Plasma exchange and immunosuppressive drug treatment in myasthenia gravis: no evidence for synergy

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SUMMARY We have investigated whether plasma exchange in myasthenia gravis synergises with additional immunosuppressive drug therapy (azathioprine, cyclophosphamide or cytosine arabinoside). Serum anti-acetylcholine receptor (AChR) antibody titres were followed over 28 days after a course of PE in 20 patients, of whom 17 were taking 20–80 mg prednisone on alternate days. No significant difference was observed in mean anti-AChR antibody recovery following plasma exchange with and without additional immunosuppressive therapy. In paired studies where patients served as their own controls, mean anti-AChR recovery with and without azathioprine or cytosine arabinoside showed no significant differences. Anti-AChR recovery rates after large and small plasma exchange courses also did not differ significantly. Prolonged administration of azathioprine reduced antibody titres independently of plasma exchange. These results fail to demonstrate significant synergy between plasma exchange and the additional immunosuppressive drugs used, and suggest that the effects of plasma exchange were transient.

The disorder of neuromuscular transmission in myasthenia gravis can be accounted for by a decrease in the number of functional acetylcholine receptors (AChRs)[1][2] and anti-AChR antibodies have been implicated in the loss of AChR.[3][4] Plasma exchange can produce a substantial reduction in serum anti-AChR and is associated with corresponding short-term clinical improvement.[5][8] When combined with immunosuppressive drug treatment, plasma exchange may be followed by prolonged remission. It has been implied that plasma exchange may play an essential part in this response,[6][9] but controlled data are lacking. Immunosuppressive drugs alone can induce remission in myasthenia gravis[10][11]

Synergy between plasma exchange and immunosuppressive drug treatment should be most evident in the period immediately following plasma

exchange, as has been demonstrated in an animal model.[12] By causing antibody producing cells to proliferate[13] plasma exchange may render them more vulnerable to immunosuppressive drug therapy.[6] In this controlled study, we have analysed the serum anti-AChR antibody responses in the period immediately following plasma exchange and have failed to demonstrate a synergistic action with immunosuppressive drug therapy.

Patients and methods

Twenty patients, 10 males and 10 females aged 18–68 years, were studied. All had the generalised acquired form of the disease (Osserman[14] grade IIB six cases, grade III six cases, grade IV eight cases); duration ranged from six months to 23 years. All were receiving acetylcholinesterase inhibitors and had elevated serum titres of anti-AChR antibody (range 0·7–120; normal <0·2 nmol/l). Eighteen patients underwent thymectomy before the period of study and two afterwards. Nine patients had thymic hyperplasia and 11 had thymomas.

Plasma exchange

Plasma exchange was performed using a Haemonetics 30 cell separator. Two to four litres of plasma were exchanged on three to 10 occasions over three to 14 days. Plasma was replaced isovolumetrically with human plasma protein fraction (PPF, Blood Products Laboratories) or Buminate (Travenol Limited) and up to 50% normal saline, Ringer lactate or Dextran 70. Calcium and potassium were added to physiological concentrations. Between eight and 32 litres of plasma were exchanged via forearm or femoral veins, arteriovenous fistula, or arteriovenous shunt. Forty-nine courses of plasma exchange were performed; 26 were classified as "small" (<12 l mean $10 \cdot 0 \pm 0 \cdot 5$ l, 2 SEM) and 13 as "large" (>14 l mean $18 \cdot 3 \pm 3 \cdot 0$ l). The remaining 10 courses formed part of a paired study in which the size of the exchange was deliberately altered and these data are analysed separately.

Anti-AChR antibody titres

Serum anti-AChR titres were measured by an immunoprecipitation method using Triton X100 solubilised AChR obtained from amputated human calf muscle, as previously described.[7] Measurements were made on single serum samples drawn immediately before exchange, 24 hours after a course of exchange, on average twice weekly for four weeks following exchange and less frequently thereafter.

Immunosuppression

Seventeen of the 20 patients received prednisone (20–80 mg on alternate days) throughout the study period. The dose was unchanged over this period in all but three. Additional immunosuppression comprised either azathioprine, cyclophosphamide or cytosine arabinoside (AraC). Ten patients received 12 courses of plasma exchange without additional immunosuppression. Twelve patients received 15 courses of plasma exchange with azathioprine $2 \cdot 5$ mg/kg per day orally. Six patients received seven courses of plasma exchange with cyclophosphamide $2 \cdot 5$ mg/kg per day orally, and five patients underwent plasma exchange followed by AraC intravenously for three to four days (see below). These groups did not differ significantly with respect to age, sex, thymic state, initial antibody titre, clinical grade, duration of disease or prednisone dosage. Immunosuppressive treatment was started on or before the first day of plasma exchange; the majority of patients had been taking the immunosuppressive drug for several months before plasma exchange. In most patients receiving cyclophosphamide, azathioprine $2 \cdot 5$ mg/kg per day orally was substituted after six weeks.

Paired comparisons

Paired comparisons in which patients served as their own controls were undertaken further to evaluate the response to plasma exchange. Three treatments were tested while all other treatment conditions were kept constant.
(i) *Azathioprine:* plasma exchange with and without azathioprine ($2 \cdot 5$ mg/kg) was carried out in six patients.
(ii) *AraC:* plasma exchange with and without AraC was investigated in five patients. The course of AraC was started on the day following a course of plasma exchange. In four patients who were also receiving prednisone and azathioprine, it was given as 1 mg/kg bolus daily for four days and in one patient (on no other treatment) 5 mg/kg was given for three days which produced a severe leucopenia.
(iii) *Size of plasma exchange:* In five patients small courses of exchange (three to five daily exchanges over five days) were compared with large courses of exchange (10 exchanges over 12 days). The size of each daily exchange was constant (volume of plasma equal to plasma volume calculated as 5% body weight) and no change in immunosuppressive treatment was made during this study.

Variables analysed

Data for individual patients was normalised for each exchange to a pre-exchange value of 100% and the following variables were analysed: (1) Serum anti-AChR antibody at 24 hours, 28 days and 112 days after the end of a course of plasma exchange, (2) the mean rate of rise in serum anti-AChR antibody titre during the four weeks following plasma exchange, derived from the anti-AChR antibody titres at 24 hours and 28 days after plasma exchange, (3) half-time ($t\frac{1}{2}$) for anti-AChR antibody recovery after plasma exchange; that is the time for antibody titre to rise to a value halfway between the pre-exchange and immediately post-exchange titres measured from individual plots of serial post-PE anti-AChR values.

Clinical assessment

Time for which arm or leg could be held outstretched, or vital capacity were measured, or both as appropriate to each patient's pattern of weakness.

Statistics

The data were analysed for variance due to drug treatment, size of exchange, age, sex, pre-exchange anti-AChR titres, clinical severity, duration of disease and interaction between age, sex, treatment and size of exchange. For paired data, paired tests were performed. Values for $t\frac{1}{2}$ were logarithmically transformed to obtain a normal distribution for analysis, and derived results are expressed as mean (with 95% confidence limits). For all other variables means (± 2 SEM) are quoted.

Results

The serum anti-AChR recovery rate after plasma exchange for all patients receiving some form of additional immunosuppression was $1 \cdot 84$ ($\pm 0 \cdot 32\%$, 2 SEM) per day (table). This was not significantly different from that in patients not receiving additional immunosuppression ($1 \cdot 94 \pm 0 \cdot 59\%$ per

Table　Effects of plasma exchange on serum anti-AChR antibody with and without additional immunosuppressive drug therapy*

	All patients	Additional immunosuppressive therapy		Azathioprine	Cyclo-phosphamide	Cytosine arabinoside	Large PE† (>14L)	Small PE† (<12L)
		Without	With					
n	39	12	27	15	7	5	13	26
Volume (litres)	12·8 (1·7)	14·4 (3·5)	12·1 (1·8)	11·8 (2·2)	13·9 (4·7)	10·3 (2·1)	18·3 (3·0)	10·0 (0·5)
Post PE anti-AChR antibody (% pre-PE)	41·0 (4·6)	35·8 (7·1)	43·4 (5·2)	43·0 (8·3)	43·6 (9·5)	44·2 (5·9)	31·5 (9·5)	45·8§ (3·9)
T½ (days)	8·5 (6·4–11·4)	10·5 (6·4–17·1)	7·8 (5·5–11·1)	7·0 (3·8–12·8)	10·5 (7·9–13·9)	7·2 (4·6–11·1)	13·2 (7·7–22·6)	6·9‡ (5·0–9·4)
Mean anti-AChR antibody recovery rate (% per day)	1·87 (0·28)	1·94 (0·59)	1·84 (0·322)	1·77 (0·28)	2·08 (1·09)	1·70 (0·42)	1·65 (0·34)	1·98 (0·39)
28 day anti-AChR antibody (% pre PE)	93·3 (9·9)	90·0 (19·1)	94·8 (11·7)	92·6 (14·4)	101·7 (34·3)	91·8 (7·4)	77·8 (14·5)	101·1‡ (11·9)

*Mean (± 2 SEM) for all variables except t½ where mean (and 95% confidence limits) are given.
†PE denotes plasma exchange course.
‡ § ¦ denotes p < 0·05, < 0·01, < 0·001 respectively.

day). Neither group differed significantly with respect to t½ or the 28 day anti-AChR titre. When the individual immunosuppressive drug regimes within the overall additional immuno-suppression group were examined none of them differed significantly in any respect from the pattern of results reported above (table). There were no significant differences between the groups with respect to volume of plasma exchange or initial percentage fall in anti-AChR titre produced by plasma exchange. No effects were attributable to age, sex, thymus status, initial antibody titres, clinical grade or duration of disease. Clinical measurements revealed an inverse relation between muscle strength and serum anti-AChR antibody titres in individual patients.

Although the differences did not reach signifi-cance, patients receiving azathioprine and AraC showed a slower mean rate of anti-AChR recovery after plasma exchange (1·77 and 1·70% per day respectively) than the control group (1·94% per day). Paired studies of the effects of these treat-ments were therefore analysed, in which the individual patients served as their own controls. The control plasma exchange was undertaken first in each case. For each individual, the time course of antibody recovery after plasma exchange with additional immunosuppressive treatment was observed to be similar to that following the control plasma exchange. Mean anti-AChR recovery rate in six patients was 1·93±1·08% per day without azathioprine and 1·56±0·38% per day with azathioprine, and for five patients who received intravenous AraC, the values were 1·83±0·34% per day and 1·70±0·42% per day (fig 1). With

neither of the treatments were these differences significant.

Larger exchanges resulted, as expected, in lower anti-AChR titres after plasma exchange than small exchanges (31·5±9·5% and 45·8±3·9% respectively; p<0·01; table). The t½ for large exchanges was significantly greater than for small exchanges, which could be accounted for by the influence of the size of the initial fall in antibody on this index. The mean anti-AChR recovery, on the other hand, appeared to be reduced although not significantly.

Paired studies were therefore undertaken in five patients to investigate further the effect of the size of PE (fig 2). Large courses of plasma exchange again resulted in significantly lower mean anti-AChR titres after exchange than small plasma exchanges (20·1±3·5 and 34·8±6·9% respectively; p<0·05, n=5), but mean anti-AChR antibody recovery rates were not significantly different (2·47±0·52 and 2·03±0·25% per day, respectively).

When it was possible to follow patients for 112 days (four months) following plasma ex-change, a biphasic effect was observed in patients receiving azathioprine (fig 3). The recovery of anti-AChR titres by 28 days to values near those before plasma exchange was followed by a gradual sustained fall. There was a signifi-cant (p<0·01) fall in anti-AChR titres between day 28 and day 112 in nine patients receiving azathioprine (minus 21·3%±11·8%); this differed significantly (p<0·05) from the antibody titres in five patients not receiving azathioprine which showed a slight increase (plus 2·2%±18·3%).
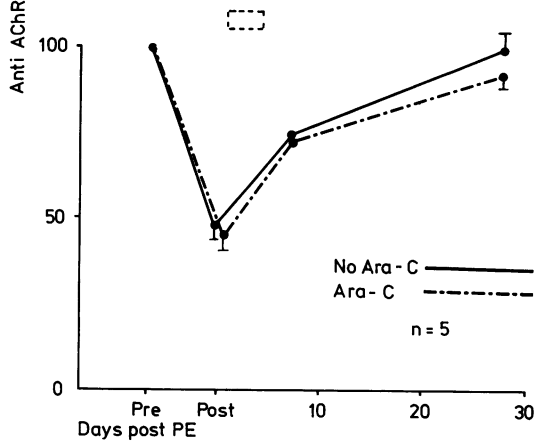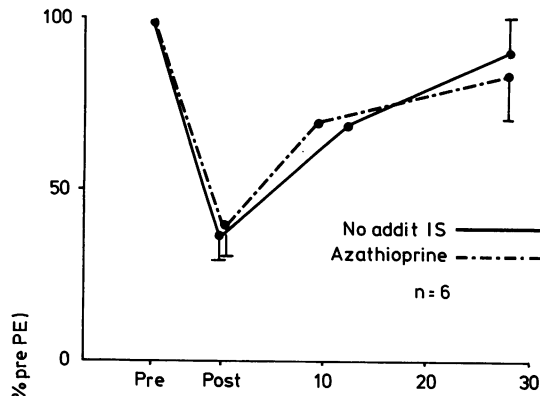
Fig 1 *Paired comparisons of the effects of a course of plasma exchange with and without azathioprine (upper panel, n=6) and AraC (lower panel, n=5). Means and SEM are shown. Each patient served as his own control. There were no significant differences attributable to the drug treatment.*
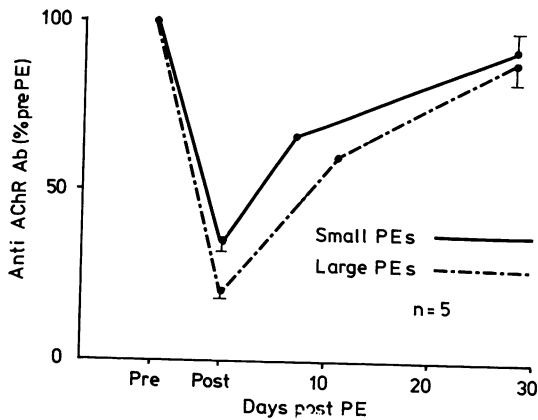
Fig 2 *Paired comparisons of the effects of large versus small courses of plasma exchange on serum anti-AChR titres (n=5). Means and SEM are shown. Large plasma exchanges produced a greater initial fall in mean anti-AChR titres, but no significant effects were observed on antibody recovery.*
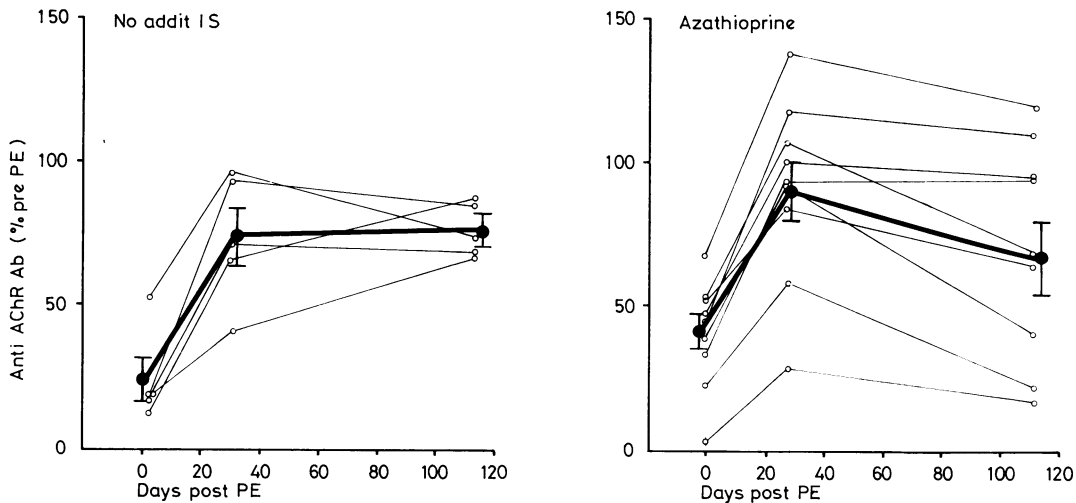
Fig 3 *Anti-AChR titres in the four month period following a course of plasma exchange in five patients receiving no additional immunosuppressive drug therapy (left-hand panel), and nine patients receiving azathioprine (right-hand panel). Heavy lines show mean values and SEMs. Note that mean antibody recovery rate during the first 28 days after plasma exchange is very similar for both groups (49·2±17·2%, 2 SEM and 50·9±11·4%). The fall in mean antibody titres between days 28 and 112 is significant for the azathioprine patients (minus 21·3±11·8%, p<0·01) and is significantly different (p<0·05) from the negligible change (plus 2·2±18·3%) in the other group.*

Discussion

Our results confirm that plasma exchange can reduce serum anti-AChR antibody by about 60% in a few days. Anti-AChR titres provided an appropriate index of clinical effectiveness because as previously reported there was a short-term inverse correlation with muscle power in individuals.[6-8] However, the benefits of plasma exchange were of limited duration and, with the exception of large exchanges, anti-AChR antibody returned to within 10% of pre-exchange values by 28 days, and there was an associated clinical decline. None of the treatments we have described in this paper significantly affected this rapid recovery of anti-AChR antibody after plasma exchange.

The use of t½ as a measure of antibody recovery after plasma exchange can be criticised on the grounds that re-equilibration between intravascular and extravascular compartments and the size of the exchange may substantially influence its value. We have therefore also evaluated the mean rate of anti-AChR antibody rise over 28 days starting 24 hours after the course of plasma exchange. This index is unaffected by the size of the exchange and the component

attributable to re-equilibration is likely to be very small.

Not surprisingly, a greater initial antibody reduction was achieved by exchanging larger volumes of plasma. However, the anti-AChR antibody recovery rate was not significantly different whether exchanges were large or small and the data do not support the suggestion[9] that the size of exchange has a critical influence on its effectiveness.

Exchange transfusions in animals can stimulate antibody synthesis[13] and this may occur when plasma exchange is used for the removal of rhesus antibody[16] as indicated by an overshoot of serum antibody titres in patients having plasma exchange without immunosuppression. This occasionally occurs in myasthenia gravis[17] and we observed an overshoot in antibody titres (more than 10% higher than pre-exchange values) in seven out of 49 courses of plasma exchange in this study. Five of these patients were receiving immunosuppressive treatment.

Synergism between exchange transfusion and immunosuppressive drug treatment has been demonstrated in an animal model[12] but only when immunosuppressive drugs were used in combination and at extremely high dosage. It is thus very

unlikely that our failure to demonstrate synergy between plasma exchange and additional immunosuppressive drugs was because synergy with the moderate or low doses of prednisone used in the control group was already maximal. A (non-significant) trend to lower antibody recovery rates was observed after plasma exchange with azathioprine or AraC, but this was of a similar order of magnitude to that seen in the absence of plasma exchange and this trend (if real) suggests that there may be a small additive, rather than synergistic, effect of plasma exchange and these immunosuppressive drugs.

Our data on the late effects of azathioprine show that this drug can have a long-term action in lowering serum anti-AChR antibody. It would be difficult to implicate plasma exchange in this effect since it occurred after the mean anti-AChR titre had risen to within 10% of the pre-exchange value. Moreover, the rate of antibody decline was similar to that reported for azathioprine without plasma exchange.[11] This group of patients was selected by exclusion of those whose anti-AChR titres rose to levels requiring further plasma exchange. Thus the control group who were not receiving azathioprine represent those who responded best in this group and it seems very improbable that the late fall seen in the azathioprine group would have occurred in the absence of this drug.

We have not been able to confirm the suggestion[6][9] that plasma exchange may synergise with immunosuppressive drug therapy in myasthenia gravis. The possibility that such an effect would occur at higher immunosuppressive dosage levels has not been excluded, but the risk of serious side effects would then further increase. Our data are consistent with earlier evidence that repeated plasma exchange courses confer no long-term cumulative benefits on patients also receiving immunosuppressive drugs.[11] Variability in the unpaired data was considerable and might have masked synergistic effects, but the lack of any significant differences in the paired data suggests that any such effect must have been very small. Given the reported possible hazards of plasma exchange, which include thromboembolism, septicaemia, hypotension, 'flu-like illness and death,[6][7][9][17] it would seem prudent at present to restrict plasma exchange in MG to those with severe disease. In such patients, plasma exchange may be useful in controlling symptoms while immunosuppressive therapy becomes effective. Our results provide no grounds for recommending the use of plasma exchange in less severely affected cases.

References

1 Fambrough DM, Drachman DB, Satyamurti S. Neurosmuscular junction in myasthenia gravis: Decreased acetylcholine receptors. *Science* 1973; **182**:293–5.

2 Ito Y, Miledi R, Vincent A, Newsom-Davis J. Acetylcholine receptors and end-plate electrophysiology in myasthenia gravis. *Brain* 1978; **101**: 345–68.

3 Engel AG, Lambert EH, Howard FM. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis. Ultrastructural and light microscopic localization and electrophysiological correlatives. *Mayo Clin Proc* 1977; **51**:267–80.

4 Toyka KV, Drachman DB, Griffin DE, *et al.* Myasthenia gravis study of humoral immune mechanisms by passive transfer to mice. *N Engl J Med* 1977; **296**:125–31.

5 Pinching AJ, Peters DK, Newsom-Davis J. Remission of myasthenia gravis following plasma exchange. *Lancet* 1976; **ii**: 1373–6.

6 Dau PC, Lindstrom JM, Cassel JK, Denys EH, Shev EE, Spitter LE. Plasmapheresis and immunosuppressive drug therapy in myasthenia gravis. *New Engl J Med* 1977; **297**:1134–40.

7 Newsom-Davis J, Pinching AJ, Vincent A, Wilson SG. Function of circulating antibody to acetylcholine receptor in myasthenia gravis investigated by plasma exchange. *Neurology (Minneap)* 1978; **28**:266–72.

8 Carter B, Harrison R, Lunt GG, Behan PO, Simpson JA. Anti-acetylcholine receptor antibody titres in the sera of myasthenia patients treated with plasma exchange combined with immunosuppressive therapy. *J Neurol, Neurosurg Psychiatry* 1980; **43**:397–402.

9 Behan PO, Shakir RA, Simpson JA, Burnett AK, Allan TL, Gasse G. Plasma exchange combined with immunosuppressive therapy in myasthenia gravis. *Lancet* 1979; **ii**:438–40.

10 Mertens HG, Balzereit F, Leipert M. The treatment of severe myasthenia gravis with immunosuppressive agents. *Europ Neurol* 1969; **2**:321–39.

11 Newsom-Davis J, Vincent A, Wilson SG, Ward

CD. Long-term effects of repeated plasma exchange in myasthenia gravis. *Lancet* 1979; i: 464–8.

12 Terman DS. Chemotherapeutic agents for specific suppression of antibody rebound after extracorporeal immunoadsorption. *Muscle Nerve* 1978; 1:341.

13 Sturgill BC, Worzniak MJ. Stimulation of proliferation of 19S antibody forming cells in the spleens of immunized guinea pigs after exchange transfusion. *Nature* 1970; 228:1304–5.

14 Osserman KE, Genkins G. Studies in myasthenia gravis: review of a twenty year experience in over 1200 patients. *Mt Sinai J Med (NY)* 1972; 38:497–537.

15 Bystryn JC, Graf MW, Uhr JW. Regulation of antibody formation by serum antibody. *J Exp Med* 1970; 132:1279–87.

16 Barclay GR, Greiss MA, Urbaniak SJ. Adverse effect of plasma exchange on anti-D production in rhesus immunisation owing to removal of inhibitory factors. *Br Med J* 1980; i:1569–71.

17 Newsom-Davis J. Plasma exchange in myasthenia gravis. *Plasmatherapy* 1979; 1:17–31.