-4 lymphoma in mice, immunoreactivity is known to be suppressed [11]. Although EL-4 cells specifically sensitize a syngeneic host, no effective immune re-

sponse develops [9]. The presence of an active suppressor mechanism, including both humoral and cellular suppressor factors, has been suggested [9, 11, 14].

Cyclophosphamide is one of the most widely used chemotherapeutic agents with an immunomodulating action [13]. Within a definite dose range CP as a rule potentiates the cellular immune response as a result of inhibition of function of suppressor cells [13, 15].

In the present experiments injection of CP during the period of remission of EL-4 lymphoma in a dose of 100 mg/kg intraperitoneally four times at weekly intervals (Fig. 1) or twice with an interval of 18 days (Fig. 2) led to prolongation of remission. In the first case MDR in the group of mice receiving CP was increased by 12 days compared with the group of mice receiving araC + dC only (30 and 18 days, respectively; $P < 0.01$), and in the second case it was increased by 14 days (29 and 15 days, respectively; $P < 0.001$). After injection of CP ceased, the relapse rate increased sharply and nearly all the mice died from leukemia. Lengthening of MDS of animals receiving CP once a week was 171% compared with 151% in the group of mice receiving araC + dC only. Injection of CP according to the second schedule was more effective: Prolongation of MDS increased compared with the group of mice not receiving CP from 144 to 218% ($P < 0.01$; Fig. 3).

The antirelapse effect of CP in this system could be due both to the direct cytotoxic action of the drug on residual tumor cells and to its ability to damage the function of suppressor cells [10, 13, 15]. The role of immunologic factors in the antirelapse action of CP is indicated by the results of a preliminary experiment in which three groups of mice, treated with araC + dC by the above scheme, received 25, 50, or 100 mg/kg of the CP in a single dose. The effect of the compound in this case was independent of dose. MDS of the mice of these three groups was virtually the same.

Dye and North [7] observed good results from the combined use of CP with *Salmonella enteritidis* endotoxin in the treatment of sarcoma SA-1 in $AB6F_1$ mice.

In the present investigation combined treatment with CP and BL of mice during remission of EL-4 leukemia led to a much stronger effect than the use of CP alone (Fig. 3, group 4). The length of the remission in mice in this experiment was 11 days in the group of mice receiving araC + dC (group 2), 21 days in mice receiving araC + dC + CP (group 3), and 44 days in mice receiving araC + dC + CP + BL (group 4). Starting with the 34th day of observation a significant difference was observed in the percentage of mice with remission between group 2 and groups 3 and 4, and this persisted until death of the last animal in the group of mice receiving araC + dC alone. Starting from the 70th day of the experiment a significant difference was found in the percentage of mice without relapse between groups 3 (araC + dC + CP) and 4 (araC + dC + CP + BL). MDS of animals of the last group was 75 days and it differed significantly from MDS of mice receiving CP only during remission. Lengthening of MDS in the group of mice receiving CP and BL amounted to 262%; 25% of the animals, moreover, were completely cured ($P_{2,4} < 0.05$).

In the present investigation BL, which by itself is inactive against EL-4 lymphoma, potentiated the antirelapse action of CP.

Administration of CP alone and, in particular, of a combination of CP with BL can thus be a promising way of maintaining remission in hemoblastoses. To elucidate the mechanisms of the combined action of these compounds further research is necessary. It can be tentatively suggested that the effect is connected with inhibition of function of suppressor cells by CP and simultaneous activation of effector cells of antitumor immunity by BL.

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CELLULAR MECHANISM OF THE ANTILEUKEMIC ACTION OF QUINOLINE DIBROMIDE

O. D. Golenko, T. D. Paramonova, and T. G. Nikolaeva

UDC 615.277.3:547.831].015.44:616.155.392

KEY WORDS: leukemia; antimetabolites; proliferation.

The possibility of enhancing the antitumor activity of cyclodependent antimetabolites of methotrexate, cytosar, and 5-fluorouracil, during combined administration with quinoline dibromide (QD), a potential inhibitor of cobalamine-dependent methionine synthetase [1, 3, 4], has been proved experimentally in recent years. QD has a marked antileukemic action and significantly lengthens the duration of survival of mice with leukemias L-1210, La, and P-388 [5, 6]. A study of the pharmacokinetics of [^{14}C]-QD in healthy animals revealed delayed excretion of the compound with the urine and a high concentration of it in the tissues, due to its strong interaction with cell protein and DNA [9]. However, the toxicity of QD limits its isolated use [4]. The cellular mechanisms of the antileukemic action of QD have not been studied.

This paper describes the results of an investigation of the action of QD on the kinetics of proliferation of ascites leukemia L-1210.

EXPERIMENTAL METHOD

Leukemia L-1210 was inoculated intraperitoneally by injection of 5×10^6 leukemic cells into DBA/2 mice weighing 20 g. On the 3rd day after inoculation of the leukemia, mice of the experimental group were given an intraperitoneal injection of QD in the maximal tolerated dose of 10 mg/kg. QD (NSC-176319) was obtained from the National Cancer Institute, USA, in accordance with the program of collaboration between the USSR and USA in the field of tumor chemotherapy. At various times after injection of QD, the following parameters of proliferation of leukemic cells were determined in mice of the experimental and control groups autoradiographically: duration of the mitotic cycle (Tc) and its individual periods ($t_{G_1} + {}^1/_2 t_M$; t_S; $t_{G_2} + {}^1/_2 t_M$), the distribution of cells by phases of the cell cycle, and their transition into the phase of DNA synthesis, and the number of cells synthesizing DNA. The duration of the mitotic cycle and its individual periods was calculated from the change in percentage of labeled mitoses at different times after a single injection of [^{3}H]thymidine [2]. QD was injected into the animals of the experimental group 1 h before injection of the isotope. [^{3}H]Thymidine was injected into mice of the experimental and control groups in a dose of 1 μCi/g (specific activity 0.94 gBq/mole). Every 2 h, between 1 and 28 h after injection of the isotope, three animals from the experimental and control groups were killed and films were made from cells of the ascites fluid. Autoradiographs were obtained by the standard method, using "M" photograph-

Laboratory of Endogenous Modifying Factors of Carcinogenesis, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 7, pp. 59-61, July, 1984. Original article submitted July 27, 1983.

 0007-4888/84/9807-0922$08.50