A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting-relapsing multiple sclerosis

Zbigniew Stelmasiak[1,2], Janusz Solski[3], Jacek Nowicki[1], Ewa Porębska-Piwowarczyk[1], Beata Jakubowska[3], Mirosław S. Ryba[4], Paweł Grieb[4]

[1] Clinical and Research Center for Demyelinating Diseases, Lublin, Poland
[2] Department of Neurology, Medical University of Lublin, Lublin, Poland
[3] Department of Clinical Analytics, Medical University, Lublin, Poland
[4] Laboratory of Experimental Pharmacology, Polish Academy of Sciences Medical Research Centre, Warsaw, Poland

key words: multiple sclerosis, cladribine, immunosuppression

SUMMARY

Lymphocytotoxic nucleoside analog cladribine (2-chlorodeoxyadenosine) has recently been reported to favourably alter the clinical course of chronic progressive multiple sclerosis (MS). In the present study 10 patients with the remitting-relapsing form of MS were treated with six courses of this drug (5 mg subcutaneously or 10 mg orally, once daily, repeated on five consecutive days) given once a month, followed by two additional courses at three month intervals. The patients were observed for two years after the initiation of the therapy. The treatment resulted in the reduction of lymphocyte counts to approx. 40% of the initial value at 6 months, with a trend toward recovery evident only at 24 months. Neurological status of the patients (expressed semi-quantitatively according to the EDSS scale) showed a significant improvement between 6 and 15 month of the study. The number of relapses, compared to the two-year period immediately before the treatment, remained unchanged in three patients, and was markedly reduced (almost five times on average) in the remaining seven patients. Patients who experienced the reduced relapse rate also seemed to show longer and more pronounced improvement in their neurological status.

Med Sci Monit, 1998; 4(1): 4-8

INTRODUCTION

According to the prevailing theory, multiple sclerosis (MS) is an autoimmune disease in which abnormalities in immune regulation lead to the lymphocyte-dependent demyelination process in the central nervous system [1,2]. The most common therapeutic approach to autoimmune diseases is based on the use of drugs producing general immunosuppression. Clinical trials with available immunosuppressants (such as cyclophosphamide, metothrexate, or cyclosporin A) evidenced at most a modest and transient benefit to MS patients [3,4]. However, until more specific treatments are discovered, general immunosuppression is considered a theoretically justified therapeutic option in this disease.

Cladribine (2-chlorodeoxyadenosine) is a purine analog with a potent and clinically useful activity against some indolent leukemias and lymphomas [5,6]. The drug displays a highly selective toxicity toward malignant lymphocytes, and normal lymphocytes are also subject to the cytotoxic effect of the drug. Cytotoxicity is mediated by cladribine phosphorylation by deoxycytidine kinase, the enzyme located predominantly in lymphoid cells [7]. Cladribine phosphates which accumulate in lymphoid cells trigger cellular events resembling to some extent the effects of irradiation, namely the accumulation of DNA strand breaks leading to programmed cell death [8].

Cladribine-induced mmunosuppression is a side effect in the therapy of lymphoid malignancies. However, immunouppressive activity of the drug may be useful in the treatment of diseases of

Received: 97.08.30 **Correspondence address:** Paweł Grieb PhD DSc, Laboratory of Experimental Pharmacology, Polish Academy of Sciences Medical Research

autoimmune etiology. Treatment with repeated doses of cladribine appeared to halt the progression of progressive multiple sclerosis [9], although a possibility of hematological toxicity of the drug (bone marrow depression) raised some concern [10]. In the present study we tested clinical efficacy of cladribine in remitting-relapsing form of MS in a two-year open-label pilot clinical trial.

MATERIAL AND METHODS

The protocol of the study was approved by the Ethical Committee of the Medical University of Lublin. Participation in the experimental trial of cladribine was offered to a limited group of multiple sclerosis patients with a definitive remitting-relapsing course of the disease, and a certain degree of neurological deficit, who were enrolled in the outpatient service of the Clinical and Research Center for Demyelinating Diseases. Clinical diagnosis of MS was additionally confirmed by brain MRI scans immediately prior to the start of the trial. Patients at relapse, or with evidence of secondary progressive course of the disease were not admitted. Further exclusion criteria were active infections, blood cytopenia of any kind, laboratory evidence of kidney or liver dysfunction, and hepatitis B antigenemia.

The study group consisted of 10 patients (eight females and two males), aged 21-51 years (median: 35 years), body weight 52-75 kg (median: 66 years). The time from the initial diagnosis of MS was 2-16 years (median: 9 years). During the two-year period immediately before entering the study the number of relapses reported by individual patients varied from 2 to 6 (3.8 per patient on average). The neurological status prior to the start of the therapy, expressed semiquantitatively in the EDSS scale [11], ranged from 1 to 6.5 (median: 4.5). The patients selected to participate in the trial were informed about the experimental drug status of cladribine, and possible side effects of therapy, and gave their written consent. Those in reproductive age were instructed to avoid pregnancy, or not to father a child, for at least two years from the beginning of the therapy.

Cladribine (2-CdA) used in the present study was synthesized and supplied free of charge by the Foundation for the Development of Diagnostics and Therapy (Warsaw, Poland). The drug was prepared as sterile solution in isotonic saline, 1 mg/ml for oral use and 2.5 mg/ml (phosphate-buffered at pH 7.4) for subcutaneous injections.

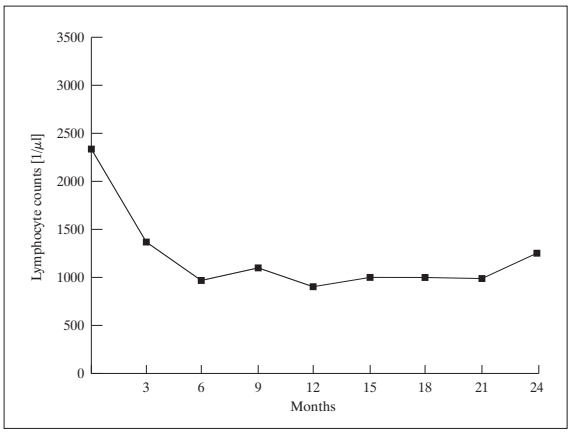
Six cladribine courses were given at monthly intervals, and two additional courses were given at 9 and 12 or 15 months. Each course consisted of five doses of the drug taken once daily on consecutive days. Cladribine was given either subcutaneously (dose 5 mg per day, six patients) or orally (dose 10 mg per day, four patients). These dosing regimens produce equivalent area under the concentration-time curve of the drug [12]. Blood counts and neurological examinations were taken at prescheduled days (preceeding the beginning of each treatment course) at monthly intervals during the first 6 months, and later every three months. The patients were instructed to maintain a close contact with supervising physicians during the study period, and to refer to them immediately in case of relapse, infection, or any other unusual event. Steroids were allowed, if severe relapse occurred.

Significance of changes in the EDSS scores were assessed by non-parametric statistical methods (Friedman ANOVA and post-hoc Wilcoxon matched pairs test), using Statistica software package.

RESULTS

Compliance of patients and tolerance of therapy was good, and hematological side effects were mild. Granulocyte counts remained relatively constant at about 4 000 per μl on average. Platelet counts dropped only slightly (to approx. 200.000 on average, and not in a single case below 100 000 per μl). Lymphocyte counts dropped from the initial count of 2 336±595 per μl (mean±S.D.) to 968±229 per μl at 6 months, and remained at approximately 1000 per μl for the next 15 months. A tendency toward normalization of lymphocyte

Figure 1. Average lymphocyte counts during and after treatment with cladribine.

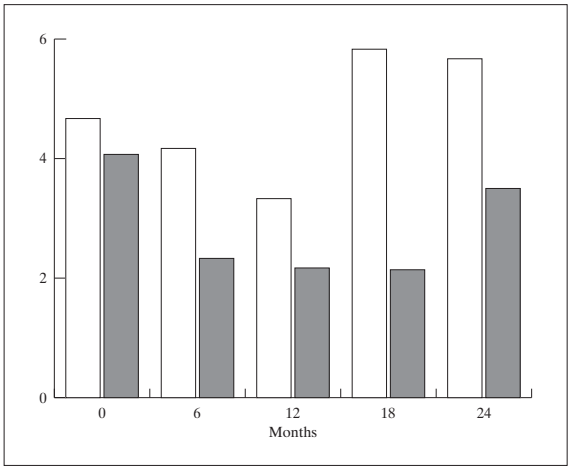


counts became evident only at the end of the 2-year observation period (Fig. 1). The magnitude of lymphocyte count reduction varied among the patientswas different in particular cases, ranging from virtually no effect in one patient to a transient drop by 80% of the initial value (to less than 500 per μl) in another one. However, there was no correlation between the depth of lymphocyte nadir, or the magnitude of lymphocyte count reduction from the initial value, and the drug dose expressed per kg of body weight (normalized for oral vs. subcutaneous route of administration). Two out of four patients taking cladribine orally complained transiently of upper abdominal pain, but the association of this effect with drug intake could not be ascertained. During the study period 17 infection episodes (most of them upper respiratory tract or urinary tract infections) occurred in the whole group (including eight in a single patient). All infections responded to standard antibiotic therapy, creating no significant threat.

Table 1. Average EDSS scores during and after the treatment.

Month after the initiation of treatment	EDSS (mean±SD)	p
0	4.3±1.8	–
3	3.4±1.5	0.01
6	2.9±1.7	0.02
9	2.7±1.9	0.02
12	2.6±1.6	0.01
15	2.4±1.7	0.02
18	3.3±2.4	0.26
21	3.4±2.5	0.14
24	4.3±2.0	0.34

Figure 2. Average EDSS scores „responders" (filled bars) and „non-responders" (open bars).



Analysis of the data for the whole group revealed that EDSS scores were significantly reduced during the treatment compared to the initial values (Friedman ANOVA $\chi^2(N=6, df=8)=18.07$, $p<0.02$, and that the decreases observed at the third to fifteenth month were significant by Wilcoxon matched pairs test (Table 1). The total number of relapses reported by the patients during the two years immediately preceeding the initiation of the treatment was 38, whereas it was only 15 during the two-year study period.

Further inspection of the data revealed that, from the point of view of the apparent efficacy of the treatment, the group under study can be divided into two sub-groups, the 'responsders' and the 'non-responders'. The 'responders' sub-group consisted of seven patients who reported a total of 29 relapses during the two preceeding years (average relapse rate 2.07 per patient per year), and a total of 6 relapses during the two-year study period (average relapse rate 0.43 per patient per year). Two of the responders reported no relapses for the entire two-year period of treatment and post-treatment follow-up, whereas during the two preceeding years one of them experienced three, and the other one four relapses. The 'non-responders' subgroup consisted of three patients whose relapse rate was the same during the two-year periods prior to and after the initiation of the therapy (total of 9 relapses, average relapse rate 1.5 per patient per year). Averaged EDSS scores for 'responders' and 'non-responders' are shown in Fig. 2. One patient who did not respond to treatment displayed only a slight and transient reduction in lymphocyte count during the treatment, but the other two showed the reduction of approximately the same magnitude as in some of the 'responders'. The average body weight was the same in both sub-groups.

DISCUSSION

Clinical trials of general immunosuppressants in multiple sclerosis produced disappointinging results. The reason may be that the depth of immunosuppression required for successful modification of the natural history of MS cannot be achieved with 'conventional' immunosuppressive drugs because of systemic side effects. Indeed, it has been shown in a double-blind placebo-controlled trial with cyclosporin A in relapsing-remit-

ting MS [13] that beneficial effect of the therapy (reduction in relapse rate) could be achieved only when high (and toxic) doses of the drug were given, so that severe concomitant side effects (hypertension, renal insufficiency and anemia) precluded such treatment.

The present trial was performed with a small number of patients, and was not placebo-controlled. Its results concerning the efficiacy of the treatment shall, therefore, be interpreted with great caution. Nevertheless, we consider them potentially important. The therapy appeared to be effective in seven patients who reported a very marked (almost five-fold on average) reduction in the relapse rate during the 2 years after the initiation of the treatment, while it seemed ineffective in the remaining three. The 'responders' showed also a somewhat more pronounced improvement in their neurological status as measured by EDSS scores. We were unable to identify any factor which would differentiate between the 'responders' and the 'non-responders'. There was no indication that a 'good response' is related to the actual cladribine dose per body weight. Although the reduction of lymphocyte count was only minute and transient in one 'non-responder', in the other two the lymphocyte drops were within the range of reductions observed in the 'responders'.

When side effects of the therapy are considered, cladribine favourably compares with other immunosuppressants. In the treatment of lymphoid malignancies, besides an increased incidence of infections, the only relatively frequent side effect is thrombocytopenia (which, at least in some cases, may be related to the marrow involvement in the disease process), but there is virtually no non-hematologic toxicity at the doses up to 0.1 mg/kg daily i.v. for five to seven days days [5,6]. The limited toxicity of cladribine is attributed to the confinement of the drug-activating enzyme (deoxycytidine kinase) to the lymphoid cells. Although dangerous marrow depression were reported in some multiple sclerosis patients treated with cladribineby Beutler et al [10], our experience (extended already to a larger group of patients [14]) indicates that in this clinical setting a two- to three-fold reduction of blood lymphocyte count can be achieved by the drug given subcutaneously without clinically significant hematological side effects.

There are some aspects of cladribine pharmacokinetics and pharmacodynamics which may be responsible for its beneficial activity in MS. The drug is able to cross the blood-brain barrier resulting in CSF: plasma ratio of 25% [15,16], so that it may exert some toxicity also toward lymphocyte clones inhabitating the CNS. It displays, at least in the in vitro conditions, immunosuppressive effects not related to the simple reduction of lymphocyte counts: it inhibits T and B cell activation and the response of T cells to co-stimulation by proteins of the extracellular matrix [17,18]. Its property of stimulating the activity of the NK cells in vitro [19] and restoring their impaired activity in vivo (observed in leukemic patients by Lauria et al [20]) may also be of some significance, because in relapsing-remitting MS new MRI-visible lesions were reported to appear only during the periods of reduction of NK functional activity [21].

CONCLUSIONS

In patients with remitting-relapsing multiple sclerosis treatment with cladribine decreases lymphocyte counts in peripheral blood, to 1/3 of the initial values on average. The relapse rate in some (but not all) patients is impressively decreased. Side effects of the therapy are mild. The reason why some patients do not respond to the treatment remains to be identified. Along with clinical evaluation in MS, the detailed pattern of cladribine-induced immunosuppression in clinical conditions deserves further study.

REFERENCES:

1. *French-Constant C: Pathogenesis of multiple sclerosis. Lancet, 1994; 343: 271-275*
2. *Hafler DA, Weiner HL (1989) MS: a CNS and systemic autoimmune disease. Immunol Today, 1989; 10: 104-107*
3. *Hughes R: Immunotherapy for multiple sclerosis (Editorial). J Neurol Neurosurg Psychiat, 1994; 57: 3-6*
4. *Noseworthy JH: Immunosuppressive therapy in multiple sclerosis: pros and cons. Int MSJ, 1994; 1: 79-89*
5. *Beutler E: Cladribine (2-chlorodeoxyadenosine). Lancet, 1992; 340: 952-956*
6. *Saven A, Piro LD: 2-Chlorodeoxyadenosine: a newer purine analog active in the treatment of indolent lymphoid malignancies. Ann Intern Med, 1994; 120: 784-791*
7. *Carson DA, Wasson DB, Taetle R, Yu A: Specific toxicity of 2-chlorodeoxyadenosine towards resting and proliferating human lymphocytes. Blood, 1983; 2: 737-743*
8. *Carrera CJ, Piro LD, Saven A, Beutler E, Terai C, Carson DA: 2-Chlorodeoxy- adenosine chemotherapy triggers programmed cell death in normal and malignant lymphocytes. In: Purine and Pyrimidine Metabolism in Man, (Harkness RA et al, eds), Plenum Press, New York, 1991, pp. 15-18*
9. *Beutler E, Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J: The treatment of chronic progressive multiple sclerosis with cladribine. Proc Natl Acad Sci USA, 1996; 76: 2430-2433*
10. *Beutler E, Koziol JA, McMillan R, Sipe JC, Romine JS, Carrera CJ: Marrow suppression produced by repeated doses of cladribine. Acta Haematol, 1994; 91: 10-15*

11. *Kurtzke JF: Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology, 1983; 33: 1444-1452*
12. *Liliemark J, Albertioni F, Hassan M, Juliusson G: On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: alternative routes of administration. J Clin Oncol, 1991; 10: 1514-1518*
13. *Rudge P, Koetsier JC, Mertin T, Mispelblombeyer JO, van Walbeek HK, Clifford Jones R, Harrison J, Robinson K, Mellein B, Poole T, Stokvis JCJM, Timonen P: Randomised double blind controlled trial of cyclosporin in multiple sclerosis. J Neurol Neurosurg Psychiat, 1989; 52: 559-565*
14. *Stelmasiak Z, Solski J, Jakubowska B, Nowicki J, Grieb P: (1995) Safety of cladribine treatment in multiple sclerosis (Abstract). J Neuroimmunol, 1995; Suppl. 1, 17*
15. *Kearns CM, Blakley RL, Santana VM, Crom WR: Pharmacokinetics of cladribine (2-chlorodeoxyadenosine) in children with acute leukemia. Cancer Res, 1994; 54: 1235-1239*
16. *Liliemark J, Juliusson G: On the pharmacokinetics of 2-chloro- 2'-deoxyadenosine (CdA) in cerebrospinal fluid (CSF). (Abstract) Blood, 1992; 80 (Suppl. 1), 471a*
17. *Górski A, Grieb P, Korczak-Kowalska G, Wierzbicki P, Stępień-Sopniewska B: Cladribine: an inhibitor of human B and T cell activation in vitro. Immunopharmacology, 1993; 26: 197-202*
18. *Górski A, Grieb P, Makula J, Stępień-Sopniewska B, Mrowiec M, Nowaczyk M: 2-Chloro-2'-deoxyadenosine - a novel immunosuppressive agent. Transplantation, 1993; 56: 1253-1257*
19. *Priebe T, Kandil O, Nakic M, Fang Pan B, Nelson JA: Selective modulation of antibody response and natural killer cell activity by purine nucleoside analogues. Cancer Res, 1988; 48: 4799-4803*
20. *Lauria F, Rondelli LF, Raspadori D, Benfenati D, Tura S: Rapid restoration of natural killer activity following treatment with 2-chlorodeoxyadenosine in 22 patients with hairy cell leukemia. Eur J Hematol, 1994; 52: 16-20*
21. *Kastrukoff LF, Morgan N, Zecchini D, Satoh J, Paty DW: (1995) Correlation of natural killer (NK) cell functional activity and phenotype with disease activity determined clinically and by serial MRIs. Effects of interferon-(-1b (Abstract). J Neuroimmunol, 1995; Suppl. 1: 64*