Recovery of 2’-Deoxycoformycin-inhibited Adenosine Deaminase of Mouse Erythrocytes and Leukemia L1210 in Vivo¹

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ABSTRACT

The antibiotic 2’-deoxycoformycin, a potent inhibitor of adenosine deaminase, has potential as a chemotherapeutic agent. Injection of 2’-deoxycoformycin i.v. (0.2 mg/kg) to mice bearing ascites L1210 leukemia cells completely inhibits adenosine deaminase in both erythrocytes and L1210 cells. The recovery of the enzymic activity is markedly different in the two tissues. The recovery is very slow in erythrocytes (13% in 48 hr), whereas 80% recovery occurs during the same time interval in L1210 cells. This marked difference in the recovery of the enzyme in different tissues may play a role in the pharmacological and chemotherapeutic behavior of this drug.

INTRODUCTION

DCF,² an antibiotic, isolated from culture broth of Streptomyces antibioticus (16) is a tightly binding inhibitor of the enzyme ADA, with a Kᵢ of about 2.5 x 10⁻¹² M (3). Our earlier studies with human erythrocytic hemolysates and partially purified human erythrocytic ADA revealed that it takes 25 to 29 hr for 50% reactivation of DCF-inhibited enzyme (4, 13). The reactivation of the inhibited enzymic activity in intact human erythrocytes and intact Sarcoma 180 cells in vitro was extremely slow (less than 10% in 48 hr), suggesting that the cell membrane plays a role in the inhibition of ADA by DCF (13). The use of ADA inhibitors, such as DCF, in combination with various adenosine analogs markedly increases the incorporation of the analogs into intracellular nucleotide pools as well as enhancing their chemotherapeutic effectiveness (2, 3, 5, 6, 8, 9). Of special interest is the report on Phase 1 clinical trials of DCF in patients with advanced cancer, currently under way in England. Patients treated with DCF have shown prolonged but reversible lymphopenia.³ Because of its possible use as an antineoplastic, as an antiviral, and as an immunosuppressive agent, DCF may soon be available in the United States for Phase 1 clinical trials. Therefore, it is important to examine the effects of DCF on ADA in vivo. This paper describes the differences in recovery of DCF-inhibited ADA in mouse erythrocytes and L1210 cells in vivo. A preliminary report of these findings has been presented (1).

MATERIALS AND METHODS

L1210 leukemia cells were maintained in CDF mice by i.p. injection of 10⁶ cells. On Day 5, the mice were treated with DCF (0.2 mg/kg) by injecting 0.2 ml drug solution in the tail vein. Controls were treated similarly with 0.9% NaCl solution. Animals were sacrificed at intervals by cervical dislocation. Ascites tumor cells were harvested by washing the peritoneal cavity with Hanks’ balanced salt solution and were counted by hemocytometer. Blood was collected in heparinized capillary tubes by retroorbital puncture.

Determination of ADA Activity in L1210 Cells and Erythrocytes. L1210 cell suspensions (10 ml) were centrifuged at 600 x g for 5 min. After the supernatant was carefully removed, the RBC present in L1210 cells were hemolyzed by adding 4 volumes of cold distilled water and vortexing for 30 sec. Isotonicity was adjusted by the addition of concentrated NaCl solution, and the supernatant was removed by centrifugation at 600 x g for 5 min. RBC-free L1210 cell pellet was suspended in 1.0 ml of Hanks’ balanced salt solution, the cell volume was determined by hematocrit, and the ADA activity was determined by measuring ammonia liberation due to deamination of adenosine as described earlier (13).

To determine ADA activity in erythrocytes, the cells were collected by centrifugation of blood at 600 x g for 5 min. Plasma and buffy coat were removed carefully and discarded. Erythrocytes were washed twice with 0.9% NaCl solution and then suspended in buffered medium containing 50 mm potassium phosphate, pH 7.4; 75 mm NaCl; 2 mm MgCl₂; 10 mm glucose; penicillin, 10,000 units/liter; and streptomycin, 10,000 μg/liter. After determination of hematocrit, the ADA was determined by measuring ammonia liberation (13).

RESULTS

Chart 1 presents the recovery of ADA activity in L1210 cells and erythrocytes after injection of DCF (0.2 mg/kg) in the tail vein of L1210 tumor-bearing mice. The enzymic activity was inhibited to 2 to 4% of initial activity in 2 hr or less (2 hr was the earliest point of ADA activity assay after DCF injection) and remained at this low level up to 8 hr in erythrocytes and 4 hr in L1210 cells. The activity then recovered slowly in erythrocytes to 8.8, 13.0, and 22.9% in

¹ This work was supported by ACS Grants IN-97B and CH-54 and National Cancer Institute Grant CA-18837.
² The abbreviations used are: DCF, 2’-deoxycoformycin (Covidarabin; 3-[2-deoxy-β-D-erythro-pentofuranosyl]-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]-diazepin-8-(R,R)-ol); ADA, adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4).
³ J. F. Smyth, personal communication.

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25, 48, and 72 hr, respectively. The recovery was significantly faster, however, in L1210 cells where 46.8 and 77.9% activity recovered in 25 and 48 hr, respectively. The enzymatic activity was not assayed in the 72-hr sample of tumor cells inasmuch as they were heavily contaminated with blood at that stage.

The total number of L1210 cells present at 0, 24, and 48 hr postinjection of DCF is presented in Table 1. The cell growth was similar in both the control (treated with 0.9% NaCl solution) and DCF-treated groups.

In a study to be published elsewhere, the long-term effects of inhibition of mouse whole blood ADA in vivo were examined. The enzyme was inhibited by a single i.p. dose of DCF (0.2 mg/kg), and enzymatic activity and other parameters were measured at intervals of 3 to 4 days. This study showed that it takes about 15 and 34 days to recover 50 and 100% ADA activity, respectively, in blood. No differences in weight, temperature, and leukocyte counts were observed between the DCF-treated and control group.

**DISCUSSION**

These studies illustrate in vivo differences in the recovery of DCF-inhibited ADA in 2 cell types, i.e., mouse erythrocytes and L1210 cells. The ADA activity was completely inhibited by injecting DCF (0.2 mg/kg) in the tail vein of L1210-tumor mice, and the recovery of the inactivated activity (units/ml packed cells) was followed in the erythrocytes and the leukemia cells at various time intervals. The recovery of the inhibited enzyme in erythrocytes was very slow, i.e., about 9 and 23% in 25 and 48 hr, respectively. Our in vivo studies of recovery of mouse erythrocytic ADA are in agreement with the in vitro studies with human erythrocytes. In the latter, the recovery was even slower (10% in 25 hr) (13). On the other hand, the recovery of the enzymic activity was rapid in L1210 cells in vivo (47 and 78% in 25 and 48 hr, respectively). The recovery in L1210 cells in vivo differed markedly from the recovery of the enzyme in intact Sarcoma 180 cells in vitro and in intact human erythrocytes in vitro (13), where recovery is very slow.

It has been hypothesized that the recovery of DCF-inhibited ADA might be slower in anucleated cells such as mature mammalian erythrocytes (which do not synthesize new protein) than in nucleated cells, i.e., L1210 cells where active protein synthesis occurs (13). In Sarcoma 180, which is a nucleated cell, the recovery was very slow in vitro (13). It should be noted that the conditions used in studies with Sarcoma 180 in vitro (13) were such that cells were not growing (and probably not synthesizing new protein), whereas in the present study L1210 cells were growing. It is evident from the cell numbers in both control and DCF-treated mice (Table 1) that their growth was slow and that the tumor was in its late stage of growth. It is possible that the recovery of enzymic activity might be even faster in exponentially growing cells. Confirmation of this speculation must await investigation of L1210 cells at different stages of growth and other actively growing normal tissues such as bone marrow. However, the observations of the present study are in agreement with the hypothesis above and further suggest the role of protein synthesis in the rapid recovery of DCF-inhibited cells. Other possibilities, such as the existence of differences in enzyme protein and membrane properties in these cell lines, cannot be ruled out. In studies to be documented elsewhere, we have found differences in the association rate constants ($k_1$) of DCF and ADA in mouse blood and L1210 cells. The importance of the cell membrane and the nucleoside transport system in the interaction of DCF and ADA in human erythrocytes has recently been emphasized (13, 14). Paterson et al. (11, 12) have indicated that differences occur in the nucleoside transport system among species and cell types. Therefore, an area of future interest is studies of effects of protein synthesis inhibitors and nucleoside transport inhibi-

### Table 1

<table>
<thead>
<tr>
<th>Time postinjection of drug (hr)</th>
<th>Control$^a$</th>
<th>DCF treated$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.50 $\times$ 10$^6$ ± 0.44$^b$</td>
<td>1.50 $\times$ 10$^6$ ± 0.44</td>
</tr>
<tr>
<td>24</td>
<td>3.09 $\times$ 10$^6$ ± 0.31</td>
<td>2.95 $\times$ 10$^6$ ± 0.25</td>
</tr>
<tr>
<td>48</td>
<td>3.34 $\times$ 10$^6$ ± 0.49</td>
<td>3.79 $\times$ 10$^6$ ± 0.64</td>
</tr>
</tbody>
</table>

$^a$ DCF in 0.2 ml (0.2 mg/kg) was injected in the tail vein. Similarly, controls were treated with 0.2 ml of 0.9% NaCl solution.

$^b$ Mean ± S.D. of 2 to 3 animals.
itators on the inhibition of ADA by DCF in mouse blood and L1210 cells.

Since the ADA inhibitors have demonstrated activity as potentiators of antitumor activity of adenosine analogs in a number of tumor lines (5, 9), as immunosuppressive agents (10), and as lymphocytotoxic agents in vivo (15), they are being considered for evaluation in clinical trials in the United States. However, the prolonged inhibition of ADA by a tight-binding inhibitor, DCF, observed in in vitro studies with human erythrocytes, Sarcoma 180 cells, and purified ADA (4, 13) has provoked questions concerning the usefulness of DCF in chemotherapy (2, 7). In this respect, the preliminary study presented here is important because it points out that significant differences occur from one tissue to another in vivo. An important consideration that must be borne in mind in future chemotherapeutic trials of DCF is that differences in tissue response may profoundly affect the pharmacological and chemotherapeutic behavior of this drug.

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REFERENCES

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