Short report

Cyclosporin A curtails the progression of free light chain synthesis in the CSF of patients with multiple sclerosis

B N McLEAN,* P RUDGE,† E J THOMPSON*

From the Departments of Neurochemistry, Institute of Neurology, and the National Hospital for Nervous Diseases,† London, UK*

SUMMARY Free light chains in the cerebrospinal fluid of patients with multiple sclerosis correlate closely with disease activity. As part of a double-blind placebo controlled trial of immunosuppression using cyclosporin A in patients with multiple sclerosis, light chain analysis was performed on pre- and post-treatment CSF samples in 19 patients, nine having received cyclosporin A and 10 placebo. The placebo treated patients showed continued immunological activity, as evidenced by an increase in both free kappa and lambda chains, but cyclosporin A treatment resulted in no change or an improvement in light chain response, indicative of suppression of continued activity. As cyclosporin A does not enter the CSF, this implies that systemic stimulation is at least partly responsible for continued disease activity in multiple sclerosis. Cyclosporin A may thus be effective in preventing the deterioration of patients with multiple sclerosis.

Multiple sclerosis is a demyelinating disorder of unknown aetiology, although considerable evidence exists for the importance of immunological mechanisms in the pathogenesis. Analysis of the CSF not only aids in diagnosis, but also has been found useful in assessing disease activity. In particular the presence of free light chains correlates significantly with recent demyelinating activity.^{1,2} There is no specific treatment for multiple sclerosis, so attention has focussed on the role of immunomodulating agents on established disease. So far only high dose steroids, in the form of oral prednisolone, or intravenous methylprednisolone, have been shown to have some clinical benefit on the acute relapse,³ but these do not influence the ultimate outcome, merely ameliorate the symptomatic period, and have no significant influence on immunological parameters.

A randomised prospective double-blind controlled trial using cyclosporin A was thus undertaken at the National Hospital for Nervous Diseases in London to

examine the effect of long term therapy on clinical outcome. As part of the study, immunological parameters in CSF were monitored, the results of which we present here.

Materials and methods

Patients fulfilling the international criteria for clinically definite multiple sclerosis⁴ were recruited and assigned randomly to receive 10 mg/kg/day cyclosporin A for 2 months then 8 mg/kg/day for the next 22 months or placebo in a double blind fashion. The mean amount of cyclosporin A received by the subjects over the final 22 months was 7.2 mg/kg/day. Blood and CSF drug levels were initially measured by radioimmunoassay (RIA) and later by high performance liquid chromatography (HPLC). Lumbar puncture was performed immediately before commencement of 2 years of therapy and again at completion. Altogether 43 patients were recruited. Of these six males and 13 females, mean age 32 and 34 yr respectively had two lumbar CSF specimens suitable for analysis. All had established disease for 6.6 and 8.6 (range 2 to 18) years.

CSF samples were spun, stored at 4°C until analysis which was within 24 h of puncture. A matched serum specimen accompanied each CSF. Kappa/Lambda immunoblotting was performed as previously described.⁵ Briefly, CSF specimens were electrophoresed on 6 mm rod polyacrylamide gels, a uniform amount of protein being loaded on each gel. Upon completion of the run (about 2 h),

Address for reprint requests: Dr Brendan McLean, Dept of Neurochemistry, Institute of Neurology, Queen Sq, London WC1N 3BG, UK.

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protein was then transferred to nitrocellulose membranes by compression, and the membranes incubated with light chain type-specific goat anti-human antisera and finally rabbit anti-goat IgG-HRP conjugate before visualising with 3-amino, 4-ethylcarbazole. The resulting light chain patterns were assessed for the presence of free and bound bands. The same investigator assessed all the immunoblots. When all the patients had completed the trial, and the analyses performed, the code was broken.

Results

Measurement of cyclosporin A by RIA and HPLC gave trough levels in blood varying between 300 and 500 ng/ml. In CSF all levels were less than 30 ng/ml., which was the limit of detection of the assays.

Of the 43 patients recruited, 10 failed to complete the trial, either because of failure of compliance or refusal of a lumbar puncture. Eleven patients entered the trial before the technique of light chain immunoblotting was developed, and although the second CSF was analysed, insufficient sample remained from the first specimen to perform any immunoblots. A further three patients failed to have a second CSF analysed for technical reasons. This left 19 patients with two suitable CSF specimens. Nine patients received cyclosporin A (six females, three males) and 10 received placebo (seven females, three males). The table shows the analysis for each group.

The pre-treatment CSF profiles did not show any significant differences between the two groups. When total numbers of free and bound light chains were considered, the increase in the placebo group is significant (1% level, Wilcoxon rank sum 2-tailed), whereas the cyclosporin group showed no significant change. A comparison of the changes in the groups shows that placebo therapy was associated with an increase in the numbers of light chains, but cyclosporin A effected no change or an improvement (<5% probability, Fisher's exact test). Considering now free light chains only, placebo treatment resulted in a significant increase in numbers (1%, Wilcoxon), whereas cyclosporin A resulted in no significant change. Again, comparing the two groups, placebo therapy resulted in increases in free light chain numbers, but cyclosporin A resulted in no change or fewer (<5%, Fisher's exact). There was no significant difference between and within placebo and cyclosporin A groups in bound light chains. When free light chain subtypes were examined, both Kappa and Lambda showed a significant increase with placebo therapy (5%, Wilcoxon, for both), and no change with cyclosporin A. Comparing the two groups, cyclosporin A therapy resulted in no change or a decrease in numbers and placebo an increase for both Lambda (<2% significance, Fisher's exact) and Kappa (<5% significance, Fisher's exact). In bound chains there

Table Summary of light chain analysis in 19 patients treated with either placebo or cyclosporin A

Patient	Light chains								Treatment
	Pre-treatment				Post-treatment				
	Free		Bound		Free		Bound		
	K	L	K	L	K	L	K	L	
5.BU	0	0	0	0	0	0	0	1	Cyclosporin
13.DU	0	0	0	0	0	0	0	0	"
17.FI	0	0	0	0	0	0	0	0	"
18.FR	0	0	2	0	0	0	0	0	"
19.HA	0	0	0	1	2	6	1	3	"
29.OC	0	0	0	0	1	0	0	0	"
34.RE	0	0	0	1	0	8	0	2	"
38.TH	2	6	2	2	2	5	2	2	"
40.WA	0	0	0	0	0	0	0	0	"
3.BE	0	0	1	2	6	7	3	0	Placebo
4.BO	0	0	0	0	0	0	0	0	"
14.ED	0	0	1	0	8	2	3	0	"
20.HI	0	1	0	0	3	7	2	3	"
26.LA	0	0	1	1	3	7	0	1	"
27.MA	0	1	1	0	3	3	1	1	"
30.PE	0	2	0	2	0	8	0	2	"
36.RU	2	5	1	1	0	2	3	6	"
39.WA	0	0	0	1	1	4	0	1	"
42.WY	0	3	2	3	6	10	3	1	"

K = Kappa, L = Lambda, numbers refer to bands seen on blots of electrophoresed CSF.

were no significant changes in either subtype on any test for both groups.

Discussion

Free light chains in the CSF of patients with neurological disease originate from intrathecal synthesis and not degradation of IgG.^{6,7} They reflect disease activity, but are not disease specific, occurring in a variety of neurological disorders, including multiple sclerosis.⁸ Increased numbers of free light chains are associated with recent antigenic stimulation in multiple sclerosis, but not apparently with clinical disability.¹ The reason for this elevation is unclear, but probably reflects increased turnover in stimulated B-lymphocytes. In our study the changes in light chain numbers were entirely due to the free fraction, and it was in the placebo treated group only that significant increases were seen. Cyclosporin A treated patients had no change, or a decrease in free light chain numbers. Since free light chains indicate active disease, this suggests that in the placebo treated group there was continuing demyelination and that cyclosporin A caused a decrease in activity. Clinical studies on the two groups also suggests that cyclosporin A appeared to halt deterioration (to be published). It has been established in this series and others, however, that cyclosporin A does not enter the CSF in therapeutic or even detectable quantities. This implies that the mechanism of action of cyclosporin is on the systemic

immune system and supports the concept that it is systemic immune activation that triggers CNS demyelination or contributes to its maintenance. Other workers have found that cyclosporin A suppresses the continued and progressive immune activation in the serum of multiple sclerosis patients, as evidenced by T-cell changes.⁹ Subtype analysis of the light chains demonstrates that there are significant increases in both Kappa and Lambda with disease progression, and that cyclosporin A affects both similarly. This does not support the findings of some workers, that there is a dominant Kappa response in multiple sclerosis,⁶ but agrees with others.⁸

This study shows that cyclosporin A is effective in reducing continued immune activity in patients with multiple sclerosis, and thus may arrest the clinical deterioration.

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