CONCISE REPORT

Inhibition of Adenosine Deaminase Activity Results in Cytotoxicity to T Lymphoblasts In Vivo

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We have treated a patient with refractory T-cell acute lymphoblastic leukemia with 2'-deoxycoformycin, a potent inhibitor of the enzyme adenosine deaminase. Inhibition of adenosine deaminase activity resulted in (1) an abrupt rise in plasma deoxyadenosine, but not adenosine, concentrations; (2) accumulation of deoxyadenosine triphosphate by lymphoblasts; (3) inhibition of the enzyme S-adenosylhomocysteine hydrolase; and (4) rapid lysis of the leukemic cells. The patient died suddenly 3 days after therapy was discontinued, and postmortem examination revealed a complete absence of leukemic cells in all organs. Pharmacologic inhibition of adenosine deaminase activity can result in the lysis of T lymphoblasts in vivo, and this effect appears to be mediated by deoxyadenosine.

HEREDITARY DEFICIENCY of the enzyme adenosine deaminase (ADA) is associated with a severe combined immunodeficiency disease characterized by lymphopenia, thymic involution, and defective T- and B-cell function.1 ADA catalyzes the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Although earlier studies focused on adenosine as a potential mediator of lymphocytotoxicity,23 more recent clinical and experimental observations suggest that deoxyadenosine may play a major role in the pathogenesis of the immune disorder.

In the absence of ADA activity, deoxyadenosine may be phosphorylated to dATP. Increased levels of this metabolite have been found in erythrocytes from ADA-deficient children2,5 and are associated with inhibition of DNA synthesis and cell death in human T lymphoblasts cultured in the presence of deoxyadenosine and an ADA inhibitor.5,8 In addition, deoxyadenosine has been shown to inactivate the enzyme S-adenosylhomocysteine hydrolase in vitro.9 S-adenosylhomocysteine hydrolase catalyzes the synthesis and hydrolysis of S-adenosylhomocysteine, and inactivation of this enzyme could lead to an accumulation of S-adenosylhomocysteine, a potentially toxic compound, in lymphocytes. Absence of S-adenosylhomocysteine hydrolase activity has also been demonstrated in erythrocytes from ADA-deficient children.10

We administered 2'-deoxycoformycin (dCF), a potent inhibitor of ADA activity, to a patient with refractory lymphoblastic leukemia of T-cell origin and achieved a dramatic lysis of the leukemic cells. We were thus able to monitor the biochemical events that were directly associated with toxicity for T lymphoblasts in vivo.

MATERIALS AND METHODS

Case Report

The patient was a 13-yr-old male who presented in October 1978, with a white blood count of 365,000/cumm (98% lymphoblasts) and a large anterior mediastinal mass. Surface marker studies on bone marrow lymphoblasts revealed 46% T cells. A complete remission was obtained with an induction regimen of prednisone, vincristine, and 1-asparaginase; prophylactic cranial irradiation (1800 rads) and intrathecal methotrexate were subsequently given. In May 1979, a testicular relapse occurred, and 86% T cells were present in a testicular biopsy. A marrow relapse was diagnosed in June and was followed by a rapidly rising peripheral lymphoblast count. The patient was then treated with vincristine and methotrexate, followed by 1-asparaginase. The white count fell to 1400, but within 3 days had risen to 284,000, 94% of which were lymphoblasts positive for T-cell surface markers. Leukapheresis was performed on two occasions prior to dCF administration, resulting in a reduction in the white count to 167,000/cumm. Leukapheresis was also performed on days 3–6 of therapy. Other medications at the time of this trial were prednisone (10 mg/day) and allopurinol (300 mg/day).

Source and Preparation of Drug

2'-Deoxycoformycin (Pentostatin) was obtained from the Parke-Davis division of Warner-Lambert, Detroit, Mich., after approval of our protocol by the Human Use Committee of the University of Michigan and by the Food and Drug Administration. Informed consent was given by the patient’s parents. The drug was reconstituted in 100 ml D2W containing 10 meq NaHCO3 (pH 8.2).

Enzyme Assays

Lymphoblasts were separated from heparinized venous blood on a Ficoll-Hypaque gradient and were washed 3 times in cold 10 mM Tris-150 mM NaCl, pH 7.4 (buffer A), and once in 10 mM Tris-140 mM NH4Cl to remove contaminating red cells. Lymphoblasts were

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RESULTS

Twenty-four hours after the first dose of 0.001 mg/kg (0.073 mg) dCF (Fig. 1A), red cell ADA activity had been inhibited by 97%, but the extremely high lymphoblast ADA activity was only inhibited by 20% (Fig. 1B and C). Consequently, we escalated the dose of dCF on a daily basis until the lymphoblast ADA activity was completely inhibited. Deoxycoformycin was then discontinued. As long as residual lymphoblast ADA activity was present, plasma deoxyadenosine levels remained undetectable (<0.1 μM). Twenty-four hours after the inhibition of residual lymphoblast ADA activity, however, the plasma deoxyadenosine concentration rose abruptly from <0.1 μM to 50 μM and reached a peak value of 104 μM on day 7 (Fig. 2A).

The rise in plasma deoxyadenosine was paralleled by an increase in lymphoblast dATP levels from 18 to 460 pmole/10^6 cells (Fig. 2B) and by complete inhib-
dition of lymphoblast S-adenosylhomocysteine hydrolase activity (Fig. 2C). Plasma adenosine levels, on the other hand, rose to only 2.1 μM on day 4 and were less than 0.1 μM on days 6–10 (data not shown). On day 7, the white blood count fell to 2200/μl with 53% lymphoblasts, and on day 9 was 200/μl with no lymphoblasts (Fig. 2D). A bone marrow aspirate revealed a paucity of normal hematopoietic precursors and no lymphoblasts.

The patient’s clinical status deteriorated following the rapid lysis of leukemic cells. Symptomatic hypocalcemia (4.6 mg/dl), hyperphosphatemia (14.5 mg/dl), and uremia (108 mg/dl) necessitated hemodialysis on day 7. The serum uric acid peaked at 10.5 mg/dl. Urinary output remained greater than 4 liters/day and the blood urea nitrogen (BUN) stabilized at 60 mg/dl after dialysis. The patient died suddenly on day 10. Postmortem examination revealed a complete absence of leukemic cells in all tissues, including the bone marrow, liver, spleen, and central nervous system. The immediate cause of death was acute pulmonary edema. The heart was normal. The kidneys showed acute tubular necrosis with early cellular regeneration. Crystalline deposits were found in the renal pelvis and tubules and were identified as xanthine based on a u.v. absorption peak at 269 nm and a shift in this peak to 291 nm (uric acid) with the addition of xanthine oxidase.

**DISCUSSION**

We have demonstrated that inhibition of ADA activity in the lymphoblasts of a patient with a highly proliferative T-cell acute lymphoblastic leukemia can result in rapid and complete lysis of the leukemic cells. The decline in white blood count was preceded by an increase in the plasma deoxyadenosine concentration and lymphoblast dATP levels, as well as by inhibition of the enzyme S-adenosylhomocysteine hydrolase. Plasma adenosine concentrations remained low. These data indicate that deoxyadenosine, rather than adenosine, mediated the toxicity to proliferating lymphoid cells of T-cell origin in vivo.

It has been postulated that the relatively high activity of deoxynucleoside kinase in lymphoid cells renders them uniquely susceptible to dATP accumulation, and hence to cell death, in the presence of increased circulating deoxyadenosine levels. It was thus hoped that an inhibitor of ADA might act as a “selective” chemotherapeutic agent for lymphoproliferative malignancies. The administration of dCF to six patients with acute lymphoblastic leukemia in England resulted in a partial response in two patients and rapid cell lysis and death in an additional patient with T-cell disease. No biochemical parameters were monitored in this study. We have treated a patient with ALL without surface markers with this drug and did not obtain a clinical response, but lymphoblast dATP levels remained low. It is becoming apparent that a true understanding of the effects of ADA inhibition in lymphoid malignancies can only be achieved through a careful correlation of clinical and biochemical data.

Although the etiologies of the renal failure and unexpected death of our patient were not fully elucidated, these events do raise major concerns about the potential toxicity of pharmacologic inhibition of ADA activity for other organs. Preliminary results from phase I clinical trials in this country now indicate that both pulmonary and renal toxicity may be associated with the use of dCF. The biochemical basis of damage to nonlymphoid cells remains to be clarified. One possibility is that very high plasma deoxyadenosine levels may cause a more generalized intracellular accumulation of dATP. Interference with the metabolism of S-adenosylhomocysteine or adenosine may also play a role. The documented response to dCF therapy in this patient should encourage further investigation into the use of this drug in refractory leukemias.

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**REFERENCES**

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