CONCISE REPORT

Effect of 2′-Deoxycoformycin on Erythroid, Granulocytic, and T-Lymphocyte Colony Growth

By M. T. Aye and J. V. Dunne

The finding of elevated intracellular levels of adenosine deaminase (ADA) in some patients with acute lymphoblastic leukemia has led to attempts to control this disease with the adenosine deaminase inhibitor 2′-deoxycoformycin (dCF). Because of clinical reports indicating its relative freedom from myelotoxicity, we have tested the effects of this drug on erythroid, granulocytic, and T-lymphocyte colony formation by normal marrow and peripheral blood cells. While clinically the drug has been found to be active at serum concentrations of approximately 10 μM, we have tested it at concentrations up to and including 1 mM. It was found that both erythroid and granulocytic colony growth was completely unaffected by 1 mM dCF, a concentration at least 2 magnitudes higher than that necessary to totally ablate intracellular ADA levels. T-lymphocyte colony growth was unaffected by 100 μM dCF, but at 1 mM some inhibition was observed. These findings therefore indicate that dCF, while able to cause leukemic cell lysis in vivo, has no inhibitory effect on the proliferative capacity of normal hematopoietic cells.

ADENOSINE DEAMINASE (adenosine amino-hydrolase EC 3.5.4.4.) (ADA) is an enzyme in the purine salvage pathway responsible for the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Recent interest in cellular ADA levels was aroused by the report of Giblett and colleagues, who found that some patients with severe combined immunodeficiency (SCID) also had an associated ADA deficiency. Investigations on the role of ADA in immune function have now revealed that (a) ADA levels are higher in T than in B lymphocytes, (b) human thymic cells contain at least 10 times more ADA than most other cells in the body, and (c) T-cell proliferation may be unduly susceptible to ADA inhibition. These findings led to studies of ADA in human acute leukemia, which showed that patients with acute lymphoblastic leukemia (ALL) and chronic myeloblastic leukemia in blast crisis had abnormally high levels of ADA in their leukemic cells. Subsequently, it was shown that patients with T-ALL had much higher ADA levels than patients with null cell ALL, and further, a positive correlation was also demonstrated between elevated ADA and terminal deoxynucleotidyl transferase (TdT) levels in patients with ALL.6,7

2′-Deoxycoformycin (dCF), an antibiotic nucleoside analog isolated from the culture broth of Streptomyces antibioticus, is a tightly binding transition state inhibitor of ADA with a K_i of approximately 2.5 × 10^{-12} M.8 It is now being tested as a possible chemotherapeutic agent in acute leukemia,9-13 has been reported to produce rapid lysis of leukemic cells with little or no associated myelosuppression. In one study it was reported that dCF, in combination with deoxyadenosine, inhibited DNA synthesis in leukemic but not in normal myeloid cells.14 In another study it was found that in vivo administration of dCF to mice did not reduce the number of spleen colony-forming units (CFU-S) in their marrow.15 These findings suggest the possibility that dCF may be cytotoxic for leukemic but not for normal myeloid cells. However, we are not aware of any reported study on the effects of dCF on normal human myeloid or lymphoid stem cells. In this article we report results of our study on the effects of dCF on erythroid, granulocytic, and T-lymphocyte colony-forming cells from human marrow and blood.

MATERIALS AND METHODS

Marrow was aspirated from the sternum or iliac crest of patients undergoing routine hematologic investigations. Peripheral blood was obtained from normal volunteers. All samples were collected in heparin or ethylene bis (ethylene oxynitriilo) tetraacetic acid (EGTA) and mononuclear cells obtained by centrifugation on a cushion of 60% Percoll (Pharmacia, Uppsala, Sweden) as previously described.16 2′-Deoxycoformycin (Pentostatin) was donated by Dr. M. L. Black of Warner-Lambert, Parke Davis Company, Ann Arbor, Mich. It was dissolved in tissue culture medium at a concentration of 10 mM/liter.

Erythroid and granulocytic colony growth was obtained using methods previously described.17 T-lymphocyte colony-forming cells in marrow and blood were assayed using a method recently developed in this laboratory and to be described in a forthcoming paper. Briefly, mononuclear cells were prepared for plating in 3-ml aliquots, each containing 0.4 ml human plasma, 0.3 ml of a 10%
bovine serum albumin solution, 1 ml of 1% agar (Difco, Detroit, Mich.) made up in medium, 0.3 ml of appropriately diluted cells, and the balance made up with medium. The cultures also contained 5% leucocyte-conditioned medium (LCM), 0.1% phytohemagglutinin (PHA; Burrough Wellcome HA 15), and 100 ng/ml 12-0-tetradecanoyl phorbol 13-acetate (TPA; Consolidated Midland Chemicals, Brewster, N.Y.). The tissue culture medium used in these experiments was modified Dulbecco’s Essential Medium (Gibco H21, Grand Island, N.Y.) prepared according to a method described by Guilbert and Iscove. LCM was prepared using 1% PHA as previously described. Cultures were plated in 35-mm plastic Petri dishes, 1 ml/plate, and incubated for 7 days in a humidified CO2 incubator; colonies were scored with an inverted microscope and all aggregates containing in excess of 50 cells were each counted as a colony. Under these conditions there is total abrogation of erythroid and granulocytic colony growth, and the T-cell nature of the colonies has been confirmed using rosetting techniques and with the OKT3 monoclonal antibody.

RESULTS

2'-Deoxycoformycin, obtained from the manufacturer, was greater than 95% pure as determined by ultraviolet spectrophotometry. We further confirmed its activity, using differential spectrophotometry, by demonstrating its ability to inhibit the ADA (partially purified from calf intestine; Boehringer Mannheim) catalyzed conversion of adenosine to inosine.

The effect of varying doses of dCF on myeloid and T-lymphocyte colony formation by marrow and peripheral blood cells is shown in Tables 1 and 2. The results show that myeloid colony growth was unaffected by dCF up to a concentration of 1 mM. For T-lymphocyte colony growth, variable inhibition was observed only at 1 mM dCF. Dose–response curves using dCF at concentrations shown in Tables 1 and 2 have been repeated in 5 additional experiments, and the results obtained fully support the data shown here.

Other ADA inhibitors, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) and Coformycin, have been reported to inhibit PHA-induced mitogenesis of human peripheral blood mononuclear cells. Our results on the effect of dCF on PHA-induced mitogenesis of human peripheral blood mononuclear cells is shown in Table 3. The table also contains data from experiments where deoxyadenosine was added to dCF-containing cultures. The results show that dCF is not inhibitory for mitogenesis; however, in agreement with findings of other investigators, dCF was found to markedly potentiate the inhibitory effects of deoxyadenosine on mitogenesis.

DISCUSSION

The increasing use of dCF in clinical trials for the treatment of acute leukemia make it imperative to know its effect on hemopoietic stem cells. The investigations described here were primarily done to answer this specific question. We are not aware of any previous work where this question has been addressed; further, the recent development in our laboratory of a unique T-cell colony assay has made it possible to determine the effect of dCF, not only on myeloid progenitors, but also on lymphoid stem cells as well.

Our data clearly show that dCF has little or no effect on cell proliferation in myeloid and T-lymphocyte populations. The occasional inhibition of T-lymphocyte colonies observed at 1 mM may not be

Table 1. Effect of Deoxycoformycin on Myeloid Colony Formation

<table>
<thead>
<tr>
<th>dCF (μM)</th>
<th>Eryth</th>
<th>Gran</th>
<th>dCF (μM)</th>
<th>Eryth</th>
<th>Gran</th>
<th>dCF (μM)</th>
<th>Eryth</th>
<th>Gran</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>237 ± 4</td>
<td>102 ± 9</td>
<td>2</td>
<td>207 ± 25</td>
<td>96 ± 10</td>
<td>2</td>
<td>695 ± 76</td>
<td>217 ± 11</td>
</tr>
<tr>
<td>1</td>
<td>280 ± 4</td>
<td>137 ± 3</td>
<td>3</td>
<td>376 ± 1</td>
<td>69 ± 2</td>
<td>3</td>
<td>758 ± 24</td>
<td>216 ± 22</td>
</tr>
<tr>
<td>2</td>
<td>255 ± 17</td>
<td>118 ± 4</td>
<td>4</td>
<td>387 ± 2</td>
<td>74 ± 4</td>
<td>4</td>
<td>727 ± 33</td>
<td>215 ± 23</td>
</tr>
<tr>
<td>5</td>
<td>226 ± 9</td>
<td>132 ± 15</td>
<td>5</td>
<td>414 ± 14</td>
<td>77 ± 4</td>
<td>5</td>
<td>850 ± 79</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>10</td>
<td>235 ± 29</td>
<td>117 ± 5</td>
<td>6</td>
<td>318 ± 2</td>
<td>31 ± 30</td>
<td>6</td>
<td>920 ± 54</td>
<td>115 ± 12</td>
</tr>
<tr>
<td>20</td>
<td>244 ± 24</td>
<td>121 ± 9</td>
<td>7</td>
<td>361 ± 27</td>
<td>53 ± 3</td>
<td>7</td>
<td>871 ± 45</td>
<td>176 ± 16</td>
</tr>
<tr>
<td>50</td>
<td>272 ± 20</td>
<td>107 ± 14</td>
<td>8</td>
<td>387 ± 14</td>
<td>77 ± 6</td>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>100</td>
<td>243 ± 10</td>
<td>116 ± 3</td>
<td>9</td>
<td>310 ± 16</td>
<td>85 ± 2</td>
<td>9</td>
<td>918 ± 14</td>
<td>166 ± 20</td>
</tr>
<tr>
<td>500</td>
<td>263 ± 4</td>
<td>126 ± 11</td>
<td>10</td>
<td>321 ± 22</td>
<td>57 ± 4</td>
<td>10</td>
<td>851 ± 23</td>
<td>176 ± 32</td>
</tr>
<tr>
<td>1,000</td>
<td>223 ± 8</td>
<td>103 ± 6</td>
<td>11</td>
<td>315 ± 13</td>
<td>60 ± 5</td>
<td>11</td>
<td>947 ± 25</td>
<td>140 ± 4</td>
</tr>
</tbody>
</table>

BM denotes bone marrow cells; Eryth denotes erythroid and Gran denotes granulocytic colonies. Cells were cultured with 1 U/ml and 5% LCM.
leukemic cells in vitro were able to accumulate dATP when incubated with dCF and deoxyadenosine; however, similar elevations of dATP could not be demonstrated when dCF was administered in vivo and the patient failed to respond to the drug. It is therefore possible that the differing sensitivity of cells to dCF-induced cytotoxicity may be dependent on their ability to accumulate dATP. Such differences may be responsible for dCF-induced toxicity in leukemic but not normal marrow cells.

Our observations on the relatively insignificant effect of dCF on T-lymphocyte colony formation is, however, more difficult to explain. Lymphopenia has been consistently observed in animals or patients administered dCF, and in animal models, dCF has been shown able to cause immunodeficiency.28 One possible reason for this difference may lie in the fact that mitogen-stimulated lymphoid cell proliferation may not accurately reflect the proliferative regulatory mechanisms prevailing in vivo. Thus, while lymphocyte proliferation may be directly stimulated in vitro by mitogens, in vivo they may be dependent on stimuli originating from cells susceptible to the action of dCF. Regardless of the mechanisms involved, our results indicate that the lymphopenia observed must be due to effects other than the ability of dCF to directly inhibit T-lymphocyte proliferation. In support of this view it is now well recognized that some individuals with ADA deficiency do not have lymphopenia or immunodeficiency.29

In conclusion, we have demonstrated that cellular proliferation in myeloid and T-lymphocyte populations is not inhibited by dCF-induced ADA deficiency. If indeed human leukemic cell proliferation is susceptible to inhibition by dCF, as has been suggested from recent clinical reports, then our findings would indicate a selective cytotoxic effect of dCF on leukemic populations. In this respect, dCF would be unique among cytotoxic agents currently in use in having this property and indicate the need for further studies to elucidate the exact mechanisms by which dCF is able to kill leukemic but not normal cells.

ACKNOWLEDGMENT

The authors wish to thank Drs. R. K. Smiley, J. Drouin, and L. Huesch for allowing us to study their patients and Dr. G. Rock of the Canadian Red Cross, Ottawa Depot, for providing normal blood samples. The assistance of S. Pellerin, N. Biggs, B. Foss, W. Yang, and J. d. Plessis is also acknowledged.

REFERENCES

3. Carson DA, Kaye J, Seegmiller JE: Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphory-


Effect of 2'-deoxycoformycin on erythroid, granulocytic, and T-lymphocyte colony growth

MT Aye and JV Dunne