The Effect of L-Asparaginase on the Nucleic Acid Metabolism and Cell Cycle of Human Leukemia Cells

By E. Fred Saunders

The effect of L-asparaginase on the cell cycle and nucleic acid synthesis of leukemic cells was studied in five children with acute lymphoblastic leukemia. Following an intravenous infusion of the drug, serial marrow samples were obtained for buffy coat volume, mitotic index, and autoradiographic assessment of DNA and RNA synthesis using tritiated thymidine and tritiated uridine, respectively. A rapid decline in buffy coat volume indicated a lytic effect on lymphoblasts. There was a greater kill of proliferative (blasts in the cell cycle) than nonproliferative (G0) leukemic cells. Mitotic indices changed little until 24 hr; in contrast, thymidine labeling indices decreased markedly to less than 50% of control by 6 hr. The changes in labeling indices prior to changes in mitotic indices indicated that L-asparaginase blocked the entrance of cells into the DNA synthesis period of the cell cycle. Cells already in DNA synthesis appeared to continue into mitosis. Uridine labeling indices decreased progressively in all patients. Uridine uptake was inhibited equally in both proliferative and nonproliferative blasts. Therefore, inhibition of RNA synthesis by L-asparaginase was independent of the proliferative activity of the marrow.

L-ASPARAGINASE is an enzyme with chemotherapeutic activity against human acute leukemia and some animal tumors. The drug acts primarily by inhibiting protein synthesis in malignant cells deficient in L-asparagine synthetase activity, and therefore, dependent on exogenous L-asparagine.1,2 The inhibition of protein synthesis is associated with inhibition of DNA and RNA synthesis in certain experimental animals tumors.3,4 I studied the effect of L-asparaginase on the cell cycle and nucleic acid metabolism of human acute leukemia cells.

MATERIALS AND METHODS

The subjects were four girls and one boy ranging in age from 5 to 11 yr. All had advanced acute lymphoblastic leukemia and had been treated with most available antileukemic drugs. All patients were in relapse with 90% or more leukemic blasts in the marrow. Previous antileukemic agents had been discontinued. No blood or platelet transfusions, or drugs other than L-asparaginase were given during the study. Three of the patients eventually achieved complete clinical and marrow remission. Informed consent of the parents was obtained.

From the Department of Pediatrics, University of Toronto, and the Research Institute, The Hospital for Sick Children, Toronto, Canada.

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E. Fred Saunders, M.D.: Assistant Professor of Pediatrics, Faculty of Medicine, University of Toronto, and Staff Physician, Division of Hematology, The Hospital for Sick Children, Toronto.
L-asparaginase (Farbenfabriken Bayer A. G.) produced from Escherichia coli was used. Studies were done at the time of the first injection of L-asparaginase. Following a control bone marrow aspiration, 2000 U/Kg of the drug was given by intravenous drip over a 30-min period. Serial marrow samples were obtained from widely separated pelvic sites during the subsequent 48 hr. Marrow was aspirated into a dry syringe and processed for routine smears, buffy coat volume using a Wintrobe hematocrit tube, and mitotic index using the acetocarmine suspension method. The mitotic index was obtained by counting 10,000 nucleated cells per sample and expressed as the number of mitotic figures per 1000 nucleated cells. The marrow needle was redirected at the same site and a heparinized marrow sample obtained. One milliliter aliquots were incubated with thymidine-5-methyl-3H (TdR3H) (0.36 Ci/mM), or uridine-5-3H (UR3H) (2.0 Ci/mM), in a concentration of 1 µCi/ml, for 1 hr at 37°C. Autoradiographs were prepared using Kodak AR10 stripping film; those with TdR3H were exposed 10 days and those with UR3H 14 days. Background labeling was light and cells with five or more grains over the nucleus were considered labeled. Two thousand leukemic blasts per sample were counted and the labeling indices expressed as a percentage.

Leukemic blasts may be classified morphologically as proliferative or nonproliferative. Dividing blast cells are large with fine nuclear chromatin; nondividing blast cells are relatively smaller with coarse nuclear chromatin. Only the dividing cells incorporate label during a 1 hr incubation with TdR3H. Both classes of cells incorporate UR3H. On the autoradiographs, leukemic blasts were designated as proliferative or nonproliferative depending on relative size and characteristics of the nuclear chromatin. Five hundred blasts per sample were so classified. Mean grain counts were determined by counting 100 consecutive labeled blasts with five or more grains over the nucleus, per sample. The significance of changes in labeling and mitotic indices, and grain counts was determined by means of Student's t test.
RESULTS

Changes in buffy coat volume are shown in Fig. 1. Two patients had marked decreases in buffy coat volume by 6 hr and two had relatively gradual responses, all reaching hypocellular levels by 48 hr. At that time the marrows consisted of from 60% to 90% leukemic blasts; most of the remaining cells were small lymphocytes. One patient started with a hypocellular marrow and changes were not significant. Because leukemic blasts disappeared rapidly, determination of mitotic and labeling indices became difficult, and the study was discontinued at 48 hr.

Initial mitotic indices ranged from 5 to 15 per 1000 nucleated cells (Fig. 2). They changed little during the first 6 hr, but then decreased steadily to very low levels by 48 hr. TdR\(^{3}H\) labeling indices of control marrows ranged from 4% to 18% (Fig. 3). In contrast to mitotic indices, labeling indices decreased markedly by 6 hr, reaching almost zero by 48 hr in all cases. To compare mean decreases in mitotic and TdR\(^{3}H\) labeling indices for the five patients (Fig. 4), means are expressed as a percentage of control values. By 6 hr, the mean labeling index decreased to less than 50% of the control, whereas the mean mitotic index was statistically unchanged. Thereafter, the two curves roughly paralleled each other.

In two patients the size and labeling of only the proliferative population of blasts in the marrow was followed. Initially, 23% and 31% of the marrow...
cells were large proliferative blasts. By 48 hr the proliferative population was reduced to 10% and 8%, respectively. The remaining 90% of the blasts were small nonproliferative cells. Labeling within the proliferative population decreased from control values of 51% and 32% to less than 10% by 48 hr.

The uptake of UR^3H into leukemic cells is shown in Fig. 5. Labeling was limited to the area over the cell nucleus. Initial labeling in 4 patients ranged from 8% to 19%, and in the other was 56%. I cannot explain the high labeling in this instance. In all patients UR^3H labeling indices decreased significantly to an average of 25% of control values by 48 hr. The pattern of response was variable; the patient with the highest initial labeling showed the greatest response. In two patients the UR^3H labeling indices were obtained separately for the proliferative and nonproliferative blast populations. Initial labeling of proliferative blasts was 32% and 41%, that of nonproliferative blasts 5% and 8%. The relative decreases in labeling to about 30% of control values by 48 hr were similar in both populations of cells.

Mean grain counts with both thymidine and uridine were not reproducible. The pattern of change varied from patient to patient and could not be interpreted statistically. However, the cells most heavily labeled with uridine (>20 grains per cell) disappeared by 48 hr in each patient.

**DISCUSSION**

L-asparaginase is a useful drug in acute lymphoblastic leukemia in children resulting in a 50-60% remission rate. Unfortunately, remissions are of short duration. In vitro testing of drug sensitivity has not been useful in predicting remission, probably because of rapid induction of L-asparagine synthetase activity in some leukemic cells. Although all the patients had an initial drug effect, only three achieved complete clinical and bone marrow remission. It was impossible to detect any unique features of the patients, of the marrow changes, or of the kinetic responses, which would allow one to predict the patients who would eventually go on to remission.

The leukemic marrow consists of two populations of blasts, one proliferative (cells in the cell cycle) and the other nonproliferative (resting blasts out of the
cell cycle; G\textsubscript{0} cells).\textsuperscript{6,9,10} The two leukemic cell populations are in equilibrium.\textsuperscript{11} Thymidine methyl-\textsuperscript{3H}, a specific DNA precursor, is incorporated into the cell nucleus only during the DNA synthesis (S) period of the cell cycle. Decreases in thymidine labeling indices could have resulted from a relative decrease in the total number of proliferative blasts in the marrow, and/or a decreased proportion of proliferative blasts in the S period of the cell cycle. The data indicated that both events had occurred. By 48 hr most of the blasts in the marrow were nonproliferative, indicating that L-asparaginase preferentially killed proliferative cells. The decrease in the percentage of labeled cells within the proliferative population indicated a decreased proportion of cells in S. These effects must have been cell-cycle dependent.

However, the drug appeared to have another action independent of the cell cycle. Nonproliferative blasts were killed. The marked decreases in buffy coat volume by 6 hr after drug administration indicated a lytic effect on leukemic cells which was too rapid to be cell-cycle dependent.

The drug reduced both thymidine labeling and mitotic indices, reflecting decreased proportions of cells in the S and mitotic (M) periods of the cell cycle. Though a reduction of cells in S was evident by 6 hr after the drug, changes in cells in M were not detected until 24 hr. The data indicated that L-asparaginase blocked the entrance of cells into the S period of the cell cycle. Cells already in S appeared to complete DNA synthesis and go on to mitosis. The proportion of cells in mitosis did not decrease until S became depleted of cells. The data fit previously calculated DNA synthesis times of about 20 hr for leukemic lymphoblasts.\textsuperscript{9} Because the number of marrow aspirations is limited in humans, more detailed curves can not be drawn. In the presence of L-asparaginase, a block in initiating DNA synthesis has been shown for Jensen sarcoma cells\textsuperscript{12} and regenerating rat liver.\textsuperscript{13} This effect is probably due to inhibition of protein synthesis which is necessary for the initiation of DNA synthesis.\textsuperscript{12-15} Protein synthesis may\textsuperscript{3,4,14} or may not\textsuperscript{12,15,16} be necessary to maintain DNA synthesis once initiated. However, various workers have studied different normal or malignant tissues in experimental animals or tissue culture. As the mean grain counts were uninterpretable, it was impossible to show whether DNA synthesis rates had changed and, therefore, whether protein synthesis was necessary to maintain DNA synthesis in human leukemia cells. Hydrocortisone and prednisone block the initiation of DNA synthesis in human leukemic lymphoblasts without inhibiting DNA synthesis already in progress.\textsuperscript{17,18}

Uridine-\textsuperscript{5-3H} is a specific RNA precursor.\textsuperscript{19} L-asparaginase gradually inhibited UR\textsuperscript{3H} incorporation into RNA in all patients. RNA synthesis was inhibited equally in proliferative and nonproliferative blasts. Therefore, this effect of the drug was independent of the proliferative activity of the marrow. Changes in mean grain counts could not be interpreted statistically. However, the disappearance of heavily labeled cells reflected a decreased rate of RNA synthesis. This study does not indicate which type of RNA was inhibited. In a short incubation, human leukocytes incorporate UR\textsuperscript{3H} into unstable, DNA-dependent, nuclear RNA, which is partly ribosomal precursor RNA.\textsuperscript{20} Mouse lymphoma depleted of L-asparagine resulted in inhibition of all classes of
RNA but especially ribosomal RNA. The inhibition of RNA synthesis is probably directly due to the inhibition of protein synthesis. The effect of inhibition of protein synthesis on RNA fractions is complex and varies depending on the drug used, the cell system studied, and the duration of protein starvation.

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REFERENCES

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