SHORT REPORT

Depletion of neutralising antibodies resensitises a secondary non-responder to botulinum A neurotoxin

M Naumann, K V Toyka, B Mansouri Taleghani, J Ahmadpour, K Reiners, H Bigalke

Abstract
The objective was to evaluate whether removal of neutralising antibodies potentially resensitises a secondary non-responder to botulinum neurotoxin A (BoNT/A). Neutralising antibodies directed against BoNT/A are produced during long term treatment with BoNT/A-hemagglutinin complex in up to 10% of patients with cervical dystonia. These patients become secondary non-responders. Other serotypes of BoNT are not yet generally available and may also bear the risk of inducing antibody formation. Plasma exchange (PE) (one treatment cycle) and immunoadsorption on a protein A column (IA-PA; three treatment cycles) was employed over 15 months to remove neutralising antibodies from a severely disabled secondary non-responder with cervical dystonia. After plasma exchange or IA-PA, BoNT/A was reinjected. Antibodies were measured with a sensitive functional toxin neutralising test.

Repeated use of plasma exchange and IA-PA depleted neutralising antibodies to below the detection limit and subsequently allowed successful BoNT/A injection into dystonic muscles. No serious side effects were found related to the depletion of IgG.

In conclusion PE or IA-PA performed before BoNT/A readministration may provide an alternative strategy in treating selected secondary non-responders who are severely disabled.

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Keywords: botulinum A neurotoxin; neutralising antibodies; dystonia; immunoadsorption

Local injections of BoNT/A-complex are now the treatment of choice for dystonia and hemifacial spasm and, more recently, have proved effective in spasticity and autonomic disorders. Not unexpectedly, up to 10% of patients with cervical dystonia produce neutralising antibodies in response to repeated toxin injections. Antibodies have led to secondary therapeutic failure particularly in patients who had previously received high doses of toxin and were reinjected within a short period. Other serotypes of BoNT (B and F) have been clinically tested but these are not yet available. Moreover, BoNT/B or F are even more likely to induce antibody formation as these toxins have a lower specific activity in humans and a shorter duration of sustained presynaptic block.

Analogous to the use of plasma exchange and IA-PA in antibody mediated autoimmune disorders such as myasthenia gravis, we examined whether the removal of neutralising antibodies might resensitise a severely disabled secondary non-responder to BoNT/A, thus allowing further treatment and avoiding surgery.

Patient and methods
PATIENT
A 42 year old woman had had idiopathic torticollis since 1992. On initial examination at Würzburg she had a painful rotatory torticollis to the left side with elevation of the left shoulder and rotation of the upper trunk. Swallowing was markedly impaired due to the extreme tonic head rotation. Apart from the dystonia the patient was healthy. Cranial MRI and various laboratory tests were all normal. For quantitative assessment of dystonia over time the Tsui score was used.

METHODS
Antibody testing
The sensitive functional toxin neutralising test (phrenic nerve hemidiaphragm preparation) used for antibody testing has been described in detail elsewhere. This test is considered at least as sensitive as the traditional mouse bioassay.

In vitro adsorption of BoNT/A antibodies on protein G column
Before plasma exchange or immunoadsorption was considered as a possibility to remove neutralising antibodies to BoNT/A we performed an in vitro test with a protein G column which binds IgGs with high affinity to see whether BoNT/A-antibodies belong mainly to the IgG isotype. Five ml serum with an antibody titre of 3 mU/ml was dialysed against Krebs-Ringer...
solution and was loaded on to the column (5 ml sepharose gel). The effluent was dialysed against Krebs-Ringer solution and was then incubated with BoNT/A. A phrenic nerve diaphragm preparation was exposed to the mixture to assess the toxin binding antibody concentration.

Plasma exchange and immunoadsorption
Despite the severe disability, the patient refused to have surgery. As no other treatment was available we decided to remove the neutralising antibodies to BoNT/A by plasma exchange, a technique used in the treatment of neuroimmunological disorders.12 15 Informed written consent was obtained from the patient. Over a period of 3 weeks nine cycles of plasma exchange were performed. In addition to neutralising antibodies8 total serum IgG was measured by a standardised immunoturbidimetric assay.

An automated plasma separator was used for plasma exchange. We exchanged 1.0 to 1.5 l plasma for an equal volume of an albumin electrolyte solution (5%) in each cycle.16

Immunoadsorption with staphylococcal protein A columns (IA-PA) (Immunosorba, Excorim AB, Lund, Sweden) preferentially binding the subclasses 1, 2, and 4 was used to remove anti-BoNT/A antibodies. A reduction of circulating antibodies >90% can potentially be achieved.17

To gain information on the efficacy of each plasma exchange and IA-PA cycle, the titres of anti-BoNT/A antibodies and the concentration of total IgG were measured immediately before and after each IA-PA.

Results
CLINICAL EFFECTS OF BoNT/A AND FORMATION OF NEUTRALISING ANTIBODIES
The first EMG guided intramuscular injection of 150 U BoNT/A-complex (40 U/ml of Botox®, Allergan, Irvine, USA) was performed in October 1993, resulting in a moderate improvement. A further improvement and the relief of cervical pain was achieved by a second injection 4 weeks later. This short re-injection interval may have accelerated an immune response to BoNT/A. After the fifth injection the relief from symptoms after the same dose of BoNT/A-complex began to decline and continued to do so despite the fact that doses of toxin were increased up to 290 U, eventually leading to total unresponsiveness. Up to 1000 U of another commercial preparation (200 U/ml Dysport®, specific activity: 40 U/ng, Speywood, Maidenhead, England) was not effective. Details of the BoNT/A induced clinical effect are shown in fig 1. The mean dose of Botox® was 209 (SD 52) U (range from 100 to 290 U) and the mean interval between injections was 8 (SD 4.3) weeks (range 4–12 weeks). A high titre of neutralising antibodies to BoNT/A (6 mU/ml serum) was found to be responsible for the therapeutic failure. This was confirmed by the injection of 50 U Botox® into the extensor digitorum brevis muscle without subsequent paralysis of this muscle.18

IN VITRO ADSORPTION OF BoNT/A ANTIBODIES ON THE PROTEIN G COLUMN
Serum was depleted from IgG after passing over a protein G activated sepharose column. The antibody titre in the eluate was below the detection limit (0.3 mU/ml), indicating that virtually all neutralising antibodies were contained in the IgG class.

Titres of anti-BoNT/A antibodies and effects of the toxin after plasma exchange treatment and immunosuppression
Antibody titres fell after each plasma exchange and reached the detection limit after the ninth plasma exchange in the functional neutralising assay. The decline in the antibody titre was reflected in the reduction of total serum IgG. As some antibodies were thought to still be present in tissue compartments a higher than usual dose of Dysport® (1900 U, 50 U/ml) was injected. To prevent the toxin from inducing a
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IA-PA cycles (nine treatments each) 3 and 6 the BoNT/A-complex injection. Two further (fig 1). Symptoms reappeared 11 weeks after proved moderately and neck pain disappeared the pre-IA-PA concentration. Three weeks the concentration of total IgG hardly reached body titre rose 3 weeks after the last IA-PA but decreased to <10 mg/ml. Anti-BoNT/A anti-ously. The concentration of total IgG was (2000 U, 50 U/ml) was injected under EMG antibodies were no longer detected, Dysport® the last IA-PA, when neutralising serum antibody depletion were needed, standard plasma exchange was replaced by IA-PA, which selectively extracts serum IgG, and no further immunosuppression was given. A total of nine IA-PA treatments were preformed on each cycle (for details see fig 2). Immediately after the last IA-PA, when neutralising serum antibodies were no longer detected, Dysport® (2000 U, 50 U/ml) was injected under EMG guidance into the same muscles as done previously. The concentration of total IgG was decreased to <10 mg/ml. Anti-BoNT/A antiboody titre rose 3 weeks after the last IA-PA but the concentration of total IgG hardly reached the pre-IA-PA concentration. Three weeks after BoNT/A treatment, head rotation improved moderately and neck pain disappeared (fig 1). Symptoms reappeared 11 weeks after the BoNT/A-complex injection. Two further IA-PA cycles (nine treatments each) 3 and 6 months later on an outpatient basis and reinjection of BoNT/A-complex led to the same beneficial effect as seen previously (fig 1). There were no serious side effects to either BoNT/A or IA-PA/plasma exchange.

Thus there has been a good response to this regime for a period of over 15 months so far. In this follow up period, despite a temporary booster effect about 3 weeks after BoNT/A reinjection, no long lasting increase of neutralising antibodies to BoNT/A was seen (antibody titre between 2.5 and 5 mU/ml at the beginning of each IA-PA treatment series).

Discussion

We have shown that both IA-PA and plasma exchange effectively and repeatedly depleted neutralising antibodies from a patient’s serum, thus allowing successful continuous treatment with BoNT/A. Because B cells, the source of these antibodies, are not removed by such an approach, this type of treatment is only temporarily effective and requires repeated treatment cycles.

Virtually all neutralising antibodies to BoNT/A were of the IgG class because the serum could be cleared completely by the staphylococcal protein G column that selectively binds IgG in vitro.

The immunosuppressive regimen used initially may have been effective in preventing a booster effect by repeated BoNT challenge. Because no long lasting increase of neutralising BoNT/A antibody titres was found even without immunosuppressive drugs repeated plasma exchange and IA-PA treatments obviously did not stimulate the immune system to a major degree.

Even after antibody depletion from serum the BoNT/A doses needed were higher than those usually applied to patients with cervical dystonia. It is likely that some antibodies were still present in muscle tissue, which neutralised part of the toxin.

Serum represents only a small fraction of the total compartment in which IgGs are distributed, and only the vascular compartment can readily be cleared of these proteins by IA-PA or plasma exchange. Therefore, repeated cycles were needed to decrease the titre of tissue antibody to a concentration that did not substantially interfere with the toxin. Redistribution of IgGs from the deep compartment into the serum is reflected by the increase in the concentration of total IgG and specific antibody titres during the period between two successive cycles. Because a successful treatment is more likely when antibodies have been removed from muscle tissue rather than from serum alone the injection of BoNT/A was suspended until the titre of neutralising antibodies ceased to increase between two successive IA-PA treatments. Although antibodies were also eluted by plasma exchange, IA-PA was more efficient, particularly during the initial treatments.

The use of plasma exchange and IA-PA saves patients from surgical intervention and permanent tissue destruction. The possibility of future therapeutic strategies is not precluded,

Figure 2 Titre of anti-BoNT/A antibodies and IgG concentration after immunoadsorption (first IA-PA treatment series). Serum samples were collected on the days indicated on the abscissa. The ordinate shows the titres of anti-BoNT/A antibodies (AB-titre) and the concentration of IgG (IgG), immediately before and after each IA-PA. The toxin was injected on day 23 after the ninth IA-PA had been terminated (arrow). No titres were measured before/after the sixth IA-PA on day 15.
such as the treatment with other serotypes of BoNT once these become available. Types B and F, however, have a relatively short duration of action (3–6 weeks) and require high doses of neurotoxin.\textsuperscript{10, 11} Short intervals between injections and administration of high doses on the other hand are likely to increase the risk of antibody production.\textsuperscript{9} In selected patients, a spontaneous decline of the BoNT/A antibody titre may occur after cessation of BoNT/A injections but re-exposure to the toxin may again stimulate antibody production.\textsuperscript{19}

We conclude that in selected and severely disabled patients, the removal of neutralising antibodies by IA-PA may be a feasible although laborious and costly procedure. A larger study is encouraged to evaluate this new treatment regimen.

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