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Immunoadsorption therapy for myasthenia gravis

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Abstract

The results of a multicentre trial were analysed to evaluate the efficacy of immunoadsorption therapy for severe generalised myasthenia gravis. Twenty patients with myasthenia gravis who were concurrently receiving high dose prednisolone and azathioprine therapy were treated with an affinity-type adsorbent, using tryptophan-linked polyvinyl alcohol gel (IM-TR), according to a standardised treatment protocol. The 20 patients received five adsorption treatments within a period of 10 days. In 11, pronounced improvement of myasthenic weakness was seen and long-term remission was maintained. The treatment was especially effective in patients with thymic hyperplasia. Circulating acetylcholine receptor (AChR) antibodies were reduced by about 60% by treating one plasma volume. There was no difference in the rate of removal of the AChR antibodies between patients with thymic hyperplasia and patients with thymoma. No serious complications occurred during 100 procedures. It was concluded that the immunoadsorption therapy with IM-TR is useful in controlling symptoms in patients with severe myasthenia gravis who are otherwise unresponsive.

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Circulating antibodies to the acetylcholine receptor (AChR) are detectable in serum in 85%–90% of patients with generalised myasthenia gravis.[1–3] Most AChR antibodies in IgG bind to the main immunogenic region of the α subunit of the AChR, and cause AChR degradation secondary to cross-linking and modulation.[2–5] Plasma exchange has been shown to induce a rapid recovery from myasthenic weakness in association with the decline of the AChR antibodies.[6–8] At a consensus development conference, plasma exchange was considered effective in the management of certain neurological disorders. A major disadvantage of currently available plasma exchange procedures is the non-selective removal of essential plasma components, necessitating a supply of plasma products to serve as a substitute fluid. Plasma exchange is costly and carries serious risks of anaphylactic reactions and viral infections.[6 9 10] It is preferable to remove pathogenic substances from the circulation selectively. A specially designed affinity-type immunoadsorbent to selectively remove AChR antibodies has been developed.[11 12] This material is a synthetic resin consisting of tryptophan-linked polyvinyl alcohol gel (IM-TR). It adsorbs a large number of the AChR antibodies through hydrophobic interaction and rapidly improves myasthenic weakness.[13–16]

The present study was carried out at six neurological hospitals, according to a standardised treatment protocol to assess the clinical usefulness of IM-TR therapy in cases of severe myasthenia gravis.

Patients and methods

Patients included in this study had severe generalised myasthenia gravis diagnosed by typical clinical signs and raised titres of antibodies to AChR (more than 0·5 nmol/l bungarotoxin binding); patients were in myasthenic crisis with rapid deterioration within less than two weeks. Criteria for exclusion were chronic severe and stable myasthenia gravis; multiple changes in the immunosuppressive medication before treatment; and malignant (invasive) thymoma as determined by a pathological diagnosis of the thymus gland.

Twenty patients, range 15–65 years (five men, 20 women), have been treated with the immunoadsorption. All were receiving acetylcholinesterase inhibitors. The dose was kept constant during the study. All patients had to have undergone thymectomy at least three months before the study. Their thymic histology showed thymic hyperplasia in 13, thymoma in six, and was indeterminate in one (table 1). The histological diagnoses were obtained from the surgical pathologists'

Table 1 Analysis of relation between clinical features and outcome of immunoadsorption therapy

Effect	*No of patients*	*Age*	*Sex*		*Duration of illness (y)*	*Time before thymectomy (y)*	*Thymic histology**		*Removal of AChR Ab on day 10 (%)*
			M	*F*			*Hyperplasia*	*Thymoma*	
Pronounced	11	40(13)	2	9	9(5)	4(3)	9	1	58(9)
Poor and unresponsive	9	36(17)	3	6	5(5)	3(4)	4	5	65(14)
p Value		NS	NS		NS	NS	<0·05		NS

Values are means (SD); NS = non-significant; p values are one-tailed.
*Thymic histology was unknown in one patient.

reports. The thymoma were of both lymphocytic and epithelial types. In the non-tumour cases the thymus showed hyperplasia of lymph follicles.

Immunosuppressive treatment with azathioprine and prednisolone was instituted on day 0. Azathioprine was given at a dose of 3 mg/kg body weight during the first week and 2·5–2 mg/kg body weight for the subsequent four weeks (if this dose was not tolerated it was reduced to 2–1·5 mg/kg body weight). Prednisolone was given at a dose of 1·5 mg/kg body weight for the first two weeks, and 1·5 mg/kg body weight on alternate days thereafter.

Immunoadsorption with IM-TR was carried out on-line with a plasma separator consisting of a cellulose diacetate membrane. The blood flow was kept at 70–80 ml/min, and the transmembrane pressure was less than 40 mm Hg. The flux rate of the filtration (the flow rate at the IM-TR column) was 20 ml/min. Adsorption was performed with about one plasma volume (an average of 2300 ml of plasma) on days 1, 3, 5, 8, and 10, for a total of five treatments. Heparin was used as an anticoagulant at an initial dose of 2000 units, then 2000 units/hour during the period of perfusion. Fluids contained in the system were reinfused into the patient at the end of the procedure. No plasma proteins were given.

Clinical assessment was based on clinical muscle testing (the myasthenia gravis score of Besinger and Toyka[17]), the titre of antibodies to AChR (a standard immunoprecipitation assay with solubilised human receptor[1]), electromyographic analysis of the neuromuscular transmission block, and side effects if any. Briefly, a four-step system for grading muscle strength was used (ranging from 0 = normal to 3 = severe weakness). In each patient, five test items for muscles of the limbs and trunk and three test items for the oropharyngeal muscles were examined. All items were given the same weight. The myasthenia gravis score was calculated as the sum of the grades in each item divided by the number of the items tested. For each patient, the treatment was judged to be efficacious if the myasthenia gravis score on day 11 was more than 35% higher than the pretreatment value. To estimate the neuromuscular transmission block quantitatively, the decline in the amplitude of successive responses to repetitive nerve stimulation at frequencies of 3/s was measured on the adductor pollicis muscle and the trapezius muscle. It was calculated as a ratio of the fifth response to the first one. These clinical and laboratory findings were obtained at 0, 4, 11, 14, and 28 days according to the study protocol. The observation period was extended up to 42 days if no change in the treatment had occurred. The effects of immunoadsorption were also analysed in relation to clinical features such as age, sex, duration of illness, time before thymectomy, and thymic histology.

The clinical and electrophysiological examinations were performed by the local neurological investigator. The examining neurologists were blind as to plasma exchange treatments. All laboratory investigations that would allow the neurological investigator to know about the treatment were kept separate and were only known to the physician performing plasma exchange. Raw data were listed in the study protocol and sent to the study coordinator for statistical analysis.

Because this study was designed as an open, one-armed treatment trial, all comparisons were within groups. Changes from baseline observed at 11 days and 28 days after treatment were also compared to analyse the time profile, because the main response variable in evaluating efficacy was the change in the clinical scores adjusted by the pre-treatment value. The baseline employed was the observation immediately before the first immunoadsorption treatment. Statistical analysis was by Student's t test or χ^2 test. Results are expressed as means (SD).

Results

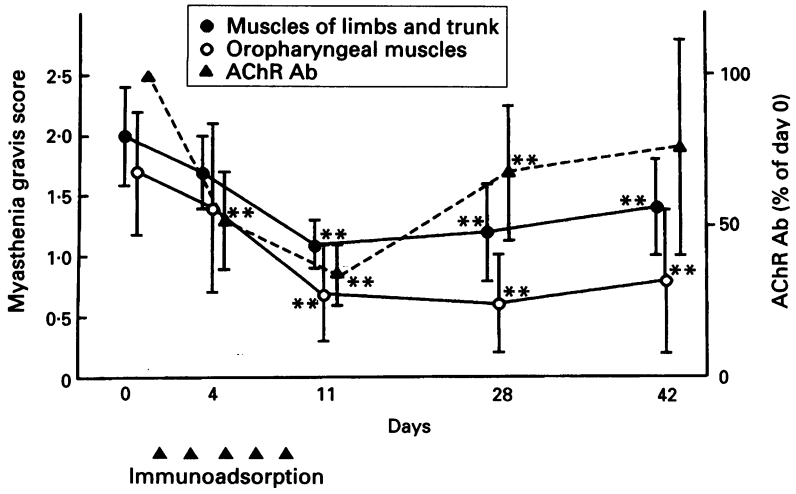
Pronounced and rapid recovery from myasthenic weakness was noted in 11 patients after a series of immunoadsorption treatments, but in the others the clinical state showed little change despite a substantial decrease in AChR antibody titre (table 1). Nine of 13 patients with thymic hyperplasia improved, although only one of six patients with thymoma benefited (table 1). The improvement was stable in nine of them for at least 32 days after termination of the immunoadsorption. The clinical effect was significantly correlated with thymic histology (tables 1 and 2), but no correlation was noted with age, sex, duration of illness, and time before thymectomy (table 1). Percentage improvement of the mean myasthenia gravis score was 39% on day 11 and 35% on day 28 in patients with thymic hyperplasia, whereas it was 15% and 11% in those with thymoma (table 2).

Serum AChR antibody was reduced by about 60% through the treatment of one plasma volume with IM-TR. No difference was noted between the thymic hyperplasia

Table 2 Analysis of relation between thymic histology and outcome of immunoadsorption therapy

Thymic histology	*No of patients*	*Age*	*Sex*		*Duration of illness (y)*	*Time before thymectomy (y)*	*Removal rate for AChR Ab on day 10 (%)*	*Mean myasthenia gravis score*			*% Improvement of myasthenia gravis score*	
			M	*F*				*Day 0*	*Day 11*	*Day 28*	*Day 11*	*Day 28*
Hyperplasia	13	37(14)	3	10	9(5)	4(3)	64(9)	1·9(0·3)	1·1(0·4)**	1·2(0·3)**	39(20)	35(19)
Thymoma	6	39(15)	2	4	5(5)	3(4)	64(14)	1·9(0·4)	1·7(0·4)	1·7(0·7)	15(20)	11(28)
p Value		NS	NS	NS	NS	NS	NS	NS	<0·05	NS	<0·05	<0·05

Values are the means (SD); NS = non-significant; p values are one-tailed.
**p < 0·01 *v* day 0.



*Mean values of myasthenia gravis score and titre of AChR antibodies in the 11 improved patients after immunoadsorption therapy. Vertical lines are SDs. Consecutive immunoadsorption treatments induced a significant fall of the myasthenia gravis score and a decrease in the titre of AChR antibodies. The myasthenia gravis score remained low even on day 42 despite the rise in AChR antibody titres. **p < 0·01 v day 0.*

and the thymoma cases in the average removal rate of AChR antibodies. In patients with remission after the treatment, the AChR antibodies were reduced by 58 (12)% (n = 53; range 33–75%) similar to the average of 61 (13)% (n = 38; range 35–85%) in patients who showed no change. Albumin fell by only 6 (3)% (n = 50).

In the 11 patients who improved, recovery usually occurred within 48 hours after the first adsoption, reaching its peak one to four days after the last adsorption. The total myasthenia gravis score (five items for muscles of limbs and trunk, and three items for oropharyngeal muscles) was significantly reduced from 1·9 (0·3) (n = 11) on day 0 to 0·9 (0·2) on day 11; to 1·0 (0·3) on day 28; and to 1·2 (0·4) on day 42 ($p < 0·01$). The mean myasthenia gravis score for oropharyngeal muscles showed the lowest value on day 28 and it was still low on day 42. The titre of AChR antibody was 44% on day 14 and 65% on day 28, in reference to the value on day 0 taken as 100% (figure). These values were significantly different from the titre of antibody on day 0 ($p < 0·01$). The AChR antibodies gradually increased after the adsorption therapies, whereas the myasthenia gravis scores remained low (figure).

Analysis of the neuromuscular transmission block was performed in nine of 11 remitted patients after the treatment. In the evoked electromyogram the decline of the fifth response compared with the first was significantly improved in the adductor pollicis muscle from 33 (13)% on day 0 to 13 (5)% on day 28 ($p < 0·01$). The mean recovery rate was 59%. The degree of decline in amplitude on the trapezius muscle was also significantly reduced from 51% to 35% ($p < 0·01$). In 100 procedures, the treatment caused no serious complications. There were eight mild hypotensive reactions and five patients had mild symptoms such as nausea, vomiting, and headache, which can accompany any type of extracorporeal circulation.

Discussion

The immunoadsorption together with the immunosuppressive drugs ameliorated myasthenic symptoms in most patients with thymic hyperplasia, and its clinical effects lasted more than five weeks, but it failed to induce clinical improvement in most patients with thymoma. It is noteworthy that the myasthenia gravis score, especially for oropharyngeal muscles, remained low even on day 42 despite the increased AChR antibody titres.

The patients with thymoma tend to have severe disease, respond poorly to thymectomy, show no HLA association, and about 90% have striated muscle antibodies.[2 3] The lack of effect of the immunoadsorption therapy in cases of thymoma despite a fall in the AChR antibodies could be because AChR antibodies are heterogeneous both with respect to binding specificity and biological function,[2 3 18 19] and because the antibody response in myasthenia gravis is polyclonal (the antibodies derived from different clones of autoimmune B-lymphocytes have variable functional activities).[2] There is no identifiable variation in antibody specificity that accounts for variation in clinical severity.[18 19] It seems likely that there are endogenous factors affecting the safety margin for neuromuscular transmission and there are different immunological and genetic factors that control differences in a patients' phenotype. Resistance to plasma exchange may also be due to irreversible changes in muscle as a consequence of the severity of the disease or to degeneration of the postsynaptic membrane due to long-term anticholinesterase therapy.[20]

Dau[8] reported that the factors correlating with the best clinical response were short duration of illness, male sex of the patient, and treatment with both prednisolone and azathioprine during plasma exchange. We could not, however, find a clear relation between the effectiveness of the immunoadsorption combined with prednisolone and azathioprine and the duration of the illness, an age and sex difference, or the timing of the thymectomy.

With immunoadsorption therapy using IM-TR, the AChR antibodies can be reduced as effectively as with conventional plasma exchange using centrifugation,[6–8] without loss of albumin and no serious complications.[13–16] The titre of the AChR antibodies began to rise soon after each immunoadsorption treatment, but its rise was slow and its titre was less than 80% of the pretreatment values even 30 days after the treatments. An inverse association of the clinical state with AChR antibody titres is usually seen just after a series of plasma exchanges.[6 7] Rapid removal of the circulating antibodies is followed by their redistribution, however,[6] and may affect the rate of synthesis by removing inhibitory feedback[21] or causing a reduction of catabolism combined with an unchanged rate of synthesis.[22] A rebound increase in the AChR antibody usually occurs within seven days, and the original titre is restored within 14 days[6 7] with accompanying clinical worsening unless azathioprine, which

may have a cytotoxic action on clones of the antigen,[6] and prednisolone, which may suppress antibody production from B-cells,[2 6] are given. Azathioprine and prednisolone are useful in the treatment of myasthenia gravis, but slow in action, and if given alone the average time required for a 50% reduction in titre is three to five months,[23 24] whereas plasma exchange alone can rapidly reduce more than 60% of the circulating antibodies. Our results showed that a combination of immunoadsorption therapy and immunosuppressive medication is necessary to maintain the beneficial effects and to avoid a rebound increase in the AChR antibodies caused by phenomena induced by IgG depletion.[21 22]

It was concluded that combined immunoadsorption therapy and immunosuppressive drug treatment is useful in controlling symptoms in patients with severe myasthenia gravis who are otherwise unresponsive.

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