Articles

Cladribine in treatment of chronic progressive multiple sclerosis

J C Sipe, J S Romine, J A Koziol, R McMillan, J Zyroff, E Beutler

Summary

Chronic progressive multiple sclerosis (MS) is a severely disabling demyelinating disease in which autoimmune processes seem to have a major role. The nucleoside drug cladribine is a potent lympholytic agent with few side-effects. We have studied its efficacy and safety in a randomised double-blind trial.

51 patients (48 entered as matched pairs) received four monthly courses of 0·7 mg/kg cladribine or placebo (saline) given through a surgically implanted central line. Neurologists with no knowledge of which medication the patient was receiving examined the patients monthly and noted two rating scale scores (Kurtzke and Scripps). Cerebrospinal fluid and brain magnetic resonance imaging (MRI) examinations were done at 6 and 12 months. Average neurological scores, demyelinated volumes on MRI, and concentrations of oligoclonal bands in cerebrospinal fluid were stable or improved in the patients receiving cladrabine but continued to deteriorate in patients on placebo. Mean paired (placebo minus matched cladribine) differences at 12 months relative to baseline were 1·0 (SE 0·4) for the Kurtzke scores, −13·9 (2·3) for the Scripps scores, 4·57 (1·17) mL for demyelinated volumes, and 7·3 (3·3) arbitrary units for concentrations of oligoclonal bands.

Cladribine was generally well tolerated and clinically significant toxicity occurred in only 1 patient, in whom severe marrow suppression developed with complete recovery after several months. 1 patient died of newly acquired hepatitis B, an event unlikely to be related to cladribine. We conclude that the immunosuppressive drug cladribine influences favourably the course of chronic progressive MS.

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Department of Molecular and Experimental Medicine, Scripps Research Institute (J C Sipe MD, J S Romine MD, Prof J A Koziol PhD, Prof R McMillan MD, Prof E Beutler MD); **and Division of Neurology and Department of Radiology, Scripps Clinic and Research Foundation** (J C Sipe, J S Romine, J Zyroff MD), **La Jolla, California, USA**

Correspondence to: Prof Ernest Beutler, Department of Molecular and Experimental Medicine (SBR-3), Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

Introduction

Although the primary cause of multiple sclerosis (MS) is unknown, there is circumstantial evidence to indicate that autoimmune mechanisms play a major part in the attack of the body's own immunocytes on central nervous system myelin to cause the symptoms in MS.

Carson and colleagues[1,2] developed cladribine (2-chlorodeoxyadenosine) as a highly specific antilymphocyte agent that mimics the accumulation of deoxynucleotides in adenosine deaminase deficiency. This drug has been found to cause death of lymphocytes by apoptosis and to have relatively low toxicity toward other tissues. Unlike most other antilymphocyte drugs, it is equally effective against resting and dividing cells.

The use of cladribine in chronic MS was considered after extensive experience had been obtained with the drug in the successful treatment of lymphoid leukaemias, notably hairy cell leukaemia and lymphomas, and some success in the treatment of autoimmune haemolytic anaemia.[3-5] Because of its relative safety and the long-lasting lymphopenia observed during its administration, we undertook a pilot study, treating 4 patients with chronic progressive MS with cladribine in 1990. Results were sufficiently encouraging for us to conduct a more extensive randomised, double-blind, placebo-controlled study in 51 patients with chronic progressive MS.

Patients and methods

51 patients, all of whom had clinically definite or laboratory-supported definite chronic progressive MS[6] for more than 2 years, were entered into the study. These patients had been followed at Scripps Clinic by the neurology group for between 6 months and 10 years. The study plan, risks, and potential benefits were explained to each patient in detail, and all patients gave informed consent.

Patients were matched according to age, sex, and severity of disease and 24 pairs of patients were identified. Each pair was randomised by the statistician (JAK) using random number tables[7] so that one patient was assigned to the group initially receiving cladribine and the other to the group originally receiving placebo. 1 other patient, for whom no suitable match was identified, was started on cladribine; this individual left the study after 8 months on protocol. 2 patients, both initially assigned to cladribine, were lost at 2 and 3 months: as discussed below, 1 patient died of acute fulminating hepatitis B during her second month on protocol and the other patient dropped out of the study at 3 months because of a traumatic hip fracture. The loss of these 2 patients seemed unattributable to cladribine so we recruited 2 additional matched patients as replacements and assigned them to cladribine. 1 patient receiving placebo withdrew from the study after 4 months on protocol for reasons unrelated to treatment, so we chose not to replace this patient. The analyses reported here refer to the 24 matched pairs of patients (table 1), and exclude the 3 patients on cladribine who did not complete a full year of the study.

Participants were permitted to continue medications to treat troublesome symptoms of MS—eg, spasticity treated with baclofen or bladder dysfunction with oxybutynin. Candidate

	Placebo	Cladribine
Sex (F/M)	16/8	16/8
Race (white/other)	22/2	24/0
Mean age (yr) (and range)	42 7 (21–54)	43 0 (28–53)
Mean duration of clinical symptoms of MS (yr) (and range)	10 5 (2–31)	12 7 (4–24)

Table 1: **Demographic characteristics of paired patients at study entry**

patients were excluded if the serum creatinine was above 132 μmol/L or if the calculated creatinine clearance was less than 80% of the age-adjusted normal value; if serum transaminases or hepatic alkaline phosphatase were more than twice the upper limit of normal; or if the neutrophil count was below 1600/μL or platelet count was less than 130 000/μL. No woman not taking adequate birth control measures or man planning to father a child for the duration of the study was included. Patients who were treated with corticosteroids or other immunosuppressive medications such as cyclophosphamide, azathioprine, or cyclosporin within the previous 6 months or who appeared to have decreased marrow reserve as manifested by leukopenia or thrombocytopenia for more than 6 weeks after conclusion of treatment with immunosuppressive agents were excluded. All patients had a central venous access device implanted for drug or placebo administration.

Study design

The investigation was designed as a 2-year double-blind crossover study. This design was ethically necessary because of the need for surgically implanted central venous lines. However, the protocol stipulated analysis as a parallel study after 1 year, crossover being decided at that time. The primary endpoint was neurological improvement, and since significant improvement was achieved at the end of 1 year this portion of the study was concluded at that time.

Evaluation

The examining neurologists, nurses, and patients were blinded to the treatment assignments. An unblinded investigator (EB or RM) monitored all the laboratory studies and patients' complaints and illnesses, if any. Every patient was examined monthly by the same neurologist for the first year of the study. Two neurological assessments, the Scripps Neurologic Rating Scale (SNRS)[8] and the Kurtzke Expanded Disability Status Scale (EDSS),[9] were scored at every examination, and at each visit blood was taken for chemical

Time	Score	
	Placebo	Cladribine
EDSS		
Baseline	4 6 (0 3)	4 7 (0 3)
6 mo	5 0 (0 3)	4 5 (0 4)
12 mo	5 6 (0 3)	4 4 (0 4)
SNRS		
Baseline	69 5 (2 1)	69 5 (2 2)
6 mo	65 3 (2 3)	73 7 (2 7)
12 mo	62 6 (2 3)	74 8 (2 1)

Table 2: **Summary statistics from neurological examinations**

analysis and complete blood count. The unblinded investigators decided, on the basis of the blood count, whether it was safe to proceed with the next dose of test medication. If these observers judged that the drug could be given safely, cladribine (or placebo) was administered by continuous 7-day intravenous infusion at the rate of 0·1 mg/kg daily. If not, the counts were repeated periodically and the decision whether or not to continue was made; planned infusions were delayed in 4 patients receiving the drug and in 2 patients on placebo.

In the original protocol six monthly doses were planned but this was modified to four courses after the study began because the thrombocytopenia was more profound than expected in some patients. 1 patient had already received five courses before the decision to reduce dosage had been made. 1 patient received only two courses and 2 patients only three because of thrombocytopenia. All other patients were given four courses.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) was performed on a 1·5 T General Electric Signa scanner. T2 and proton density weighted images were obtained using a conventional spin-echo sequence with repetition times of 2500 ms and echo delay times of 30 and 90 ms. Sections were 4 mm thick with a 1 mm interslice gap. T1 weighted scans were obtained in the sagittal and axial planes. Axial scans of 3 mm thickness and 0 interslice gap were done about 10 min after gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, New Jersey, USA) injection to ensure optimal time for transmigration of the contrast agent across the blood-brain barrier. Special attention was given to careful repositioning of patients to guarantee reproducible slice positions. Regions of demyelination on proton density weighted scans and contrast enhancement on T1 weighted scans were outlined by hand

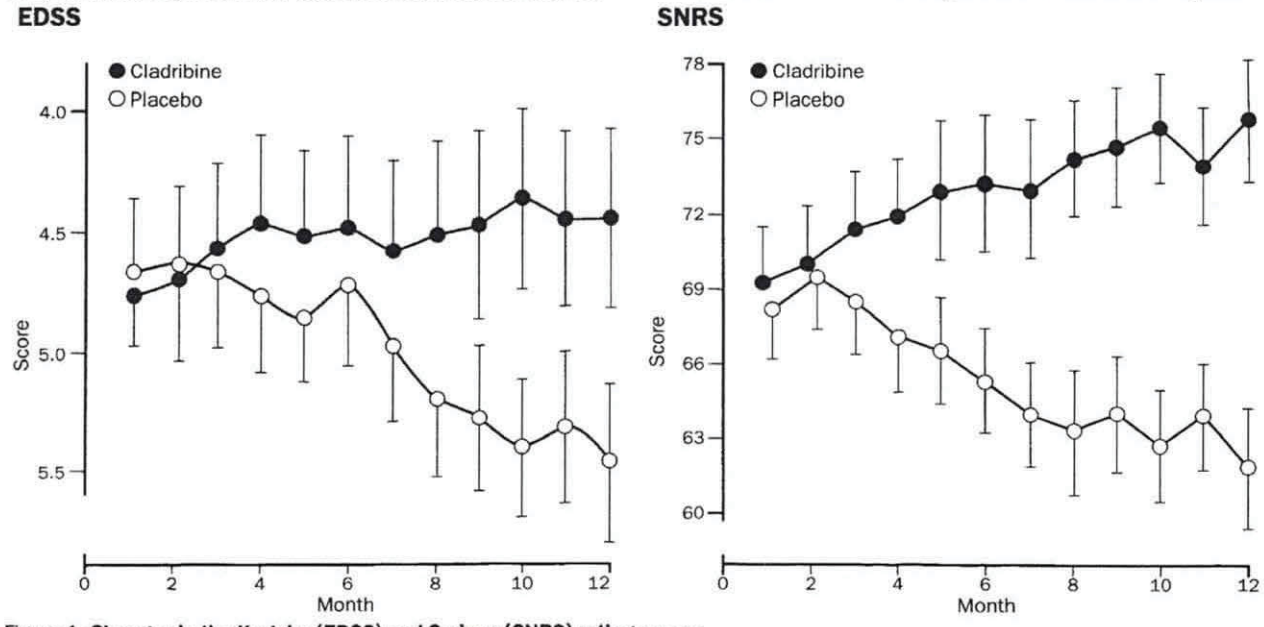

Figure 1: **Changes in the Kurtzke (EDSS) and Scripps (SNRS) rating scores**
Mean (SE bars) shown.

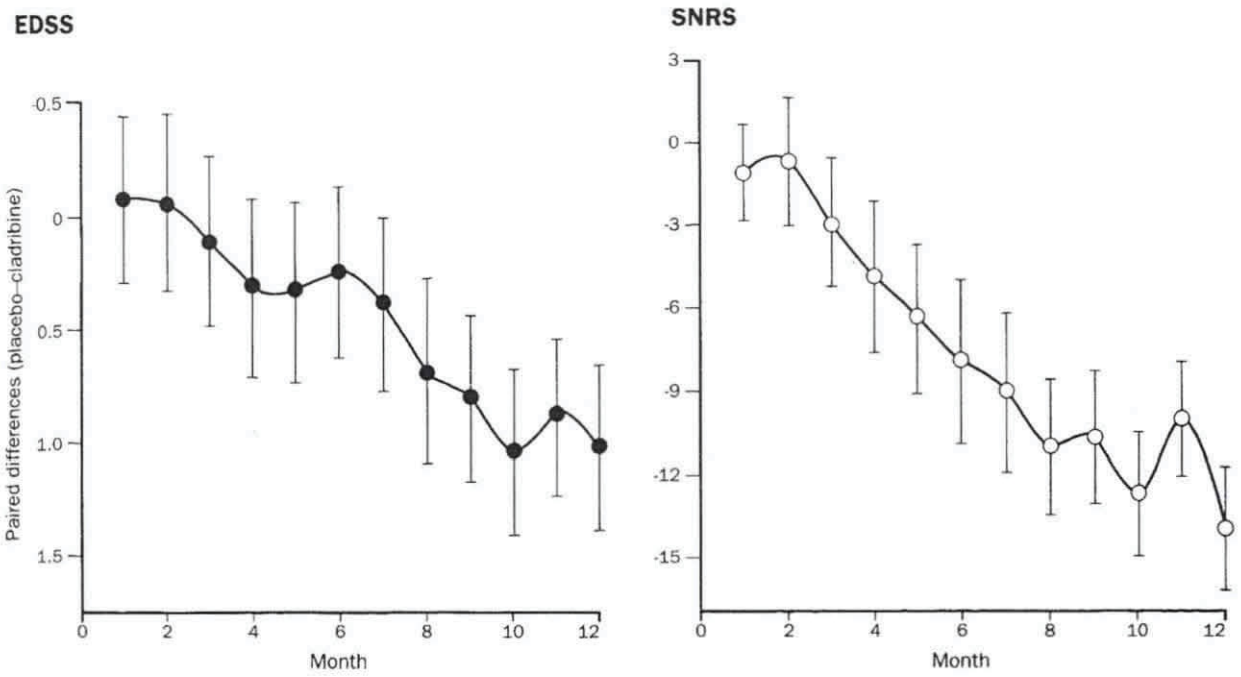

Figure 2: **Differences between Kurtzke (EDSS) and Scripps (SNRS) rating scores in matched pairs (placebo minus cladribine)**
Mean paired differences (SE bars) shown. Paired differences were significantly greater than zero (EDSS $p < 0.01$, SNRS $p < 0.001$).

on filmed images. All scans were interpreted and marked by the same neuroradiologist (JZ) who had no knowledge of treatment protocols. These were then duplicated by a technologist using the taped raw data and a computer work station (CEMAX, Santa Clara, California). Pixel counts for each slice were converted into volumes by a volume rendering software program. All the volumetric analyses were done by the same technologist.

Cerebrospinal fluid (CSF) examination

CSF was tested for total protein and immunoglobulin concentration, and samples were frozen for assessment of changes in oligoclonal bands. At the end of one year the baseline and 6 and 12 month samples of CSF were labelled with ^{125}I-albumin and concentrated 40-fold in a Centricon-10 (Amicon) centrifugal concentrators, and electrophoresis was performed on Corning high resolution protein agarose plates. After staining with acid violet, the strips were scanned with a Zeineh soft laser scanning densitometer (Biomed Instruments, Fullerton, California). The height of oligoclonal bands was measured in millimetres as arbitrary units by a technician with no knowledge of the treatment. The radioactivity of the albumin band was determined to correct for inter-sample differences in concentration and sample application. When the concentration of any sample in a series was 150% or more of any other sample electrophoresis was repeated, the volumes applied being changed so that nearly the same amounts of each original CSF was applied to the gel. A reliability experiment, on 12 random samples run on three different gels, yielded an intra-class correlation coefficient[10] of 0·95 for the replicates across the gels.

Statistical methods

The design was a double-blind crossover trial, with one planned interim analysis at 12 months, before crossover. Our primary endpoint was neurological improvement as assessed by the two rating scales. Our pilot study suggested that, relative to placebo, cladribine would improve the status of patients with chronic progressive MS. We estimated that a sample size of 44 patients (22 per arm) would be sufficient to detect a 15% SNRS improvement in patients on cladribine if there was no improvement on placebo, with a statistical power of 0·90 and a one-sided statistical test at conventional alpha level 0·05. Our stopping rule at 12 months was significant improvement in the SNRS for the cladribine group compared with the placebo arm, at an alpha level of 0·01. Since this improvement was achieved, the statistical analysis was done as for a conventional randomised parallel design.

Comparisons between treatment arms were based on the underlying matching of 24 pairs of patients; the last available observations were carried forward for the patient who had not completed 12 months in the study. Similar analyses were done in which these missing data remained missing and in which they were modelled under the representation that they were missing at random. Also, an unpaired, intent-to-treat analysis was done on data from all 51 patients; and a paired analysis incorporating data from the 2 cladribine patients lost by month 3 was also undertaken. All additional analyses yielded results similar to those reported here.

Summary statistics are based on the 24 matched pairs of patients and are reported as mean (and standard error, SEM). The paired differences in neurological scores were analysed with a non-parametric repeated measures analysis of variance.[11] Other paired comparisons between the treatment arms were made with both parametric and non-parametric one-sample procedures, with two-sided p values reported throughout.

Results

Neurological examinations

Both examining neurologists (JR and JS) participated in a study of inter-rater and intra-rater reliability. 20 patients (10 primarily followed by each neurologist) were independently assessed by each examiner on the same day. Inter-rater agreement[2] was high: the weighted κ coefficient was 0·976 for EDSS and 0·828 for SNRS. Inter-rater agreement on the EDSS was 100% for all sets of examinations when agreement was defined as a difference less than or equal to 1·0. This compared favourably with agreements reported in other clinical trials of therapeutic agents in MS.[13] Inter-rater agreement on the SNRS was 85%, with agreement defined as difference of no more than 10 points. Separately, 18 patients (JR, 8, and JS, 10) were assessed by the same examiner twice on the same day, the period between examinations ranging from 135 to 240 min.

Time	Mean (SE) volume (mL)	
	Placebo	Cladribine
Baseline	18 32 (2 85)	23 46 (4 35)
6 mo	20 91 (3 37)	24 35 (4 81)
12 mo	22 46 (3 39)	22 66 (4 32)

Table 3: **Total lesion volumes (MRI)**

Intra-rater agreement on the EDSS was perfect; weighted κ coefficients of agreement between the two SNRS scores were 0·978 (JR) and 0·998 (JS).

SNRS and EDSS scores are shown in figure 1 with summary statistics in table 2. Both scores indicated progressive deterioration in patients randomised to placebo. Modest improvement in mean scores was found in patients on cladribine. The average matched-pair differences (placebo minus cladribine) in scores during the study are shown in figure 2. A distribution-free procedure[11] was used to assess whether individual paired differences were consistently positive or negative. These directional statistics were highly significant ($\chi^2_1 = 8{\cdot}38$, $p < 0{\cdot}004$ for EDSS, $\chi^2_1 = 13{\cdot}26$, $p < 0{\cdot}001$ for SNRS). The mean paired differences in EDSS and SNRS scores at 6 months are 0·6 (0·3) and −8·9 (2·2), with respective 95% confidence intervals of 0–1·2 and −13·5 to −4·3). At 12 months, the mean paired differences in EDSS and SNRS scores are 1·3 (0·3) and −12·5 (2·0), respective 95% CIs being 0·6–2·0 and −16·7 to −8·2.

We also analysed the number of patients experiencing a change in EDSS score of 1 or more points at 1 year. Among the 23 patients receiving placebo who were evaluable at 12 months the EDSS score of 7 of them had progressed by at least 1 point, the score had improved (decreased) at least a point in only 1, and 15 patients remained within 1 point of baseline. Among the 24 evaluable patients receiving cladribine, the EDSS score of only 1 patient had worsened by at least a point, the EDSS had improved (decreased) by at least a point in 4 patients, and 19 patients had an EDSS score that changed less than 1 point. These proportions are significantly different ($p < 0{\cdot}02$, Terpstra-Jonckheere test).

MRI data analyses

Summary statistics relating to demyelinated and enhancing volumes are given in table 3. Paired differences (placebo minus cladribine) of demyelinated volumes at 6 months and at 12 months, relative to baseline values, were different from zero ($T^2_{2,25} = 17{\cdot}01$, $p < 0{\cdot}002$). This can be attributed primarily to the changes at 12 months relative to baseline. The mean paired difference (placebo minus cladribine) in demyelinated volumes at 6 months relative to baseline was 1·47 (1·32) mL (95% CI −1·26 to 4·19); at 12 months the mean difference was 4·42 (1·10) (95% CI 2·16–6·69).

We dichotomised the enhancing volume findings by calling the elimination of enhancing volumes or their continued absence a favourable outcome and labelling the emergence of or continued presence of enhancing volumes as an unfavourable outcome. A paired analysis of the 12-month findings relative to baseline yields 13 pairs with jointly favourable outcomes, 3 pairs with jointly unfavourable outcomes, 1 pair with placebo favourable and cladribine unfavourable, and 10 pairs with placebo unfavourable, cladribine favourable ($p < 0{\cdot}001$, McNemar test).

CSF examination

The number of oligoclonal bands did not change in any patient. For the cladribine group, relative oligoclonal concentrations at baseline and at 6 months and 12 months averaged 29·9 (4·2), 26·5 (3·4), and 25·0 (3·3) a significant decline ($F_{2,38} = 5{\cdot}17$, $P < 0{\cdot}02$). Corresponding values for the placebo group were 26·2 (3·8), 29·9 (3·8), and 29·9 (4·7), a modest but non-significant increase ($F_{2,38} = 1{\cdot}81$, $p = 0{\cdot}18$). Among the matched pairs, the placebo patients tended to have higher values than their counterparts on cladribine: The mean paired difference (placebo minus matched cladribine) in relative oligoclonal immunoglobulin concentrations at 6 months relative to baseline is 4·3 (2·0) (95% CI 0·1–8·5); at 12 months it was 7·3 (3·3) (95% CI 0·5–14·1).

Side-effects and complications

In general, cladribine was well-tolerated by all patients and there were no untoward side-effects or symptoms in MS patients that could have systematically affected the double-blindedness of the protocol. Marrow suppression occurred in several patients but was clinically significant in only 1, a patient who was also taking large doses of phenytoin for trigeminal neuralgia. This patient's marrow function recovered fully over a period of several months after conclusion of therapy with cladribine. Details of the haematological data collected in this study are summarised elsewhere.[14]

Two serious medical events occurred. A 40-year-old woman received her second dose of cladribine when her blood count and blood chemistry, including liver function tests, were normal. Hepatitis B serology had been negative on study entry. 3 days after the infusion, she presented with fever, abdominal pain, and transaminase levels exceeding 15 000 U/mL. Peripheral blood counts were normal except for modest lymphopenia. There was a history of probable exposure to hepatitis B infection, and liver biopsy revealed acute necrosis that was demonstrated to be due to hepatitis B virus. The clinical course was fulminant, and the patient died 5 days after admission. The fulminant course is unlikely to be related to cladribine (see Discussion). A second patient developed abdominal pain and low grade fever 2 weeks after conclusion of her second course of placebo. She declined hospital admission and did not attend for follow-up the next day. 2 days later she was admitted to the hospital with severe lower abdominal pain, decreased bowel sounds, and rebound tenderness. Surgery was considered, but a culture revealed *Salmonella enteritidis* in her stools, and the patient responded promptly to antibiotic therapy.

2 patients who had received cladribine had mild episodes of herpes zoster restricted to one or two dermatomes, and these subsided rapidly on treatment with oral acyclovir.

Discussion

Immunosuppressive therapy in MS has previously involved treatment primarily with cyclophosphamide, azathioprine, and cyclosporin. Evidence of modest efficacy[15] has been tempered by significant side-effects in some patients on long-term therapy. Plasmapheresis and lymphocytapheresis have been tried but there is no clear evidence of sustained clinical benefit.[16] Monoclonal antibodies directed against specific T-cell subsets have also been used in MS but the primary obstacle to long-term treatment has been the development of antibody to the monoclonal.[17] Although interferon beta has been found to be effective in relapsing-remitting MS—with significant reductions in exacerbation rate, in severity of exacerbations

and in accumulation of MRI lesions—no satisfactory treatment has been found for progressive MS.

Cladribine in our study was associated with highly significant improvements in neurological ratings. The MS status of patients on placebo continued to decline, and this neurological deterioration was not only significant but also was sufficiently severe to affect the patients' disability status.

In a double-blind study the aim is for both patients and evaluating physicians to be blinded to the treatment being given but sometimes there are clues that make blinding less than perfect. For example, although the study of interferon was blinded[18,19] this agent does cause malaise, among other prominent side-effects, and some patients may have known whether or not they were on active drug. With cladribine there are no such clues[5] but unexpected thrombocytopenia did lead to a delay in drug dosage in some patients, 4 on active drug and 2 on placebo. This may have provided a clue to the patient and, possibly, the neurologist that active drug was being given but exclusion of data on these 6 patients does not affect the significance of the findings. Moreover, the neuroradiologist knew nothing about the drugs administered and the MRI data indicate that new or active MS lesions decreased in patients on cladribine. The overall volume of demyelinated lesions did not significantly change. In addition, there was a significant difference in the concentration of oligoclonal protein in the CSF of patients on cladribine.

In general, cladribine was well tolerated, but marrow suppression with platelet counts below 80 000/μL was documented in 4 patients and between 80 000/μL and 100 000 in another 3.[14] The frequency of thrombocytopenia was high enough for us to plan an additional trial at a lower dose, including also patients with remitting relapsing disease. The death from hepatitis B requires comment. Liver damage in chronic hepatitis B seems to have an immunological mechanism[20] and treatment with immunosuppressive drugs seems to ameliorate the course of both acutely acquired and chronic hepatitis. "Rebound" exacerbation of infection may occur when immunosuppression is withdrawn[21,22] but our patient had fulminating hepatic necrosis during and immediately after the infusion of cladribine. Cladribine has no known liver toxicity and liver-function tests of the other patients in this study revealed no difference between drug and placebo patients. More than 1000 patients have received cladribine at this institution, and at least 1 has developed viral hepatitis with a benign course. Over 5000 patients have received this drug worldwide and no other case of fulminant hepatitis infection has been recorded. Indeed, a patient with subfulminant hepatitis C from blood transfusion given 18 days earlier showed rapid recovery in the face of the cladribine therapy.[23] We consider it unlikely that the fulminant course in our patient was related to cladribine.

Cladribine is incorporated into DNA.[1] Although our first patients were treated a decade ago[2] most had far advanced leukaemia or lymphoma and did not survive long enough to permit assessment of long-term toxicity or oncogenicity. Large numbers of patients with hairy cell leukaemia have been treated in the past 5 years, but these generally received a total dose of only 0·7 mg/kg body weight. None of the 4 patients with MS we treated with a total dose of 2·5 mg/kg 4 years ago in a pilot study has had adverse effects long-term.

In this study surgical implantation of a catheter was required because no information about the efficacy of other routes of administration was available. We now recognise that the drug may be given by the much more convenient subcutaneous route,[24] and in future trials this is what we plan to use. Preliminary results suggest that a lower dose is less likely to produce marrow suppression and is effective. Cladribine may become a useful agent for the management of chronic progressive MS.

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