Effect of Uridine on Response of 5-Azacytidine-resistant Human Leukemic Cells to Inhibitors of de Novo Pyrimidine Synthesis

S. Grant, K. Bhalla, and M. Gleyzer

Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York 10032

ABSTRACT

A uridine-cytidine kinase-deficient, human promyelocytic leukemia subline (HL-60-5-aza-Cyd) has been isolated which is highly resistant to the antileukemic agent 5-azacytidine. Resistant cells exposed to $10^{-8}$ M 5-azacytidine for 2 hr exhibit a marked reduction in both the total intracellular accumulation of 5-azacytidine (11.9 versus 156.0 pmol/10$^6$ cells) as well as its incorporation into RNA (3.1 versus 43.4 pmol/10$^6$ cells) compared to the parent line. These biochemical changes are associated with nearly a 100-fold decrease in sensitivity to the growth inhibition of 5-azacytidine (concentration of drug associated with a 50% reduction in cell growth, $3.5 \times 10^{-5}$ versus $5.0 \times 10^{-7}$ M). 5-Azacytidine-resistant cells exhibit cross-resistance to 3-deazaadenine, 6-azauridine, and 5-fluorouridine, but not to 1-deoxy-2-fluoro-5-azacytidine, 2′-deoxyazacytidine, or 5-aza-1-deoxy-2-fluoro-5-azacytidine. Coadministration of 50 μM uridine prevented depletions of pyrimidine nucleoside triphosphates and inhibition of colony formation of HL-60 cells exposed to 3 mM N-(phosphonacetyl)-L-aspartate or $5 \times 10^{-8}$ M pyrazofurin but was not capable of protecting HL-60-5-azacytidine under the same conditions. This uridine concentration was also able to restore control colony formation to normal human bone marrow progenitor cells (granulocyte-macrophage colony-forming units) exposed to de novo pyrimidine biosynthetic blockade. These in vitro studies suggest that 5-azacytidine-resistant cells lacking the pyrimidine salvage pathway enzyme uridine-cytidine kinase may be selectively vulnerable to a regimen using pyrimidine antagonists administered in conjunction with the naturally occurring nucleoside uridine.

INTRODUCTION

The nucleoside analogue 5-aza-Cyd (NSC 102816) is an effective agent in the treatment of acute myelogenous leukemia in humans (29). After conversion to its nucleoside monophosphate derivative by the pyrimidine salvage pathway enzyme uridine-cytidine kinase (21), 5-aza-Cyd is ultimately metabolized to its lethal derivative 5-aza-Cyd-CTP, which is incorporated into leukemic cell RNA and to a lesser extent DNA (17). The bulk of evidence suggests that 5-aza-Cyd-mediated cytotoxicity results from interference with RNA production and processing and inhibition of protein synthesis (20). Although no information is available concerning the mechanism by which leukemic patients become refractory to this agent, loss of uridine-cytidine kinase activity is the most commonly encountered mode of resistance in animal systems (28).

We have recently isolated a uridine-cytidine kinase-deficient, highly 5-aza-Cyd-resistant human promyelocytic leukemia subline (HL-60-5-aza-Cyd) (8) which is capable of surviving 5-aza-Cyd concentrations ($10^{-4}$ M) that exceed peak plasma levels in humans (27). The purpose of the present studies was to assess the metabolism of 5-aza-Cyd in these resistant cells and to examine their response to a variety of clinically available inhibitors of de novo pyrimidine synthesis. Of the latter agents, PALA, an inhibitor of aspartate transcarbamylase (26), and pyrazofurin, an inhibitor of orotidylate decarboxylase (5), are of particular interest, since their in vitro as well as in vivo effects have been shown to be reversible by coadministration of exogenous uridine (4, 16). This raises the theoretical possibility that drug-resistant cells impaired in their capacity to metabolize a naturally occurring nucleoside might be particularly vulnerable to a regimen using such a nucleoside in conjunction with an appropriate pyrimidine antagonist. Finally, examination of the effect of uridine on the response of normal and leukemic myeloid cells to inhibitors of de novo pyrimidine biosynthesis might shed light on the role that salvage pathway enzymes play in overcoming the effects of de novo pyrimidine biosynthetic blockade.

MATERIALS AND METHODS

HL-60 cells are derived from the line originally isolated by Collins and Gallo (7) and are maintained in RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) supplemeneted with 10% heat-inactivated fetal calf serum (Grand Island Biological Co.). Cells are subcultured twice weekly and examined routinely for Mycoplasma contamination. HL-60-5-aza-Cyd were obtained by subcultureing HL-60 cells in progressively higher concentrations of 5-aza-Cyd until a 37°, 5% CO$_2$ incubator. Initially, 200 ml of HL-60 cells (approximate cell density, $10^9$ cells/ml) were exposed to $5 \times 10^{-8}$ M 5-aza-Cyd for 4 days in a 37°, 5% CO$_2$ incubator. The cells were then washed free of medium and resuspended in 200 ml of fresh RPMI medium containing $5 \times 10^{-8}$ M 5-aza-Cyd. After an additional 4-day incubation, the cell suspension was layered over a cushion of 3 ml lymphocyte separation medium (specific gravity, 1.077 to 1.081; Bionetics, Kensington, MD) in sterile 50-ml centrifuge tubes. The tubes were centrifuged at 400 x g for 30 min. Viable cells at the interface layer were extracted with a sterile Pasteur pipet and resuspended in fresh RPMI medium containing 10% fetal calf serum at an approximate concentration of 2 x 10$^8$ cells/ml. The cells were plated in sterile tissue culture flasks (Corning Glass Works, Corning, NY), to which was added sufficient 5-aza-Cyd to yield a final concentration of $10^{-7}$ M. The flasks were placed in the incubator, and the medium (containing fresh 5-aza-Cyd) was changed twice weekly. At approximately 2- to 3-week intervals, the 5-aza-Cyd concentration was doubled and maintained at that level until cells reached a growth rate nearly equivalent to that of the parent line. After 6 months of subculturing, HL-60-5-aza are currently being maintained in $10^{-4}$ M 5-aza-Cyd, which is added to the medium twice weekly. Cells maintained separately in drug-free medium for 3 months retained 75% of their resistance to 5-aza-Cyd. More than 90% of the cells in...
suspension culture are viable as determined by trypan blue exclusion. Drugs and Chemicals. PALA and 2,3-dihydro-1H-imidazo-[1,2-b]pyrazole were furnished by Dr. Robert Engle, Drug Development Branch, National Cancer Institute (Bethesda, MD). 5-Aza-1-β-o-arabinofuranosylcytosine was provided by Dr. John Douros, Drug Development Branch, National Cancer Institute. Acivicin (AT-125) was provided by the Upjohn Co., Kalamazoo, MI. Pyrazofurin was purchased from Calbiochem, La Jolla, CA. Deoxyazacytidine, 5-aza-Cyd, 1-β-o-arabinofuranosylcytosine, hydroxyurea, thymidine, 3-deazauridine, 6-aza-cytidine, 5-fluorouridine, uridine, and cytidine were purchased from Sigma Chemical Co., St. Louis, MO. All agents were stored as dry powders at -20°C. Prior to use, drugs were reconstituted in RPMI medium and filtered sterilized utilizing 0.1 μm microfilters (Millipore Corp., Bedford, MA).

Values for each cell type are expressed as cpm [3H]leucine per 10^6 cells. Details of this procedure have been described in detail elsewhere (9).

Values for each condition were expressed as a percentage of untreated control.

Intracellular Drug Accumulation Studies. The intracellular accumulation of [3H]uridine, [3H]cytidine, and [3H]5-aza-Cyd was determined in HL-60-5-aza-Cyd cells by a rapid centrifugation technique (10). Logarithmically growing cells were exposed to nucleoside or nucleoside analogues at concentrations ranging from 0.1 to 50 μM for intervals of up to 2 hr. At the end of the incubation period, 200-μl aliquots of the reaction mixture were pipetted into 400-μl microfuge tubes containing 40 μl of 0.5 M HClO4 and 100 μl of an 84:16 silicon oil: mineral oil overlay. The tubes were centrifuged at 10,000 x g for 30 sec, and the total intracellular accumulation of labeled nucleoside was determined as described previously (10) and expressed as pmol nucleoside (or analogue) per 10^6 cells.

Inhibition of Protein Synthesis. The ability of 5-aza-Cyd to inhibit protein synthesis in HL-60 and HL-60-5-aza-Cyd was determined by measuring the incorporation of [3H]leucine into acid-precipitable material during a 2-hr exposure to varying concentrations of 5-aza-Cyd. The details of this procedure have been described in detail elsewhere (9). Values for each cell type are expressed as cpm [3H]leucine per 10^6 cells.

Nucleoside Triphosphate Determinations. Logarithmically growing HL-60 or HL-60-5-aza-Cyd cells were exposed to pyrazofurin (5 x 10^-6 M; PALA (3 μM), or both for 18 hr along with uridine at concentrations ranging from 0 to 10^-4 M. The cells were precipitated with cold 0.5 M HClO4, the acid-soluble extracts were neutralized with 4 N KOH, and pyrimidine nucleoside triphosphates were determined by a previously described high-pressure liquid chromatographic method (12). Values for each condition were expressed as the percentage of control cell UTP or CTP levels.

Enzyme Activities. Uridine-cytidine kinase activity of partially purified enzyme preparations obtained from HL-60 and HL-60-5-aza-Cyd cells was determined according to the method of Ahmed and Welch (1). Cytidine deaminase activity was determined by the method of Stewart and Burke (25).

Suspension Culture Studies. The growth-inhibitory effects of various antimetabolites toward HL-60 and HL-60-5-aza-Cyd was determined by comparing the suspension culture growth of drug-treated and control cells. Five ml of logarithmically growing cells were seeded into 25-cm² sterile tissue culture flasks (Corning) at a initial cell density of 10⁶ cells/ml. Drugs were added to the flask at concentrations ranging from 10^-10 to 10^-4 M, and the flasks were placed in the 37°C, 5% CO₂ incubator for 72 hr. At the end of the incubation period, cell density determinations were obtained with a Coulter Multisizer. The highest 5-aza-Cyd concentration tested (10^-4 M) re-

RESULTS

Biological Characteristics. HL-60 and HL-60-5-aza-Cyd were similar with respect to a variety of biological and growth characteristics. Both cell lines were predominantly comprised of promyelocytes which exhibited the same karyotype (45; -X, -5, -8, -16). HL-60-5-aza-Cyd displayed a somewhat slower doubling time than did HL-60 in suspension culture (32 versus 20 hr) and contained a lower percentage of cells in S-phase (50 versus 60%). HL-60 and HL-60-5-aza-Cyd were similar in their cloning efficiency in soft agar (10 to 12%), response to colony-stimulating activity, and ability to undergo terminal differentiation in response to dimethyl sulfoxide, 12-O-tetradecanoylphorbol-13-acetate, and cis-retinoic acid.

Biochemical Characteristics. Biochemical characteristics of HL-60 and HL-60-5-aza-Cyd are contrasted in Table 1. There was approximately a 20-fold decrease in the activity of uridine-cytidine kinase activity in HL-60-5-aza-Cyd (0.52 versus 8.8 pmol UMP/10 min/μg protein). This was associated with similar decreases in total intracellular accumulation of [3H]cytidine and [3H]uridine when cells were exposed to the nucleosides for 2 hr at a concentration of 10^-4 M. HL-60-5-aza-Cyd cells accumulated 11.9 versus 156.0 pmol 5-aza-Cyd/10^6 cells for HL-60 following a 2-hr exposure to 10^-4 M [3H]-5-aza-Cyd. Similar relative decreases in the total intracellular accumulation of [3H]cytidine and [3H]uridine when cells were exposed to the nucleosides for 2 hr at concentrations of 10^-4 to 5 x 10^-5 M (data not shown). HL-60-5-aza-Cyd cells exposed to 10^-5 M [3H]-5-aza-Cyd for 2 hr incorporated considerably less nucleoside analogue into RNA than did parent cells (3.1 versus 43.4 pmol 5-aza-Cyd/μg ribose). Levels of the enzyme cytidine deaminase were equivalent in the 2 cell lines (0.20 and 0.24 pmol uridine/min/μg protein).

Protein Synthesis Inhibition. Protein synthetic rates in HL-60-5-aza-Cyd were considerably less sensitive to the effects of 5-aza-Cyd than were those in parent cells (Chart 1). A 4-hr exposure to 10^-5 M 5-aza-Cyd reduced incorporation of [3H]-leucine by approximately 88% of control values in HL-60 cells but was associated with only a 10% reduction in HL-60-5-aza-Cyd. The highest 5-aza-Cyd concentration tested (10^-4 M) re-

SOFT-AGAR COLONY GROWTH: NORMAL AND LEUKEMIC CELLS. The technique for culturing human leukemic cells in soft agar has been described in detail elsewhere (11). Logarithmically growing HL-60 and HL-60-5-aza-Cyd were plated in the presence of 5 x 10^-4 M pyrazofurin:3 μM PALA or both combined, along with uridine at concentrations ranging from 0 to 10^-4 M. At the end of a 10-day incubation in a 37°C, 5% CO₂ fully humidified incubator, colonies consisting of groups of 50 or more cells were enumerated with the aid of an inverted microscope. Values for each condition were expressed as a percentage of untreated control cell colony formation.

Parallel studies were performed utilizing a previously described method for culturing normal human bone marrow myeloid progenitors (CFU-GM) (11). Samples were obtained with informed consent from patients undergoing routine diagnostic bone marrow aspirations for nonneoplastic disorders. These studies have been sanctioned by the Human Investigation Institutional Review Board Committee, Columbia University College of Physicians and Surgeons. Cells were plated in the presence of pyrazofurin, PALA, and uridine in the same manner as cultured leukemic cells, and colonies were scored at Day 10. The ability of uridine to reverse the inhibitory effect of pyrazofurin or PALA on normal bone marrow progenitors was determined as for HL-60 and HL-60-5-aza-Cyd cells.

CANCER RESEARCH VOL. 44 DECEMBER 1984
4-hr incorporation of [3H]5-aza-Cyd

Intracellular accumulation (pmol/10^8 cells)

- [3H]Juridine (10^{-8} M) 1074.0 ± 130.0
- [3H]Cytidine (10^{-8} M) 904.0 ± 82.0
- [3H]5-aza-Cyd (10^{-8} M) 12.6 ± 3.4
- [3H]5-aza-Cyd (5 x 10^{-8} M) 156.0 ± 36.0
- [3H]5-aza-Cyd (1 x 10^{-7} M) 768.0 ± 112.0

4-hr incorporation of [3H]5-aza-Cyd

- RNA (pmol/µg p-ribose) 43.4 ± 6.8
- DNA (pmol/µg deoxyriboside) 3.8 ± 1.8
- Uridine-cytidine kinase activity (pmol UMP/10 min/µg protein) 8.8 ± 2.4
- Cytidine deaminase activity (pmol uridine/min/µg protein) 0.20 ± 0.07

Mean ± S.D. of at least 3 experiments performed in duplicate.

Biochemical features of HL-60 versus HL-60-5-aza-Cyd cells

Table 1

<table>
<thead>
<tr>
<th></th>
<th>HL-60</th>
<th>HL-60-5-aza-Cyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTP (pmol/10^6 cells)</td>
<td>1.2 ± 0.4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>CTP (pmol/10^6 cells)</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>UMP (µM)</td>
<td>8.8 ± 2.4</td>
<td>6.2 ± 1.8</td>
</tr>
<tr>
<td>CIMP (µM)</td>
<td>11.9 ± 3.9</td>
<td>9.3 ± 2.4</td>
</tr>
<tr>
<td>DNA (µg/cell)</td>
<td>90.4 ± 12.0</td>
<td>76.8 ± 11.2</td>
</tr>
<tr>
<td>DNA (µg/pDNA)</td>
<td>9.0 ± 1.2</td>
<td>7.6 ± 1.1</td>
</tr>
<tr>
<td>Urd (µg/cell)</td>
<td>6.8 ± 1.2</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>Cyt (µg/cell)</td>
<td>5.4 ± 0.9</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Leu (µg/cell)</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

5-aza-Cyd-RESISTANT CELLS

Table 2

<table>
<thead>
<tr>
<th>Antimetabolite</th>
<th>I.C.50 (µM) HL-60</th>
<th>I.C.50 (µM) HL-60-5-aza-Cyd</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-aza-Cyd</td>
<td>5.0 x 10^-7</td>
<td>3.5 x 10^-9</td>
<td>0.016</td>
</tr>
<tr>
<td>3-Denazuridine</td>
<td>2.2 x 10^-5</td>
<td>1.6 x 10^-7</td>
<td>0.076</td>
</tr>
<tr>
<td>6-Azauridine</td>
<td>8.1 x 10^-6</td>
<td>4.0 x 10^-9</td>
<td>0.020</td>
</tr>
<tr>
<td>5-Furid</td>
<td>5.5 x 10^-6</td>
<td>7.5 x 10^-9</td>
<td>0.073</td>
</tr>
<tr>
<td>1-β-D-Arabinofuranosylositosine</td>
<td>4.6 x 10^-6</td>
<td>6.3 x 10^-9</td>
<td>0.760</td>
</tr>
<tr>
<td>2-Deoxy-aza-cytidine</td>
<td>7.5 x 10^-6</td>
<td>8.0 x 10^-9</td>
<td>0.930</td>
</tr>
<tr>
<td>5-Aza-1-β-D-arabinofuranosylositosine</td>
<td>1.2 x 10^-6</td>
<td>1.8 x 10^-9</td>
<td>0.880</td>
</tr>
<tr>
<td>PALA</td>
<td>1.0 x 10^-9</td>
<td>2.5 x 10^-11</td>
<td>0.040</td>
</tr>
<tr>
<td>Pyrazofurin</td>
<td>5.0 x 10^-9</td>
<td>1.0 x 10^-11</td>
<td>0.050</td>
</tr>
<tr>
<td>IMPY</td>
<td>7.8 x 10^-9</td>
<td>7.6 x 10^-9</td>
<td>1.020</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>4.5 x 10^-6</td>
<td>5.8 x 10^-9</td>
<td>0.770</td>
</tr>
<tr>
<td>Acivicin</td>
<td>7.2 x 10^-7</td>
<td>8.7 x 10^-7</td>
<td>0.820</td>
</tr>
</tbody>
</table>

I.C.50, that concentration of drug associated with a 50% reduction in control-cell growth; FUrd, 5-fluorouridine; IMPY, 2,3-dihydro-1H-Imidazo[1,2b]pyrazole.

Chart 1. 5-aza-Cyd (5 AzA)-mediated protein synthesis inhibition was compared in HL-60 and HL-60-5-aza-Cyd. Logarithmically growing cells were exposed to 10^{-4} M 5-aza-Cyd for 4 hr, and separating RNA and DNA by alkaline hydrolysis. The techniques for assaying uridine-cytidine kinase and cytidine deaminase activity are cited in Materials and Methods.
Pyrimidine nucleoside triphosphate levels of HL-60 and HL-60-5-aza-Cyd following exposure to pyrimidine antagonists in the presence of 0, 10, or 50 µM uridine. Logarithmically growing cells were incubated with pyrazofurin (5 × 10⁻⁸ M), PALA (3 mM) or both combined for 18 hr in the presence or absence of uridine (10 or 50 µM). At the end of this period, pyrimidine nucleoside triphosphate levels were assayed in cold acid-soluble cell extracts by high-pressure liquid chromatography as described in "Materials and Methods." Values for each condition correspond to the percentage of control CTP and UTP levels.

<table>
<thead>
<tr>
<th>Uridine (µM)</th>
<th>HL-60</th>
<th>HL-60/5-aza-Cyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazofurin (5 × 10⁻⁸ M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>10</td>
<td>19 ± 4</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>50</td>
<td>110 ± 15</td>
<td>89 ± 12</td>
</tr>
<tr>
<td>PALA (3 mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>17 ± 2</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>24 ± 6</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>50</td>
<td>124 ± 28</td>
<td>101 ± 21</td>
</tr>
<tr>
<td>PALA (3 mM) + pyrazofurin (5 × 10⁻⁸ M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 ± 1</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>10</td>
<td>16 ± 4</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>50</td>
<td>91 ± 16</td>
<td>86 ± 14</td>
</tr>
</tbody>
</table>

*Mean ± S.D. of at least 2 experiments performed in duplicate.*

In recent years, there has been interest in attempts to utilize naturally occurring nucleosides in order to improve the therapeutic efficacy of available antimetabolites. For example, low-dose thymidine has been used in humans as a potentially selective means to protect normal tissues from the lethal effects of methotrexate (13). Administration of deoxycytidine to NMRI mice inoculated with L1210 leukemia appeared to improve the therapeutic index of subsequently administered 1-β-D-arabinofuranosylcytosine (3). More recently, animal studies have suggested that coadministration of uridine might selectively protect bone marrow and gastrointestinal epithelial cells from the toxicity of 5-fluorouracil (19, 22). Although normal plasma levels of uridine in humans are low (2.6 to 8.2 × 10⁻⁸ M) (18), uridine infusions yielding molar plasma levels have been safely achieved (14). In contrast to these previous studies which involve the use of naturally occurring nucleosides in unperturbed tumor cells, the present data suggest that drug-resistant cells lacking a pyrimidine salvage pathway enzyme might represent an alternative target for nucleoside-containing regimens.

As in the case of a previously described murine tumor cell line...
decreased activity of uridine-cytidine kinase conferred high degrees of 5-aza-Cyd resistance to HL-60-5-aza-Cyd. It is of interest that a relatively modest decrease (e.g., 20-fold) in enzyme activity as well as 5-aza-Cyd nucleotide formation and incorporation into nucleic acids was associated with a 100-fold decrease in sensitivity to this agent. An explanation for this nonlinearity is not readily apparent. The pharmacokinetics of 5-aza-Cyd are complex since the agent rapidly undergoes ring scission in aqueous solution to metabolites which may retain antitumor activity (2, 23). It is conceivable that a threshold quantity of 5-aza-Cyd or one of its metabolites must be incorporated into nucleic acids for cell killing to occur, thereby allowing a small decrease in drug activation to produce a substantial increase in drug resistance.

As might be predicted, HL-60-5-aza-Cyd were cross-resistant to agents requiring uridine-cytidine kinase for activation, such as 3-dezaauridine, 5-fluorouridine, and 6-azaauridine, but not to analogues such as 1-β-D-arabinofuranosylcytosine, deoxyazacytidine, and arabinosyl 5-aza-Cyd all of which are phosphorylated to 3-deazauridine, 5-fluorouridine, and 6-azauridine, but not to an agent requiring uridine-cytidine kinase for activation, such as 3-dezaauridine, 5-fluorouridine, and 6-azaauridine. It is noteworthy that when uridine was administered to agents requiring uridine-cytidine kinase to produce a substantial decrease in drug activation to produce a substantial increase in drug resistance.

These in vitro data raise the possibility that loss of a pyrimidine salvage pathway enzyme conferring high degrees of drug resistance may be exploited by administering a naturally occurring nucleoside in conjunction with the appropriate pyrimidine antagonist. Previous studies have established that the Km of uridine-cytidine kinase obtained from human cells with respect to uridine is approximately 50 μM (6). This uridine concentration has been shown previously to be capable of preventing pyrimidine antagonist-mediated growth inhibition in murine lymphoblasts such as L1210 and L5178Y (7). Our data suggest that exposure to 50 μM uridine may replete UTP and CTP levels in parent HL-60 cells exposed to lethal concentrations of PALA and pyrazofurin but not in a 5-aza-Cyd-resistant variant deficient in uridine-cytidine kinase. Moreover, 50 μM uridine was capable of preventing PALA- and pyrazofurin-mediated growth inhibition in normal human bone marrow myeloid progenitors but not in drug-resistant leukemic cells. It is of interest that when uridine was administered at concentrations approximating maximal plasma levels (e.g., 10 μM), reversal of PALA- and pyrazofurin-mediated growth inhibition did not occur in any of the cells tested. This raises the possibility that in the intact organism, circulating levels of uridine may be insufficient to overcome the effects of agents which inhibit de novo pyrimidine biosynthesis. However, elevation of uridine concentrations to supranormal levels may permit certain normal tissues to survive pyrimidine antagonist exposure but not drug-resistant cells deficient in the appropriate salvage pathway enzyme.

Additional information will be required in order to establish the in vivo relevance of these in vitro observations. For example, the successful implementation of a pyrimidine antagonist-uridine regimen will depend upon the ability to achieve plasma levels of agents such as PALA or pyrazofurin that are capable of eradicating resistant leukemic cells (15, 24). It is possible that coadministration of uridine might increase the maximally tolerable levels of these agents, but evidence for this hypothesis is not currently available. In addition, further documentation that uridine would prevent high-dose pyrimidine antagonist-mediated toxicity in an intact organism will be necessary. Finally, critical to this approach is the role that decreased uridine-cytidine kinase activity plays in the development of 5-aza-Cyd resistance in humans. While such a strategy may be successful against a subset of leukemic cells deficient in this enzyme, it might be less successful against a heterogeneous population of cells displaying various modes of resistance. For this reason, it would be worthwhile to assess uridine-cytidine kinase activity in leukemic myeloblasts obtained from patients both sensitive and resistant to 5-aza-Cyd. Such studies are currently underway in our laboratory.

ACKNOWLEDGMENTS

We would like to express our appreciation to Drs. F. Rapoport, M. Oster, and M. Stooper for supplying bone marrow samples for these studies.

REFERENCES

5-aza-Cyd-RESISTANT CELLS

2938–2940, 1980.


Effect of Uridine on Response of 5-Azacytidine-resistant Human Leukemic Cells to Inhibitors of de Novo Pyrimidine Synthesis

S. Grant, K. Bhalla and M. Gleyzer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/12_Part_1/5505

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs.aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.