A double-blind controlled trial of high dose methylprednisolone in patients with multiple sclerosis: 2. laboratory results

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SUMMARY Laboratory measurements were compared in paired samples from 50 patients included in a double-blind placebo controlled trial of methylprednisolone in the treatment of multiple sclerosis. Cerebrospinal fluid total cell count, IgG and C9 indices, and percentage of peripheral blood OKT8 positive cells were abnormal at entry and returned closer to the normal range after active than placebo treatment, but the differences were not statistically significant. The percentage of peripheral blood OKT4 positive cells was normal at entry as was the amplitude of visual evoked potentials, whereas their latency was prolonged; these measurements were each uninfluenced by methylprednisolone. Corticosteroids might act merely by influencing oedema, but the laboratory results suggest that methylprednisolone affects immunological events which underly rapid onset and recovery of symptoms in patients with multiple sclerosis; additional forms of treatment are needed to maintain these clinical and immunological effects.

The development of strategies for treatment in multiple sclerosis is limited by poor understanding of the sequence of events leading to production of symptoms. High dose intravenous methylprednisolone improves acute and chronic symptoms in multiple sclerosis compared with placebo treated controls and in most patients these effects occur within one week of starting treatment suggesting that corticosteroids influence rapidly reversible mechanisms of myelin injury. Although this group of drugs has been used in the treatment of multiple sclerosis for many years the mode of action has rarely been investigated. Symptomatic improvement in demyelination could result from resolution of oedema, direct effects on central conduction or modification of immunological events involved in the pathogenesis of myelin injury. Imaging, electrophysiological and immunological methods can be used to investigate patients with multiple sclerosis but no one abnormality correlates well with the time course of clinical symptoms and in turn, the clinical method may be a poor guide to disease activity. In order indirectly to investigate the mechanism of action of methylprednisolone in multiple sclerosis, we have recorded the amplitude and latency of visual evoked potentials, enumerated circulating T cell sub-population phenotypes, measured cerebrospinal fluid (CSF) total cell count and derived IgG and C9 indices, before and after active or placebo treatment with methylprednisolone in patients with acute relapsing and chronic progressive forms of the disease.

Methods

Fifty patients were treated for 5 days with high dose intravenous methylprednisolone or a placebo preparation, preceded by measurement of circulating lymphocyte sub-population phenotypes and CSF total cell count. IgG, albumin and C9 were measured in plasma and CSF. Visual evoked potentials were recorded. Laboratory assessments were repeated 28 days after the start of active or placebo treatment. All observations were made blind with respect to treatment randomisation and the clinical course.

Samples of CSF and plasma in 10 mM EDTA were stored in aliquots at −70°C within 2 hours of collection. IgG and albumin were measured by rate immunonephelometry using commercially available anti-sera. C9 was estimated by a modified version of the previously described assay using a...
Kemtek 3000 automated immunoassay system. Bound radio-activity was automatically separated and counted after 2 hours incubation of 100 µl samples of diluted CSF or plasma in triplicate with 200 µl diluting buffer containing monoclonal antibodies C9-34 solid phase and radiolabelled monoclonal antibody C9-47. Any samples with C9 concentrations outside the working range of 0.1–4.4 µg/ml and 5–200 µg/ml for CSF and plasma respectively were reassayed at different dilutions. The IgG index was derived from the ratios of IgG and albumin concentrations in CSF and plasma. An analogous ratio, the C9 index, was calculated in an identical way substituting C9 concentration for IgG. CSF cell counts were estimated on fresh samples using a haemocytometer; differential cell counts were performed using morphological criteria but no sample contained more than three polymorphonuclear cells and the total cell count has been used for analysis. Lymphocytes from 15 ml of heparinised blood were separated on ficoll-hypaque by density centrifugation and cryopreserved in liquid nitrogen for up to 9 months. After rapid thawing, 1 x 10⁸ cells were incubated with 5 µl of undiluted OKT8 or OKT4 antibody (Orthomune Diagnostics) or 5 µl of phosphate buffered saline at 4°C for 30 minutes and then with 10 µl of undiluted fluorescein isothiocyanate conjugated goat anti-mouse immunoglobulin (Hybritech) at 20°C for a further 6 minutes. Cells were re-suspended in phosphate buffer for counting by flow cytometry on a Becton Dickinson FACS III. The number of fluorescent cells/1000 was estimated after subtraction of background counts which ranged from <0.5–2%, and were expressed as a percentage of total mononuclear cells. The latency and amplitude of the P100 response were recorded from an active electrode placed 5 cm above the inion with a reference electrode 10 cm above the nasion, after pattern reversal stimulus using an OTE myograph/evoked potential system (BASIS); 100 responses were averaged. Our normal range for the amplitude and latency of the P100 response is 3.2–15.5 mV and 88–105 ms respectively.

Statistics
Percentages of OKT8 and OKT4 lymphocytes and visual evoked potential latencies were analysed without transformation of scale, and summarised by mean ± standard deviation. CSF cell count, IgG and C9 indices, and amplitude of visual evoked potentials were log transformed prior to analysis; results for these variables are expressed as geometric means together with 2½ and 97½ percentiles fitted by the lognormal model.

Differences in response between active and placebo groups were assessed using analysis of covariance, the corresponding pre-treatment value being used as covariate. The adjusted difference in response is expressed on the appropriate scale of measurement, as an absolute or percentage difference, together with a t value to assess statistical significance.

Results
Cerebrospinal fluid cell count (fig 1)
Total CSF cell count was available in paired samples from 47 patients (active 24, placebo 23). The geometric mean cell count before treatment in all 47 individuals was 5 cells (95% of cell counts are in the range 0.5–46). The effect of treatment with methylprednisolone was to lower the cell count to 33-4% less than expected on placebo treatment (t = 1.62). CSF cell counts fell by ≥2 cells in 25, (13 active, 12 placebo), remained stable in 10 (eight active, two placebo) and rose by this number in 12 (three active, nine placebo) cases. The differences are not significant.

IgG index (fig 2)
Plasma and CSF concentration of IgG and albumin were available in paired samples from 42 patients (active 23, placebo 19) and used to calculate an IgG index. The geometric mean IgG index in all 42 individuals before treatment was 1.18 (95% of IgG indices are in the range 0.45–3.13). The effect of treatment with methylprednisolone was to lower the IgG index to 11.3% less than expected on placebo treatment (t = 1.07). IgG index fell by ≥0.1 in 19 (15 active, four placebo), remained stable in 16 (six active, 10 placebo) and rose by this amount in 7 (two active, five placebo) cases. The differences are not significant.

C9 index (fig 3)
Plasma and CSF concentration of C9 and albumin...
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Fig 2  See fig 1 for legend and meaning of symbols.

Fig 3  See fig 1 for legend and meaning of symbols.

was available in paired samples from 14 patients (seven active, seven placebo) and used to calculate a C9 index. The geometric mean C9 index in all 14 patients before treatment was 0.36 (95% of C9 indices are in the range 0.06–2.14). The effect of treatment with methylprednisolone was to raise the C9 index to 68.7% more than expected on placebo treatment ($t = 2.14$, $p < 0.06$). C9 index rose by $0.05$ in 12 (active seven, placebo five), remained stable in one (placebo) and fell by this amount in one (placebo) patients.

T8 cells (fig 4)

Peripheral blood circulating OKT8 positive cells were measured in paired samples from 34 patients (15 active, 19 placebo). The mean (±SD) percentage of OKT8 positive cells in all 34 patients before treatment was 20.8 (±6.9). The effect of methylprednisolone treatment was to increase the OKT8 positive cell percentage to 4.2 more than expected on placebo treatment ($t = 1.85$). OKT8 positive cells rose by $\geq 3\%$ in 18 (active nine, placebo nine), remained stable in nine (active four, placebo five) and fell by this number in seven (active two, placebo five) patients. The differences are not significant.

T4 cells (fig 5)

Peripheral blood circulating OKT4 positive cells were measured in paired samples from 34 patients (15 active, 19 placebo). The mean (±SD) percentage of OKT4 positive cells in all 34 patients before treatment was 39 (±7.3). The effect of methylprednisolone treatment was to increase the OKT4 positive cell percentage to 2.2 more than expected on placebo treatment ($t = 0.83$). OKT4 positive cells rose by $\geq 3\%$ in 20 (active 10, placebo 10), remained stable in six (active one, placebo five) and fell by this number in eight (active four, placebo four) individuals. The differences are not significant.
Visual evoked potentials (figs 6, 7)

Paired recordings of amplitude and latency of visual evoked potential were made in 52 eyes from 30 patients. Since any alteration as a result of treatment in one patient is likely to affect both eyes, we have analysed responses from the right and left eye of each patient separately; in neither case were significant differences in latency or amplitude observed following active treatment.

The mean latency from 27 right eyes (14 active, 13 placebo) before treatment was 110.8 (±13.1) ms and the geometric mean amplitude 5.69 mV (95% of the amplitudes are in the range 2.73–11.9 mV). The effect of treatment was to increase the latency to 0.7 ms more than expected on placebo treatment ($t = 0.46$); the equivalent change in amplitude was +12.3% ($t = 0.53$). The right eye latency increased by $\geq 5$ ms in four (active three, placebo one), remained stable in 19 (active eight, placebo 11) and fell by this amount in four (active three, placebo one) patients. The right eye amplitude rose by $\geq 0.05$ mV in eight (active five, placebo three), remained stable in seven (active three, placebo four) and fell by this amount in 12 (active six, placebo six) individuals. The mean latency from 25 left eyes was 114 ($\pm 14.9$) ms before treatment and the geometric mean amplitude 5.82 mV (95% of the amplitudes are in the range 2.69–12.6 mV). The effect of treatment was to increase the latency to 1.1 ms more than expected on placebo treatment ($t = 0.67$); the equivalent change in amplitude was $-0.8%$ ($t = 0.04$). The left eye latency increased by $\geq 5$ ms in three (active), remained stable in 19 (active nine, placebo 10) and fell by this amount in three (active one, placebo two) individuals. The left eye amplitude fell by $\geq 0.05$ mV in 10 (active six, placebo four) remained stable in seven (active two, placebo five) and rose by this amount in eight (active five, placebo three) individuals (not shown in figures). These differences are not significant.

**Discussion**

Patients with acute relapsing and chronic progressive multiple sclerosis improved clinically following treatment with high dose methylprednisolone in a double-blind placebo controlled trial, IgG and C9 indices and the percentage of peripheral blood OKT8 positive cells were abnormal at entry and returned closer to the normal range after active than placebo treatment but the differences were not statistically significant. The percentage of peripheral blood OKT4 positive cells was normal at entry, as was the amplitude of visual evoked potentials but not their latency; each was uninfluenced by methylprednisolone.

Difficulties arise in interpreting these laboratory results not least because the physical basis for symptoms is poorly understood during both the relapsing/remitting and progressive phases of the disease. Morphologically there is an orderly progression from lymphocytic infiltration of the early lesion to development of severe demyelination, axonal loss, oligodendrocyte depletion, astrocytosis and insignificant remyelination seen in chronic plaques; conversely, the symptoms in multiple sclerosis are usually intermittent and recover spontaneously in the early stages. Factors which determine progressive symptoms, whether from onset or after a period of relapses, are unknown but the available evidence suggests that the switch to progression does not signify a change in the disease process; for this reason we have assessed the laboratory effects of methylprednisolone in multiple
sclerosis patients irrespective of clinical classification.

Several mechanisms have been proposed for symptomatic recovery including resolution of oedema, synaptic reorganisation and removal of complement-dependent neuro-electric blocking factors. But none of these can explain all the clinical and pathological observations. One interpretation is that the symptoms of multiple sclerosis are frequently not due to demyelination defined morphologically but arise from mechanisms of myelin injury which are fully reversible. We have suggested that complement activation leading to formation of membrane attack complexes mediates reversible and irreversible lesions which in combination, could account for the time course of symptoms during the relapsing and progressive phases.

Corticosteroid treatment in patients with multiple sclerosis reduces intrathecal IgG production, reflected by changes in CSF IgG concentration, IgG index, IgG synthesis rate and intensity or number of oligoclonal bands; one or other of these changes is observed following oral, intramuscular, intravenous or intrathecal treatment but there is no effect on CSF cell count and no clear pattern emerges from comparing the reported effects of corticosteroids, or other immunological agents, on number or function of B and T lymphocytes and their subpopulations. A reduction in latency of abnormal visual evoked potentials has been observed during serial studies of untreated patients and during combined immunosuppressive treatment but the electrophysiological effects of methylprednisolone are unknown. In our study, corticosteroids produced rapid clinical improvement during relapse and in the progressive phase and there were simultaneous changes in several laboratory indices shown to be abnormal before treatment. The possibility exists that these changes would have been statistically significant if we had obtained paired samples from all participants or made post-treatment assessments on completion of the five day course.

Although the latency of visual evoked responses does not necessarily reflect changes in conduction throughout the nervous system, our results provide no evidence that rapid symptomatic changes occur primarily as a result of altered physiological properties in demyelinated axons. The rapid clinical improvement could be accounted for by resolution of oedema, present at least in acute plaques, in which case no alteration in immunological abnormalities would be expected; none were observed in our study. The selective effect of methylprednisolone in patients with multiple sclerosis could be due to a direct effect on skeletal muscle, similar to the action of dantrium sodium. But collectively the trend towards correction of abnormal peripheral blood OKT8 positive cells, CSF pleocytosis and IgG or C9 indices suggests that the mechanism of action may depend partly on a modification of immunological events directly involved in tissue injury. Specifically, the most significant effect of methylprednisolone we observed was an increase in C9 index; this finding is consistent with inhibition of intrathecal complement activation, an event which normally leads to cell lysis. The rapidly reversible consequences of membrane attack complex formation on cell injury may be mediated by a rise in intracellular calcium and in the special situation of partially demyelinated axons, this would be expected temporarily to interfere with the propagation of nerve impulses. It has recently been demonstrated that conduction in the central nervous system, reflected by latency of visual evoked potentials, improved in patients with multiple sclerosis during infusion of the calcium channel blocker verapamil. High-dose intravenous methylprednisolone might achieve similar, but less immediate effects by inhibiting complement activation, thereby preventing transient changes in the calcium ion environment of myelinated axons.

Corticosteroids appear temporarily to influence the disease process in multiple sclerosis but they do not produce a lasting effect on the clinical or immunological manifestations of the disease. Our clinical and laboratory results endorse the immunological approach to treatment, but the use of corticosteroids may require maintenance treatment with other immunological agents in order to influence the long-term course of the disease.

References

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