Biochemical Correlates of the Differential Sensitivity of Subtypes of Human Leukemia to Deoxyadenosine and Deoxycoformycin

By Steven S. Matsumoto, Alice L. Yu, Linda C. Bleeker, Bohdan Bakay, Faith H. Kung, and William L. Nyhan

From the Department of Pediatrics, University of California, San Diego, Calif 92103.

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Address reprint requests to Alice L. Yu, M.D., Ph.D., University of California Medical Center, Pediatrics/Hematology-Oncology, San Diego, Calif 92103.

1096 Blood.

MATERIALS AND METHODS

Leukemic Cells

Peripheral blood or bone marrow specimens were obtained from 54 patients (dAdo) plus an inhibitor of adenosine deaminase, 2'-deoxy-coformycin,2 to mimic the hereditary disease have led to the selective impairment of lymphocyte functions observed in children with an inherited deficiency of adenosine deaminase and ecto-5'-nucleotidase. The clinical response to DCF therapy of a patient with T-ALL and another with pre-B-ALL was consistent with the in vitro metabolic response of their cells to DCF and dAdo.

Materials

2'-Deoxycoformycin (Covidarabine, Pentostatin) was kindly provided by Warner-Lambert/Parke-Davis (Detroit, Mich.) and the Investigational Drug Branch, National Cancer Institute. 8-'4C-inosine (23.9 Ci/mmole), 8-'4C-deoxyadenosine (40.7 mCi/mmole), 2,8-3H-ATP (61 mCi/mmole) and U-14C-deoxyAMP (560 mCi/mmole) were obtained from New England Nuclear, Boston, Mass. 3H-methyl-thymidine, incubation was continued for 2 hr. and the 5% of deoxyadenosine was incubated for 20 hr at 37#{176}C in an atmosphere of carbon dioxide in air. To each well was added 1 zCi of

Leukemic cells in the presence of 20 SFD were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine. Some leukemic cell mononuclear cells were separated using Ficoll-paque (Phamacia, Uppsala, Sweden) and culture in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine. Some leukemic cell mononuclear cells were separated using Ficoll-paque (Phamacia, Uppsala, Sweden) and culture in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine.

Thymidine Incorporation

Leukemic cells incubated in vitro with 2'-deoxyadenosine and investigated the biochemical basis of the variability in results, but some significant correlation between the LD50 and the ratio of 50% inhibition of the incorporation of 3H-thymidine into DNA was observed between the LD50 and the ratio of the metabolic response of their cells to DCF and dAdo.

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ENHANCED FORMATION OF THE POTENT ANTIPROLIFERATIVE PROSTAGLANDIN (PG) BY AORTA OF THE SPONTANEOUSLY HYPERTENSIVE (SH) RAT. C. B. Pace-Asciak*, M. C. Carreras* and G. Ramgara* (SPON: B. P. Schimmer). Research Institute, Hospital for Sick Children, Toronto, ON, M5G 1X8, CAN.

We recently observed that PG1_2 has potent BP lowering properties, being twice as active as PG1_2 in the normal rat and 3-5 times as active in the SH rat. Later, PG1_2 is increased due to an increase in the formation of the isoprostane (2.5-10ng) after intracardiot and intrajugular administration due to its complete lack of catabolism by the lungs. Incubation at 37°C of SH aortas with 20 ng/ml PG1_2 plus 10 ng/ml of PG1_2 showed the same release of PG1_2 as did the normal rat aortas.


depressed substance. This substance was formed by the SH aortas in 3-7 times greater quantity (1312ng/mg aorta) than normal. Identification of this substance as PG1_2 was shown by (1) its similar activity after intraperitoneal injection (2) its instability at pH 7 and (3) by its typical delayed onset of action. Incubation of homogenates of aortas from SH rats with 3H-AD gave one major product indistinguishable from 6-keto-PGF1_2, the stable degradation product of PG1_2. This study can be unified with biochemical and pharmacological evidence of enhanced synthesis of PG1_2 by the SH aorta. Enhanced formation of PG1_2 is likely an adaptive response to increased levels of catecholamines during hypertension since these are known to stimulate PG1_2 synthesis (Biochin. Biophys. Acta 280, 161, 1972). (Supported by a grant to CPA from the Medical Research Council of Canada).


PMA stimulates secretion of a number of cellular products from phagocytic cells. These include colony stimulating factor, lysosomal hydrolases, and neutral protease. PMA stimulates the release of 6-HA/acidic rich (6-A) from pre-labeled target cells obtained by pretreatment with either collagenase or dispase in a dose-dependent manner. Using fatty acid free albumin as a trap, 10-9M PMA stimulated 6-RA release by 26%. In comparison, 50 (50 µg/ml) serving as a phagocytic stimulus, caused a 200% increase in 6-RA release. Prelabelled cells exposed to PMA in serum-free medium synthesize and release at least two prostaglandins, namely, PG1_2 and 6-keto-PGF1_2. This activity of PMA is additive with that of soyasosan, which also stimulates PG synthesis and release by macrophages. PMA also causes the selective release of a lysosomal enzyme, N-acetyl-p-D-glucosaminidase. Again additivity with soyasosan was observed. It is therefore clear that both soluble and particulate stimuli of inflammation cause PG synthesis and release by macrophages.


PG1_2 appears to be the leukotriene in sheep, guinea pig, rats and cows. Previous studies have indicated that the fluctuations in uterine PGF1_2, secretion that occur during the estrous cycle are related to changes in the activity of the uterine cycloxygenase that catalyzes the first step in prostaglandin formation. We have now obtained enzymatic and immunological evidence that cyclical changes occur in uterine cycloxygenase activities in the ewe and that these changes result from fluctuations in the level of enzyme protein while the catalytic efficiency of the cycloxygenase remains unaltered. Cycloxygenase activities of detergent-solubilized uterine microsome prepared from ewes in Days 5, 13, 14 and 15 of the cycle were measured stereochemically and found to increase toward the end of the cycle. Thus, cycloxygenase activities were 1.7 ± 0.3 x 10³ (mean) versus 15.5 ± 4.8 x 10³ units per mg of protein for animals in Days 5 and 15, respectively. In contrast, the immunocytochemical equivalence points determined using rabbit anti-sheep uterine gland cycloxygenase serum were the same: 13.9 ± 3.6 (Day 5) versus 11.2 ± 2.6 (Day 14) cycloxygenase units precipitated at the equivalence point. Immunohistochemical studies indicate that all uterine cell types contain cycloxygenase antigenicity but no changes were detected in the cyclooxygenase content of any tissue cells during the cycle. (Supported in part by NIH Grant HD 10013.)


The effect of unilateral obstruction on prostaglandin (PG) metabolism was studied. 1. Tissue slices from normal and hydrometraotic rabbit renal papillae and cortices were incubated, and the release of PG1_2 into the medium was measured. 2. Microsomal fractions from the above kidney segments were incubated with radiolabeled arachidonic acid, and the levels of PG1_2 and PG1_2, thromboxane B_2 and PGD_2 were determined. 3. The activity of prostaglandin synthase was determined by measuring formation of PG 15-keto from the radiolabeled substrate, PG1_2. Our results indicate that the obstructed cortex exhibits higher specific activity than the contralateral or normal in the tissue slices and in the microsomal fraction and that this activity increases with days obstructed. This suggests that the recovery shows higher PGD_2 activity than the contralateral or normal. Tissue slices from the contralateral papilla released more PG1_2 than did tissue slices from the obstructed kidney. There was little difference in the synthetic capacity of the papillae microsomal fractions. The excessive formation of PG1_2 by the obstructed cortex may play a role in tissue alterations in regional blood flow and in renal vascular resistance. (Supported by NIH Grant HL-17889-03)


The major metabolite of prostacyclin (PGI2) thus far identified is 6-keto-PGF1_2 (6-kPG1_2). We have shown that a prostacyclin metabolizing enzyme(s) is present in the cytoplasmic fraction of tissues of rat and rabbit. This enzyme(s) appears to be a tentative designated 15-hydroxyprostacyclin-dehydrogenase (PGIDH), converts PG1_2 to a product identified by thin-layer chromatography (TLC) and GC-MS as 6,15-diketo-PGF1_2. (6,15DkopGF1_2). (6,15DkopGF1_2) was prepared chemically from (6,15)PGF1_2 and used as substrate for the PG1_2 radiometric assay. The cytoplasmic fraction of the tissues was incubated at 37°C for 45 min with (6,15)PG1_2 and either NAD* or NADP*. The reaction was stopped by acidification to pH 3 and the metabolite extracted with ethyl acetate and separated by TLC using authentic 6,15 Dk-PGF1_2 as a standard. The specific activity of PGIDH in the rat is highest in the lung and kidney cortex, followed by the heart, kidney medulla, stomach fundus, spleen and liver. In the rabbit a similar rank order of specific activity was found. A similar activity of PG1_2 may be a major determinant of PG1_2 levels in an organ or tissue. Further, estimation of PG1_2 generation by a tissue may be misleading if only 6-kPG1_2 is measured. (Supported by Memphis Heart Assoc. American Heart Assoc. 77-387, and USPHS - HL - 18845-78H-0235).


Purified human neutrophils were incubated with 14C-arachidonic acid in the presence and absence of latex particles, zymosan granules, sodium fluoride and ionophore A23187 for 15 minutes at 37°C. Cell and medium extracts were chromatographed in four different solvent systems in parallel with reference standards. A23187 (5 µg/ml) and sodium fluoride (20 mM) increased the Incorporation of 14C radioactivity into 1,6-hydroxy-6,8,11,14-eicosatetraenoate (ET-HETE) 30-fold and into 12-L-hydroxy-5,8,10,14-eicosatetraenoate (12-HETE) three fold. Latex particles (particle:cell ratio 50:1) increased 12-HETE radioactivity three fold but did not affect 5-HETE radioactivity. Several of the stimuli also increased thromboxane A_2 biosynthesis, indomethacin and eicosatetrayclic acid (ETYA) did not inhibit the formation of TxA_2. ETYA completely inhibited 12-HETE formation. These results suggest that both the lipoxigenase and cycloxygenase pathways may be important in the modulation of neutrophil stimulation. Support: PHS T32 AI-001012.
Deoxyadenosine (pM)

Fig. 1. Inhibition of thymidine incorporation in vitro by 2'-deoxyadenosine (dAdo) plus 20 μM 2'-deoxycoformycin (DCF). (♀) Normal bone marrow mononuclear cells. (♂) Pre-B-ALL cells from patient AK. (△) T-ALL cells from patient DR. Cells were incubated for 20 hr with dAdo plus DCF, then incubated for 2 hr with [3H]-thymidine. The control value is for cells incubated without dAdo. DCF had no effect by itself. Results are means of 3 determinations ± SD. These graphs were used to obtain the LD₅₀, which is the concentration of dAdo corresponding to 50% of control incorporation.

Table 1. In Vitro Sensitivity of Leukemic Cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number of Patients</th>
<th>LD₅₀ (μM dAdo)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-ALL</td>
<td>4</td>
<td>0.8 ± 0.2</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>Pre-T-ALL</td>
<td>2</td>
<td>0.9 ± 0.1</td>
<td>0.8–0.9</td>
</tr>
<tr>
<td>T-cell Lymphoma</td>
<td>1</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Pre-T-cell Lymphoma</td>
<td>1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>T-CLL</td>
<td>2</td>
<td>0.8 ± 0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Pre-B-ALL</td>
<td>4</td>
<td>5.7 ± 4.9</td>
<td>2–12.5</td>
</tr>
<tr>
<td>Pre-B-ALL</td>
<td>2</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>B-cell Lymphoma</td>
<td>1</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>B-CLL</td>
<td>1</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>Null-ALL</td>
<td>30</td>
<td>8.1 ± 6.3</td>
<td>0.8–20</td>
</tr>
<tr>
<td>Null-ALL</td>
<td>12</td>
<td>&gt;20</td>
<td></td>
</tr>
<tr>
<td>AML, ANLL, AMoL, CML</td>
<td>3</td>
<td>7.8 ± 2.5</td>
<td>5.1–10</td>
</tr>
<tr>
<td>AML, ANLL, AMoL, CML</td>
<td>6</td>
<td>&gt;20</td>
<td></td>
</tr>
</tbody>
</table>

The sensitivity of leukemic cell specimens to in vitro incubation with deoxyadenosine plus 20 μM deoxycoformycin is indicated by the LD₅₀, the concentration of deoxyadenosine which gives 50% of control [3H]-thymidine incorporation. Some specimens have been listed separately because their LD₅₀ values were greater than 20 μM, the highest concentration of deoxyadenosine employed.

TdT−, and who had a bone marrow and CNS relapse 45 mo later while on treatment) was similar to the T-leukemic cells in its high sensitivity to deoxyadenosine, while the other specimens of null cell leukemia exhibited intermediate or low sensitivity. This appears to reflect heterogeneity within the null cell grouping.

**Accumulation of ¹⁴C-deoxyATP and ATP Depletion**

When leukemic cells were incubated in vitro with ¹⁴C-deoxyadenosine and deoxycoformycin, radioactive deoxyATP was formed intracellularly. Leukemic cells with very low values of LD₅₀ (including every T-cell specimen and one null cell specimen) accumulated the highest concentrations of ¹⁴C-deoxyATP per cell (Fig. 2A). Leukemic cells that were less sensitive to deoxyadenosine accumulated lower concentrations of ¹⁴C-deoxyATP per cell. The correlation coefficient for the fit of Fig. 2A to a straight line is −0.730 and for the fit to a hyperbola is 0.634. The absolute amount of ATP per cell varied 2–4 fold in a manner that was not highly correlated with LD₅₀ or cell type (Fig. 2B, correlation coefficient = 0.550 for the fit to a straight line).

However, there was a greater depletion of ATP following incubation with deoxyadenosine plus deoxycoformycin in the sensitive leukemic cell specimens compared to the less sensitive cell specimens (Fig. 2C, correlation coefficient for the fit to a straight line = 0.924). The value of the ratio of μCi of ¹⁴C-deoxyATP divided by μmole of ATP is affected by both the accumulation of deoxyATP and the depletion of ATP.³ For all 31 leukemic cell specimens studied, there was a definite correlation between the LD₅₀ and the ratio of ¹⁴C-deoxyATP to ATP (Fig. 3, correlation coefficient for the fit to a straight line = 0.633, for the fit to a hyperbola = 0.853).

**Enzyme Activities**

To investigate the enzymatic basis for the different responses of leukemic cells to deoxyadenosine plus deoxycoformycin, the activities of several purine metabolizing enzymes were measured (Fig. 4A, B, C, D). Not every patient specimen was assayed for enzyme activity. The experimental variability in measurement and the heterogeneity of the leukemic cells with respect to the enzyme activities are shown by the scattering of the data points. Nevertheless, some generalizations can be made. In leukemic T cells, the specific activity of ADA was higher than that of most other cell types (Fig. 4A). However, one specimen of T-CLL and one of null-ALL accumulated high concentrations of deoxyATP and had low specific activities of ADA, showing that there may be a lack of correlation between these features. The correlation coefficient for the fit of Fig. 4A to a straight line is

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Fig. 2. Correlation between in vitro toxicity of dAdo and cellular nucleotides. (T) Cell specimen from T-ALL, pre-T-ALL, or T-CLL. (B) Cell specimen from pre-B-ALL. (N) Cell specimen from null-ALL (c) Other cell specimens including AML, CML. Toxicity is expressed by the LD_{50}. Nucleotides were measured by HPLC in extracts of cells that were incubated for 20 hr with ^{14}C-dAdo and DCF. (A) The relative amount of ^{14}C-deoxyATP per intact cell after incubation. (B) The relative amount of ATP per intact cell after incubation. (C) The ATP in cells incubated with dAdo plus DCF is expressed as a percentage of the ATP in cells incubated in medium alone.

The activity of ecto-ATPase was correlated with the ratio of ^{14}C-deoxyATP to ATP (Fig. 4B) with a correlation coefficient for the fit to a straight line of −0.642 and for the fit to hyperbola of 0.612. The activity of ecto-5'-nucleotidase was not correlated with either cell type or deoxyATP accumulation (Fig. 4C) as the correlation coefficient for the fit to a straight line is −0.067. A low degree of correlation was observed between deoxyATP accumulation and the activities of cytoplasmic nucleotidase (Fig. 4C) and of deoxyadenosine kinase with correlation coefficients for the fit to a straight line of −0.410 and −0.358, respectively.

Clinical Results

Two patients who were refractory to conventional therapy became candidates for experimental treatment with deoxycoformycin. The leukemic cells of both patients, one T-ALL and the other pre-B-ALL, were sensitive to deoxyadenosine plus deoxycoformycin inhibition of thymidine incorporation in vitro with LD_{50} values of 1.2 μM and 6.0 μM, respectively. However, the ratio of ^{14}C-deoxyATP to ATP in the T-ALL cells was 100 times higher than in the pre-B-ALL cells. Deoxycoformycin therapy resulted in a complete inhibition of adenosine deaminase in the cells of peripheral blood and bone marrow in both patients. The T-ALL patient responded with a decrease from approximately 3000 to 200 peripheral lymphoid cells/cu mm that was maintained for 24 days after the first course (total 80 mg/sq m), and a decrease from approximately 14,000 to 100 peripheral lymphoid cells that lasted for 15 days after the second course (40 mg/sq m). The pre-B-ALL patient responded with a decrease from approximately 2000 to 900 peripheral lymphoid cells/cu mm that lasted only 1 day (total dose 35 mg/sq m). There was a transient decrease in the number of granulocytes in both patients for several days.

DISCUSSION

The sensitivity of a given type of leukemic cell to deoxyadenosine plus deoxycoformycin as measured by the in vitro inhibition of DNA synthesis and in vivo cell
lation is correlated with the accumulation of high concentrations of deoxyATP within the leukemic cell. Furthermore, low activities of the cellular enzymes, ecto-ATPase and cytoplasmic purine 5'-nucleotidase, were found in cells that accumulated high concentrations of deoxyATP. With respect to all of these features, T-cell leukemias (5 of 5) form a unique, highly sensitive group. A single null-cell specimen (1 of 13) had properties similar to those of the T-cell specimens.

The other leukemic cell specimens had intermediate or low values of sensitivity to deoxyadenosine and accumulation of deoxyATP.

In 15 published reports of deoxycoformycin therapy, most, but not all, T-cell malignancies and a few null-cell and myeloid malignancies have responded to treatment.5-17 In those studies that included lymphoid malignancies of diverse immunologic cell types, variable responses were demonstrated among all types. A method for predicting which patient will respond to therapy would have potential clinical usefulness.

Studies of established human lymphoblastoid cell lines have shown that T-cell lines are much more sensitive than B-cell lines to the inhibition of growth by deoxyadenosine plus deoxycoformycin,24-30 and every null cell line tested had the same sensitivity as did the T-cell lines. The sensitivity of these cell lines to inhibition of growth was correlated with their ability to degrade intracellular deoxynucleotides,28,29 and their low activities of cytoplasmic purine 5'-nucleotidase and ecto-ATPase.22 The activity of ecto-purine 5'-nucleotidase was not correlated with sensitivity, as it was found to be high on some B-cell lines, low on other B-cell lines, low on T-cell lines, and high on sensitive null cell lines.24,28,30

The present study provides further evidence that T lymphoblasts are highly sensitive to deoxyadenosine plus deoxycoformycin in vitro and in vivo. It is of interest to note that pre-T-ALL, T-ALL, and T-CLL lymphoblasts have equal sensitivity. This suggests that cells of T-cell lineage, regardless of the stage of differentiation and the degree of maturation, had a common biochemical response to deoxycoformycin plus deoxyadenosine. The sensitive leukemic cells accumulate high concentrations of deoxyATP, and this accumulation could be related to the low activity of ecto-ATPase.22 Previous work24,25 has demonstrated a much higher degree of correlation between deoxyATP accumulation and the activities of cytoplasmic nucleotidase and deoxyadenosine kinase than was observed here. Other studies have supported the generalization that the activity of ADA is higher and the activity of ecto-5'-nucleotidase is lower in T cells as compared to B cells and null cells, but heterogeneity with respect to these enzymes also has been reported for T, B, and null cells.31-38 In the present work the activities of ADA and ecto 5'-nucleotidase did not correlate with sensitivity to deoxyadenosine. Recently, it was reported that a case of null-ALL with a high activity of ecto-5'-nucleotidase responded to deoxycoformycin therapy.44

Certain specimens of null, pre-B, and myeloid leukemic cells had values of LD50 for inhibition of thymidine incorporation that were less than 8 µM, whereas
29. Wortmann RL, Mitchell BS, Edwards NL, Fox IH: Biochemical basis for the differential deoxyadenosine toxicity to T and B lymphoblasts: Role for 5'-nucleotidase. Proc Natl Acad Sci USA 76:2434, 1979
Biochemical correlates of the differential sensitivity of subtypes of human leukemia to deoxyadenosine and deoxycoformycin

SS Matsumoto, AL Yu, LC Bleeker, B Bakay, FH Kung and WL Nyhan