Archivum Immunologiae et Therapiae Experimentalis, 1995, **43**, 323 – 327
PL ISSN 0004-069X

Effect of Repeated Treatments with Cladribine (2-Chlorodeoxyadenosine) on Blood Counts in Multiple Sclerosis Patients

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Abstract. We report the results of blood morphology monitoring of 11 remitting-relapsing multiple sclerosis patients who received repeated treatments with cladribine (2-chlorodeoxyadenosine). The drug was given once, daily, subcutaneously (5 mg) or orally (10 mg) for 5 consecutive days, as 6 monthly courses followed by one or two additional courses at 3 or 6 month intervals. The treatments were well tolerated, although many patients suffered from incidental upper respiratory tract infections, most of which occured during the last 6 months of the observation period. One patient had recurrent infections, including an episode of urosepsis. All infections responded to standard therapy with antibiotics. Progressive lymphocyte reduction to 1000/μl on average, and clear but clinically insignificant drop in thrombocytes, was observed. Granulocyte counts were sometimes markedly elevated. A few patients developed macrocytosis, but none required transfusion. With our dosing and schedule, cladribine seems relatively safe in multiple sclerosis patients.

Key words: 2-chlorodeoxyadenosine; blood counts; toxicity.

Introduction

Cladribine (2-chlorodeoxyadenosine, 2-CdA) is a deoxyadenosine analog selectively toxic against human lymphocytes (both resting and proliferating) and monocytes[6,8]. It is also highly active against many leukemia cell lines *in vitro*[1,18]. The drug proved to be a remarkably effective treatment of hairy cell leukemia (response rate > 90% after a single course of treatment)[9,20], and promising results have been reported in other indolent lymphoproliferative diseases, mainly in B cell chronic lymphocytic leukemia (B-CLL) and low grade non-Hodgkin lymphomas[2]. Its immunosuppressive potential has been recognized in an early study when a severe autoimmune hemolytic anemia, concomitant with a diffuse lymphoma, was abated following treatment[7]; this preliminary observation was confirmed in further clinical trials[4]. A recent report[22] suggests that cladribine may favourably influence the natural history of progressive multiple sclerosis, and our preliminary data suggest also some beneficial effect in remitting-relapsing type of this disease[11,23]. Future clinical evaluation of this drug also in other autoimmune disorders seems warranted.

Cladribine displays a marked toxicity toward bone marrow progenitor cells *in vitro*[5,19]. In agreement with these observations, main dose-limiting side effects of the drug in patients with both lymphoid[2] and non-hematological[21] tumors are thrombocytopenia and, less fre-

quently, anemia and granulocytopenia (with consequential infections). Cytopenias, usually more severe in heavily pretreated patients with advanced lymphoid malignancies[1,2], could be attributed to bone marrow involvement in a disease process and/or cumulative toxicity of the drug with previous chemotherapy. However, marrow suppression and prolonged macrocytosis following repeated doses of cladribine was observed also in multiple sclerosis patients, rising concern of a delayed and long lasting hematologic toxicity of the drug even when bone marrow is unaffected[3].

In a pilot study of cladribine in remitting-relapsing multiple sclerosis we have monitored blood morphology of patients taking repeated treatments with the drug over 18 months.

Materials and Methods

Cladribine (purity > 98% by HPLC) was synthesized by Z. Kazimierczuk (Department of Biophysics, University of Warsaw). The drug was prepared as isotonic saline solution, 1 mg/kg for oral use, and 2.5 mg/kg (phosphate-buffered at pH 7.4) for subcutaneous injections. Eleven patients (8 females and 3 males, age 21–51, body weight 62 ± 8 SD kg, range 52–75 kg) suffering from remitting-relapsing multiple sclerosis, with normal blood counts, and normal kidney and liver function tests, were treated with multiple courses of the drug. Each course consisted of 5 daily doses, 10 mg orally in seven patients and 5 mg subcutaneously in the remaining four. The patients received 6 monthly courses, and additional courses were given at 9, and (in some patients) also at 12 or 15 months. Blood morphology was monitored before each treatment course, and later every three months. Statistical significance of deviations from control values was assessed by *t*-test for paired data and assumed to be statistically significant if $p < 0.05$.

Results

The treatments were well tolerated. Although two study participants taking cladribine orally complained of upper abdominal pain, its relationship to the treatment cannot be ascertained, since none of a considerable number of patients treated with cladribine given orally for B-CLL reported such complaints (J. Liliemark, Department of Clinical Pharmacology, Karolinska Hospital, Stockholm, personal communication). Several patients reported increased frequency of upper respiratory tract infections, most of which occurred during the last 6 months of the observation period. One patient suffered from recurrent infections, including one episode of urinary tract infection which occured during the third month of the study. All infections were succesfully treated with standard antibiotic therapy.

Averaged hematological data of all patients are displayed in the graphs as means ± 1 SEM. Following treatments, lymphocyte counts decreased sharply, reaching the nadir after the sixth course (Fig. 1).

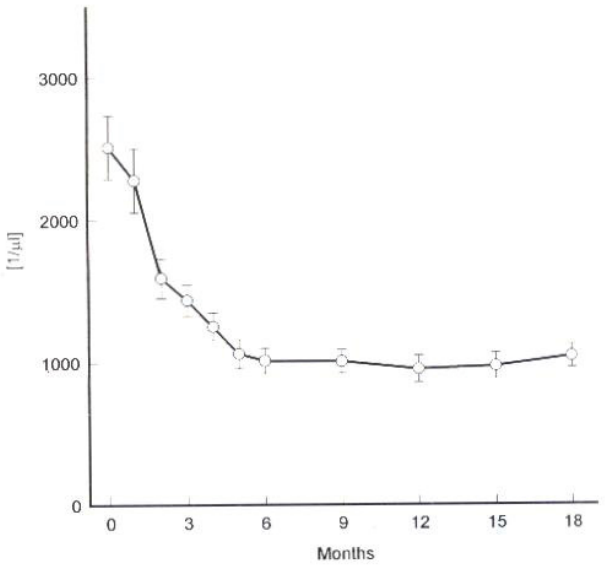

Fig. 1. Average lymphocyte counts during treatment with cladribine. The decrease is statistically significant from the second month of therapy

The degree of lymphocytopenia (its measure being the nadir of lymphocyte counts after 6 months of the study) was not related to the dose of the drug per body weight (Fig. 2), which may reflect differing individual susceptibility of lymphocytes to the cytotoxic effect of the drug. There was no appreciable drop in hemoglobin content (data not shown). Platelet counts decreased significantly (Fig. 3), but never dropped below 100 000/µl. A slight macrocytosis developed in some patients, but was not significant in the whole group, except at 18 months (Fig. 4). Averaged granulocyte count did not significantly deviate from the control level, except of the drop at 18 months (Fig. 5), but transient granulocytosis appeared in some patients (Figs. 6, 7).

Discussion

Until recently 2-chlorodeoxyadenosine had been given by continuous 7-day intravenous infusions and repeated monthly, if required. This mode of admini-

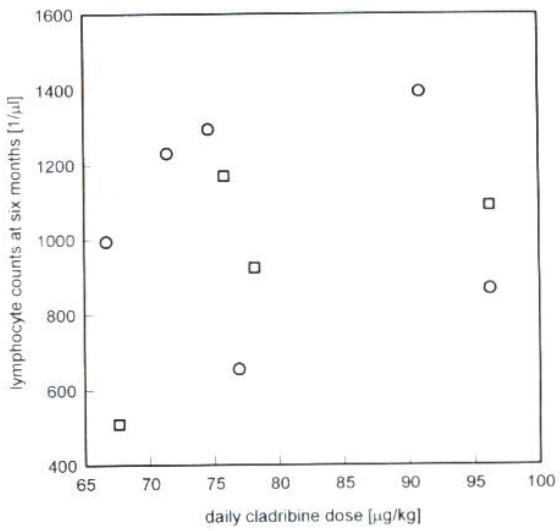

Fig. 2. The lack of correlation between the lymphocyte counts at 6 months of therapy and daily cladribine dose per kg body weight. Squares – the drug taken subcutaneously; circlets – drug taken orally (dose normalized to subcutaneous route assuming 50% oral bioavailability). Data available for 10 patients

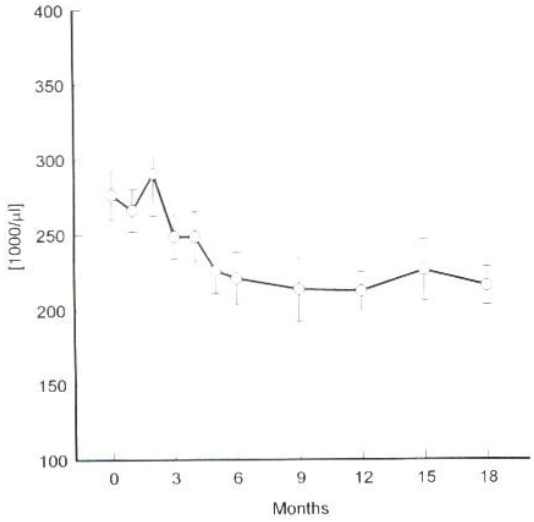

Fig. 3. Average platelet counts during treatment with cladribine. The decrease is statistically significant from the third month of therapy

stration was established on the basis of the *in vitro* studies suggesting that multi-day exposure to nanomolar concentrations of the drug is required to kill resting human peripheral blood lymphocytes[8]. In this mode of administration a single course of 0.09 mg/kg daily (i.e., total dose 0.63 mg/kg) is usually well tolerated by leukemia patients[2,4], but the probability of cytopenia increases when treatments are repeated.

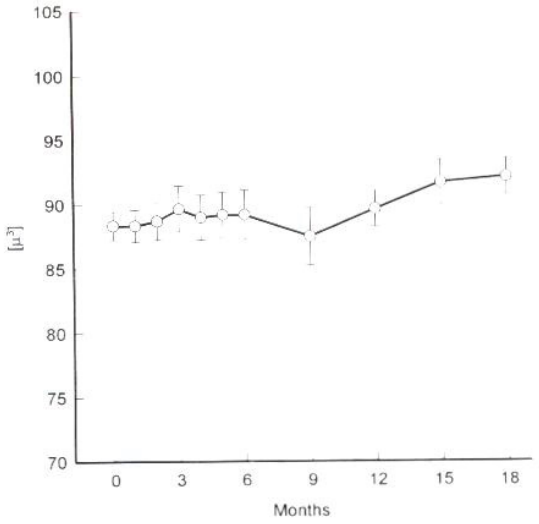

Fig. 4. Average MCV during treatment with cladribine. The rise is statistically significant at 18 months

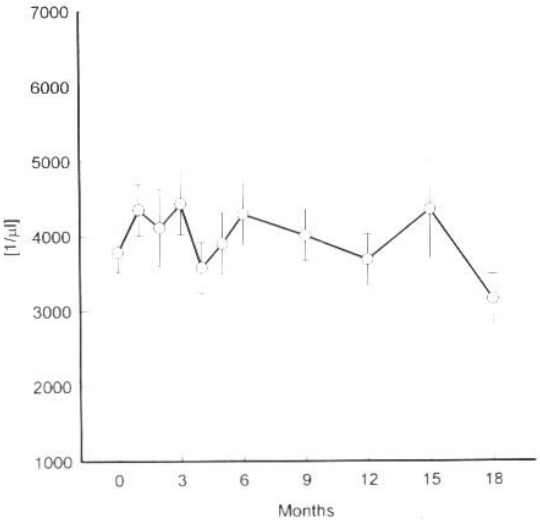

Fig. 5. Average granulocyte counts during treatment with cladribine. The change is statistically significant only at 18 months

Recent pharmacokinetic studies have shown that the area under the plasma concentration versus time curve (AUC), which is a measure of the exposure of target cells (peripheral blood lymphocytes) to the drug, is practically of the same size when the dose is given as continuous 24 h i.v. infusion, and when it is given once daily, either as an i.v. infusion lasting 2 h, or subcutaneously, or when a doubled dose is given orally (oral bioavailability is approximately 50%)[14,15]. In the treatment of lymphoid malignancies intermittent dosing schedules proved to be at least as effective as week-long intravenous infusions[13].

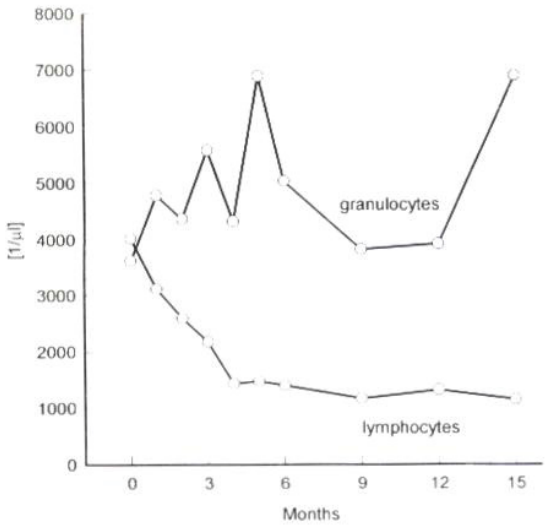

Fig. 6. Lymphocyte and granulocyte counts of the patient no. 9 (a female, the drug given subcutaneously, daily dose 0.07 mg/kg)

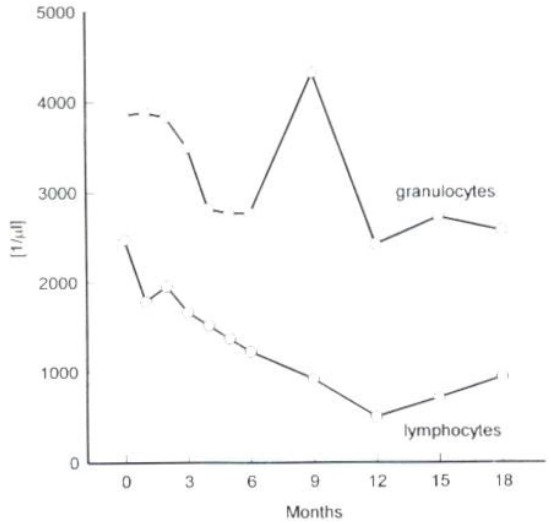

Fig. 7. Lymphocyte and granulocyte counts of the patient no. 4 (a female, the drug given orally, daily dose 0.18 mg/kg)

Cladribine is a pro-drug requiring intracellular enzymatic phosphorylation to exert cytotoxicity[8]. In B-CLL patients at the end of 2 h i.v. infusion intracellular concentration of CdA-phosphates in lymphocytes was two orders of magnitude higher than the peak plasma level of the drug. Its decay had $t_{1/2}$ of approximately 24 h, and lymphocytic intracellular AUC's of CdA phosphates after intermittent and continuous infusion were similar[16,17]. Although no data are available on the intracellular kinetics of CdA-phosphates in non-leukemic lymphocytes *in vivo*, one may expect it to be similar to that in B-CLL cells. Therefore, intermittent (e.g., subcutaneous, or oral) dosing of cladribine should also be effective for reduction of lymphocyte population and induction of immunosuppression. The results of the present study suggest, that it may also produce lower hematologic toxicity.

In the Scripps study, chronic progressive multiple sclerosis patients were treated with 4 to 6 monthly courses of cladribine, dose 0.087 – 0.1 mg/kg per day for 7 days, given as continuous i.v. infusion. The nadir of lymphocyte counts (ca. 500/µl) occurred after the last treatment, and the rebound was slow and incomplete (even during more than 3 years). While severe hematologic toxicity was observed in only 2 out of more than 20 patients and it could have been related to prior or concomitant intake of other marrow-depressing drugs, all patients on average displayed marked signs of marrow toxicity: a progressive, although moderate and reversible fall in hemoglobin concentration, a distinct macrocytosis and a moderate thrombocytopenia (these changes were not reversing after the treatment was discontinued), and a modest, but noticeable drop in granulocyte counts. Those hematological side effects were markedly more severe than these observed in our patients.

The design our present study and that of Scripps[3,22] differ in some points: 1) patients suffered from different form of the disease (in our group it was remitting-relapsing, while in the Scripps study it was chronic progressive multiple sclerosis); 2) while the daily doses of the drug received by our patients (0.067 – 0.096 mg/kg) were similar, the total dose in our study was lower on average by approximately 30% because the course of treatment lasted 5 instead of 7 days; 3) we used intermittent dosing (once daily orally or subcutaneously) instead of a week-long continuous infusion. We hypothesise that the last factor is the most important one, i.e., that hematological side effects of cladribine depend on its dosing schedule and are more severe when the drug is given in continuous infusion. A possible explanation may be that the entry of cladribine to the marrow cells is slower than to lymphocytes, and reducing exposure to 5 days and utilizing intermittent drug delivery may to some extent spare marrow progenitors, while still acting on lymphocytes. In agreement with this hypothesis are the data of PETZER et al.[19] who showed that the inhibition by cladribine of proliferation of bone marrow progenitors *in vitro* is achieved at high concentrations (> 300 nM) and it develops over a few days. In some of our patients granulocyte counts increased markedly (supposedly as a response

to infections), which clearly indicates that with our dosing and schedule the drug is toxic neither to granulocytes nor to granulocyte progenitors.

The reduction of lymphocyte counts in our study was on average only about half of that observed in the Scripps study. Would the severity of lymphocytopenia be an index of therapeutic efficiacy of cladribine, one might expect the Scripps protocol be more effective. However, the depletion of lymphocytes, while certainly being a sort of measure of the effect of cladribine on the lymphatic component of the immune system, may not be the only factor contributing to therapeutic activity of this drug in autoimmune diseases. *In vitro* cladribine, acting by a yet unknown mechanism, is a potent inhibitor of lymphocyte activation[10], and this property may well contribute to its immunosuppressive efficiacy *in vivo*. Functional properties of lymphocytes following *in vivo* treatment with the drug remain be described.

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Received in March 1995
Accepted in May 1995