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CELLULAR MECHANISM OF THE ANTILEUKEMIC ACTION OF QUINOLINE DIBROMIDE

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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-

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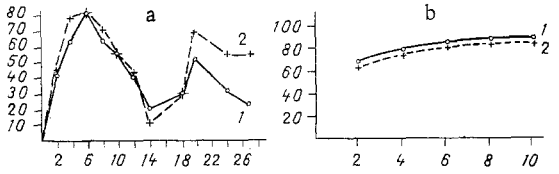

Fig. 1. Change in percentage of labeled mitoses in leukemia cells under influence of QD (a), and entry of leukemic cells into phase of DNA synthesis under the influence of QD (b). Abscissa, time after first injection of [^{3}H]-thymidine (in h), ordinate, percentage of labeled mitoses (a) or cells after repeated injections of [^{3}H]thymidine with 2-hourly intervals (b). QD was injected in a dose of 10 mg/kg on 3rd day after inoculation of leukemia, 2) control, 3rd day after inoculation of leukemia L-1210.

TABLE 1. Effect of QD on Passage of Leukemic Cells through Cell Cycle (M ± m, n = 4)

Group of mice	Time after injection of QD, h	Index of labeled cells, %	Distribution of cells by phases of cell cycle, %		
			G_1/g_0	S	G_2/M
Control	5	51,2±3,0	55,8±2,0	36,1±1,7	8,1±1,4
Experiment	—	50,3±1,6	55,3±2,7	36,6±2,0	8,1±1,2
Control	16	51,7±2,0	—	—	—
Experiment	—	43,4±3,9*	—	—	—
Control	20	—	48,5±1,4	42,6±0,4	8,9±1,4
Experiment	—	—	55,3±2,7	36,6±2,0*	8,1±1,2
Control	36	46,5±2,7	—	—	—
Experiment	—	36,8±2,8*	—	—	—

Legend. *P ⩽ 0.05.

ic emulsion. When the effect of QD on transition of the cells into the phase of DNA synthesis was studied, [^{3}H]thymidine was injected five times at intervals of 2 h. QD was injected 30 min after the first injection of the isotope.

The distribution of cells by phases of the cell cycle was studied 5 and 20 h after injection of QD, on a fluorescent-activated ICP-22 flow cytofluorometer (Phywe, West Germany). Leukemic cells were separated from ascites fluid in a Ficoll—Verografin density gradient (sp. gr. 1.07) during centrifugation (400g, 25 min). Cells fixed in 70% ethanol were washed with phosphate buffer and treated with a 0.1% solution of RNase and a 0.5% solution of pepsin. After enzyme treatment the cells were stained with mithramycin and ethidium bromide [7]. The percentage distribution of cells by phases of the cell cycle was determined by analysis of cells stained for DNA [8]. These DNA cytograms were obtained by analysis of at least 50,000-100,000 cells. The significance of differences between the parameters was determined by Student's t test.

EXPERIMENTAL RESULTS

According to the results of autoradiography and fluorescent-activated DNA cytofluorometry, QD had no significant effect on the course of the mitotic cycle or generation time of the cells immediately after its injection. The duration of phases of the mitotic cycle ($t_{G_1} + \frac{1}{2}t_M$; t_S; $t_{G_2} + \frac{1}{2}t_M$) showed no significant change under these circumstances and was 6, 8.5, and 1.5 h, respectively (Fig. 1). The first rise in the percentage of labeled mitoses in the population of leukemic cells after injection of QD was virtually indistinguishable from the control, i.e., cells which at the time of injection of the compound were at the end of the phase of DNA synthesis reached the phases of mitosis in equal time. The considerable number of labeled mitoses and the high mitotic index 2 h after injection of QD also were evidence of the absence of any primary blocking of cells in the postsynthetic period and in mitosis of the cell cycle. Transition of the cells from the presynthetic period into the phase of DNA synthesis was confirmed by experiments with repeated injections of [^{3}H]thymidine. An increase in the index of labeled cells in the leukemic population was observed in the course of 10 h and it did not differ significantly from that observed after injection of QD (Fig. 1). The intensity of DNA synthesis in the cells was not reduced under these circumstances. The distribution of leukemic cells by phases of the cell cycle likewise was not disturbed during this period of observation (Table 1). Under the influence of QD there was therefore no primary blocking of cells in the various phases of the mitotic cycle.

However, in the late stages after injection of QD (16-20 h) substantial changes were found in the distribution of leukemic cells among phases of the cell cycle. The number of cells in the phase of DNA synthesis 20 h after injection of the compound was 6% greater than in the control. The number of cells only in the presynthetic period was reduced. An increase in the number of cells during this period of observation also was confirmed by autoradiographic data. The index of labeled cells was considerably increased, and its value remained high in the leu-

kemic population during the next 20 h (Table 1). These changes were evidently due to the blocking action of QD on cells in the late phase of DNA synthesis in the second division cycle. An important result of secondary blocking by QD of transition of the cells from the phase of DNA synthesis into the postsynthetic period was a considerable decrease in the rise of the second wave of labeled mitoses and of the mitotic index. The mechanism of action of the inhibitor of cobalamine-dependent methionine synthetase which we have examined thus determines the increased effectiveness of cyclodependent antimetabolites, and above all of the S-phase-specific preparation cytosar during combined treatment of L-1210 leukemia.

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MODIFICATION OF AMINO-ACID RESIDUES OF BLOOD ALBUMIN FROM CANCER PATIENTS

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The writers previously have described changes in the blood albumin in various diseases. These changes were recorded by many different physicochemical methods, including peptide mapping [5]. When the structural changes observed in albumin were discussed, the possibility of post-translation modification of this protein under pathological conditions was considered. Later experiments showed that besides the native form of albumin, a conformationally changed (modified) form also circulates in the patients' blood, and the structural changes observed largely depend on the ratio between these forms [6]. This paper describes the study of this modified form of albumin with a view to the possible discovery of amino-acid residues modified as a result of disease.

EXPERIMENTAL METHOD

Preparations of blood albumin from patients and normal subjects were obtained by preparative electrophoresis in polyacrylamide gel [2]. The purity of the isolated preparations was tested by analytical electrophoresis in the same gel and by an immunochemical method. The modified form of albumin (A_m) was separated from the native form (A_n) from the electrophoretic fraction (A_e) by the method described in [3]. Free SH-groups, ionization of free hydroxyl groups, and N- and C-terminal amino acids were determined in the isolated albumin preparations [1]. The N-terminal sequence as far as the 6th residue in the albumin preparation was determined on a Model 890 "Sequenator" (Beckman, USA) [1]. Mass-spectrometric analysis also was used in the investigation [4].

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