Biological inhibition of deoxycytidine kinase, deoxycoformycin (DCF), show different metabolic responses depending on the histologic and immunologic type of the leukemic cell specimens. The clinical correlation of the differential sensitivity was obtained from the LD50, the concentration of dAdo that caused 0.8-20% inhibition of the incorporation of 3H-thymidine into DNA in 20% or more of the leukemic blast cells. When 20% or more of the leukemic blast cells expressed a given marker, the leukemic specimen was judged to be positive for that marker.

In this study all leukemic specimens that were positive for one or more markers expressed these markers on at least 50% of the monoclonal antibody, 0KM1 (Ortho Pharmaceuticals, Raritan, N.J.). The expression of the five markers was correlated best with the accumulation of deoxyATP by the leukemic cells in the presence of 20sM deoxycoformycin and with 0-20sM deoxyadenosine. The accumulation of deoxyATP was not inhibited by 20sM adenosine deaminase and ecto-5'-nucleotidase. The clinical correlation of the differential sensitivity was observed between the LD50 and the ratio of the accumulation of deoxyATP by leukemic cells incubated in vitro with 14C-dAdo plus DCF. The correlation coefficient for the fit to a hyperbola was 0.853. The accumulation of deoxyATP by the leukemic cells was also not inhibited by 20sM adenosine deaminase and ecto-ATPase.
ENHANCED FORMATION OF THE POTENT ANTIPROLIFERATIVE PROTAGLANDIN (PGLa) BY AORCS OF THE SPONTANEOUSLY HYPERTENSIVE (SH) RAT. C. G. Pace-Asciak*, N. C. Carreras* and G. Ramaglia* (SPON: B. P. Schimmer), Research Institute, Hospital for Sick Children, Toronto, Ontario, M5G 1X8, CANADA.

We recently observed that PGLa has potent BP lowering properties, being twice as active as PGF2a in the normal rat and 3-8 times as active in the SH rat. However, PGF2a is inactive (2.5-10ng) after intracardiot and intr Jugular administration due to its complete lack of catalysis by the lungs. Incubation at 37° C for 1 hr of hepatic aorta of non-hypertensive and hypertensive rats in the absence and presence of PGE1, PGF2a and PGLa showed that the latter is more potent than PGE1 and PGF2a.

3. The activity of PGLa in the SH rat is inhibited by the PGE1 inducing agent, but is not affected by the PGE1 inhibiting agent, therefore, the latter is not likely to be involved in the HTG effect of PGLa.

4. The long lasting antihypertensive effect of PGLa is not due to a primary action of PGLa on the lungs, but rather to an effect of PGLa on the periphery. This is consistent with the finding that PGLa is a potent arteriolar dilator in the hypertensive rat, but not in the normal rat.

5. PGLa is a potent inhibitor of the release of vasoactive substances from the lung, which may explain its long lasting antihypertensive effect.

6. The long lasting antihypertensive effect of PGLa may be due to its ability to inhibit the release of vasoconstrictor substances from the lung, which may explain its long lasting antihypertensive effect.

7. PGLa is a potent inhibitor of the release of vasoactive substances from the lung, which may explain its long lasting antihypertensive effect.

8. The long lasting antihypertensive effect of PGLa may be due to its ability to inhibit the release of vasoconstrictor substances from the lung, which may explain its long lasting antihypertensive effect.

9. PGLa is a potent inhibitor of the release of vasoactive substances from the lung, which may explain its long lasting antihypertensive effect.
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 FK. (▲) 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>Mean ± SD</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>
<td></td>
</tr>
<tr>
<td>Pre-T-ALL</td>
<td>2</td>
<td>0.9 ± 0.1</td>
<td>0.8–0.9</td>
<td></td>
</tr>
<tr>
<td>T-cell Lymphoma</td>
<td>1</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T-cell Lymphoma</td>
<td>1</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-CLL</td>
<td>2</td>
<td>0.8 ± 0.2</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Pre-B-ALL</td>
<td>4</td>
<td>5.7 ± 4.9</td>
<td>2–12.5</td>
<td></td>
</tr>
<tr>
<td>Pre-B-ALL</td>
<td>2</td>
<td>&gt;20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell Lymphoma</td>
<td>1</td>
<td>&gt;20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-CLL</td>
<td>1</td>
<td>&gt;20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null-ALL</td>
<td>30</td>
<td>8.1 ± 6.3</td>
<td>0.8–20</td>
<td></td>
</tr>
<tr>
<td>Null-ALL</td>
<td>12</td>
<td>&gt;20</td>
<td></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>
<td></td>
</tr>
<tr>
<td>AML, ANLL, AMoL, CML</td>
<td>6</td>
<td>&gt;20</td>
<td></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.

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

**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. 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 hyperbola = 0.853).
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 \mu M and 6.0 \mu M, 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...
lysis 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. 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, 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, and their low activities of cytoplasmic purine 5'-nucleotidase and ecto-ATPase. 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.

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 ecto-ATPase. Previous work 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.

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. 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 deoxyribonucleoside 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