Selective Toxicity of Deoxyguanosine and Arabinosyl Guanine for T-Leukemic Cells

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Deoxyguanosine is selectively cytotoxic to leukemic cells from patients with T-acute lymphoblastic leukemia (T-ALL), whereas all other leukemic cell types were significantly less sensitive. Arabinosylguanine, a deoxyguanosine analog resistant to cleavage by purine nucleoside phosphorylase, is a more potent inhibitor of DNA synthesis in T-leukemic cells than deoxyguanosine and retains a selective cytotoxic activity for T-leukemic cells. Deoxyguanosine and arabinosylguanine are phosphorylated to deoxyGTP and arabinosylGTP, respectively, by T cells but not by other cell types. The phosphorylation and the cytotoxicity of arabinosylguanine are prevented by deoxycytidine. The selectivity of arabinosylguanine for malignant T cells, the exquisite sensitivity of these cells to the drug, and the failure of PNP to cleave the nucleoside indicate its potential in the treatment of T-ALL.

The DISCOVERIES of the association of deficiencies of the purine metabolic enzymes adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) with immune deficiencies in humans has stimulated interest in the potential use of inhibitors or substrates of ADA and PNP for selective immunosuppression and in the treatment of malignant lymphoproliferative diseases. Previous approaches to the selective pharmacologic control of lymphoproliferation have focused on the use of inhibitors of ADA. However, as expected from the pathogenesis of ADA deficiency, inhibition of ADA results in loss of both cellular immunity and humoral immunity. In addition, the administration of 2'-deoxycoformycin, a potent inhibitor of ADA, to patients with acute lymphoblastic leukemia (ALL) produced not only a marked reduction in neoplastic and normal B and T lymphoid cells but also serious toxicity involving the kidneys, lungs, and central nervous system.

In contrast to the combined humoral and cell-mediated immunodeficiency accompanying ADA deficiency, PNP deficiency results in selective abnormalities of T-lymphocyte function, resulting in loss of cellular immunity without compromising humoral immunity. Thus, an inhibitor or a substrate of PNP would potentially be a more selective pharmacologic agent for the treatment of T-cell malignant lymphoproliferative diseases or the modulation of specific T-cell functions, such as the prevention of tissue rejection after organ transplantation.

Of the four PNP substrates accumulated in PNP-deficient patients, only deoxyguanosine is further phosphorylated by mammalian deoxycytidine kinase. Indeed erythrocytes from PNP-deficient patients accumulate increased amounts of deoxyguanosine triphosphate (deoxyGTP). Previous studies have demonstrated that immature T lymphocytes and T-lymphoblastoid cells are exquisitely sensitive to low concentrations of deoxyguanosine. Thymocytes, but not B lymphocytes, incubated in the presence of deoxyguanosine accumulate large amounts of deoxyGTP. The basis for the increased ability of thymocytes to accumulate deoxyGTP appears to be related to the high activity of deoxycytidine kinase, the enzyme responsible for deoxyguanosine phosphorylation, and to a decreased ability of thymocytes to degrade deoxyGTP. The accumulation of deoxyGTP in T cells inhibits ribonucleotide reductase, the enzyme that catalyzes the reduction of nucleoside diphosphates to their corresponding 2'-deoxy derivatives; depletion of other deoxynucleoside triphosphates results in inhibition of DNA synthesis and cell death.

The pharmacologic use of PNP inhibitors is limited because of their relatively low affinity. On the other hand, the in vivo administration of deoxyguanosine without inhibitor is ineffective, since deoxyguanosine is immediately degraded by PNP activity in the red blood cells. A third approach is the use of deoxyguanosine analogs that are resistant to cleavage by PNP. To test this possibility we began by comparing the toxicity of deoxyguanosine and arabinosylguanine and their metabolism in leukemic cells from patients with different types of leukemia.

MATERIALS AND METHODS

Chemicals

DeoxyGTP, deoxyCTP, deoxyguanosine, and deoxycytidine were purchased from Sigma Chemicals (St. Louis, Mo.). Arabinosylguanine was a gift from Dr. G. Elion of Burroughs Wellcome (Research Triangle, N.C.), and from Dr. M.J. Robins, University of Alberta (Edmonton, Alberta).
**Cell Preparations**

Peripheral blood and bone marrow were obtained from the patients or peripheral blood from normals and mononuclear cells harvested following Hypaque-Ficoll gradient centrifugation. Leukemic blasts comprised more than 60%–70% in all suspensions and often were greater than 90%. Patients with T-ALL were defined as those with greater than 40% E-rosetting cells, and non-T, non-B as those that had less than 5% E-rosetting cells, were slg-negative, and stained positively for 

**Cell Lines**

The human T-lymphoblastoid cell lines (Jurkat and Molt-3)18 and the non-T, non-B (null) cell lines (HOON-1B, HYON-2B)19 were derived from patients with ALL. The human B lymphoblast lines (HSC-3 and HSC-58) were derived from normal individuals.

**Cell Cultures**

The cells were maintained in exponential growth phase in RPMI 1640 containing 10% heat-inactivated fetal calf serum. Cell proliferation studies were performed during a 24-hr period. Cells (1–2 x 10^6) were cultured at 37°C in 5% CO2 in microtest II plates (Falcon, Oxnard, Calif.) in 0.2 ml RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS). After 20 hr, the cells were washed 3 times in fresh medium and incubated for a further 4 hr in the presence of 1 μCi 3H-thymidine (6.7 Ci/mmole, New England Nuclear, Boston, Mass.). Thymidine incorporation into DNA was assayed by harvesting the cells with an automated harvester on fiberglass filters, drying, and measuring radioactivity in a Beckman liquid scintillation counter. In studies with peripheral blood lymphocytes, the cells were incubated with 10 μg/ml phytohemagglutinin (PHA) and DNA synthesis measured by pulsing with 3H-thymidine after 3 days.

Deoxyguanosine or the different analogs were added at the initiation of culture. In some experiments, parallel cell cultures were incubated for 48 hr with deoxyguanosine or analogs, and cell growth was determined by counting viable cells in the presence of trypan blue. There was a good correlation between cell growth and 3H-thymidine incorporation.

**Intracellular Nucleotide Measurements**

Cells (2 x 10^6/ml) were cultured in RPMI 1640 with 10% heat-inactivated FCS containing various concentrations of deoxyguanosine or arabinosylguanine. At the end of the incubation period, the cells were washed in phosphate-buffered saline (PBS, pH 7.2), extracted with cold 0.5 M HC104, and neutralized with 3 N KOH, and the precipitate KC104 was removed. Ribonucleotides, deoxyribo- nucleotides, and arabinonucleotides were chromatographed on a Whatman SAX-10/25 partisil column using a Waters high performance liquid chromatography system. DeoxyGTP, arabinosylGTP (araGTP) and GTP were eluted with 0.5 M ammonium phosphate (pH 3.5)13,14 and were well separated from each other (GTP elutes after 32 min, deoxyGTP after 39 min, and araGTP after 43 min). The flow rate was 1 ml/min and u.v. absorbance was monitored at 254 and 280 nm.

**Phosphorolysis of Arabinosylguanine**

Phosphorolysis was measured by incubating 0.5 mM arabinosylguanine with various amounts of purified purine nucleoside phosphorolase from spleen (Sigma Chemical Co., St. Louis, Mo.) in 0.05 M potassium phosphate buffer, pH 7.5, at 37°C. The incubation was stopped by the addition of cold perchloric acid (0.5 M final concentration), mixed, neutralized by KOH, and centrifuged. The superna-

tant was frozen at -20°C until analysis by HPLC. Analyses were carried out using a 5-μ Radial-PAK C8 reverse phase column (Waters Associates, Inc., Milford, Mass.); arabinosylguanine and guanine were eluted by 10% methanol in 20 mM ammonium phosphate, pH 3.5; and u.v. absorption at 254 nm and 280 nm was monitored and compared to known standards. The activity of spleen purine nucleoside phosphorolase preparation was also verified using deoxyguanosine as substrate by the method described above.

**3H-Deoxyguanosine Metabolism**

For measurements of 3H-deoxyguanosine incorporation, nucleo-
tides were extracted and chromatographed as described above. The fractions containing GTP and deoxyGTP were collected, 10 ml of scintillation fluid (Ready-Solv EP, Beckman Instruments, Inc., Irvine, Calif.) added to 1 ml of the pooled nucleotide peak, and radioactivity counted in a scintillation counter.

**RESULTS**

Figure 1 shows the effect of the addition of deoxy-
guanosine on thymidine incorporation into DNA by human T lymphocytes and leukemic cells of various lineages. It can be seen that thymocytes and leukemic cells from four patients with T-cell ALL are sensitive to the lowest concentration of deoxyguanosine (50% inhibition between <1 μM and 30 μM deoxyguanosine). Some variation in the degree of sensitivity among T-ALL was observed, but as a group, they were much more sensitive than any other cell type. In this group, there was no obvious correlation between initial peripheral blood white cell count, degree of spontaneous thymidine incorporation, and sensitivity to deoxyguanosine. Cells from other leukemic patients with chronic and acute myeloblastic leukemia and non-B, non-T ALL (null-ALL) are not sensitive to inhibition by deoxyguanosine (50% inhibition >400 μM deoxyguanosine). Peripheral blood T lymphocytes, responsive to phytohemagglutinin, are sensitive to intermediate deoxyguanosine concentrations (50% inhibition at 150 μM deoxyguanosine).

We have previously shown that the cytotoxic effects of deoxyguanosine toward human thymocytes and peripheral blood cells depend on the cell's capacity to phosphorylate the nucleoside to deoxyGTP.12 The accumulation of deoxyGTP in T-ALL and null-ALL cells is shown in Fig. 2. In the presence of exogenous deoxyguanosine, the degree of deoxyGTP accumulation was significantly higher in T-ALL cells. In the presence of as little as 10–25 μM deoxyguanosine, T-ALL cells contained 7–20-fold more deoxyGTP than the null-ALL cells. In order to determine whether deoxyGTP accumulation correlates with deoxyguanosine toxicity, we have compared the metabolism of 3H-deoxyguanosine in B and T lymphocytes. From the results summarized in Table 1, it can be seen that although both B- and T-cell lines incorporate substantial amounts of the radiolabeled deoxyguanosine into
GTP pools via guanine formed by purine nucleoside phosphorylase action, deoxyGTP accumulates only in the T-cell line. Moreover, deoxycytidine, which prevents deoxyguanosine cytotoxicity in thymocytes and in T-cell lines (see below), only prevents the accumulation of deoxyGTP but not GTP (Table 1). These results demonstrate that deoxyGTP accumulation and not guanine and GTP levels correlate with deoxyguanosine cytotoxicity.

Deoxyguanosine can be degraded by purine nucleoside phosphorylase (PNP), thus preventing deoxyguanosine phosphorylation to deoxyGTP. Deoxyguanosine analogs, which are resistant to cleavage by PNP but can still be phosphorylated by deoxycytidine kinase, may have increased cytotoxicity. An example of such an analog is arabinosylguanine (Fig. 3). Structurally, the two molecules are very similar except for the hydroxyl at the 2’ position. However, arabinosylguanine is resistant to cleavage by bovine spleen purine nucleoside phosphorylase ($V_{max} \approx 1\%$ that of deoxyguanosine, data not shown).

Figure 4 illustrates the effect of arabinosylguanine on thymidine incorporation into DNA by lymphoblas-

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**Fig. 2.** DeoxyGTP accumulation T-ALL and null-ALL cells. Deoxyguanosine was added to peripheral blood leukemic cells from patients with T-ALL or null-ALL; cells were incubated at a density of $2 \times 10^6$ cells/ml. The cells were harvested after 15 hr and deoxyGTP levels measured as described in Materials and Methods. (O-O) T-ALL cells, (O-O) null-ALL cells.

**Fig. 1.** Effect of deoxyguanosine on $^3$H-thymidine incorporation. Peripheral blood cells ($10^6$) were cultured for 72 hr (PBL) or 20 hr (all others) in the presence of the indicated deoxyguanosine concentrations. Four hours before termination of culture, the cells were washed and incubated with 1 µCi $^3$H-thymidine without deoxyguanosine. Results are expressed as the percent of untreated control ($^3$H-thymidine uptake). Each point represents the mean of 3 determinations. The SD in all experiments never exceeded 10%. Mean control values for PHA-induced proliferation of PBL was 63,000 cpm, for spontaneously dividing thymocytes 6500 cpm, for T-ALL cells 7600 cpm (range 5800-12,400 cpm, 4 patients), for null-ALL cells 6300 cpm (3 patients), and for AML cells 4500 cpm (1 patient). All studies were carried out prior to the initiation of treatment. (A---A) Thymocytes, (A---A) PBL, (O-O) T-ALL, (O-O) null-ALL, (O-O) AML.

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**Table 1.** Deoxyguanosine Metabolism in B and T Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Deoxycytidine (10 µM)</th>
<th>$^3$H-Deoxyguanosine Incorporation (cpm/10^6 Cells)</th>
<th>Percent Guanine Formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GTP</td>
<td>DeoxyGTP</td>
</tr>
<tr>
<td>HSC-3 (B)</td>
<td>-</td>
<td>17,680</td>
<td>527</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>19,085</td>
<td>206</td>
</tr>
<tr>
<td>Molt-3 (T)</td>
<td>-</td>
<td>9,734</td>
<td>6,756</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>13,435</td>
<td>630</td>
</tr>
</tbody>
</table>

B or T cell lines (2 x 10^6 cells/2 ml) were incubated in RPMI 1640 with 10% heat-inactivated fetal calf serum in the presence of 100 µM $^3$H-deoxyguanosine (10 µCi) with or without 10 µM deoxycytidine at 37°C. After 2 hr, the cells were washed and nucleotides extracted, separated, and the incorporation of radioactivity into GTP and deoxyGTP analyzed as described in the Methods section. The medium was analyzed for cleavage of deoxyguanosine to quanine as described in the Methods. The data are representative of 3 separate experiments with similar results.
Selective Toxicity of Arabinosyl Guanine

Fig. 3. Structure of 2'-deoxyguanosine and arabinosylguanine.

Fig. 4. Effect of arabinosylguanine on 3H-thymidine incorporation into various lymphoblastoid cells. Cells (2 x 10⁶) were cultured in the presence of the indicated arabinosylguanine concentration for 24 hr. Results are expressed as the percent of untreated control. Each point represents the mean of 3 separate experiments carried out in triplicate. The SD in all experiments was less than 10%. Mean control values were: Molt-83480 cpm, Jurkat-94490 cpm, HSC-58-93050 cpm, HSC-3-550 cpm, HOON-1B-42860 cpm, and HYOB-2B-14830 cpm. (A—A) HSC-3 (B cell), (O—O) HSC-58 (B cell), (+—+) HOON-1B (null-cell), (x—x) HYOB-2B (null-cell), (Δ—Δ) Molt-3 (T-cell), (O—O) Jurkat-3 (T-cell).

T-cell lines are the most sensitive to inhibition by arabinosylguanine; 50% inhibition of thymidine incorporation occurred at concentrations of 1 µM of the drug. The null cell lines exhibited an intermediate sensitivity to arabinosylguanine (50% inhibition at approximately 10 µM), whereas DNA synthesis of B-lymphoblastoid cells is not inhibited significantly except at much higher concentrations (50% inhibition >30 µM). Thus, arabinosylguanine appears to be a more potent inhibitor of DNA synthesis than deoxyguanosine, while maintaining its relative specificity toward T-lymphoblastoid cells.

We next compared the effects of deoxyguanosine and arabinosylguanine on the inhibition of DNA synthesis in patients with T-ALL. As shown in Fig. 5, T-leukemic blasts are very sensitive to low concentrations of both nucleosides. For T-ALL cells, as with the T-lymphoblastoid lines, arabinosylguanine inhibits DNA synthesis at lower concentrations than deoxyguanosine (roughly 100-fold differences at the level of 50% inhibition).

Since phosphorylation to deoxyGTP appears crucial to the expression of toxicity, we determined if arabinosylguanine cytotoxicity involves its phosphorylation to araGTP. For this purpose we determined the degree of araGTP accumulation in the T-lymphoblastoid cells and two cases of T-ALL. As can be seen from the results summarized in Table 2, measurable amounts of araGTP accumulate only in the T-lymphoblastoid cells and in the T-ALL cells but not in the B-lymphoblastoid cells. These results parallel previous studies showing the selectivity of deoxyGTP accumulation in the presence of deoxyguanosine (Table 1). Furthermore, deoxycytidine, which can compete with the phosphorylation of deoxyguanosine to deoxyGTP (Table 1), may also prevent the phosphorylation of arabinosylguanine to araGTP (Table 2). Deoxycytidine, by competing with nucleoside phosphorylation, can thereby relieve the inhibition of DNA synthesis by either deoxyguanosine or arabinosylguanine (Fig. 6). Addition of 2–5 µM deoxycytidine completely prevented the effect of 100 µM deoxyguanosine on DNA synthesis in T-lymphoblastoid cells. Similar concentrations of deoxycytidine were also capable of...
abrogating the inhibition of DNA synthesis by arabinosylguanine.

**DISCUSSION**

Deoxyguanosine has been shown to be selectively cytotoxic to thymocytes and to T-lymphoblastoid cell lines. We have now extended these observations to leukemic cells from patients with T-ALL and have distinguished them from other forms of leukemia (Fig. 1). Some heterogeneity in the degree of sensitivity of leukemic cells from different T-ALL patients was observed. Overall, the T-leukemic cells constitute a distinct group expressing deoxyguanosine sensitivity (50% inhibition of DNA synthesis in the presence of 1–30 μM deoxyguanosine), whereas all other types of leukemic cells are sensitive to deoxyguanosine concentrations only above 300 μM (Fig. 1). This selectivity of deoxyguanosine indicates the potential use of deoxyguanosine in the treatment of T-ALL. However, administration of deoxyguanosine in vivo may be inefficient, since deoxyguanosine is cleaved by purine nucleoside phosphorylase (PNP) present in red blood cells. For this reason it was thought advantageous to select a deoxyguanosine analog that is resistant to cleavage by PNP. We have selected the analog arabinosylguanine, which is structurally similar to deoxyguanosine (Fig. 3), but is a poor PNP substrate. Arabinosylguanine, like deoxyguanosine, is selectively cytotoxic to T cells and is essentially not cytotoxic to B-lymphoblastoid cells (Fig. 4). Arabinosylguanine is also inhibitory to T-ALL and at significantly lower concentrations than deoxyguanosine (Fig. 5).

The biochemical basis for the selective T-cell cyto-
toxicity is not clear, it may be due to both increased deoxyguanosine phosphorylation and to decreased deoxynucleotide degradation activity in T cells. Immature T cells and T-leukemic cells accumulate more deoxyGTP when incubated in the presence of deoxyguanosine (Table I and Fig. 2). Arabinosylguanine may also be phosphorylated more rapidly by T lymphoblasts. Indeed, the phosphorylation product of arabinosylguanine, araGTP, is found only in T-lymphoblastoid or T-ALL cells (Table 2). Null-leukemic cells and null-cell lines appear to exhibit an intermediate sensitivity (when compared to B-cell lines) to both arabinosylguanine and deoxyguanosine. Null cells also tend to have intermediate levels of several purine metabolic enzymes.22

Addition of deoxycytidine at low concentrations prevents the cytotoxicity of both deoxyguanosine and arabinosylguanine to T-cell lines (Fig. 6). In parallel, deoxycytidine prevents the accumulation of arabinosylGTP and deoxyGTP from arabinosylguanine and deoxyguanosine respectively (Tables 1 and 2). The mechanism by which deoxycytidine prevents accumulation of deoxyGTP and arabinosylGTP is not clear. Deoxycytidine may directly compete with deoxyguanosine and arabinosylguanine transport or phosphorylation, or deoxyCTP, formed from deoxycytidine, may inhibit deoxycytidine kinase. The mode of action of deoxyGTP is believed to be via inhibition of ribonucleotide reductase, which results in depletion of deoxyCTP pools and inhibition of DNA synthesis.15,23 Arabinosylguanine, on the other hand, is an inhibitor of DNA polymerase-α.24 The increased sensitivity of T-ALL cells to deoxyguanosine may reflect not only increased accumulation of arabinosylGTP but also increased cytotoxicity of the accumulated arabinosylGTP as compared to deoxyGTP.

The particular selectivity and sensitivity of T-ALL blasts demonstrated here, coupled with their resistance to cleavage by PNP, are important technological advantages for considering the therapeutic use of arabinosylguanine in T-ALL.

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Selective toxicity of deoxyguanosine and arabinosyl guanine for T-leukemic cells

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