Differential Effect of N-(Phosphonacetyl)-L-aspartate on 1-ß-D-Arabinofuranosycytosine Metabolism and Cytotoxicity in Human Leukemia and Normal Bone Marrow Progenitors

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ABSTRACT

The effect of N-[phosphonacetyl]-L-aspartate (PALA) on the metabolism and cytotoxicity of 1-ß-D-arabinofuranosycytosine (ara-C) was studied in the human promyelocytic leukemic cell line, HL-60, and in normal human bone marrow. HL-60 cells exposed to 0.1 mM PALA for 12 hr accumulated 58.7 pmol ara-C per 10^6 cells after a 45-min exposure to 1 μM ara-C, compared to 27.8 pmol ara-C per 10^6 cells in untreated control cells. This PALA concentration and exposure interval was associated with a greater than 2-fold increase in both the 45-min generation and 4-hr retention of 1-ß-D-arabinofuranosycytosine 5’-triphosphate compared to untreated control HL-60 cells. Exposure of HL-60 cells to PALA followed by ara-C produced greater than additive effects on the inhibition of DNA synthesis, the inhibition of cell growth, and clonogenicity. In contrast, exposure of normal human bone marrow to the same PALA-ara-C schedule was not associated with a synergistic inhibition of colony-forming units in soft agar. If these perturbations also occur in vivo, an improvement in the therapeutic index of ara-C in patients with acute leukemia might result.

INTRODUCTION

ARA-C is a deoxycytidine analog which is effective for the treatment of human acute myelogenous leukemia (5, 15). The mechanism of action of ara-C is controversial. ara-CTP is considered the lethal nucleotide form of ara-C, exerting its antitumor effect through inhibition of DNA polymerase (16, 18). However, the degree of ara-C incorporation into DNA has also been associated with cytotoxicity (35). Resistance of tumor cells to ara-C, both in vivo (53) and in vitro (49), has been associated with decreased levels of deoxycytidine kinase, the enzyme catalyzing the conversion of ara-C to its nucleotide form, 1-ß-D-arabinofuranosycytosine 5’-monophosphate (40). Since this enzyme is under regulatory feedback control by dCTP (26), biochemical perturbations resulting in reductions in intracellular levels of this metabolite might be expected to enhance the phosphorylation and activation of ara-C. Pretreatment of murine tumor cells with inhibitors of de novo pyrimidine synthesis have resulted in an augmentation of ara-C metabolism (44). For example, dThd, which after conversion to its triphosphate derivative, dTTP, serves as an inhibitor of ribonucleotide reductase (3), is capable of increasing ara-C sensitivity in vitro (22, 31) and in vivo (12). The degree of augmentation of ara-C metabolism and cytotoxicity following dThd treatment has been correlated with reductions in dCTP pool sizes (20). Similarly, 3-deazauridine, an inhibitor of CTP synthetase (38), produces reductions in intracellular dCTP levels (17) and enhanced ara-C activation (39). The study of these antimitabolite interactions forms the scientific foundation of ongoing clinical trials in humans involving the administration of dThd (4) and 3-deazauridine (1) in conjunction with ara-C. While the influence of dCTP pool size on deoxycytidine kinase activity is important, other nucleotides can also alter the activity of this enzyme (34). In addition, other possible sites of ara-C metabolic regulation might be important in some cell lines (14).

PALA is a transition state analog inhibitor of L-aspartate transcarbamylase, the enzyme catalyzing the second step in the de novo pyrimidine-synthetic pathway (24, 51, 56), with limited antitumor activity (28, 29, 54). PALA treatment will result in a reduction of the intracellular levels of UTP, CTP (42), and their respective deoxyribonucleotides (41). Resistance of tumor cells to PALA has generally been associated with elevated levels of aspartate transcarbamylase (27), although increased activity of carbamyl phosphate synthetase II or salvage metabolic regulation might be important in some cell lines (14). The purpose of the present studies was to determine whether PALA pretreatment, through pyrimidine-biosynthetic blockade and exhaustion of intracellular deoxyribonucleotide pools, could enhance the metabolism and cytotoxicity of subsequently administered ara-C in the human leukemic cells, HL-60 (17), which closely approximate those of human leukemic myeloblasts in vivo (32). Since an improvement in the therapeutic index of ara-C will depend upon differential inhibitory effects on clonogenicity of normal versus leukemic stem cells, the effect of prior PALA exposure on ara-C sensitivity was also determined for normal bone marrow myeloid progenitors (CFU-
In general, single agents have exhibited similar patterns of cytotoxicity with respect to normal and leukemic cells. If normal bone marrow progenitors are relatively resistant to the effects of PALA, as has been suggested by clinical studies, then this agent might be capable of selectively enhancing ara-C metabolism and cytotoxicity in leukemic precursors. The interaction between ara-C metabolism and inhibitors of de novo pyrimidine-biosynthetic pathways is illustrated in Chart 1.

**MATERIALS AND METHODS**

**Cells**

Suspension cultures of HL-60 cells were maintained in RPMI 1640 supplemented with 1% sodium pyruvate, 1% nonessential amino acids, and 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) at 37° and 5% CO2. Cells were passaged twice weekly and routinely screened for Mycoplasma contamination. All experiments were performed utilizing cells in mid-logarithmic growth (concentration, 2 to 4 × 105 cells/ml).

**Drugs and Chemicals**

PALA (NSC-224131) was provided by Dr. Robert Engle, Developmental Therapeutics Program, National Cancer Institute, Bethesda, Md. It was stored as a dry powder at 0°, and reconstituted in sterile 0.9% NaCl solution prior to each use. ara-C and deoxycytidine were purchased from Sigma Chemical Co., St. Louis, Mo. [5-3H]ara-C (26 Ci/mmol), [6-3H]dThd (22 Ci/mmol), and [5-3H]deoxycytidine (20 Ci/mmol) were purchased from Amersham Radiochemicals, Arlington Heights, Ill.

**Intracellular Drug Accumulation Studies**

HL-60 cells were placed in 25-cm3 sterile tissue culture flasks (Corning Glass Works, Corning, N. Y.) and were exposed to concentrations of PALA ranging from 0.01 to 10.0 mm. The flasks were then placed in a 37°, 5% CO2 humidified incubator. At the end of the incubation period, the cells were transferred to sterile, disposable 15-ml centrifuge tubes, and spun at 1200 rpm for 8 min, the drug-containing medium was discarded, and the cell pellet was washed twice with fresh medium. The cells were then resuspended in 2 ml of fresh RPMI 1640 with 20 μl of 1% HEPES buffer and placed in a metabolic shaking water bath at 37°. To each flask was added [3H]ara-C at a final concentration of 1 μM, with a final specific activity of 0.5 Ci/μmol. At the end of a 45-min incubation period, 200-μl samples were taken and the intracellular accumulation of drug was determined by a method which was modified and described previously (15). Briefly, 200 μl of the cell suspension were placed in a microfuge tube containing 40 μl of HClO4, underneath 100 μl of silicon: mineral oil (84:16). The tubes were then centrifuged at 10,000 × g in a Beckman Microfuge (Somerset, N. J.) for 30 sec. Under these conditions, 99.99% of the free radiolabeled drug remained in the top layer, and 99.25% of the cells entered the bottom layer where they were lysed by the HClO4. The tubes were frozen, sectioned into separate compartments, and placed in scintillation vials containing 10 ml of aqueous scintillation fluid (National Diagnostics, Somerville, N. J.), and the radioactivity was determined, using a Uniflux II scintillation counter (Nuclear Chicago, Chicago, Ill.). Intracellularly incorporated drug was expressed as pmol/106 cells and increased linearly over 45 min.

**DNA Synthesis Inhibition**

After exposure of cells to PALA, cells were washed free of drug and resuspended in 2 ml of fresh medium containing 20 μl of 1% HEPES buffer. The reaction flasks were placed in the shaking metabolic water bath at 37°, and the cells were exposed to the appropriate concentration of ara-C in the presence of 5 μCi of [3H]dThd (22 Ci/mmol). At the end of a 45-min incubation period, 750 μl of the cell suspension were filtered through glass fiber filter paper (Whatman, Inc., Clifton, N. J.), utilizing a 12-channel filtration manifold. The cells were lysed with cold 10% HClO4, and washed 3 times with cold distilled water alternating with cold 5% HClO4. The filters were then air dried and placed in scintillation vials containing 10 ml of nonaqueous scintillation fluid (Betafluor, National Diagnostics), and the radioactivity was determined. Inhibition of DNA synthesis, reflected by a decrease in [3H]dThd incorporated into the acid-precipitable HL-60 cell fractions relative to control cells, is expressed as cpm/106 cells. ara-C-induced inhibition of DNA synthesis is compared for control and PALA-treated cells.

**ara-CTP Generation and Retention**

The generation of ara-CTP in HL-60 cells following PALA pretreatment was determined as we have described previously (20). After PALA exposure, cells were washed twice with fresh medium, resuspended in 2 ml of RPMI 1640 containing 10% fetal calf serum and 20 μl 1% HEPES buffer, and placed in a 37° water bath. After a 45-min exposure to 1 μM [3H]ara-C, the cells were centrifuged at 1200 rpm for 8 min at 4°. The cell pellet was washed twice in cold RPMI 1640 and precipitated with 0.75 ml of cold 0.5 M HClO4. After centrifugation at 1200 rpm for 8 min at 4°, the acid-soluble extract, containing the ribonucleotides, was neutralized with cold 4 N KOH. After repeated centrifugation and removal of the precipitated KClO4, the acid-soluble extracts were stored at −20° until all samples could be analyzed at the same time. Separations were performed on an Altex Model 332 high-pressure liquid chromatographic system (Altex Instruments, Berkeley, Calif.), utilizing a Partisil-10SAX anion exchange resin (4.6 mm x 0.25 cm; Whatman, Inc.) and an isocratic 0.4 M Na2HPO4 buffer (pH 3.3) at a flow rate of 1.5 ml/min. Absorbances were recorded at 254 and 280 nm, and 30-sec fractions were collected. The amount of ara-CTP was determined by quantitating the amount of radioactivity coeluting with the nonradiolabeled ara-CTP standard. Significant quantities of 1-β-D-arabinofuranosylcytosine 5′-triphosphate were not noted under conditions of this experiment.

For determination of ara-CTP retention, cells exposed sequentially...
to PALA and [3H]ara-C were washed free of all drugs after the 45-min treatment of [3H]ara-C. The drug-free cell suspension was kept in the water bath for 4 hr more before determining the ara-CTP levels.

Cloning Studies

**HL-60 Cells.** The effect of PALA on ara-C-induced inhibition of clonogenicity of HL-60 cells in semisorb agar was determined, utilizing a modification of the technique of Galleghe et al. (17). Cells exposed to PALA were washed twice, resuspended in fresh medium, and plated at a concentration of 2 x 10^3 cells/plate in 35-mm sq Petri dishes. Each plate contained a bottom layer made up of 1 ml of RPMI 1640 with 15% fetal calf serum and 0.5% Bacto agar (Difco, Detroit, Mich.). The top layer, containing the cells, consisted of 1 ml of RPMI 1640 with 15% fetal calf serum, 0.3% Bacto agar, and the indicated concentration of ara-C. After the agar solidified, 0.1 ml of GCT-conditioned medium (Grand Island Biological Co.) was added to each plate as a source of colony-stimulating factor (13). The plates were then placed in the 5% CO2 humidified incubator at 37°C. After 10 to 12 days, colonies consisting of groups of 40 or more cells were scored with the use of an Olympus Model CK inverted microscope (Olympus Corporation of America, New Hyde Park, N. Y.). Each condition was performed in quadruplicate and each experiment was repeated at least three times. Under these conditions, untreated HL-60 cells form colonies with a cloning efficiency of 10 to 15%.

**Normal Human Marrow Cells.** The effect of sequential antimetabolite therapy on normal bone marrow myeloid progenitors (CFU-GM) was determined, utilizing a culturing technique based on a minor modification (13) of the method of Broxmeyer (8). Bone marrow aspirates were obtained, after obtaining informed consent, from the posterior iliac crests of patients without hematological cancers undergoing routine diagnostic studies. The aspirates were placed in sterile tissue culture tubes containing 500 IU of preservative-free heparin and passed through 25-gauge needles to disperse clumps. The bone marrow sample was diluted 1:3 with McCoy's Medium 5A and layered over lymphocyte separation medium (Litton Bionetics, Waltham, Mass.) and the radioactivity incorporated into newly synthesized DNA was determined.

**RESULTS**

**Intracellular Accumulation of [3H]ara-C**

The effect of various PALA concentrations and exposure intervals on the intracellular accumulation of ara-C in HL-60 cells is represented in Table 1. A 12-hr exposure to 0.1 mM PALA was associated with the intracellular accumulation of 58.7 pmol ara-C per 10^6 cells, compared to 27.8 pmol ara-C per 10^6 cells in untreated control cells. Lower PALA concentrations were obtained, after obtaining informed consent, from the posterior iliac crests of patients without hematological cancers undergoing routine diagnostic studies. The aspirates were placed in sterile tissue culture tubes containing 500 IU of preservative-free heparin and passed through 25-gauge needles to disperse clumps. The bone marrow sample was diluted 1:3 with McCoy's Medium 5A and layered over lymphocyte separation medium (Litton Bionetics, Kensington, Md.) with a density of 1.077 to 1.080. The cells were centrifuged at 400 x g for 40 min according to the method of Boyum (6). The mononuclear cell layer was extracted with a sterile Pasteur pipet and washed twice with McCoy's Medium 5A. The cells were then resuspended in McCoy's Medium 5A supplemented with additional essential and nonessential amino acids, glutamine, serine, asparagine, and sodium pyruvate, plus 10% fetal calf serum at a concentration of approximately 5 x 10^6 cells/ml. The cells were then pipetted into sterile tissue culture flasks, exposed to PALA at varying concentrations, and placed in a 37°C, 5% CO2 incubator for periods up to 24 hr. At the end of the incubation period, the cells were centrifuged for 8 min at 1200 rpm, washed twice with McCoy's Medium 5A and resuspended. Trypan blue hematocytometer counts were obtained to determine the number of viable cells. The cells were then adjusted to a concentration of 2 x 10^6 cells/ml in McCoy's Medium 5A supplemented with 15% heat-inactivated fetal calf serum and 0.3% Bacto agar. One ml aliquots of this mixture, containing the appropriate concentration of ara-C, were layered over a 1-ml bottom layer consisting of McCoy's Medium 5A with 15% fetal calf serum and 0.5% agar in a 35-mm sq Petri dish. After the agar hardened, 0.1 ml of GCT-conditioned media was pipetted onto each plate. The plates were then placed in a 37°C, fully humidified 5% CO2 incubator. At the end of 10 days, the number of colonies, consisting of groups of 40 or more cells, was determined. Each condition was performed in quadruplicate for each experiment. The ability of a prior exposure of PALA to alter the ara-C sensitivity of normal bone marrow progenitor cells was compared to its effect on ara-C sensitivity of the human leukemic HL-60 cells. The low continuous concentration of ara-C (10 nM) was chosen to mimic the situation which would commonly result from the standard clinical use of ara-C at 100 mg/sq m every 8 to 12 hr. Higher concentrations of ara-C for shorter periods following PALA pretreatment produced similar results.

### **Table 1**

<table>
<thead>
<tr>
<th>PALA (mM)</th>
<th>Exposure (hr)</th>
<th>Intracellular ara-C (pmol/10^6 cells)</th>
</tr>
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<tr>
<td>0</td>
<td>0</td>
<td>27.8 ± 4.3*</td>
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<td>0.05</td>
<td>6</td>
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<td>24</td>
<td>30.4 ± 3.8</td>
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<tr>
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<td>6</td>
<td>36.9 ± 5.2</td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
<td>58.7 ± 5.4</td>
</tr>
<tr>
<td>0.1</td>
<td>24</td>
<td>48.7 ± 4.2</td>
</tr>
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<td>6</td>
<td>32.7 ± 3.8</td>
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<td>1.0</td>
<td>12</td>
<td>42.1 ± 5.3</td>
</tr>
<tr>
<td>1.0</td>
<td>24</td>
<td>34.2 ± 4.2</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
trations (0.05 mM) and shorter exposure intervals (6 hr) resulted in small increments in ara-C accumulation. Longer exposure intervals (24 hr) and higher PALA concentration (0.2 and 1.0 mM) also resulted in increments in intracellular ara-C accumulation which were generally less than those associated with a 12-hr exposure to 0.1 mM PALA.

**DNA Inhibition**

The effects of PALA on ara-C-induced DNA inhibition is shown in Chart 2. Exposure of HL-60 cells to 0.1 mM PALA for 12 hr and 50 nM ara-C for 45 min produced 8% and 24% decrements in the acid-precipitable incorporation of \(^{3}\text{H}\)dTd, respectively. Sequential exposure of cells to 0.1 mM PALA for 12 hr followed by 50 nM ara-C for 45 min resulted in an 88% inhibition of DNA synthesis. Because of the sharp dose-response curve of ara-C-induced DNA inhibition, the effects of PALA in conjunction with higher (≥100 nM) or lower (<25 nM) concentrations of ara-C were only additive (not shown).

**ara-CTP Generation and Retention**

The effect of PALA on the generation and retention of ara-CTP is illustrated in Table 2. Cells exposed to 0.1 mM PALA for 12 hr accumulated 36.9 ± 2.4 pmol ara-CTP per 10⁶ cells, compared to 16.4 ± 1.8 pmol ara-CTP per 10⁶ cells for untreated control cells. The corresponding 4-hr retentions of ara-CTP were 11.5 ± 1.6 and 4.6 ± 0.8 pmol/10⁶ cells, respectively. As with ara-C intracellular accumulation, lower (0.5 mM) and higher (1 mM) PALA concentration, as well as longer incubation intervals (24 hr), produced smaller increments in ara-CTP formation and retention.

**Cloning Studies**

**HL-60 Cells.** The effects of prior exposure to PALA on the soft-agar clonogenicity of HL-60 cells in the continuous presence of ara-C is shown in Table 3. A 12-hr exposure to 0.1 mM PALA resulted in an 8% reduction in colony relative to untreated control cells. The continuous presence of 10 nM ara-C was associated with a 24% reduction in cloning efficiency. The sequential exposure of HL-60 to 0.1 mM PALA for 12 hr, followed by plating in the presence of 10 nM ara-C, resulted in an 86% reduction in colony formation. Prior exposure to PALA in conjunction with higher concentrations of ara-C (e.g., 20 nM) produced a greater inhibition of cloning efficiency, but the degree of drug synergism was reduced (not shown).

**Human Bone Marrow Cells.** Table 4 illustrates the effect of pretreatment with PALA on ara-C-induced inhibition of colony formation of normal granulocyte-monocyte bone marrow progenitor cells (CFU-GM) in soft agar. In 8 separate experiments, exposure of cells to 0.1 mM PALA for 12 hr resulted in 73.1 ± 17.4% of control colony formation, whereas a continuous exposure to 10 nM ara-C resulted in 47.9 ± 12.9% of control cells. In cells exposed to 0.1 mM PALA for 12 hr, followed by continuous exposure to 10 nM ara-C, colony formation was reduced to 35.5 ± 14.2%. This sequence was only additive.
for inhibition of bone marrow colony formation (73.1 × 47.9 = 35.0). These results contrast sharply to those obtained in HL-60 cells, where similar PALA and ara-C doses and exposure intervals resulted in greater than additive inhibitory effects. A 24-hr exposure of normal bone marrow cells to 0.1 mM PALA, followed by continuous exposure to 10 mM ara-C, also resulted in only additive effects on colony formation. In other experiments utilizing higher concentrations of ara-C (e.g., ≥20 mM), PALA either had no or little effect on further reduction in cloning efficiency of normal bone marrow progenitors. However, the substantial inhibition of clonogenicity associated with these higher ara-C concentrations (≥96%) makes interpretation of these results difficult.

Deoxyrribonucleotide Levels

Alterations in dNTPs in HL-60 cells following PALA exposure are illustrated in Table 5. A 12- or 24-hr exposure to PALA concentrations of 0.5 mM or higher reduced dCTP pools below or to barely detectable levels (less than 0.5 pmol/10⁶ cells). A 0.1 mM PALA concentration also reduced intracellular levels of dCTP and dTTP but not to the extent of the higher PALA concentrations. Shorter PALA exposure intervals (e.g., 6 hr) were associated with small decreases in intracellular dCTP levels (not shown).

DISCUSSION

Efforts to improve the efficacy of nucleoside analogs such as ara-C by combining them with inhibitors of de novo pyrimidine synthesis have a rational biochemical basis. Early studies by Potter (46) have shown that combinations of agents which block separate steps in a linear metabolic pathway are likely to produce synergistic tumoricidal effects. If, in addition, one such agent were capable of enhancing the metabolism of another, further antitumor activity might be expected to occur. Previous work has suggested that diverse agents, such as dThd (12, 20, 31, 44), hydroxyurea (44, 55), 3-deazauridine (7, 44), pyrazofurin (44), and methotrexate (10), are capable of enhancing ara-C metabolism in murine tumor cells, in some cases resulting in synergistic antitumor effects. The common mechanism underlying these interactions is believed to be the depletion of intracellular levels of dCTP, the naturally occurring nucleotide which antagonizes the activation of ara-C by inhibiting deoxycytidine kinase (26). Based on considerations such as these, clinical trials involving the sequential administration of 3-deazauridine or dThd in conjunction with ara-C have been designed for patients with refractory acute nonlymphocytic leukemia (1, 4).

PALA, a transition state inhibitor of aspartate transcarbamylase, is similarly capable of enhancing ara-C metabolism and cytotoxicity in a human promyelocytic cell line. In contrast to agents such as dThd, hydroxyurea, or dihydro-IH-pyrazolo imidazole, which are inhibitors of ribonucleotide reductase, PALA inhibits at a considerably more proximal step in de novo pyrimidine synthesis. Exposure of HL-60 cells to 0.1 mM PALA for 12 or 24 hr produced a decrease in intracellular dCTP levels, a result which is comparable to that seen in L1210 cells following dThd exposure (12, 20, 31). This decrease was associated with an increase in the total intracellular accumulation of ara-C, as well as the generation and retention of the lethal ara-C metabolite, ara-CTP. The latter perturbation may be of particular significance, since recent clinical studies in patients with acute myelogenous leukemia have shown that leukemic cell retention of ara-CTP may correlate closely with response to this agent (48). Another possible determinant of ara-C cytotoxicity is its incorporation into nucleic acids, specifically DNA, with resultant inhibition of chain elongation or the formation of abnormal DNA (35). It is likely that PALA-induced enhancement of ara-C metabolism would be associated with an increase in DNA incorporation of the analog, and this might in part account for the increased cytotoxicity of these agents when administered in sequence. PALA-induced perturbations in ara-C metabolism are reflected biologically by enhanced inhibition of DNA synthesis and clonogenicity in soft agar. These effects are most pronounced at low (e.g., ≤50 mM) ara-C concentrations and may represent enhancement of ara-C metabolism within a kinetically susceptible subgroup of cells. Resistance of leukemic cells to ara-C may exist on either a biochemical (reduced formation of ara-C metabolites) or on a kinetic basis (decreased percentage of G-S-phase cells) (47). Increased ara-C activation following PALA administration may in part overcome resistance secondary to biochemical factors but would not be expected to increase susceptibility in the kinetically resistant subgroup of cells.

The time course of optimal drug scheduling in HL-60 cells differs from that observed in the rapidly dividing murine leukemia L1210. Whereas a short (e.g., 3 to 6 hr) antimetabolite incubation period may produce maximal biochemical alterations in the murine line (20), a considerably longer exposure interval (e.g., 12 to 24 hr) may be required in human leukemic cells, whose doubling time is approximately 36 hr (32). The depletion of intracellular dCTP may depend upon multiple factors, including degree of inhibition of de novo synthetic pathways, availability of salvage pyrimidines, activity of catalytic enzymes such as deoxycytidine deaminase, and rate of utilization of nucleotides in DNA synthesis (37). In slowly replicating human leukemia cells such as HL-60, longer PALA exposure intervals may be required to allow sufficient numbers of cells to pass through the DNA-synthetic phase, resulting in depletion of available dCTP. These scheduling considerations may have complications for the design of in vivo trials involving antimetabolites such as PALA.

The ability of one antimetabolite to enhance the metabolism

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Table 5

Effect of PALA treatment on HL-60 deoxynucleoside triphosphate pools

<table>
<thead>
<tr>
<th>Exposure (hr)</th>
<th>PALA (mM)</th>
<th>Nucleotide</th>
<th>(pmol/10⁶ cells)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>dTTP</td>
<td>dCTP</td>
</tr>
<tr>
<td>0</td>
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<td>22 ± 6</td>
<td>15 ± 6</td>
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<tr>
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<tr>
<td>1.0</td>
<td>1.5</td>
<td>24 ± 10</td>
<td>UD ± 0</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
* UD, undetectable.
REFERENCES


17. Grant, S., Ellison, R. R., and Cadman, E. PALA induced enhancement of ara-C in a human promyelocytic cell relative to normal bone marrow precursors. In early clinical trials, PALA has demonstrated little myelosuppression (23). It is possible that elevated levels of aspartate transcarbamylase in myeloid progenitors may protect the cells from the action of PALA, although the inability to obtain sufficient numbers of bone marrow stem cells to perform enzymatic assays makes it appear difficult to confirm. Whether these in vitro results will produce improved selectivity of ara-C in vivo will depend on multiple factors, including the ability to achieve adequate and sustained plasma levels of PALA, the development of leukemic cells resistant to PALA, the response of normal bone marrow progenitors to PALA in an in vivo setting, and cytotoxic considerations, which may in some instances antagonize biochemical effects. Nevertheless, selective enhancement of ara-C by PALA in leukemic cells would appear to be a theoretical possibility, and in vivo trials to determine optimal schedules of these agents in sequence appear warranted.

18. Graham, S. Grant et al. (11). Cadman, E., Heimer, R., and Benz, C. The influence of methotrexate pre-
Effect of PALA on ara-C Metabolism

Differential Effect of \( N \)-(Phosphonacetyl)-l-aspartate on 1-\( \beta \)-d-Arabinofuranosylcytosine Metabolism and Cytotoxicity in Human Leukemia and Normal Bone Marrow Progenitors

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