Cyclophosphamide in exacerbations of multiple sclerosis

Therapeutic trial and a strategy for pilot drug studies

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SYNOPSIS Cyclophosphamide (CY) has been shown to reverse the signs of experimental allergic encephalomyelitis (EAE) even after the onset of neurological deficits. Because of the analogy of EAE to exacerbations of multiple sclerosis (MS) a clinical trial of CY in acute MS exacerbations was undertaken. A 'sequential criterion' method was used to minimize the size of sample needed for this pilot study. CY failed to alter significantly the course of acute exacerbations of MS. Possible reasons for this failure, and the value of the sequential criterion method in pilot studies, are discussed.

It is well known that after acute exacerbations of multiple sclerosis (MS) some of the acute manifestations may improve spontaneously. For this reason, neurologists have inferred that the neurological deficits occurring during acute exacerbations of MS reflect the potentially reversible component of the disease. A form of treatment limiting the severity of exacerbations and facilitating more complete remissions might theoretically prevent or reduce permanent damage to the central nervous system. Such treatment could arrest further progress of the disease, although reversal of longstanding previously-incurred damage to the central nervous system would not be expected.

In recent years some guidance in the choice of treatment for MS has been derived from the study of possible animal models of the disease. In particular, experimental allergic encephalomyelitis (EAE) has been considered by many investigators to represent the closest analogue to multiple sclerosis, similar in pathology and possibly also in pathogenesis. Many drugs with anti-inflammatory and immunosuppressant action, such as corticosteroids, methotrexate, etc., have been shown to inhibit the development of EAE, but only when administered before the

development of clinical signs (Brandriss *et al.*, 1965). Unfortunately, the comparable use of these drugs in MS is limited by the inability to anticipate the appearance of a new exacerbation in order to administer preventative treatment. However, one drug—cyclophosphamide—has been shown by several workers (Field, 1969; Paterson and Drobish, 1969; Paterson, 1971) to reverse the course of EAE even when administered after signs of nervous system dysfunction have appeared. In these studies cyclophosphamide produced prompt and striking reversal of EAE in a large majority of animals, even if treatment was delayed for as long as five days after the appearance of paralysis, and continued treatment successfully prevented the re-appearance of clinical EAE. More dramatically, histological examination of the brain and spinal cord showed that cyclophosphamide reduced the histopathological changes in treated animals compared with controls (Paterson, 1971).

Since the *raison d'être* of this investigation was the parallel between EAE and MS, the present study sought to determine whether cyclophosphamide might benefit patients with multiple sclerosis when administered in a fashion as closely similar as possible to its effective use in EAE. However, cyclophosphamide is an alkylating agent with significant risks and side-effects, requiring that the investigation be designed as a

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pilot study capable of evaluating the potential usefulness of this agent without using an excessive number of patients. For these reasons, certain stringent criteria of 'success'—comparable with the results observed in EAE—were predetermined; and a 'sequential criterion' design was employed to permit us to study the effectiveness of the drug with the smallest number of patients.

The lack of success of cyclophosphamide in reversing exacerbations of multiple sclerosis, and the usefulness of a sequential criterion design for pilot drug studies in MS, form the basis of this report.

METHODS

PATIENTS Patients were drawn from a large group of known MS patients fulfilling the criteria set forth by Schumacher *et al.* (1965) for 'definite multiple sclerosis'. Only those patients with an acute exacerbation producing new objective neurological signs within the previous four days or with a subacute attack of MS showing significant progression during the four days before treatment were admitted to the study. All patients were screened for haematological disorders, and for pregnancy in females. No patient who had received ACTH or corticosteroids within the preceding six months was considered for acceptance in the study. All patients gave informed consent for treatment after the risks and possible benefits were explained.

In all, patients were planned to be admitted to the study as they presented with new symptoms up to a maximum sample size of 20 patients, or until the 'sequential criterion' described below was reached, indicating either success or failure of the treatment.

TREATMENT Patients were hospitalized for approximately three weeks for treatment and subsequent observation. Cyclophosphamide was given intravenously in single daily doses of 4–5 mg/kg for 10 successive days, beginning no later than five days after the occurrence of new symptoms. In no case did side-effects of medication require reduction of dose.

LABORATORY TESTS Each patient received a peripheral blood count, urinalysis, bone marrow evaluation, and lumbar puncture on admission. Haematological and urine examination were frequently repeated during treatment and subsequent hospital observation; lumbar punctures were repeated at the end of three weeks. In five patients lymphocyte transformation studies were carried out. At the

beginning of treatment and eight to 13 days afterwards observations were made on the incorporation of tritiated thymidine into cell-cultures of lymphocytes exposed *in vitro* to a nonspecific mitogen (phytohaemagglutinin-M (PHA-M)), and to other common antigens (varidase; monilia). Results were expressed as disintegrations per minute (d.p.m.)

NEUROLOGICAL EVALUATION Complete neurological evaluations were carried out on admission and at three day intervals for three weeks, with special attention to the areas of recent neurological deficits that led to treatment, and to the development of any new neurological signs or symptoms.

DEFINITION OF SUCCESS OR FAILURE Neurological symptoms and signs were divided into phase 1 deficits, present at the beginning of therapy, and phase 2 deficits which developed between the fifth and 21st days of treatment. The criteria for clinical success were that no phase 1 deficits should progress after the fifth day of treatment and no phase 2 deficits develop after the fifth day of treatment. These arbitrary limits were based specifically on the observed time of the arrest of neurological deficits in cyclophosphamide-treated animals with EAE (Paterson and Drobish, 1969; Paterson, 1971); and on evidence that cyclophosphamide produces immunosuppression in humans within this period (Santos *et al.*, 1970).

STATISTICAL METHODS Since this was designed as a pilot study, arbitrary decisions were made for an 'acceptable' success rate and a 'reasonable' sample size. It was decided arbitrarily that if significantly more than 50% of patients showed arrest of exacerbations of MS this would be sufficient preliminary evidence on which to plan a more definitive study. The maximum sample size was set at 20 patients, based on estimates of the rate at which patients within the available clinical population presented with new exacerbations; and the justification for a trial of a drug of known toxicity and unknown efficacy in volunteer MS patients.

A sequential criterion method was used to inspect the data periodically (Armitage, 1960, 1971). This method utilizes a 'stopping rule', which enables the investigator to determine the earliest point at which the data reach statistical significance, or the first point at which, within a maximum sample size (20 patients in this study) criterion can no longer be reached. Using this technique, if treatment were successful in all of the first seven patients, a significant majority could be recognized as showing arrest of exacerbations ($P < 0.05$); with one failure, 9/10 successes would be needed to satisfy the same

criterion. Other stopping points are 10/12, 12/15, 14/18, 15/20. The accumulation of six treatment failures would indicate that success for a significant majority (> 50%) of patients could not be achieved within a group of 20 patients, and the study would be terminated.

RESULTS

CLINICAL COURSE Patients were treated individually during an experimental period lasting 18 months. All of the first six patients showed either progression of phase 1 deficits on the fifth day of treatment or later, or the development of phase 2 deficits after four days, or both; and thus all were considered treatment failures (Table 1).

In the four patients in whom the phase 1 deficits progressed, increasing symptoms and signs were noted throughout the course of the hospital portion of the study—that is, for 21 days. In two patients, phase 1 deficits were arrested by the third and fourth days respectively.

TABLE 1
CYCLOPHOSPHAMIDE TRIAL IN ACUTE MS

Patient	Age (yr)	Sex	Neurological deficits		Success or failure
			Phase 1: progress	Phase 2: development	
1	20	Female	0	+	Failure
2	31	Male	+	+	Failure
3	26	Female	+	+	Failure
4	47	Male	+	0	Failure
5	49	Female	+	+	Failure
6	25	Female	0	+	Failure
Totals			4	5	6 Failures/6 0 Successes/6

In five patients, phase 2 deficits appeared after the fifth day of treatment, beginning on the fifth day in three and the seventh day in two patients, and continuing for between five and nine days subsequently.

Since all of the first six patients were treatment failures by the predetermined criteria, the study was discontinued according to the sequential stopping rule; the possibility of statistically-significant success within sample size of 20 patients had been eliminated.

TABLE 2
LYMPHOCYTE TRANSFORMATION DURING
CYCLOPHOSPHAMIDE TREATMENT

Patient	PHA*		Varidase†		Monilia‡	
	Pre	Post	Pre	Post	Pre	Post
1	—	—	—	—	—	—
2	+	0 (22%)‡	±	0 (20%)	—	—
3	+	0 (37%)	0	+	+	+
4	+	0 (15%)	+	0 (0.4%)	—	—
5	±	0 (45%)	—	—	—	—
6	+	0 (10%)	+	0 (7%)	—	—

* + = > 100 000 d.p.m.; ± = 80 000–100 000 d.p.m.

† + = > 10 000 d.p.m.; ± = 8 000–10 000 d.p.m.

‡ = % of pretreatment d.p.m.

— = Missing data.

Despite the failure of the treatment according to the predetermined criteria, an effort was made to see whether any unanticipated overall benefit to the patients, either on a long or short-term basis, could be detected. Only one patient appeared better overall after the course of treatment (patient 1), and she had been regarded as a treatment failure because of the development of new (phase 2) abnormalities during therapy. One other patient underwent a dramatic and rapid downhill course both during and after treatment, developing a severe tetraparesis due to a major brain-stem lesion. The other patients showed no distinctive neurological benefits or deterioration as the consequence of treatment.

LABORATORY FINDINGS In all, the white blood counts of six patients fell below 4 000/mm³, with a mean minimum of 2 850 WBC/mm³. In most patients the nadir occurred within a week after cessation of therapy.

In five patients lymphocyte transformation to a variety of stimulating agents was studied. In each case marked inhibition of lymphocyte transformation to the non-specific mitogenic stimulus, phytohaemagglutinin-M, was clearly demonstrated. In one patient a significant lymphocyte response to monilia was present at the beginning of treatment and showed no suppression during treatment. In two other patients initial positive responses to varidase were inhibited during the course of immunosuppression, while one patient developed an increased response (possibly artefactual).

Lumbar cerebrospinal fluid was obtained on admission in all patients and at the end of the period of observation in four. On admission, the cerebrospinal fluid protein showed a mean of 0.375 g/l with a mean immunoglobulin G (IgG) of 20.2%; post-therapy mean total protein was 0.478 g/l, with a mean IgG of 26.1%, showing no significant change after treatment.

SIDE-EFFECTS Almost total alopecia occurred in two patients after treatment, despite the use of a scalp tourniquet during the administration of cyclophosphamide. Lesser degrees of partial alopecia occurred in all patients treated, but did not represent a significant problem. Cystitis developed in one patient, possibly related to treatment. Nausea occurred in all patients after several days of treatment, but was readily controlled with antinauseant medication. In no patient did significant intercurrent infection occur.

DISCUSSION

The present study of the effect of cyclophosphamide on acute exacerbations of MS was, both in principle and in methodology, a direct outgrowth of the demonstration of successful reversal of exacerbations of EAE in experimental animals (Field, 1969; Paterson and Drobish, 1969; Paterson, 1971). However, we could find no pattern of change in neurological signs and symptoms to suggest that cyclophosphamide altered the clinical course of the patients, and it appeared that the acute manifestations of MS progressed in a typical fashion. The results of the pilot study contrasted sharply with the results obtained by the use of cyclophosphamide in EAE.

The possible relation of EAE to MS has been strongly suggested for many years, largely based on the similarity of the pathological lesion in the two conditions (Wolf, 1963; Levine, 1971). The relationship has been emphasized by studies of Bornstein and coworkers demonstrating the presence of presumptive antibodies both in animals with EAE and in patients with MS that, with the aid of complement, prevent myelination in neuronal tissue culture, and interfere with synaptic transmission (Bornstein and Appel,

1961, 1965; Bornstein, 1972). Other findings, including the presence of IgG in the lesion of MS (Tourtellotte and Parker, 1967) give further support to this immunological relationship. Reservations about the similarities of the two conditions arise from the inability of many investigators to demonstrate cutaneous hypersensitivity, circulating antibodies of conventional types, or *in vitro* cellular responses to presumptive antigenic substances (Mackay *et al.*, 1973; Paterson, 1973).

Presumptions that MS has an immunological basis have led to trials of several immunosuppressive agents using a variety of regimens, with generally unsuccessful results. In most of these studies the immunosuppressive agents were given chronically in an effort to prevent progression or exacerbations of MS (Millac and Miller, 1969; MacFadyen *et al.*, 1973; Silberberg *et al.*, 1973). Silberberg *et al.* (1973) found no benefit in the chronic use of azathioprine in 15 patients with MS; nor did Millac and Miller (1969) find that long-term cyclophosphamide treatment altered the course of MS in nine patients maintained for a year on this drug. MacFadyen *et al.* (1973) noted no improvement or alteration in course in four patients treated with antilymphocyte globulin (ALG) for brief periods. Only in the large scale studies of ACTH has any anti-inflammatory or immunosuppressive regimen shown a consistent pattern of benefit, and that was noted to be a temporary one (Rose *et al.*, 1970).

The present trial of cyclophosphamide in MS differs from previous studies in several particulars. Only patients with acute exacerbations of very recent onset, or those with recently progressing signs, were included in the study. The use of this highly selected patient group provided the opportunity to observe whether rapid institution of effective immunosuppression could halt the progress of disease or prevent the development of new manifestations during a period of MS 'activity', in a manner closely comparable with the effective use of cyclophosphamide in EAE. Failure to reproduce the results seen in EAE in these ideal circumstances further weakens the possibility that other—either acute or chronic—immunosuppressive regimens would realistically hold out hope for benefit.

Several explanations should be considered for the failure of this acute immunosuppressive

regimen to halt the progress of exacerbations of MS despite its effectiveness in reversing EAE:

1. Cyclophosphamide may fail to penetrate the blood-brain barrier in MS patients, even at the site of recent involvement and, presumably, histopathological change.

2. Since an exacerbation of MS represents a recurrence of disease, 'memory' immunocytes would be involved in any immunological response, in contrast with the primary-type response to nervous tissue antigen in EAE. Cyclophosphamide is well known to be more effective in suppressing primary than secondary immune responses (Gabrielsen and Good, 1967); this immunological difference might explain the effectiveness of treatment in EAE and its failure in MS. Illustrating this point, one patient (after cyclophosphamide treatment) showed loss of *in vitro* lymphocyte transformation to the non-specific mitogen, PHA; while lymphocyte transformation to a previously-experienced antigen—monilia—persisted despite treatment.

3. Alternatively, a purely immunological model may be an incorrect explanation for clinical MS. The human disease may be the result of several interactive mechanisms—such as persistent virus infection with a host immune response (Johnson and Weiner, 1972; Adams and Dickinson, 1974)—or it may result from a primarily non-immunological process.

The use of a pilot study with the sequential analysis method of determining a 'stopping rule' seems a particularly appropriate method for evaluating drug treatment in this disease. From a practical standpoint, we were not interested in determining whether cyclophosphamide treatment provided a minimal percentage benefit over non-treatment; only a major alteration of the course of the disease in a majority of patients would justify further investigation or treatment with this agent. Sequential analysis permitted us to draw a practical, negative conclusion with a relatively small group of patients. It may be recommended as an economical method of conducting other pilot studies, with sample size and criteria for success set appropriately for the experimental design.

This study, like all clinical investigations of the response of MS patients to a treatment regimen, is hampered by the absence of a reliable indicator of disease activity, and the need to rely

on indirect measures of disease, as shown by clinical symptoms and signs. The finding of a more reliable indicator of MS activity—perhaps the macrophage migration inhibition factor recently reported by Sheremata *et al.* (1974)—could markedly simplify the process of evaluating therapy in this tantalizing yet frustrating disease.

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