Therapeutic and Neurotoxic Effects of 2-Chlorodeoxyadenosine in Adults With Acute Myeloid Leukemia

By Linda Vahdat, Eric T. Wong, Michael J. Wile, Marc Rosenblum, Kathleen M. Foley, and Raymond P. Warrell, Jr

Despite expectations that 2-chlorodeoxyadenosine (2-CdA) would prove active primarily in lymphoproliferative diseases, early reports suggested unexpectedly high activity of this drug in heavily pretreated children with acute myeloblastic leukemia (AML) at a maximally tolerated dose of 8.9 mg/m²/day for 5 days. In view of these findings, we conducted an escalating dose trial of 2-CdA in adult patients with relapsed or resistant AML. Thirty-six patients who had received extensive prior therapy were treated at 9 dose levels of 2-CdA at daily doses ranging from 5 to 21 mg/m² for 5 days. 2-CdA eliminated leukemic blasts from the peripheral blood in 32 of 36 cases; however, bone marrow hypoplasia was seen only at daily dose levels ≥15 mg/m². We observed a total of 3 complete remissions: 1 at the 15 mg/m²/d dose level and 2 at the 21 mg/m²/d dose level; these responses persisted for 3, 2, and 3 months, respectively. Although prolonged myelosuppression would have been dose-limiting at 21 mg/m²/d for 5 days, the most important adverse effect was the development of a sensorimotor peripheral neuropathy. This reaction, whose onset was substantially delayed after completion of drug treatment, was observed in 2 of 5 patients at the 19 mg/m²/d level and in 4 of 4 evaluable patients at the 21 mg/m²/d level. Pathologically, this process was characterized by axonal degeneration and secondary demyelination. Other side effects included reactivation of a posttransplant Epstein-Barr virus-related lymphoma in 1 patient and tumor lysis syndrome. We conclude that the maximally tolerated dose of 2-CdA in adult patients (17 mg/m²/d for 5 days) is approximately twofold in excess of that previously reported in children and that the limiting toxic effect is a degenerative neuropathic disorder. We confirm that this drug has definite activity in AML, but the magnitude of this effect needs to be determined in larger numbers of patients who have received less extensive therapy. This agent deserves further evaluation in patients with both AML and acute lymphoblastic leukemia at these higher doses and perhaps as part of a preparative regimen for patients undergoing bone marrow transplantation.

© 1994 by The American Society of Hematology.

Recent studies have shown that 2-chlorodeoxyadenosine (cladribine; 2-CdA) has substantial activity in several lymphoid neoplasms, notably hairy cell leukemia, chronic lymphocytic leukemia, and low-grade lymphomas.\(^1\)\(^2\) 2-CdA is an adenosine deaminase-resistant purine analog that is taken up intracellularly and phosphorylated at the 5' position by deoxycytidine kinase (dCK). Incorporation of 2-chloro-2'-deoxyadenosine triphosphate into DNA terminates chain elongation by DNA polymerase β and causes single-stranded DNA breakage, inhibition of DNA repair, depletion of NAD, and ultimately cell death.\(^5\) 2-CdA is toxic to both dividing and nondividing cells.\(^6\) Lymphoid cells typically have higher levels of dCK activity than nonlymphoid cells and therefore were presumed to be more sensitive to the toxic effects of these halogenated deoxyribonucleotides.\(^7\)

Recently, Santana et al\(^8\) examined the clinical activity of 2-CdA in children with acute leukemia. Although no major responses were observed in patients with acute lymphoblastic leukemia (ALL), 2-CdA proved strikingly active in heavily pretreated children with acute myeloblastic leukemia (AML). In the initial study, 31 patients were treated with escalating doses of 2-CdA and antileukemic effects were observed in all patients treated with doses of 6.2 mg/m²/day for 5 days. One partial and two complete remissions were attained in patients with AML.\(^8\)

These findings were confirmed in a subsequent trial by this group in which 8 of 17 children (47%) with relapsed AML achieved complete remission using their previously defined maximum tolerated dose of 8.9 mg/m²/d.\(^9\) By contrast, only 1 of 7 patients with ALL achieved remission. Given these data in children and the drug's minimal hematologic toxicity, we initiated a study of 2-CdA in adults with myeloid leukemias.

**MATERIALS AND METHODS**

**Patients.** Patients with AML or chronic myelocytic leukemia (CML) in blast crisis who had failed to respond to conventional chemotherapy or who had relapsed after achieving remission were eligible for entry. Serum creatinine and bilirubin concentrations ≤2.5 mg/dL were required. Pretreatment evaluation included a bone marrow aspirate, complete blood count, and biochemical screening profile. There was no restriction on the amount of prior therapy. Signed informed consent was required, and the study was approved in advance by this center's Institutional Review Board.

**Monitoring during study.** Patients were monitored with frequent determinations of blood counts and serum biochemical screening profiles. The antileukemic response to 2-CdA was evaluated at two points. First, the ability of the drug to induce bone marrow hypoplasia without evidence of leukemic cells was evaluated by a bone marrow aspirate performed 10 to 14 days after completion of therapy. Second, remission status was evaluated by serial bone marrow aspirations performed every 7 to 14 days thereafter. Complete remission (CR) was defined as a cellular bone marrow containing less than 5% blasts, a peripheral leukocyte count greater than 3,000/μL, and a platelet count greater than 100,000/μL.

**Dosing schedule.** The prescribed daily dose of 2-CdA was diluted in 1,000 mL of normal saline solution and was administered as a continuous intravenous infusion over 24 hours for 5 days using an infusion pump. The initial dose level was 5 mg/m²/d, which approximated the total cumulative dose approved in the United States for the Developmental Chemotherapy and Leukemia Services, Department of Medicine, and the Departments of Neurology and Pathology, Memorial Sloan-Kettering Cancer Center, and the Cornell University Medical College, New York, NY. Submitted May 9, 1994; accepted July 12, 1994. Supported in part by Grant No. CA-05826 from the National Cancer Institute. Presented in part at the annual meeting of the American Society of Hematology, St Louis, MO, December 5, 1993. Address reprint requests to Raymond P. Warrell Jr, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology. 0006-4971/94/8410-0015$3.00/0

**Blood, Vol 84, No 10 (November 15), 1994: pp 3429-3434**
for hairy cell leukemia and which was about 40% of the effective dose in the antecedent pediatric AML studies. The daily dose was increased by 2 mg/m² increments until the maximally tolerated dose (MTD) was achieved. The MTD was defined as that dose that caused grade 2 neurologic toxicity or nonhematologic toxicity of grade 3 or 4 in more than 2 of 6 (or >30%) of patients.

At least 3 evaluable patients were entered at each dose level. Escalation to subsequent dose levels was continued until either a consistently effective antileukemic dose was observed and/or the MTD was reached.

Toxicity assessment. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria. After a drug-related neuropathy was suspected, nerve conduction studies were performed in selected patients treated at the highest dose levels. In addition, measurement of F-waves and H-reflexes were used to evaluate proximal nerve segments. Neuroradiologic studies were performed at autopsy in one individual who expired after experiencing a debilitating clinical neuropathy. We performed gross and microscopic examinations of the brain, spinal cord, and peripheral nerves. All immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tissues using an enzyme-labeled avidin-biotin system. Monoclonal antibodies to neurofilament (Signet, Dedham, MA) and myelin basic protein (DAKO, Carpinteria, CA) were used to show both axonal and myelin loss, respectively. Special stains, including luxol fast blue, Bielschowsky, and hematoxylin and eosin, were also performed.

RESULTS

Patients treated. Thirty-six patients entered the study whose relevant characteristics are shown in Table 1. As a group, these patients had received quite extensive prior therapy and had proved resistant to multiple drugs. Twenty-one patients (58%) had never achieved remission despite administration of multiple courses of therapy. Five patients had a prior myelodysplastic syndrome (MDS) and 4 patients had CML in blastic crisis. Only 7 patients (19%) were treated had a prior myelodysplastic syndrome (MDS) and 4 patients were also performed. All patients entered at each dose level (2, 7, 9, 11, 13, 15, 17, 19, 21 mg/m²/d) were treated in first relapse. The median number of prior treatment regimens was 2 (range, 1 to 10). Only 2 patients (at the 17 and 19 mg/m²/d dose levels, respectively) were observed for durations of 3, 2, and 3 months, respectively. There were no obvious similarities among the three complete responders with respect to French-American-British (FAB) subtype or their response to prior treatments.

2-CdA had no effect on the marrow in 3 of 4 patients with blastic CML even though they were treated at the higher dose levels (17, 19, and 21 mg/m²/d). In fact, the single patient who did not experience prolonged myelosuppression at the highest dose level had blastic CML.

Adverse effects. Acute toxic reactions to 2-CdA were minimal. One patient experienced mild nausea and headache during the infusion at the top dose level. However, in general, most patients experienced no gastrointestinal side effects. Mild-to-moderate tumor lysis syndrome was seen in 3 patients but resolved without sequelae. One patient who received 2-CdA at the 15 mg/m²/d dose level had reactivation of a posttransplant-associated Epstein-Barr virus (EBV) lymphoma 4 days after completion of the infusion. This problem regressed with acyclovir therapy. At the highest dose level, the myelosuppression was markedly prolonged in 4 of 5 patients with delayed recovery of the peripheral blood leukocyte count to ≥1,000/µL at 38, 47, and 60 days. One patient at the 19 mg/m²/d dose level took 44 days to recover.

Two patients died of fungal sepsis during a period of aplasia after 3 and 6 weeks from the start of therapy at the 15 and 21 mg/m²/d dose levels, respectively. Because of protracted myelosuppression and delayed-onset neurotoxicity, a planned “consolidation” course of 2-CdA could not be administered to the 2 patients who achieved CRs at the highest dose level. The other complete responder (at 15 mg/m²/d) was not consolidated because of postoperative complications after a cholecystectomy, during which time he re-
lapsed. A second induction course in this individual at the same dose level failed to recapture the clinical response.

Neurotoxicity. Six patients experienced severe motor weakness after treatment with 2-CdA: 4 who had received 21 mg/m²/d and 2 who had received 19 mg/m²/d. None of these individuals had received other concurrent therapy that was known to be neurotoxic. Four to 7 weeks after treatment with 2-CdA, severe leg weakness with inability to walk developed in all 6 patients. The weakness was more evident proximally than distally, and it was most severe in the lower extremities. Three patients reported sensory symptoms with paresthesia, dysesthesia, and numbness that were greater in the feet than in the hands. Serum levels of vitamin B₁₂, folate, creatine phosphokinase (CPK), and thyroxin were normal in the patients in whom these tests were performed. Two patients had a gadolinium-enhanced magnetic resonance imaging (MRI) performed of the spine that showed no abnormal meningeal or nerve root enhancement. One other patient underwent lumbar puncture that showed a slight elevation of cerebrospinal fluid protein (52 mg/dL) without abnormal cells; however, lumbar punctures were generally not performed because of thrombocytopenia. Electromyography (EMG) in 1 patient showed few low-amplitude polyphasic potentials without myopathic discharges in the proximal leg muscles. In 5 patients, nerve conduction velocities (NCVs) were mildly affected, but compound muscle action potentials (CMAPs) of the peroneal, posterior tibial, and sural nerves were severely diminished (Fig 1). F-waves and H-reflexes were absent or delayed in the legs. NCVs and CMAPs were minimally affected in upper extremities. Overall, these results strongly suggested an axonal peripheral polyneuropathy.

All but 1 of the 6 patients regained some motor function in their legs 8 to 10 weeks after onset of the motor weakness. One patient has required a walker to assist with ambulation, and a second patient has persistent paresthesias in the feet. One patient expired from sepsis before neurologic recovery. Because the onset of the neurotoxic symptoms occurred 4 to 7 weeks after completion of drug infusion, we reevaluated all patients who were not previously identified as having had neurotoxic symptoms and who had been treated at the 4 highest dose levels (ie, ≥15 mg/m²/d). Eight of 10 potentially evaluable patients were examined 4 weeks after completion of the infusion, and none manifested neurotoxic symptoms attributable to 2-CdA. (One patient was inevaluable secondary to ongoing peripheral neuropathy from pre-existing meningeal leukemia, and the other patient had expired before the 4 week follow-up point.)

Neuropathology. A postmortem exam was performed on the 1 patient who expired without neurologic recovery at day 42 and who had been treated at the 19 mg/m²/d dose level. This patient developed a progressive sensorimotor peripheral neuropathy that was prominent in both lower extremities. Pathologic examination (Fig 2A and B) showed a chronic peripheral neuropathy with active axonal degeneration that was most prominent distally (ie, in the sural nerve), with mild axonal degeneration present more proximally (in the femoral nerve and brachial plexus). There was no leukemic infiltration of the peripheral nerves; however, multiple granulocytic sarcomas were noted on the dura mater. The brain and spinal cord were otherwise unremarkable.

DISCUSSION

2-CdA has proven to be a major advance in the treatment of certain lymphoid neoplasms and it is now the drug of choice for initial therapy of hairy cell leukemia. In this dis-
ease, response rates exceed 90% and the majority of these responses are complete, occurring after only a single 7-day course of therapy.\textsuperscript{4,11} A considerably lower incidence of response (25% to 50%, mostly partial in magnitude) has been observed in patients with chronic lymphocytic leukemia, low-grade lymphoma, and Waldenstrom’s macroglobulinemia.\textsuperscript{12,13} The surprising degree of activity reported in pediatric AML prompted us to initiate a confirmatory trial in adults with this illness.

In an earlier experience, our group had shown that another halogenated adenosine derivative, fludarabine, also displayed quite high antileukemic activity in AML. Unfortunately, that benefit was more than offset by the development of marked neurotoxicity.\textsuperscript{14} Like 2-CdA, the neurotoxicity of fludarabine was substantially delayed in onset, occurring 3 to 6 weeks after cessation of treatment. However, the toxic neurologic effects of fludarabine were primarily central rather than peripheral in origin and the reactions began as cortical blindness that progressed to extensive demyelination and death. In view of this experience, along with reports that another group had encountered severe weakness after treatment with high doses of 2-CdA, we elected to begin our study at a dose of 5 mg/m\textsuperscript{2}/d for 5 days. This dose is approximately equivalent to the single-course cumulative dose used for treatment of hairy cell leukemia (0.1 mg/kg/d for 7 days). Thereafter, the dose levels were cautiously
2-CdA IN ADULT AML

escalated to avoid overlooking potentially delayed neurotoxic effects.

Unlike the experience in pediatric AML, we observed no substantial antileukemic effect at doses that were reported to be effective in children. However, substantial activity was noted at doses that were considerably in excess of the pediatric MTD. At the highest doses used in this study we observed prolonged myelosuppression (in excess of 8 weeks in some patients). Therefore, 21 mg/m²/d would have been in excess of the MTD based solely on this reaction and without the added toxic dimension of the neuropathy. At the highest dose levels, moderate-to-severe weakness was observed in most patients, and this effect was characterized by a peripheral axonal polyneuropathy.

Neurotoxic effects, predominantly cerebral dysfunction, have been associated with the use of other purine derivatives. In addition to the toxic CNS effects of fludarabine previously noted, deoxycoformycin, an inhibitor of adenosine deaminase, has been associated with both severe ocular toxicity as well as lethal encephalopathy. Adenine arabinoside, a compound closely related to fludarabine, has produced fatal CNS reactions preceded by tremors, myoclonus, dysarthria, and coma. However, recently, this class of drugs has also been implicated in a variety of peripheral neuropathies.

For example, high doses of the antiviral compounds 2',3'-dideoxycytidine (ddC), 2',3'-dideoxyinosine (ddI), and 2',3'-dideoxy-3'-deoxythymidine (d4T) have been associated with burning dysesthesias in the feet and loss of deep tendon reflexes in human immunodeficiency virus (HIV)-infected patients; however, only about 2% of these patients have reported frank motor weakness. NCV/EMG studies on some of these individuals confirmed the presence of an axonal sensorimotor peripheral neuropathy. Nevertheless, it was unclear whether these agents themselves caused a de novo peripheral neuropathy or whether they simply exacerbated a pre-existing asymptomatic HIV-related problem.

In a trial at the Scripps Clinic, 2-CdA at a dose of 0.4 to 0.5 mg/kg/d for 7 to 14 days (roughly equivalent to 20 to 50 mg/m²/d for 5 days) was used as part of a conditioning regimen that included cyclophosphamide and total body irradiation. Of 31 patients with resistant leukemia or lymphoma, 12 patients (39%) developed delayed neurologic symptoms, including 11 who manifested paraparesis and 1 who developed quadraparesis. Although a cause and effect relationship was suspected, various clinical circumstances suggested that those symptoms might not be solely attributable to the drug. Our data indicate that 2-CdA itself is capable of inducing these effects.

The mechanism of 2-CdA–induced axonal toxicity is unclear. The axonal damage has been presumed to be a delayed toxic effect of the agent or its metabolites, a phenomenon known as “coasting” in the toxic neuropathies. Both adenosine deaminase and 5' nucleotidase are present in neuronal tissue, however, the distribution and activities of these enzymes are unknown in motor and sensory neurons. Although 2-CdA may block neuronal DNA and RNA synthesis, this effect may not be the only mechanism responsible for axonal damage. Patients with niacin deficiency also have depleted nicotinamide (NAD) stores and are known to develop a polyneuritis. Depletion of NAD, a cofactor necessary for DNA repair, also occurs with 2-CdA. Thus, drug-induced depletion of NAD may also have contributed to our observations (and may suggest a future method of amelioration, if relevant).

Given the severity of the neuropathic disorder, we have designated 17 mg/m²/d for 5 days as maximally tolerable for future studies of this drug in AML. However, we note that this dose is substantially higher (ie, almost twofold) than the dose reported effective in children. Moreover, an ongoing pediatric study at this center also confirms that children can tolerate these substantially higher doses of 2-CdA (Dr C. Tan, personal communication, September 1994). It is conceivable that the surprising lack of activity in pediatric ALL may perhaps be explained by inadequate dosing.

The reason for the discrepancy in therapeutic effects in AML is unclear. Similar to the pediatric study, adult patients in this study had received extensive prior therapy and most had proved resistant to many other drugs. Another possible explanation would be that the drug formulation used in this study (and now approved in the United States) differs from that used by the St Jude’s group (which was formulated locally). Nonetheless, we confirm that 2-CdA is an active agent in patients with AML, a finding that agrees with recent in vitro sensitivity studies using freshly aspirated human myeloblasts. The degree of this therapeutic activity must now be determined in disease-oriented studies encompassing larger numbers of patients who have received less extensive treatment. Finally, the immunosuppressive activity of 2-CdA suggests that this agent may also be useful for incorporation into conditioning regimens of patients with leukemia who are undergoing bone marrow transplantation.

ACKNOWLEDGMENT

We are indebted to the R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ) for supplying the drug used in this study.

REFERENCES

8. Santana VM, Mirro J, Harwood FC, Cherrie J, Schell M, Kal-


Therapeutic and neurotoxic effects of 2-chlorodeoxyadenosine in adults with acute myeloid leukemia

L Vahdat, ET Wong, MJ Wile, M Rosenblum, KM Foley and RP Jr Warrell