A Clinical Evaluation of a Prolonged Schedule of Cytosine Arabinoside (NSC 63878) 1

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SUMMARY

The effect of repeated 10-day courses of s.c. administered arabinosyl cytosine was evaluated in 85 patients with metastatic cancer. This study attempted to exploit the presumed kinetic characteristics of slow-growing tumors and the pharmacological properties of a cell cycle-dependent drug. A dose of 0.4 mg/kg given every 8 hr for 30 doses consistently produced tolerable hematological toxicity without other untoward effects. Myelosuppression was less with the 2nd course. A biphasic white blood cell depression was noted.

Tumor regression was transient and occurred in less than 10% of the cases.

INTRODUCTION

The biological activity of ara-C2 (Cytosar) is determined by the amount of the nondeaminated intracellular phosphorylated synthetic nucleoside available to inhibit DNA polymerase during DNA synthesis (S phase) (5-7, 11, 13).

In human and animal leukemias, schedules resulting in high concentrations of drug maintained only briefly have demonstrated antitumor effect and a high therapeutic index (1, 3, 20). Similar regimens have not produced significant effects in solid tumors (4, 10). However, an S-phase-linked compound might exert a greater effect on tumors composed of a small proportion of proliferating cells if its concentration were maintained over a more prolonged period.

This study was devised to explore the effect of multiple frequent s.c. doses of ara-C on normal bone marrow and tumor cells.

MATERIALS AND METHODS

The initial dose-finding study was conducted at Johns Hopkins University School of Medicine in previously untreated patients with inoperable lung carcinoma. After a tolerable dose and schedule were determined, members of the Eastern Cooperative Oncology Group treated patients with a tissue diagnosis of metastatic carcinoma of the colon and breast and malignant melanoma. Although some of these patients had been treated previously, none had received myelosuppressive treatment during the preceding month, nor had they any renal, hepatic, or hematopoietic function abnormalities.

Studies obtained prior to treatment and 3 times each week during the study included hemocrit, white blood count, differential count, and reticulocyte count. Blood and serum determinations for urea nitrogen, alkaline phosphatase, serum transaminase (glutamic oxaloproparic and pyruvic), prothrombin time, total protein, uric acid, calcium, phosphorus, and bilirubin were completed weekly. Tumor measurements were also made weekly. ara-C was diluted with “water for injection” to 50 mg/ml and injected s.c. in the deltoid region.

The initial dose and schedule of ara-C was 0.4 mg/kg every 8 hr for 30 doses given s.c. (1.2 mg/kg daily for 10 days in divided doses). Due to severe platelet depression, reduction of this dose to 1.0 mg/kg daily for 10 days was required in patients who had received previous myelosuppressive treatment.

A course of therapy was defined as a period of 28 days. The regimen was repeated on Day 29, or with hematopoietic “recovery” if myelosuppression was of longer duration.

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The abbreviations used are: ara-C, arabinosylcytosine (1-β-D-arabinofuranosylcytosine) (Cytosar); BCNU, 1,3-bis-(2-chloroethyl)-1-nitrosourea.

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RESULTS

Antitumor Effect

The distribution of patients by diagnosis is presented in Table 1. Eighty-five patients with measurable disease who had been followed for at least 1 full 28-day course of therapy and observation qualified for antitumor and kinetic evaluation. Three patients with colon carcinoma had a decrease in the size of enlarged livers as measured by scan and physical examination. One patient with breast cancer had a decrease in the size of axillary nodes and symptomatic improvement. None of these responses were of significant duration.

Toxicity

Route of Administration. The site of the s.c. injection of ara-C was without local reaction. Occasional patients noted a “stinging” sensation at the time of administration, but classical signs of inflammation were absent.

Gastrointestinal. In most patients mild nausea and anorexia were encountered during the period of drug administration. However, effects on the gastrointestinal mucosa were not of sufficient magnitude to produce mucositis, ulceration, or diarrhea.

Liver. Major hepatic and/or renal toxicity was not manifested, although moderate and transient elevations of liver cellular function tests occurred in some patients.

Bone Marrow. Hematopoietic suppression was the limiting toxicity of ara-C. Thrombocytopenia was noted in all cases and was most marked in patients who had received previous chemotherapy. Subsequent to the reduction of dosage from 1.2 mg/kg/day to 1.0 mg/kg/day, platelet depression of less than 50,000/cu mm was rare. The nadir occurred on Day 14, followed by rapid recovery and rebound.

A biphasic pattern of white blood cell depression was noted (Chart 1). At both dose levels, the time of occurrence and duration were similar. However, the nadir of white count depression was less at the lower dose (mean, $1.95 \times 10^{-3}$ for the high dose and $2.9 \times 10^{-3}$ for the low dose; $p < 0.01$). In those patients who received 2 full

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Table 1

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. of cases which could be evaluated for tumor response</th>
<th>No. of courses of therapy</th>
<th>Tumor response</th>
<th>Extent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma of colon</td>
<td>37</td>
<td>15 22</td>
<td>3</td>
<td>&gt;50% decrease in liver size</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>17</td>
<td>11 6</td>
<td>1</td>
<td>&gt;50% decrease in axillary lymph nodes</td>
</tr>
<tr>
<td>Carcinoma of breast</td>
<td>14</td>
<td>6 8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>11</td>
<td>7 4</td>
<td>4</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>Carcinoma of urethra</td>
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<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma of pancreas</td>
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<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Carcinoid</td>
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<td></td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
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</tr>
<tr>
<td>Totals</td>
<td>85</td>
<td>41 44</td>
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</table>

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Chart 2. Mean white blood cell counts of patients who received 2 sequential courses of ara-C. Mean white blood cell count nadirs are plotted with 1 S.D.
courses of drug on schedule, the magnitude of white count depression with the 2nd course was significantly less than that which occurred with the 1st course ($p < 0.01$) (Chart 2).

Reticulocytopenia occurred in all patients. Counts fell rapidly, with complete absence of reticulocytes from the blood by Day 11. Return, with rebound, began on Day 16.

**DISCUSSION**

The *in vivo* biological activity of ara-C is determined by several factors. ara-C is rapidly deaminated, and the urinary excretion of pharmacologically inert arabinosyl uridine begins within minutes of injection. Plasma clearance half-times in man range from 30 to 60 min (8). During this brief period of availability, ara-C must diffuse across the cell membrane. The intracellular nucleoside is phosphorylated by deoxycytidine kinase (5, 6, 13), and, as ara-C triphosphate, inhibits DNA polymerase (11). Although a deaminating enzyme also exists within target cells, the cytotoxic effect of ara-C, once intracellular, is determined principally by the level of deoxycytidine kinase activity.

The antacellular effects of ara-C are also a function of cell kinetics. Previous studies have indicated that the major effect of ara-C on normal hematopoietic and leukemic cells is related to the proportion of cells presumed to be in the proliferating pool during drug administration (2, 4, 10). This is the relationship expected with a drug exerting its effects only during the period of DNA synthesis.

Tyrer *et al.* (19) have cured L1210 leukemia by treatment with the "cell-cycle-stage nonspecific" drug BCNU followed by ara-C. A sequential schedule of BCNU and ara-C has also been successful in the treatment of human acute leukemia (12). Theoretically, BCNU reduces the pool of dividing and nondividing cells and causes surviving cells in $G_0$ or prolonged $G_1$ to move into proliferative activity. Thus, during the period of DNA synthesis, they become susceptible to S-phase-active agents (15, 17). Similar results have been achieved in the solid tumor Ca 755 by Laster *et al.* (15), using cyclophosphamide and 6-mercaptopurine.

In man, the administration of ara-C by infusion for periods encompassing 2 cell generation times has induced remissions in acute leukemia (1, 3, 12). This result parallels the optimal dose schedule of ara-C as defined in the treatment of L1210 leukemia in mice (18). On the other hand, when ara-C was given in 2- or 5-day courses to patients with solid tumors, marrow suppression was encountered regularly and useful antitumor effects were encountered infrequently (4, 10).

In this study, the dose schedule used was designed to produce frequent peak concentrations of drug in the blood over a 10-day period. The s.c. route was chosen to facilitate administration and to produce high concentrations of drug, in contrast to the lower blood levels resulting from continuous infusion. The dose level was limited by tolerable bone marrow suppression. It was hoped that this technique would enhance the uptake of ara-C by mitotically active tumor cells over a moderately prolonged period. If, as the result of this treatment, these slow-growing tumors had a greater growth fraction 1 month later, the second 10-day course of ara-C would be expected to produce a more profound antitumor effect (14). In contrast, the more rapidly regenerating marrow might be expected to return to its basal proliferative state and show no enhanced response to the 2nd course of ara-C.

The 1st course of ara-C was followed by a depression of all formed elements in the peripheral blood (Chart 1). The kinetics of the fall and recovery of the reticulocyte and platelet counts and the 2nd white blood cell response are consistent with current knowledge of their morphogenesis and maturation (9, 16). The biphasic response of the white blood cell count primarily reflects changes in the circulating granulocytes and has been described previously (4). Assuming a homogeneous population of granulocytes and precursors, it is difficult to attribute the initial phase of this double response solely to a mitotically linked drug effect. Chu and Fischer (5) previously have suggested that ara-C also exerts a direct unexplained cytotoxic effect on cells irrespective of their proliferative status.

In those patients who received a 2nd course of therapy, the white count depression was less than after the 1st course ($p < 0.01$) (Chart 2). The 2nd course of therapy was begun at the time of peripheral hematopoietic recovery, just prior to the overshoot. At this time myelopoiesis would be suppressed, allowing cells protection from an S-active drug. However, this effect may not relate to a decreased growth fraction alone. Alternate explanations include increased deamination of ara-C, decreased marrow cell uptake of drug, a lesser level of kinase activity, or other unidentified factors. No effects on the gastrointestinal mucosa were noted.

The paucity of significant antitumor activity was disappointing. The deterrent to greater aggressiveness with this regimen was marrow toxicity. Alternate approaches utilizing newer knowledge of the pharmacology of ara-C and tumor cell kinetics will be required to increase the clinical utility of this agent in the treatment of solid tumors.

**REFERENCES**
