Immunological aspects of Botox®, Dysport® and Myobloc™/NeuroBloc®

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In some patients treated with botulinum toxin (BT), antibodies are produced in association with certain treatment parameters, patient characteristics and immunological properties of the BT preparation used. Therapeutic BT preparations are comprised of botulinum neurotoxin, non-toxic proteins and excipients. Antibodies formed against botulinum neurotoxin can block BT's biological activity. The antigenicity of a BT preparation depends on the amount of botulinum neurotoxin presented to the immune system. This amount is determined by the specific biological activity, the relationship between the biological activity and the amount of botulinum neurotoxin contained in the preparation. For Botox® the specific biological activity is 60 MU-EV/ng neurotoxin, for Dysport® 100 MU-EV/ng neurotoxin and for Myobloc™/NeuroBloc® 5 MU-EV/ng neurotoxin. For Myobloc™/NeuroBloc® this translates into an antibody-induced therapy failure rate of 44% in patients treated for cervical dystonia, whereas for BT type A preparations this figure is approximately 5%. No obvious differences in antigenicity of BT type A preparations have been detected thus far. For the current formulation of Botox®, the rate of antibody-induced therapy failure is reportedly less than 1%. To determine the antigenicity of different BT preparations in more detail, prospective studies on large series of unbiased patients with sensitive and specific BT antibody tests are necessary.

Introduction

Botulinum toxin (BT) has been used since 1980 to treat strabismus [1], blepharospasm and hemifacial spasm [2]. Other dystonias, various other muscle hyperactivity syndromes and exocrine gland hyperactivity syndromes are now also indications [3,4]. Generally, results of BT therapy are so impressive that it is considered the treatment of choice for most of its indications. In some patients, however, formation of BT antibodies occurs. These BT antibodies may not interfere with BT's therapeutic effect when their titres are low. However, when their titres are high, partial or complete therapy failure can occur. Formation of BT antibodies has been associated with various risk factors, including certain treatment parameters, patient characteristics and immunological properties of the therapeutic BT preparations used. This article will explore and compare the immunological properties of the different therapeutic BT preparations that are currently available.

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History

Immunological aspects of BT were first studied when protection against accidental environmental or laboratory intoxications or against intended biological warfare or terrorist intoxications were sought. When BT was first used therapeutically, the chronic nature of the disorders treated and the symptomatic character of the treatment (i.e. the expected chronic application) together with BT's protein structure immediately raised concerns about potential immune responses against BT. After mouse lethality assays failed to demonstrate BT antibodies in patients receiving BT therapy for blepharospasm [5,6], it was believed that BT doses used, overall BT antigenicity, immunoreactivity of the target tissues and other therapy parameters were such that formation of BT antibodies would not be stimulated. It was concluded that lack of BT antibodies formation in the therapeutic setting reflected difficulty in producing sufficiently high and sufficiently stable BT antibody titres as opposed to what was seen in immunization attempts using pentavalent toxoids [7].

Shortly afterwards, however, the first reports of BT therapy failure in the presence of BT antibodies detected by mouse lethality assay occurred [8], indicating – amongst other parameters – a potentially higher

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antigenicity of the therapeutic BT preparation than of the pentavalent toxoid. Robust studies on BT antibody formation or on antibody-induced therapy failure have been rare. Most studies have been hampered by the low frequency of antibody-induced therapy failure (i.e. by lack of sufficient cases available), methodological problems in detecting BT antibodies, problems in monitoring the clinical effectiveness of BT therapy, and fluctuations of the patient pool, especially when therapy failure occurs.

Treatment parameters as risk factors for botulinum toxin antibody formation

By comparing patient groups with and without antibody-induced therapy failure, several treatment parameters have been identified as risk factors for BT antibody formation. Most of the early studies were done with Botox® (Allergan, Inc., Irvine, CA, USA). In an initial attempt to study risk factors, no definite conclusions could be drawn because only 36% of the 21 patients with cervical dystonia and therapy failure had BT antibodies [9]. In another study five of 105 patients with cervical dystonia presented with secondary therapy failure, and in three of those patients, BT antibodies were detected in the mouse lethality assay (Anderson *et al*., 1992). In a third study BT antibodies were detected in three of 96 patients with cervical dystonia [8]. In those three patients the interinjection interval was significantly shorter than in the other patients. Another study compared eight patients with cervical dystonia and secondary therapy failure with 68 patients without therapy failure [11]. Secondary therapy failure could be verified by mouse lethality assay in only some of the patients. In other patients with secondary therapy failure, the mouse lethality assay was not performed. Comparing both groups, patients with therapy failure had significantly shorter interinjection intervals, more booster injections (injection series with interinjection intervals of less than 2 weeks) and higher BT dosages at each injection series.

In another study, 86 patients with focal dystonia were tested for BT antibodies [9]. In 20 of them BT antibodies were detected in the mouse lethality assay. All of those patients presented with complete secondary therapy failure. In the remaining 66 patients BT antibodies could not be detected. When the 20 patients with BT antibodies were compared to a matched group of 22 patients without BT antibodies, the BT antibody carriers had an earlier onset of BT therapy, higher BT dosage at each injection series and a higher cumulative BT dosage. When risk factors for BT antibodies were studied with a more elaborate multivariate logistic regression model [12], only the BT dosage given at each injection series and the interinjection interval were detected as independent risk factors. Booster injections and cumulative BT dose were not identified as independent risk factors. Female gender reached borderline statistical significance.

Patient characteristics as risk factors for botulinum toxin antibody formation

Although various treatment parameters have been identified as risk factors for antibody-induced therapy failure, neither the general nor the individual weight of those risk factors has ever been established. With numerous patients receiving large BT dosages and not forming BT antibodies and with some patients forming BT antibodies after few injection series and in minute BT dosages [13,14], additional risk factors seem likely. Analogies with immune reactions to environmental antigens suggest that those additional risk factors might include the overall reactivity of the patient's immune system and priming of BT antibodies formation by structurally similar environmental antigens. However, in our experience, allergies do not seem to be over-represented in BT antibody carriers, and tetanus immunization does not appear to predispose for BT antibodies formation. Formal studies on special patient characteristics have not been performed as yet.

Botulinum toxin preparations as risk factors for botulinum toxin antibody formation

Therapeutic BT preparations comprise complex mixture of various proteins and excipients (Fig. 1). The biologically, i.e. the therapeutically and toxicologically active compound, is the 150 kDa botulinum neurotoxin consisting of the 100 kDa heavy chain and the 50 kDa light chain. Additionally, this mixture contains

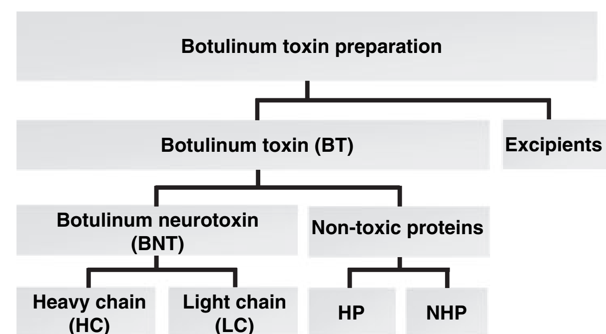

Figure 1 Therapeutic botulinum toxin preparations, constituents.

hemagglutinizing and non-hemagglutinizing non-toxic proteins, sometimes also referred to as complexing proteins. As shown in Fig. 2 for Botox® and Dysport® (Ipsen Ltd., Slough, Berkshire, UK), these complexing proteins have a molecular weight of 300 kDa, whereas for Myobloc™/NeuroBloc® (Solstice Neurosciences, Inc., South San Francisco, CA, USA) their weight is 150 kDa. In all therapeutic preparations the botulinum neurotoxin/non-toxic protein complex (NC) aggregates to dimers with a molecular weight of 600 kDa as in Myobloc™/NeuroBloc® or 900 kDa as in Botox® and Dysport®. Excipients, such as human serum albumin, lactose, NaCl or buffer are added for pharmaceutical purposes.

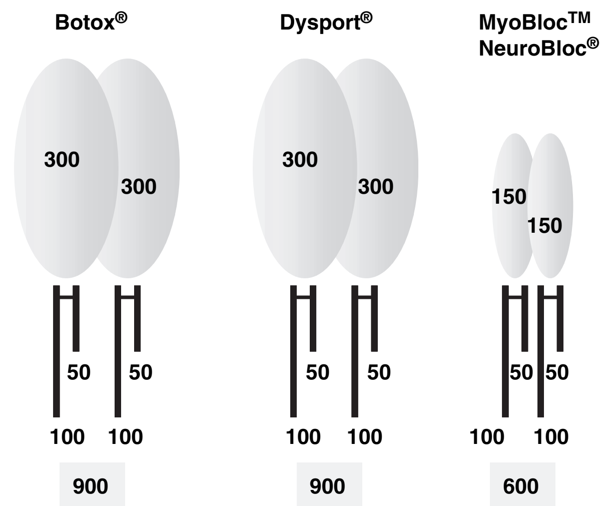

Figure 2 Metastructure of botulinum toxins. Botulinum toxin consists of the botulinum neurotoxin (150 kDa) and of non-toxic proteins. The non-toxic proteins have a molecular weight of 300 kDa in Botox® and Dysport® and of 150 kDa in Myobloc™/NeuroBloc®. All botulinum toxins are dimers producing conglomerates of 900 kDa as in Botox® and Dysport® and 600 kDa as in Myobloc™/NeuroBloc®.

In principle, all non-human protein components of this mixture can act as antigens, stimulating antibody formation. When antibodies are formed against botulinum neurotoxin, they can block its biological activity and hence they are called neutralizing or blocking BT antibodies. Antibodies formed against non-toxic proteins do not interfere with BT's biological activity and are therefore called non-neutralizing BT antibodies.

The functionally relevant antigenicity of a BT preparation depends on the amount of antigen, in this case the amount of botulinum neurotoxin, presented to the immune system. This amount depends on the specific biological activity describing the relationship between the biological activity and the amount of botulinum neurotoxin contained in a particular therapeutic BT preparation. When therapeutic BT preparations are manufactured, stored and handled, botulinum neurotoxin may be inactivated. Therefore, all therapeutic BT preparations contain some degree of biologically inactive botulinum neurotoxin. Although inactivated botulinum neurotoxin – by definition – does not exert a therapeutic effect, it may act as an antigen for BT antibody formation. High immunological quality, i.e. low antigenicity, is therefore described by a high specific biological activity and a low protein load.

Table 1 lists the specific biological activities for Botox®, Dysport® and Myobloc™/NeuroBloc®. They are reported as 20 MU-A/ngNC-B for Botox® [14], 100 MU-I/ngNC-D for Dysport® [15] and 100 MU-E/ngNC-M for Myobloc™/NeuroBloc® [16] (MU-A: mouse units as measured by the Allergan mouse assay, MU-I: mouse units as measured by the Ipsen mouse assay, MU-E: mouse units as measured by the Elan mouse assay, NC-B: Botox® neurotoxin/non-toxic

Table 1 Properties of different therapeutic botulinum toxin preparations

Parameter	Botox®	Dysport®	Myobloc™/NeuroBloc®
Serotype	A	A	B
SNARE target	SNAP25	SNAP25	VAMP
pH	7.4	7.4	5.6
Excipients	Albumin 500 μg/vial	Albumin 125 μg/vial	?
	NaCl 900 μg/vial	Lactose 2500 μg/vial	
Biological potency (MU/vial)	100	500	1.0 k/2.5 k/10.0 k
Potency conversion factor	1	1/3	1/40
Complex size	900 kDa	900 kD	600 kD
Specific biological activity	20 MU-A/ngNC-B	100 MU-I/ngNC-D	100 MU-E/ngNC-N
Corrected* specific biological activity	60 MU-EV/ngBNT	100 MU-EV/ngBNT	5 MU-EV/ngBNT

BNT: botulinum neurotoxin; MU-A: mouse units as measured by the Allergan mouse assay; MU-E: mouse units as measured by the Elan mouse assay; MU-EV: equivalent mouse units, 1 MU-EV = 1 MU-A = 3 MU-I = 40 MU-E; MU-I: mouse units as measured by the Ipsen mouse assay; NC-B: Botox® neurotoxin/non-toxic protein complex; NC-D: Dysport® neurotoxin/non-toxic protein complex; NC-M: Myobloc™/NeuroBloc® neurotoxin/non-toxic protein complex.

*Corrected for different potency units and for different neurotoxin/non-toxic complex protein sizes.

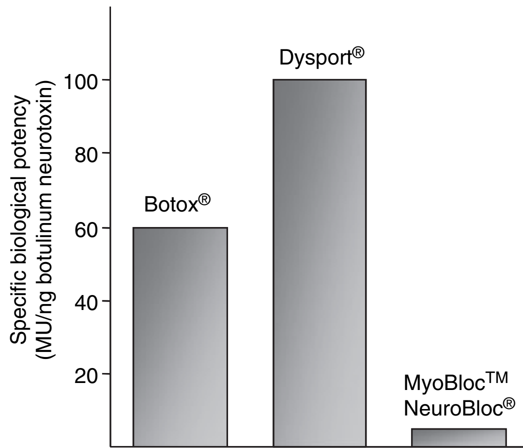

Figure 3 Specific biological activities of different therapeutic botulinum toxin preparations corrected for different potency units and for different botulinum neurotoxin/non-toxic protein complexes.

protein complex, NC-D: Dysport® neurotoxin/non-toxic protein complex, NC-M: Myobloc™/NeuroBloc® neurotoxin/non-toxic protein complex).

When these figures are corrected for the different potency units used and for the different NC the specific biological activities are 60 MU-EV/ngBNT for Botox®, 100 MU-EV/ngBNT for Dysport® and 5 MU-EV/ngBNT for Myobloc™/NeuroBloc® (MU-EV: equivalent mouse units, 1 MU-EV = 1 MU-A = 3 MU-I = 40 MU-E, BNT: botulinum neurotoxin) (Fig. 3). With this, Botox® and Dysport® have similar corrected specific biological activities, whereas Myobloc™/NeuroBloc® has a substantially lower one.

Up to 1998, when the product was formulated from its original 79-11 batch, the specific biological activity of Botox® was 4 MU-A/ngNC-B. When a continuous production process was adopted, the specified biological activity could be increased to its current value of 20 MU-A/ngNC-B. For Dysport® the specific biological activity used to be reported as 40 MU-I/ngNC-D [18, 19, 20]. Occasionally, however, figures as low as 20 MU-I/ngNC-D were reported [21]. The figure currently claimed by the manufacturer [16] was published in 2003 without giving further explanations as to the origin of these discrepancies.

With different corrected specific biological activities, the antigenicity of the different therapeutic BT preparations should be different and the rate of antibody-induced therapy failure should be different, too. Indeed, for BT-B, recent data suggest that Myobloc™/NeuroBloc® with its particularly low corrected specific biological activity may produce complete antibody-induced therapy failure in as many as 44% of patients treated for cervical dystonia within a relatively short time-period [22]. Suspicion of a higher antigenicity of Myobloc™/NeuroBloc® when compared with BTA preparations is supported by results of studies #301, #302, #351 and #352 submitted by Elan Pharmaceuticals to the Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Biologics Evaluation and Research, Division of Clinical Trial Design and Analysis as part of the drug registration process [23]. These studies included 468 patients with cervical dystonia who received between 5000 and 15 000 MU of Myobloc™/NeuroBloc®. Testing for neutralizing antibodies against BTB using a mouse protection assay revealed BT antibodies in 9.6% of the patients after 1 year, in 18.2% after 18 months and in 22.6% after 610 days of treatment. The Food and Drug Administration comments that 'these results indicate that there is substantial formation of antibodies in response to treatment with BT type B, and that many patients will convert to having neutralizing antibodies within 2 years of beginning treatment' [23].

For BTA preparations used to treat cervical dystonia, the rate of antibody-induced therapy failure is in the order of 5% [24]. So far, no studies have compared the antigenicity of different BTA preparations. For studies performed in the US (9,10, 25), the only available BTA preparation has been Botox®, whereas studies performed in Europe (8,10) only used Dysport®. Direct comparisons between Botox® and Dysport® can, therefore, not be performed. In only one study [12], treatments with both BT-A preparations were reported. No obvious differences in antigenicity can be detected when these data are reviewed. This impression also reflects further experience in our centres as well as experience from other centres performing BT therapy with both BTA preparations.

When a new formulation of Botox® was introduced in the US in 1998 and 1999 in Europe, this situation changed. For the first time a prospective study of antibody formation was attempted with this preparation [15]. So far, in the patient group receiving the new Botox® formulation no BT antibodies could be detected with a mouse lethality assay, whereas in the patient group receiving the old Botox® preparation 9.5% of patients had BT antibodies. Although the new Botox® preparation decreased the risk of BT antibody formation this risk still exists, as was recently demonstrated [14].

To determine the antigenicity of different therapeutic BT preparations in more detail, large prospective series of unbiased patients must be tested with sensitive and specific BT antibody tests. With new therapeutic BT preparations currently being developed in various parts of the world, antigenicity testing will remain important.

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