Megaloblastosis Produced by a Cytosine Antagonist
1-3-D-arabinofuranosylecytosine

By R. W. Talley and V. K. Vaitkevicius

CYTOSINE ARABINOSIDE (1-3-D-arabinofuranosylecytosine hydrochloride, fig. 1) is a recently synthesized pyrimidine nucleoside. Johnston reported that this compound produced hematopoietic toxicity in dogs at a dose of 50 mg./Kg. given daily for 14 days. The major depression was noted in the neutrophilic leukocytes and platelets. Three cebus monkeys treated with a similar dose for 8 days developed less severe hematopoietic toxicity; however, platelet values were not reported.

J. S. Evans et al. reported cytosine arabinoside to inhibit growth of mouse Sarcoma 180, Ehrlich carcinoma, azaserine-resistant Sarcoma 180, and L-1210 leukemia. Because of our interest in exploring new chemotherapeutic agents and especially nucleosides in which the abnormality resides in the sugar moiety, evaluation of this drug in humans with malignant disease was undertaken. The hematopoietic toxicity we observed was qualitatively different than in animals.

METHODS

Thirteen patients with proven disseminated malignant disease were selected for this study. All patients had lesions which could be measured either by roentgenograms or by direct palpation except for four moribund patients in whom only acute toxicity could be studied. Cytosine arabinoside was given in one daily intravenous dose of 3-10 mg./Kg. for 4-9 days for a total dose of no more than 50 mg./Kg. or a single 30-50 mg./Kg. dose at 7- to 10-day intervals. Five patients received cytosine arabinoside in both dosage regimens. Pretreatment hemograms, bone marrow aspirations (when possible), urinalyses, and tests of liver and cardiac function were made and repeated at intervals during the study. Routine clinical observations for gastrointestinal, neurologic, and peripheral vascular toxicity were carried out in all patients.

RESULTS

Therapeutic Effect

The majority of patients included in the study had such far-advanced disease that evaluation of therapeutic effect of any agent would be difficult. However, three patients with lymphosarcoma and one patient with carcinoma of the bladder had definite but temporary regression of tumor masses. In two of the three patients with lymphosarcoma, the objective changes occurred after the patient had become unresponsive to alkylating agent and adrenal steroid therapy.
Fig. 1.—Comparative structural formulas of cytosine arabinoside (left) and cytosine deoxyribose (right).

One patient had clearing of oculomotor palsy, as well as a marked decrease in palpable adenopathy following two courses of cytosine arabinoside. The first course was a single dose at 30 mg./Kg, and the second course was given three weeks later and consisted of 10 mg./Kg. for 5 days. Severe leukopenia and thrombocytopenia were evident after the second course of the drug. Following recovery from the toxicity, the patient developed Escherichia coli pneumonia which was fatal. The second patient with lymphosarcoma developed a leukemic picture terminally with a 66,000 peripheral white cell count, 90 per cent of which were atypical lymphocytes. The patient was moribund with bronchopneumonia at onset of therapy. Following a single dose of 20 mg./Kg., the peripheral white count fell to 3,100 with 40 per cent atypical lymphocytes at 4 days after treatment. Also the platelets increased from 25,000 per cu. mm. of blood to 100,000 per cu. mm. The patient expired from bronchopneumonia 4 days after therapy.

The third patient with lymphosarcoma, previously untreated, had a significant regression in nodes and subjective improvement which persisted for 8 weeks after two 40 mg./Kg. doses of cytosine arabinoside at 14-day intervals. This patient did not develop significant hematopoietic toxicity.

A patient with carcinoma of the bladder had disappearance or decrease in size of 6 of 12 pulmonary nodules for a short period of time; however, there was no change in appearance or size of two subcutaneous nodules. A patient with testicular carcinoma also had a decrease in pulmonary metastases after two courses of therapy. However, the development of increased intracranial pressure caused by brain metastases was fatal to the patient 3 weeks after the second course of therapy.

Hematopoietic Changes

Peripheral blood changes are summarized in table 1. This table presents the changes in peripheral blood data at the time of greatest depression of either white cells, platelets, or hemoglobin. Only patients observed for 2 weeks or more after therapy are included in this table. Four patients did not
Table 1.—Peripheral Blood Studies during Cytosine Arabinoside Therapy

A. Patients Receiving 3-10 mg./Kg. Cytosine Arabinoside Daily for 4-10 Days (Maximal Dose 50 mg./Kg.)

<table>
<thead>
<tr>
<th>Number of Observations</th>
<th>Hemoglobin (Gm./100 cc.)</th>
<th>WBC/mm.³</th>
<th>Platelets/mm.³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pretreatment</td>
<td>8</td>
<td>10.8</td>
<td>9,995</td>
</tr>
<tr>
<td>Average maximal depression</td>
<td>8</td>
<td>7.9</td>
<td>3,850</td>
</tr>
</tbody>
</table>

B. Patients Receiving 30-50 mg./Kg. in Single Dose

<table>
<thead>
<tr>
<th>Number of Observations</th>
<th>Hemoglobin (Gm./100 cc.)</th>
<th>WBC/mm.³</th>
<th>Platelets/mm.³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pretreatment</td>
<td>9</td>
<td>10.6</td>
<td>6,630</td>
</tr>
<tr>
<td>Average maximal depression</td>
<td>9</td>
<td>9.5</td>
<td>4,794</td>
</tr>
</tbody>
</table>

*Patients observed for at least 2 weeks after therapy are included.
†Four patients were studied on both dosage regimens, two patients were studied in regimen A on two occasions, and two patients were studied in regimen B on two occasions.
‡Determined by the technic of Brecker and Cronkite.²⁷

survive this 2-week period. There appeared to be a slight difference in all of the parameters studied in patients receiving the drug as a single dose, as shown in table 1. Those receiving daily small doses appeared to have greater depression of the peripheral blood elements than those receiving essentially the same amount of drug in a single large dose. Although the difference is not statistically significant, the trend suggests that in a larger group of patients the difference might be significant. This is particularly true of the difference in the platelet values where the “p” value is equal to 0.15. A hemoglobin depression of 1 Gm. or more was observed in every patient followed for 2 weeks or more and was also more marked in the group receiving daily small doses. Reticulocyte depressions were noted in patients in whom they were counted, with equal depression of granulocytic and lymphocytic cells. The reticulocytes fell to very low levels of 0.1 per cent or less by 10 days after therapy. Leukocyte depression was variable. In one patient the white cell count reached a low of 300 white blood cells/cu. mm. after two courses of therapy. This patient also manifested the lowest hemoglobin and platelet count seen. Thrombocytopenia with levels below 100,000 was observed in all but one patient in the daily treatment program but in only one of the patients on the single large dose program.

The most striking alteration noted was the rapid induction of significant megaloblastic changes in the bone marrow. The bone marrow findings are summarized in table 2. The first patient to whom the drug was given developed megaloblastic changes in 26 per cent of nucleated erythrocytes 48 hours after administration of 10 mg./Kg. There was a decrease in the peripheral leukocyte count from 27,500 to 12,500, which probably was not significant as the patient was receiving antibiotics for a urinary tract infection. However, the platelet count was decreased from 130,000 to 42,500 at the time of death, 6 days after the first injection.

Subsequently, varying numbers of megaloblastic cells were found in the marrow of all patients so studied. This change was most marked in the hemoglobinated red cell precursors. The cells became very large with loose
Table 2.—Bone Marrow Changes Induced by Cytosine Arabinoside

<table>
<thead>
<tr>
<th>Patient</th>
<th>Drug Dosage*</th>
<th>Day of Observation</th>
<th>Per Cent Megaloblasts of Nucleated Erythrocytes</th>
<th>Per Cent of P.A. Neutrophils</th>
<th>Megakaryocytes</th>
<th>Myeloid/Erythroid Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 mg./Kg.</td>
<td>days 1, 2, 3</td>
<td>28%</td>
<td>5%</td>
<td>absent</td>
<td>12.5:1</td>
</tr>
<tr>
<td>2</td>
<td>3 mg./Kg.</td>
<td>days 1, 2</td>
<td>0%</td>
<td>0%</td>
<td>normal</td>
<td>3.2:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 8-17</td>
<td>27%</td>
<td>5%</td>
<td>decreased</td>
<td>6.4:1</td>
</tr>
<tr>
<td>3</td>
<td>6 mg./Kg.</td>
<td>days 1, 2, 3</td>
<td>2%</td>
<td>0.1%</td>
<td>decreased</td>
<td>46:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 8-17</td>
<td>40%</td>
<td>1%</td>
<td>absent</td>
<td>78:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 10-15</td>
<td>5%</td>
<td>1%</td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>30 mg./Kg.</td>
<td>days 1, 11, 18</td>
<td>78%</td>
<td>4%</td>
<td>normal</td>
<td>3.5:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 1, 11, 18</td>
<td>78%</td>
<td>10%</td>
<td>normal</td>
<td>1.1:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 1, 2, 3</td>
<td>70%</td>
<td>8%</td>
<td>normal</td>
<td>1.9:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 10-15</td>
<td>86%</td>
<td>25%</td>
<td>decreased</td>
<td>3:1</td>
</tr>
<tr>
<td>5</td>
<td>30 mg./Kg.</td>
<td>days 1, 11, 18</td>
<td>53%</td>
<td>1%</td>
<td>decreased</td>
<td>46:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 10-15</td>
<td>40%</td>
<td>0.5%</td>
<td>decreased</td>
<td>28:1</td>
</tr>
<tr>
<td>6</td>
<td>10 mg./Kg.</td>
<td>days 1, 2, 3</td>
<td>1%</td>
<td>0%</td>
<td>normal</td>
<td>1.8:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 10-15</td>
<td>36%</td>
<td>4%</td>
<td>decreased</td>
<td>6:1</td>
</tr>
<tr>
<td>7</td>
<td>10 mg./Kg.</td>
<td>days 1</td>
<td>0%</td>
<td>0%</td>
<td>normal</td>
<td>2.1:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 1</td>
<td>1%</td>
<td>0%</td>
<td>decreased</td>
<td>46:1</td>
</tr>
<tr>
<td>8</td>
<td>30 mg./Kg.</td>
<td>days 1, 5</td>
<td>2%</td>
<td>1%</td>
<td>normal</td>
<td>2.6:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 1</td>
<td>73%</td>
<td>4%</td>
<td>normal</td>
<td>2.6:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 10-15</td>
<td>11%</td>
<td>0%</td>
<td>absent</td>
<td>18.8:1</td>
</tr>
<tr>
<td>9</td>
<td>30 mg./Kg.</td>
<td>days 1, 5</td>
<td>0%</td>
<td>0%</td>
<td>normal</td>
<td>2.8:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 10-25</td>
<td>17%</td>
<td>2.2%</td>
<td>decreased</td>
<td>48:1</td>
</tr>
</tbody>
</table>

*Bone marrow studies were not performed or were not adequate for this study on four patients (5, 6, 10, 12).

chromatin structure of the nuclei and prominent parachromatin (fig. 2). The non-hemoglobinated red cell precursors showed a less striking increase in the cell size, and loosening of the chromatin.

Patient 4 had several episodes of massive hematemesis prior to the study, resulting in erythroid hyperplasia of the bone marrow with markedly increased mitotic activity. Because of this the effect of cytosine arabinoside on the mitotic process could be easily studied. Unfortunately, a pretreatment bone marrow was not available in this patient; however, 48 hours after therapy 78 per cent of the erythroid series consisted of megaloblasts, subsequently rising to 86 per cent. Also well demonstrated in this patient was the development of large metamyelocytes, and multilobed mature neutrophils. Both the megaloblasts and P.A. type neutrophils are demonstrated in figure 3.
Fig 2—Bone marrow changes in patient 8 after cytosine arabinoside. a) Pro-treatment marrow (5800); b) megaloblast 48 hours after cytosine arabinoside (5800); c) giant metamyelocytes and megaloblasts seen at 48 hours.
Corresponding changes were seen in the peripheral blood also (fig. 4). The abnormalities of mitosis are shown in figure 5, and demonstrate the following:
1. Abnormal stickiness of the chromosomes with resultant bridge formation;
2. Lagging chromosomes, uneven distribution of nuclear material between daughter cells, resulting in production of micronuclei;
3. Multicentric mitotic figures, and polyploidy resulting in multinucleated and lobulated megaloblasts.

**Discussion**

Cytosine arabinoside is unique among available pyrimidine or purine antimetabolites in that both of its moieties—base and sugar—occur naturally: cytosine as a normal constituent of all living cells and arabinose as a sugar present in some foods. It is also one of the few nucleoside antimetabolites with abnormal type sugars tested in the chemotherapy of malignant disease. Although clinical results presented are insufficient to warrant any conclusions as to the value of this compound in treatment of human neoplasms, induction of tumor regression, even in a small number of patients, with a representative of a new class of chemicals suggests further clinical studies would be worthwhile.

Our interest in this compound was aroused, however, by our hematologic observations. The exact pathogenesis of megaloblast production in pernicious as well as in other megaloblastic anemias is not known. However, Vilter’s concept of deoxyribonucleic acid deficiency being responsible for the cytologic abnormalities seen in these anemias appears to be the best explanation avail-
Fig. 4—Peripheral blood changes in patient 472 hours after second dose of cytosine arabinoside. a) Hypertrimucleated myeloblasts; b) hypersegmented neutrophils and macrocytes; c) megakaryocytes; d) erythroblasts (×800).
CYTOSINE ARABINOSIDE-INDUCED MEGALOBLASTOSIS

Fig. 5.—Alterations in mitosis observed in bone marrow of patient 4 48 hours after cytosine arabinoside. a) Late prophase of basophilic megaloblast; b) trinucleated megaloblast and cell in metaphase with scattered chromosomes; c) anaphase with sticking chromosomes and tripolar distribution of chromosomes; d) late anaphase with lagging chromosomes; e) late metaphase with poor alignment of mitotic figure; f) metaphase with increased number of chromosomes which will result in polyploid nucleus.

able, even though his concepts are not accepted by all. Davidson found that the amount of deoxyribonucleic acid was increased in bone marrow of pernicious anemia by approximately 40 per cent (from $8.69 \times 10^{-7}$ to $12.57 \times 10^{-7}$ µg, per cell), but the content of ribonucleic acid was increased by almost 100 per cent (from $6.9 \times 10^{-7}$ µg, to $13.38 \times 10^{-7}$ µg, per cell). Similar changes were found by Lajtha and Kuniatori using autoradiographic techniques.6
Although DNA and RNA were found increased by the workers, the DNA/RNA ratio was decreased, probably indicating a relative DNA deficiency.

The increased number of polyploid or multinucleated cells found in megaloblastic bone marrows can account for the elevation of DNA content per cell. This is especially true if one accepts, as Reisner does, that one of the reasons of anemia in megaloblastic states is prolongation of the intermitotic phase.

The prolongation of DNA synthetic period (S period) and postsynthetic period (G2) when cells have tetraploid amount of DNA would increase apparent DNA concentration per cell even in the presence of relative DNA deficiency. Although usually intermitotic phase prolongation is mainly due to G1 prolongation, selective prolongation of the G2 period has been reported.

Thymine, thymidine, orotic acid, uracil and partially hydrolyzed DNA have been reported to completely or partially repair the megaloblastic defect in pernicious anemia. Spray and Witts were unable to induce remission in pernicious anemia with 250 mg. of thymidine, which was considerably less than the 15 Gm. of thymine used by Vilter et al. Other disturbances of nucleic acid metabolism were associated with megaloblastic anemia such as orotic aciduria and 6-MP intoxication. Unlike 5-fluorouracil or folic acid antagonist intoxications, there is no need to postulate selective disturbance in DNA metabolism in orotic aciduria or in 6-MP intoxication. While ribonucleic acid synthesis occurs during the entire intermitotic period, DNA synthesis is limited to the S period. Therefore, DNA deficiency should occur earlier than RNA deficiency when nucleic acid precursors or the coenzymes needed for this synthesis are limited in amounts.

Mitotic abnormalities observed in this study have been described in spontaneous megaloblastic anemias by several authors and have been produced in vitro not only with purine antimetabolites but also with unsubstituted purine, and in excessive concentration by adenine and adenosine. Erythroblasts with multiple and abnormally shaped nuclei occur even in normal individuals; however, they are not found as commonly as in megaloblastic bone marrows.

Cytosine arabinoside has been shown by Slechta to inhibit uridylic acid and cytidylic acid synthesis in E. coli. Chu and Fisher have shown that in murine lymphoblast cells (L-5178Y), the conversion of 1-β-D-ribofuranosylcytosine-5' phosphate to 1-β-D-2' deoxyribofuranosylcytosine-5' phosphate was inhibited by cytosine arabinoside with resulting inhibition of DNA synthesis. Because of rapid and regular development of megaloblastosis in our patients, it appears that in intact humans this disturbance in nucleic acid metabolism also occurs. It is worthy of speculation that arabinose, when linked with a pyrimidine or purine, because of its steric configuration (fig. 1) could interfere with intracellular cytidylic acid utilization for DNA synthesis.

**Summary**

1. Cytosine arabinoside induced objective, but temporary, decrease of tumor masses in three patients with lymphosarcoma and slight decrease in some lesions in two out of ten treated patients with disseminated carcinomatosis.

2. In doses of 3 to 50 mg./Kg. given at varying intervals, cytosine arabinoside...
induced definite megaloblastic changes in the marrow of all patients studied. Mitotic abnormalities similar to those found in other megaloblastic anemias also occurred.

3. Associated with bone marrow changes, depressions of hemoglobin, white blood cells and platelets in the peripheral blood were observed.

4. The exact mechanism of action of cytosine arabinoside has not been elucidated. It is speculated that because of the close structural similarity between cytidylate acid, cytosine arabinoside could interfere with DNA synthesis.

**REFERENCES**


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