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Antileukemic and immunosuppressive activity of 2-chloro-2′-deoxyadenosine*

(deoxyadenosine/chemotherapy/adenosine deaminase)

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ABSTRACT **The adenosine deaminase-resistant purine deoxynucleoside 2-chloro-2′-deoxyadenosine (CdA) is markedly toxic *in vitro* to nondividing and proliferating normal human lymphocytes and to many leukemia cell specimens. The CdA is also effective against mouse L1210 leukemia *in vivo*. The present investigations have examined the pharmacology, chemotherapeutic activity, and toxicity of CdA in nine patients with advanced hematologic malignancies refractory to conventional therapy. When administered by continuous intravenous infusion, the deoxyadenosine analog was well tolerated. As monitored by radioimmunoassay, plasma CdA levels rose gradually during the infusions. The CdA was not deaminated significantly. In all patients with leukemia, the CdA lowered the blast count by at least 50%. In one patient with a T-cell leukemia-lymphoma, and in another patient with chronic myelogenous leukemia in blast crisis, the CdA infusion eliminated all detectable blasts from the blood and bone marrow. In a patient with a diffuse lymphoma complicated by severe autoimmune hemolytic anemia, CdA treatment quickly terminated the hemolytic process. Bone marrow suppression represented the dose-limiting toxicity, and was related to plasma CdA levels, cumulative drug dosage, and the rapid release of CdA that accompanied tumor cell lysis.**

A genetic deficiency of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) produces combined immunodeficiency disease in humans (1). The profound lymphopenia observed in affected children has been attributed to the toxic effects of deoxyadenosine (2–7). These findings have prompted the development of adenosine deaminase inhibitors and deoxyadenosine congeners resistant to deamination, in the hope that these agents might possess antileukemic and antilymphocyte activity (8, 9).

Several studies have now shown that the high-affinity adenosine deaminase inhibitor deoxycoformycin can increase plasma deoxyadenosine levels in patients with malignant disease, with resulting toxicity to normal and neoplastic lymphocytes (10–16). In an earlier study, we identified a deoxyadenosine congener, 2-chloro-2′-deoxyadenosine (CdA; see Fig. 1) (17), that was toxic to human lymphoblastoid cell lines at concentrations in the nanomolar range (8). The CdA was not deaminated significantly, but instead was actively phosphorylated by deoxycytidine kinase (EC 2.7.1.74), converted to the 5′-triphosphate derivative, and incorporated into DNA (8, 18). The CdA was also an effective agent for the treatment of murine L1210 lymphoid leukemia *in vivo* (18). Notably, *in vitro* it was exquisitely toxic to normal resting peripheral blood lymphocytes and to slowly dividing malignant T cells. This ability to kill nondividing lymphocytes distinguished CdA from other commonly used chemotherapeutic agents affecting purine and pyrimidine metabolism (18, 19).

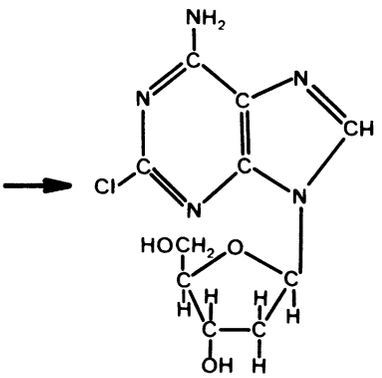

FIG. 1. Structure of CdA. The substitution of chlorine for hydrogen in the 2 position of the purine ring, indicated by the arrow, renders the compound insensitive to adenosine deaminase.

We also tested the anti-proliferative effects of CdA toward more than 40 bone marrow specimens from patients with acute leukemia, as compared to 20 normal bone marrow specimens (18). The deoxynucleoside inhibited the proliferation and survival of many leukemia cells at concentrations that did not significantly impair spontaneous thymidine uptake or granulocyte–monocyte colony formation by normal bone marrow cells.

On the basis of (*i*) the marked toxicity of CdA toward many human malignant hematologic cell lines but not toward cell lines derived from solid tissues, (*ii*) the efficacy of the drug in the L1210 leukemia system, and (*iii*) the toxicity of the deoxynucleoside toward fresh human leukemia cells at concentrations that did not similarly suppress bone marrow colony formation, we initiated CdA toxicity studies in primates and a Phase I clinical trial in patients with intractable hematologic malignancies. The results of these investigations indicate that the deoxyadenosine analog has significant antileukemic and immunosuppressive activity.

MATERIALS AND METHODS

Synthesis of CdA. CdA was prepared as a single lot by transfer of the deoxyribose moiety from thymidine to 2-chloroadenine, catalyzed by a partially purified transdeoxyribosylase from *Lactobacillus helveticus* (8, 18). The drug was isolated by ion-exchange chromatography as described (18). Purity was confirmed by spectral analysis, melting point, and both thin-layer chromatography and high-performance liquid chromatography. The CdA was sterilized by ultrafiltration and stored at −20°C as a 1 mg/ml solution in isotonic saline. Under these conditions there was no detectable breakdown of the deoxynucleoside over 1 month. The CdA was nonpyrogenic in rabbits and was free of bacterial or

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Abbreviation: CdA, 2-chloro-2′-deoxyadenosine.

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fungal contamination, as determined by routine microbiological culture.

Radioactive CdA was prepared by transfer of the deoxyribose moiety from thymidine to 2-chloro[8-^{3}H]adenine. The latter compound had been prepared from 2-chloro[8-^{3}H]-adenosine (11 Ci/mmol, Moravek Biochemicals, City of Industry, CA; 1 Ci = 37 GBq) as described (18).

Radioimmunoassay of CdA. 2-Chloroadenosine (Sigma) was coupled to bovine serum albumin, as described by Erlanger *et al.* for adenosine (20). Two rabbits received two injections of the conjugate emulsified in complete Freund's adjuvant, at 3-week intervals. One week after the last injection, the animals were bled, and the sera were pooled and stored at −20°C.

In the radioimmunoassay for CdA, increasing concentrations of CdA were added to 400-μl aliquots of normal human plasma or the plasma from CdA-treated patients. Then 100 μl of a 1:66 dilution of anti-CdA antibody in phosphate-buffered saline (0.01 M phosphate, 0.15 M sodium chloride pH 7.4), containing 2% normal rabbit serum, was added to each tube. Control tubes contained normal rabbit serum only. After 1-hr incubation at 37°C, 100 μl of [8-^{3}H]CdA (10,000 cpm) was added to each tube. One hour subsequently, the tubes were placed on ice, and 0.6 ml of saturated ammonium sulfate solution was added to each sample. Thirty minutes later, the tubes were centrifuged (1000 × *g*, 30 min, 4°C), washed once in 50% saturated ammonium sulfate, and recentrifuged. The resulting precipitates were dispensed into scintillation fluid and their radioactivities were measured in a spectrometer.

To minimize fluctuations in the measurement of CdA levels in the plasmas of patients receiving the drug, pretreatment plasma from each individual subject was used as a diluent to construct a standard curve. Plasma CdA levels were derived by extrapolation from the standard curves that were included in each assay.

***In Vitro* Sensitivity of Leukemia Cells to CdA.** Fresh leukemic cells dispersed at a density of 10^6 per ml in RPMI 1640 medium supplemented with 2 mM L-glutamine and 20% autologous plasma were incubated with increasing doses of CdA or 9-β-D-arabinofuranosylcytosine ("cytosine arabinoside"; cytosine arabinonucleoside) for 3 days. At that time, cell viability was estimated microscopically by the trypan blue exclusion method (19).

Phase I Trial. The Phase I treatment with CdA was offered to patients with far-advanced hematologic malignancies who had exhausted all conventional modes of therapy. The protocol was approved by the Human Subjects Review Committee and by the General Clinical Research Center Advisory Committee at the Scripps Clinic and Research Foundation. Informed and voluntary consent was obtained from each patient prior to initiation of the experimental treatment.

Patients were hospitalized in the General Clinical Research Center. The CdA (1 mg/ml in isotonic saline) was administered by continuous intravenous infusion at dosages of 0.1–1.0 mg/kg per day for 5–14 days. Plasma CdA levels were monitored by radioimmunoassay. Complete blood counts, platelet counts, and urinalysis were performed daily. Liver and kidney function, serum electrolytes, blood clotting parameters, and uric acid were measured every other day. Other medications were administered as dictated by the patients' clinical status. Some patients, in whom neither a therapeutic response nor a toxic response was demonstrable after the initial CdA infusion, received a second or third course of CdA with a 0.1 mg/kg per day increment in dosage.

RESULTS

Pharmacokinetics of CdA. A radioimmunoassay was developed for the estimation of CdA levels in plasma (Fig. 2). The rabbit anti-CdA antiserum did not react appreciably with other purine bases or nucleosides. When autologous pretreatment plasma was used as a diluent, the radioimmunoassay detected CdA at concentrations ≥5 nM.

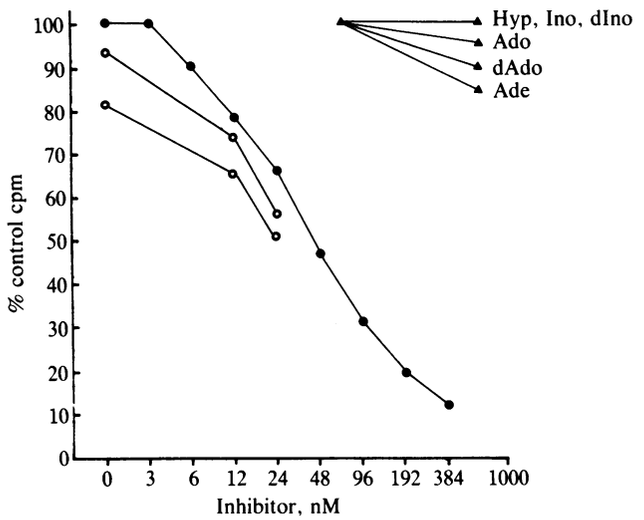

FIG. 2. Radioimmunoassay of CdA in plasma. CdA at increasing concentrations was added to duplicate specimens of normal plasma (●) or the plasmas from two patients treated with CdA at 0.1 mg/kg per day (○). After the addition to each sample of rabbit anti-CdA antibody and 10^4 cpm of [8-^{3}H]CdA, antibody-bound CdA was precipitated and the radioactivity in the pellet was measured. % control cpm = [(cpm in pellet with additional CdA)/(cpm in pellet without additional CdA)] × 100. As shown, 1 μM concentrations of hypoxanthine (Hyp), inosine (Ino), deoxyinosine (dIno), adenosine (Ado), deoxyadenosine (dAdo), and adenine (Ade) did not inhibit significantly the CdA–anti-CdA antigen–antibody reaction.

In general, increasing dosages of CdA given via continuous intravenous infusion yielded increasing plasma concentrations. For uncertain reasons that may relate to baseline renal tubular function or to tumor burden, the plasma levels varied up to 3-fold among patients who received the same dosage. In each subject, the plasma CdA concentrations rose gradually during the drug infusion. At the time of rapid tumor cell lysis, plasma CdA levels increased abruptly (Fig. 3 *Upper*). With termination of the CdA infusion, the nucleoside was cleared from the plasma over a period of 1–3 days.

The fractionation of deproteinized plasma from CdA-treated patients by high-performance liquid chromatography revealed primarily CdA, with lesser amounts of 2-chloroadenine (results not shown). Chromatographic analysis of spot urine specimens taken during treatment yielded basically similar results. No deaminated products of the drug were detected.

Antileukemic and Immunosuppressive Effects of CdA. Nine patients with far advanced hematologic malignancies received CdA at 0.1–1.0 mg/kg per day by continuous intravenous infusion for 4–14 days (Table 1). The drug was well tolerated. Only the patient who received the highest dosage experienced significant nausea and vomiting during the infusion. None had drug-related fevers or changes in sensorium.

Several of the patients who participated in the protocol had received prior chemotherapy with cytosine arabinonucleoside. As illustrated in Fig. 3 *Lower*, leukemic cells resistant to cytosine arabinonucleoside remained sensitive to the *in vitro* toxic effects of CdA.

In all patients with leukemia, the CdA lowered the peripheral blood blast count by at least 50%. Responses were observed at various dosages from 0.1 to 1.0 mg/kg per day. In one patient with a refractory T-cell leukemia-lymphoma and in one patient with chronic myelogenous leukemia in blast crisis the CdA eliminated detectable malignant cells from the blood and bone marrow (Fig. 3 *Upper*). Unfortunately, both

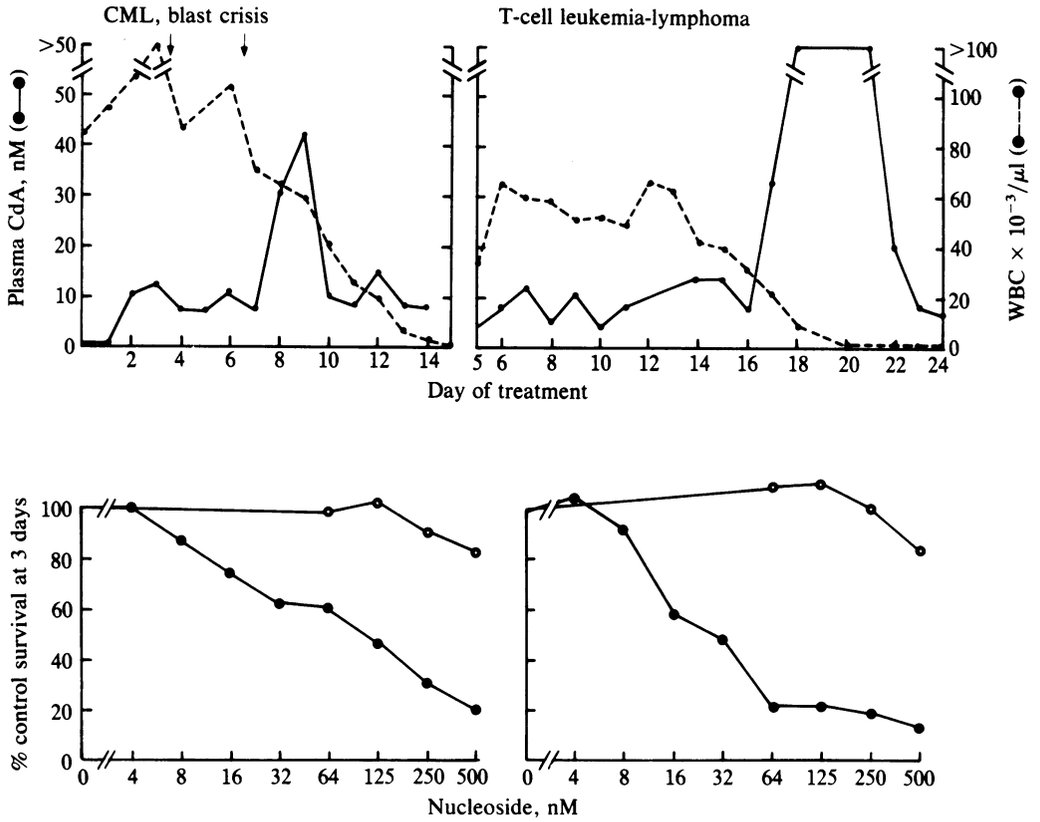

FIG. 3. Effects of CdA infusions in two patients with advanced hematologic malignancies. A patient with chronic myelogenous leukemia in blast crisis (CML, *Upper Left*) and a patient with refractory T-cell leukemia-lymphoma (*Upper Right*) received CdA at 0.2 mg/kg per day for 7 days and 14 days, respectively. The CML patient also received intermittent hydroxyurea (arrows). In both cases a precipitous fall in the peripheral blood blast (WBC) count (●---●) was followed by a massive rise in plasma CdA levels (●—●). (*Lower*) *In vitro* toxicity of CdA (●) and cytosine arabinonucleoside (○) toward the leukemia cells from these two patients.

Table 1. Effects of CdA infusion in a Phase I trial

Diagnosis	CdA dose, mg/kg per day	Response	Toxicity
Acute nonlymphocytic leukemia	1.0	Decrease in blast count	Bone marrow suppression*
Plasma cell leukemia	0.1	Decrease in blast count	Bone marrow suppression*
Immunoblastic sarcoma	0.1	None	None
Diffuse histiocytic lymphoma	0.1–0.5	None	Bone marrow suppression
Lymphocytic lymphoma	0.1–0.3	Hemolytic anemia abated	Bone marrow suppression
Chronic lymphocytic leukemia	0.1–0.3	Decrease in lymphocyte count	Bone marrow suppression*
T-lymphocyte leukemia-lymphoma	0.1–0.3	Disappearance of tumor	Bone marrow suppression*
Chronic myelogenous leukemia in blast crisis	0.1	Decrease in blast count	None
Chronic myelogenous leukemia in blast crisis	0.1–0.15	Loss of detectable tumor	Bone marrow suppression

A decrease in blast count = >50% reduction in the number of blasts in the peripheral blood during or subsequent to the infusion.
*These patients required erythrocyte and platelet support prior to therapy, making bone marrow suppression difficult to evaluate.

patients succumbed from opportunistic infection during the prolonged post-treatment aplastic phase, during which, however, leukemia cells did not reappear despite the fact that CdA therapy had been discontinued. Postmortem examination revealed no microscopic evidence of tumors.

One patient with a diffuse histiocytic lymphoma had a severe and massive autoimmune hemolytic anemia, complicated by a cerebrovascular accident. The hemolytic anemia was refractory to conventional chemotherapy with prednisone, alkylating agents, and antimetabolites. As illustrated in Fig.

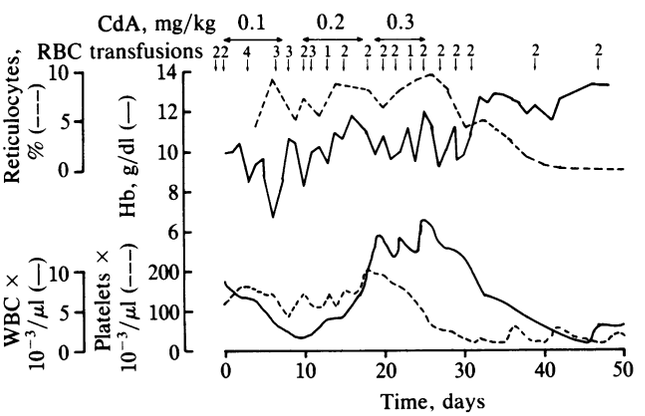

FIG. 4. Effect of CdA in a patient with diffuse lymphoma and autoimmune hemolytic anemia. As indicated this patient required 7-10 units of packed erythrocytes (RBC) per week. The CdA treatment terminated the hemolytic process and produced sustained leukopenia and thrombocytopenia.

4, treatment with CdA terminated the hemolytic process. The patient was discharged from the hospital, but remained persistently thrombocytopenic and ultimately succumbed from a second cerebrovascular accident occurring several months after the discontinuation of chemotherapy. An autopsy could not be performed.

Toxicity of CdA in Humans. Bone marrow suppression was the dose-limiting toxicity. Some degree of leukopenia, thrombocytopenia, or both was observed in all patients who received CdA at a dose of at least 0.2 mg/kg per day for 5 days (Table 1). The degree of bone marrow suppression was related not only to the rate of administration but also to the cumulative dosage and to the tumor burden at the start of chemotherapy.

Three patients had mild and transient increases of serum glutamic-oxaloacetic transaminase during the CdA infusion. All three had abnormal liver function tests prior to treatment. CdA did not induce significant changes in serum urea nitrogen or creatinine.

DISCUSSION

The present studies document the antileukemic and immunosuppressive properties of CdA. This adenosine deaminase-resistant deoxyadenosine analog was administered by continuous intravenous infusion to nine patients with advanced hematologic malignancies refractory to conventional chemotherapy. The CdA infusions were well tolerated clinically. In all the leukemia patients, the drug lowered the number of circulating blasts. In one patient with a T-cell leukemia-lymphoma, and in one patient with chronic myelogenous leukemia in blast crisis, the CdA eliminated detectable blasts from the blood and bone marrow. In a patient with advanced lymphoma, the treatment halted a severe autoimmune hemolytic process.

Bone marrow suppression represented the dose-limiting toxic effect of the deoxynucleoside. Leukopenia, thrombocytopenia, or both occurred in all patients who received 0.2 mg/kg per day for at least 5 days. Such dosages usually produced plasma CdA levels $\geq$20 nM as measured by radioimmunoassay. These results are consistent with previous *in vitro* studies, in which similar CdA concentrations inhibited spontaneous thymidine uptake and granulocyte-monocyte colony formation by normal human bone marrow cells (18).

The continuous administration of CdA for several days at even modest dosages (0.1–0.2 mg/kg per day) induced the lysis of susceptible hematologic neoplasms. Accompanying tumor destruction was a sudden increase in plasma CdA to concentrations capable of causing sustained bone marrow suppression. The synchronous discharge and subsequent dephosphorylation in the plasma of CdA 5′-phosphates, previously trapped in the tumor cells, offers a plausible explanation for the phenomenon. The predicament might be averted by vigorous cytoreduction with conventional chemotherapeutic agents prior to, or concurrent with, treatment.

Although analogs of deoxyadenosine are more toxic to lymphocytes than to other cell types, they can potentially harm all normal somatic cells. In this regard, the high-affinity adenosine deaminase inhibitor deoxycoformycin has demonstrated considerable chemotherapeutic activity against leukemias and lymphomas but has produced idiosyncratic and sometimes unacceptable damage to normal tissues (10–16). The toxicity correlated with sustained increases in the plasma concentrations of endogenously generated deoxyadenosine and adenosine. With CdA, as opposed to deoxycoformycin, the chemotherapist can more easily regulate the plasma levels of the active deoxynucleoside moiety. Conceivably, the toxicity of CdA toward normal cells could be reduced by altering the mode or schedule of administration.

Among human malignant lymphoblastoid cell lines, resistance to CdA is achieved easily by a change in the nucleoside transport system, the loss of deoxycytidine kinase, or an increase in the endogenous synthetic rate of deoxynucleosides (ref. 21 and unpublished data). In particular, the latter event may require only a single dominantly inherited metabolic alteration and does not impair cell growth. These same changes also render malignant cells resistant to the antiproliferative effects of cytosine arabinonucleoside. As shown here, and also in our earlier study (18), leukemic cells clinically resistant to cytosine arabinonucleoside often remain sensitive to CdA.

Besides CdA, other deoxynucleosides active in the L1210 mouse leukemia model include the 2-fluoro and 2-bromo derivatives of deoxyadenosine, and 9-β-D-arabinofuranosyl-2-fluoroadenine (9, 22–24). On a molar basis, CdA is the most potent of these agents *in vitro*. Although the metabolisms of the various deoxyadenosine congeners are basically similar, the exact modes of action, pharmacokinetics, and potential toxic effects of the drugs may differ. Thus, the results of the present *in vivo* trial may not apply to these other potentially promising antileukemic agents.

The accumulated data suggest that CdA possesses the following favorable chemotherapeutic properties: (*i*) antileukemic and immunosuppressive activity, (*ii*) high degree of patient compliance, (*iii*) resistance to deamination, (*iv*) relative selectivity toward malignant lymphocytes and hematologic cells, and (*v*) ability to kill cells arrested in prolonged G1 phase. However, the agent may depress bone marrow function, trigger sudden tumor lysis, and engender early drug resistance.

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