2-Chlorodeoxyadenosine: An Effective New Agent for the Treatment of Chronic Lymphocytic Leukemia

By Lawrence D. Piro, Carlos J. Carrera, Ernest Beutler, and Dennis A. Carson

2-Chlorodeoxyadenosine, a new lymphocyte-selective, anti-neoplastic drug was administered to 18 patients with advanced chronic lymphocytic leukemia of B-cell origin. All patients were resistant to conventional treatment. A total of 44 courses of 2-chlorodeoxyadenosine were completed with minimal toxicity. An overall response rate of 55% was achieved with four of 18 patients demonstrating partial response and six of 18 patients experiencing clinical improvement. Only minor bone marrow suppression occurred during administration of the drug, indicating a high degree of lymphocyte selectivity. Reduction of lymphocyte infiltration in bone marrow occurred in treated patients including one patient who experienced normalization of the bone marrow. Three of four patients with concurrent autoimmune hemolytic anemia experienced resolution of hemolysis, as indicated by elimination of transfusion requirement, fall in reticulocyte count, elevation of hemoglobin, and ability to taper prednisone without recurrence of hemolysis. Duration of responses ranged from 2 to 15 months without maintenance therapy.

CHRONIC LYMPHOCYTIC leukemia is a malignant proliferation of monoclonal lymphocytes of B-cell origin, in most cases characterized by the presence of surface membrane immunoglobulins, mouse erythrocyte receptors, and the CD5 antigen. Patients with early stage disease are usually not treated and have a median survival of 8 to 12 years. Patients with advanced disease having anemia, thrombocytopenia, splenomegaly, and organ infiltration have a much shorter survival. Approximately 10% of patients with chronic lymphocytic leukemia develop autoimmune phenomena including hemolytic anemia and thrombocytopenia, which can be life-threatening. Treatment of chronic lymphocytic leukemia generally consists of alkylating agents and steroids with response in up to 60% of cases. Intensive combination chemotherapy programs are often used when alkylating agents fail. Treatment with these intensive programs is associated with considerable toxicity and results in responses that are often short-lived.

There is no second line therapy that is considered standard at this time. The development of new agents effective for the treatment of this disease is particularly important because chronic lymphocytic leukemia is the most commonly encountered leukemia in the western world. The enzyme adenosine deaminase plays an important role in purine nucleoside catabolism. Inherited deficiency of the enzyme occurs in approximately 50% of children with severe combined immunodeficiency disease and has been associated with lymphopenia and defects in both humoral and cellular immunity. The observation that deficiency of this enzyme could be associated with lymphocyte incompetence led to the investigation of agents that inhibit adenosine deaminase activity.

Deoxycoformycin, a product of the bacterium Streptomyces antibioticus, is a tight-binding inhibitor of adenosine deaminase that has activity in lymphoid malignancies including chronic lymphocytic leukemia. Initially, its clinical use was associated with considerable toxicity, including nausea, vomiting, conjunctivitis, neurotoxicity, hepatotoxicity, and nephrotoxicity although more recently when used at lower doses toxicity has been reduced.

We have synthesized a pure analog, 2-chlorodeoxyadenosine, which unlike deoxycoformycin is not an adenosine deaminase inhibitor but rather is a substrate analog resistant to the action of adenosine deaminase. Thus, phosphorylated derivatives of 2-chlorodeoxyadenosine accumulate in cells with high deoxycytidine kinase activity, such as lymphocytes, conferring considerable tissue specificity on the drug. Our phase I trial of this agent has shown the drug to be safe for administration to humans with acceptable toxicity and established that 2-chlorodeoxyadenosine is potently lympholytic in T-cell neoplasms. We also observed an excellent response in a patient with life-threatening idiopathic autoimmune hemolytic anemia. Accordingly, we treated a patient with severe, refractory autoimmune hemolytic anemia secondary to chronic lymphocytic leukemia of B-cell origin with 2-chlorodeoxyadenosine, and were surprised that not only the hemolytic anemia but also the chronic lymphocytic leukemia appeared to respond to therapy. We have now treated 18 patients with advanced chronic lymphocytic leukemia in a phase II trial of 2-chlorodeoxyadenosine.

MATERIALS AND METHODS

Eligibility. A diagnosis of chronic lymphocytic leukemia of International Stage B or C with associated autoimmune hemolytic anemia as well as patient life expectancy of > 3 months was required for entry into the study. Cell marker studies were performed to confirm B-cell origin. Patients accepted into the study were those who had undergone prior treatment with conventional therapy, including at least an alkylating agent and prednisone and either failed to respond or developed progressive disease after an initial response. Patients were required to be > 18 years of age. Absence of active infection, and adequate renal and hepatic function were confirmed by Lawrence D. Piro, MD, Department of Basic and Clinical Research, Research Institute of Scripps Clinic, La Jolla, CA.


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Address reprint requests to Lawrence D. Piro, MD, Department of Basic and Clinical Research, Research Institute of Scripps Clinic, 10666 N Torrey Pines Rd, BCR3, La Jolla, CA 92037.

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required for entry. This study was approved by our institutional review board and informed consent was given in writing by each patient. All 18 patients registered into the study were evaluable.

**Response criteria.** Response criteria used were modifications of those proposed for clinical trials in chronic lymphocytic leukemia in the guidelines of Silver et al. Complete response required complete disappearance of all objective and subjective evidence of disease. Specifically, a bone marrow aspirate must have demonstrated <20% lymphocytes. Peripheral blood absolute lymphocyte count was <4,000/µL, and absolute neutrophil count was >2,500/µL. Hemoglobin levels and platelet counts were required to be normal.

The definition of partial response required maintenance of the hemoglobin level >12 g/dL, platelet count >100,000/µL, peripheral blood neutrophil count >1,500/µL, and a decrease in absolute lymphocyte count to <15,000/µL. Lymph node enlargement and spleen size must have been reduced by >50% of the pretreatment measurement.

The definition of clinical improvement required peripheral absolute lymphocyte count to be decreased to <50% of the pretreatment count as well as absolute neutrophil count increased to >1,500/µL. The hemoglobin level was to be maintained at >9 g/dL and the platelet count at >75,000/µL. Reduction of lymph node size by >50% was designated clinical improvement if hematologic improvement was observed simultaneously.

Disease progression was determined when any single parameter, absolute lymphocyte count, lymph node size, spleen size or marrow infiltration with lymphocytes increased by 50% over the value at the time of response determination, or when hemoglobin or platelet count decreased 25% below the level at the time of response determination.

When present, autoimmune hemolytic anemia was evaluated by hemoglobin level, reticulocyte count, Coombs test, and transfusion requirement. Response was defined as a fall in reticulocyte count associated with the loss of transfusion requirement, and rise in hemoglobin level, which was maintained despite the discontinuance of prednisone.

**Drug therapy.** 2-Chlorodeoxyadenosine was synthesized and purified as described.15,19 It was supplied to the pharmacy as a 0.1% solution (1 mg/mL) of pyrogen-free 2-chlorodeoxyadenosine in sterile 0.9% NaCl. The desired dosage (0.05 to 0.2 mg/kg) was added to 500 mL of 0.9% NaCl solution and infused intravenously (IV) over a 24-hour period. A course consisted of 0.5 to 0.2 mg/kg/d for seven days by continuous infusion. A seven-day continuous infusion was chosen because of in vitro data showing that adequate cell toxicity depends on a continuous exposure of lymphocytes to 2-chlorodeoxyadenosine.19 Patients were given between one and four courses of 2-chlorodeoxyadenosine in our General Clinical Research Center. If after an initial course of treatment the absolute lymphocyte count had become normal, no further treatment was administered. If improvement had occurred but was not sufficient to bring the WBC count to normal, a second, third, or fourth course was administered. If no response was seen after two courses, no further therapy was administered. One patient received a third course despite a normal absolute lymphocyte count because of a frequent transfusion requirement, and a bone marrow that was essentially replaced by lymphocytes. No patient received more than four courses. Starting dosage was 0.05 mg/kg/d if platelet count was <60,000 and 0.1 mg/kg/d if platelet count was >60,000. Dosage was increased for each subsequent course by 0.05 mg/kg/d if <25% response was observed and repeated at same dosage if >25% response was observed. While receiving therapy many patients received allopurinol 300 mg daily as prophylaxis against hyperuricemia.

**Toxicity monitoring.** Toxic effects were monitored and assessed according to standardized criteria.20 In addition to the blood counts, the creatinine, bilirubin, SGOT, EKG, urinalysis, and general physical exam were serially evaluated and recorded.

**Serum 2-chlorodeoxyadenosine levels.** 2-Chlorodeoxyadenosine levels were measured in the serum daily during drug infusion by radioimmunoassay using techniques that have been previously described.16

**In vitro studies.** Peripheral blood chronic lymphocytic leukemia cells were obtained from 16 of the patients before 2-chlorodeoxyadenosine treatment and were partially purified by Hypaque-Ficoll density gradient centrifugation. Cells were cultured in nicotinamide-free RPMI 1640 medium (Select-Amine Kit; GIBCO, Grand Island, NY) supplemented with 2 mmol/L L-glutamine, 50 µmol/L 2-mercaptoethanol, and 20% autologous plasma. The in vitro sensitivity to 2-chlorodeoxyadenosine was determined by erythrosin B dye exclusion after three days.21 The effect of 2-chlorodeoxyadenosine on DNA integrity of the leukemic cells was measured by the fluorescent assay for DNA unwinding described by Birnboim and Jevcak.22 In this assay, the number of DNA strand breaks induced during short-term culture with 2-chlorodeoxyadenosine is proportional to the ethidium bromide fluorescence of residual double-stranded DNA after a controlled period of DNA unwinding in alkali.

**RESULTS**

Forty-four courses of 2-chlorodeoxyadenosine were administered to 18 patients, 15 men and three women, with chronic lymphocytic leukemia. All patients had B-cell disease confirmed by cell marker studies. Four patients had autoimmune hemolytic anemia at the time of entry into the study. Seventeen patients had International Stage B or C chronic lymphocytic leukemia. One patient had International Stage A disease but had active autoimmune hemolytic anemia as an indication for treatment.

**Response.** Four of 18 patients (22%) had a partial response, six of 18 patients (33%) experienced clinical improvement, while no patient had a complete response. The overall improvement rate is 55%. Of the eight patients who were scored nonresponders because they did not meet the strict response criteria used, four (50%) demonstrated a decrease in the absolute lymphocyte count >50%. Some of these showed other evidence of decrease in tumor mass such as decrease in lymph node and spleen size and diminished marrow infiltration with lymphocytes although not sufficient to qualify as a response as defined in the study. Of the four nonresponders who did not have a decrease in lymphocyte count, three had stable disease and one had disease progression. There was one bone marrow complete response by histologic criteria (Table 1).

**Duration of response.** Treatment with 2-chlorodeoxyadenosine was discontinued when response was observed. The median duration of response was 4.5 months with a range of 2 to 15 months. Five of ten responders are in unmaintained response status at present with a mean duration of 8.0 months.

**Response of autoimmune hemolytic anemia.** Four patients had concomitant autoimmune hemolytic anemia, which was the initial indication for treatment. We were able to confirm the hemolytic process in three of the four patients by performing autologous chromium-51 RBC survival studies and demonstrating significantly decreased red cell survival. In three of the four patients we observed remission of hemolysis as defined previously.23 One of the...
Fig 1. Effects of 2-chlorodeoxyadenosine on hematologic parameters in three patients with autoimmune hemolytic anemia associated with chronic lymphocytic leukemia.

Table 1. Patient Demographics and Response Characteristics of Eighteen Patients With Chronic Lymphocytic Leukemia Treated with 2-Chlorodeoxyadenosine

<table>
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Abbreviations: CI, clinical improvement; NR, no response; PR, partial response.

The patients (no. 2) reverted to negative, while in the other two responders it remained positive. The hematologic parameters of these three patients before, during and after 2-chlorodeoxyadenosine therapy are shown in Fig 1. One patient developed autoimmune hemolytic anemia during the interval on 2-chlorodeoxyadenosine therapy and was therefore scored a nonresponder.

Toxic effects. Toxicity was minimal. No nausea or vomiting occurred in any patient during or following the administration of 2-chlorodeoxyadenosine. No neurological complications, skin eruptions, renal insufficiency, or hepatic insufficiency were observed.

One patient developed disseminated herpes zoster following treatment. Whether this is related to treatment or to the existing risk from chronic lymphocytic leukemia is not known. Two patients (no. 1 and 5) had pulmonary infiltrates that were undiagnosable before treatment. These infiltrates persisted, and following initiation of treatment were ultimately documented in both patients to be aspergillus pulmonary infections. Both patients had been on sustained high dosages of prednisone and the infiltrates antedated treatment. These infiltrates were related to the chronic steroid exposure, but may have been exacerbated by 2-chlorodeoxyadenosine administration. One patient (no. 3), developed acute hepatitis B. No other significant infections occurred.

Five of 18 (28%) patients experienced a reduction in platelet count during or following therapy. In one of the five patients (no. 17) whose platelet count was 15,000 to 20,000/μL before treatment, the platelet count fell to 3,000 to 3,800/μL following the second course of 2-chlorodeoxyadenosine. The number of megakaryocytes in the marrow was increased, however, and after splenectomy the platelet count increased to 150,000, where it remained. Three of the five patients died of progressive disease without improvement of thrombocytopenia, suggesting that bone marrow infiltration with chronic lymphocytic leukemia may have been the cause. The fifth patient experienced only mild transient thrombocytopenia.

Neutropenia was not observed. Rather, in most patients an increase in the neutrophil count was noted.

2-Chlorodeoxyadenosine drug levels. During drug infusion 2-chlorodeoxyadenosine levels were estimated daily in the serum of all patients. In most cases the drug levels were <10 nmol/L. No patients demonstrated an abrupt increase in plasma 2-chlorodeoxyadenosine levels after tumor response as was documented in some patients in our phase I studies when sudden tumor lysis occurred.

Effects of 2-chlorodeoxyadenosine on cultured chronic lymphocytic leukemia cells. In all but one sample studied for in vitro toxicity, 2-chlorodeoxyadenosine, at concentrations ≤1 μmol/L, lysed 50% of the cells during a three-day culture. However, there was no apparent correlation between the in vitro sensitivity of the patients' cells and the clinical response to 2-chlorodeoxyadenosine therapy. In part, this finding may be attributed to the small number of patients in...
each clinical group, and to the longer duration of exposure of leukemic cells to the drug in vivo.

Incubation of all of 16 chronic lymphocytic leukemia cell samples with 2-chlorodeoxyadenosine 0.2 to 1.0 μmol/L in vitro caused the appearance of DNA strand breaks. The DNA damage was dose-dependent, and occurred within 24 hours, before a decrease in the viability of the cultured cells. Figure 2 illustrates the effects of the drug on DNA integrity in the leukemic cells from two representative patients.

**DISCUSSION**

The effectiveness of a new antilymphocyte agent, 2-chlorodeoxyadenosine was tested in 18 patients with chronic lymphocytic leukemia. The results show that the drug can be safely administered to humans with acceptably low toxicity and that it has potent in vivo activity against chronic lymphocytic leukemia cells. Following only one to four courses of the drug, 14 of 18 (78%) patients had a reduction in the circulating lymphocyte count by >50%, and 55% of patients demonstrated objective clinical responses. These treatment results are especially encouraging because the patients had failed prior treatments yet responses were seen with only a few courses of 2-chlorodeoxyadenosine. Additionally, responses persisted for 2 to 15 months after 2-chlorodeoxyadenosine therapy was discontinued. This is unusual as patients with advanced disease usually require ongoing therapy to maintain remission. Perhaps complete responses may have been observed if we had treated patients more extensively. In fact, one of the later patients studied (no. 15) was given a third course of treatment despite a normal absolute lymphocyte count because of persistent bone marrow infiltration and transfusion requirement. In this case, the bone marrow, initially 100% infiltrated with lymphocytes demonstrated dramatic clinical improvement, with only scattered lymphoid nodules seen after the second course of treatment. The transfusion requirement in this patient was abolished and the hemoglobin spontaneously rose to 14 g/dl where it remains on no maintenance therapy. This lends support to the speculation that better results may be observed with more aggressive treatment with 2-chlorodeoxyadenosine. Similarly, since in vitro studies suggest that the drug is slow acting and must be present continuously to exert a lympholytic effect, it is also conceivable that the response might be greater with a more prolonged drug administration schedule.

One of the most interesting observations of this study is the striking action of the drug in hemolytic anemia. We observed resolution of severe autoimmune hemolytic anemia associated with chronic lymphocytic leukemia in three of four patients who presented with hemolysis. Resolution of hemolysis may have been related to modulation of T-cell and macrophage function following drug treatment as well as to a direct effect on the malignant lymphocytes. The clinical course of the one patient who presented with autoimmune hemolytic anemia but did not respond lends support to this possibility. In this case the size of lymph nodes was reduced, the lymphocyte count fell and the bone marrow eventually normalized, with no evidence of residual chronic lymphocytic leukemia, but hemolysis persisted. Thus the tumor responded but the autoimmune process continued.

With the small number of patients in each response group, we were unable to correlate the in vitro sensitivity of the chronic lymphocytic leukemia cells to 2-chlorodeoxyadenosine with the outcome of therapy. In every case studied, exposure of the malignant lymphocytes to 2-chlorodeoxyadenosine in vitro caused accumulation of DNA strand breaks. However, excellent clinical responses were observed with plasma drug levels below those required to kill the patients' tumor cells in culture. As mentioned earlier, it is possible that the cytoreductive effect of 2-chlorodeoxyadenosine is exerted on the neoplastic cells only after prolonged exposure, and that short-term in vitro measurements imprecisely predict in vitro sensitivity. Alternatively, the tumoricidal effect of 2-chlorodeoxyadenosine may actually be indirect: its primary effect may be on regulatory cells that are required for the proliferation of the clone of neoplastic B lymphocytes.

The toxicity observed was minimal with only limited bone marrow suppression manifested primarily as reversible thrombocytopenia. Because bone marrow aspiration and biopsy in these cases revealed the presence of megakaryocytes, the exact nature of this thrombocytopenia is unclear. The possibility exists that the persistent thrombocytopenia observed was secondary to continued marrow infiltration with chronic lymphocytic leukemia cells and not to 2-
chlorodeoxyadenosine therapy. It is also conceivable that the decrease in platelet count could also be related to a cytoregulatory effect of 2-chlorodeoxyadenosine. There was no evidence to suggest a high rate of infectious complications. It is notable that the drug is devoid of the side effects typically associated with chemotherapy agents such as nausea, vomiting, and alopecia.

Further clinical trials with 2-chlorodeoxyadenosine, including more intensive treatment schedules for chronic lymphocytic leukemia, as well as trials in hairy cell leukemia, non-Hodgkin's lymphoma, autoimmune hemolytic anemia, and cutaneous T-cell lymphomas appear to be warranted and are now underway with this promising new agent.

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