

Overview of Botulinum Toxin

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1.1 Introduction

Botulinum toxin (BNT) is a fascinating drug which specifically targets the release of acetylcholine. BNT is produced by the anaerobic bacterium *Clostridium botulinum*. In order to be used as a drug the toxin has to be isolated, purified and stabilized (Huang et al. 2000) (Table 1.1).

1.2 Different Subtypes of Botulinum Toxin

Seven distinct antigenic botulinum toxins (BNT-A, -B, -C, -D, -E, -F, and -G) produced by different strains of *Clostridium botulinum* have been described. The human nervous system is susceptible to five toxin serotypes (BNT-A, -B, -E, -F, -G) and unaffected by 2 (BNT-C, -D). Although all toxins have different molecular targets, their action leads to the blockade of the cholinergic nerves. However, only the A and B toxins are available as drugs. In aesthetic medicine, the BNT predominately used has been of type A so far, even though some trials have been published utilizing type B BNT (Baumann et al. 2003).

1.3 Mode of Action

BNT blocks the action of acetylcholine. Acetylcholine is a common neural transmitter and

Table 1.1. Pharmacological aspects of therapeutic botulinum toxin preparations (modified from Dressler 2006)

	Botox/Vistabel	Dysport	Xeomin	Myobloc/NeuroBloc
Manufacturer	Allergan, Inc Irvine, CA, USA	Ipsen Ltd. Slough, Berks, UK	Merz Pharmaceuticals Frankfurt/M, Germany	Elan Plc. Dublin, Ireland
Pharmaceutical form	powder	powder	powder	solution for injection
Storage precautions	below 8°C	below 8°C	below 25°C	below 8°C
Shelf life	24 months	15 months	36 months	24 months
Botulinum-toxin-serotype	A	A	A	B
<i>Clostridium-botulinum</i> -strain	Hall A	Ipsen strain	Hall A	Bean B
SNARE-target of action	SNAP25	SNAP25	SNAP25	VAMP
Purification	precipitation and chroma- tography	precipitation and chroma- tography	precipitation and chroma- tography	precipitation and chroma- tography
pH-value of the reconsti- tuted preparation	7.4	7.4	7.4	5.6
Stabilization	vacuum drying	freeze drying (lyophiliza- tion)	vacuum drying	pH-reduction
Excipients	human serum albumin 500 µg/vial	human serum albumin 125 µg/vial	human serum albumin 1 mg/vial	human serum albumin 500 µg/ml
Biological activity	NaCl 900 µg/vial 100 MU-A/vial or 50 MU-A/vial	lactose 2500 µg/vial 500 MU-I/vial	sucrose 5 mg/vial 100 MU-M/vial	NaCl 6 mg/ml 5.0 kMU-E/ml as 2.5/5.0/10.0 kMU-E /vial
Biological activity in relation to Botox	1	1/3	1	1/40
Molecular weight of the BNT component	900 kD	900 kD	150 kD	600 kD

BNT botulinum-neurotoxin, MU-A mouse-unit in the Allergan-mouse lethality assay; MU-E mouse-unit in the Elan-mouse lethality assay, MU-I mouse-unit in the Ipsen-mouse lethality assay, MU-M mouse-unit in the Merz-mouse lethality assay

stimulates striated as well as smooth muscles and the secretion of glands such as sweat glands.

After BNT has been ingested or injected, it diffuses into the human tissue until it selectively and irreversibly binds to the presynaptic terminal of the neuromuscular or neuroglandular junction, where it exerts its actions by cleaving specific membrane proteins responsible for acetylcholine excretion.

It is important to understand that the action of the BNT does not occur immediately. Usually the maximum effect can be seen after a couple of weeks. The first effects might be visible after 48 hours. Depending on the strength of the muscles treated and the dosages used, the duration of the effect varies from a couple of months to several months.

The action of the drug slowly decreases over time as the affected axons sprout new nerve terminals which continually restore the impaired transmission. During this phase the damaged synapse itself will regenerate its function (de Paiva et al. 1999).

Botulinum toxin only acts after ingestion or injection. Topical application is insufficient.

Claims of creams that induce botulinum toxin A effects have to be questioned.

1.4 Antidote

Although a BNT antidote exists, it is unable to reverse any drug effects that have arisen. Once symptoms become visible, the toxin has already bound to the synapse and the late application of antibodies has no effects. Please note that antibodies are nevertheless quite helpful in botulism occurring after accidental ingestion of contaminated foods when BNT might still diffuse in the body from the gastrointestinal tract.

1.5 Different Products

So far, there are several BNT-A products and one BNT-B product on the market.

The BNT-A products differ in their amount of protein as well as in the amount of albumin added (Table 1.1). At the moment Botox, also marketed in some countries as Botox Aesthetic/Vistabel/Vistabex for aesthetic indications, and Dysport share the majority of the aesthetic market. The new German BNT-A preparation Xeomin is only available in a few countries so far, and lacks clinical data on its efficacy in aesthetic medicine. NeuroBloc (also marketed as Myobloc) is the only commercially available type B BNT. Although there is some data on its efficacy in aesthetic indications, it is not often used for these indications (Baumann et al. 2003).

Botox may be marketed as Botox Aesthetics, Vistabel or Vistabex. For simplification in this book we will talk only about Botox when referring to dosages.

1.6 Units of Botulinum Toxin

The concept of calculating the dosage units for the different products Botox and Dysport is not easy to understand and may not be necessary. The user must only be aware that the dosage units of different products do not relate to each other. There are some attempts to offer ratios for these products. However, apart from one trial with severe methodological shortcomings (Lowe et al. 2005) there are no comparative clinical trials for aesthetic indications. For Botox and Dysport, based on the available data from placebo controlled clinical trials and dosages recommended at consensus conferences, the ratio is close to 1:2.5 – 1:3. The manufacturer claims that Xeomin has a 1:1 ratio to Botox. However, we have little

experience and no published data on aesthetic indications for this BNT-A formulation so far to support this claim (Table 1.1).

Therefore, when in doubt, instead of using ratios we would recommend the treating physician to go back to the data from clinical trials or consensus conferences.

Do not get confused by units or ratios between different products. In case of doubt one should go back to the clinical trial data or data from consensus conferences.

In this book the dosages recommended are the dosages that in our experience have the best effect in the majority of patients. For some indications these recommended dosages are based on clinical trials. However, for most indications no clinical trials have been performed so far.

1.7 Off-Label Use

Botox and Dysport are not licensed for aesthetic indications in all countries. In addition, the license is usually limited to the glabella area. In cases where no labelling or a limited labelling exists, the physician has to deal with off-label use. The patient must be informed if the product is used for an off-label indication.

As is sometimes the case with licensing of an other indication, the drug name is changed: basically the same brand may be available for off-label as well as labelled use. For example, in Germany Botox is listed for various neurological indications but not for aesthetic indications. However, for the treatment of the glabella area, the same drug is available as Vistabel. Both drugs contain exactly the same BNT, but Botox comes in 100 U vials and Vistabel in 50 U vials.

All the companies are trying to obtain licenses for aesthetic indications, therefore, it seems quite likely that the number of countries where

the major aesthetic indications are still off-label will decrease over time. Nevertheless, it is also clear that for the present time in most countries only some indications will be licensed, such as the treatment of the glabella.

Do not worry too much about off-label use. For Botox and Dysport there are enough studies proving efficacy and safety. The patient, however, must be informed when the product is used for an off-label indication.

1.8 New Drugs

At the moment several companies are working on new BNT preparations. These new products should be carefully evaluated and compared with the products presently on the market. It is always important to consider the evidence behind these new drugs. Randomized controlled clinical trials based on aesthetic indications should be the gold standard which new BNT preparations have to match. A *'This brand of botulinum toxin is comparable or even better than that brand of botulinum toxin.'* without good supporting data is not enough.

1.9 Evidence Behind the Use of BNT-A

In contrast to injectable fillers, the evidence behind the use of BNT-A in aesthetic medicine is much larger – at least for the two leading brands Botox and Dysport.

In the following chapter the evidence for the efficacy and safety of the different BNT-A preparations will be discussed for some key questions. In order to reduce bias only large studies, e.g. only studies of more than 50 patients will be included in this review.

1.10 Efficacy: Optimal Dosage

Key question 1: *What is the optimal dosage for treating the glabella?*

This is an important question. The glabella is probably the most frequently treated area. Fortunately there are several clinical trials available that try to answer this question. The question will be discussed for both brands separately.

What should efficacy measure? BNT targets the activity of the mimic muscles. Therefore, the ability of the toxin to reduce muscular movements should be measured. Usually it is not the muscular strength itself, but the effect of the reduction of muscular strength on the severity of wrinkles, which is measured by clinical scales. In most clinical trials four-point rating scales (with 0 for no and 3 for severe wrinkles) have been used to measure efficacy (Honeck et al. 2003).

In addition, subjective improvement is an important outcome measure. Here several scales have been used.

1.10.1 Botox

There are several trials focusing on the optimal dosage of Botox in the area of the glabella. The standard dosage used is 20 Botox U. In the first large placebo-controlled trial, patients with moderate to severe glabellar lines at maximum frown received intramuscular injections of 20 U BNT-A or placebo into five glabellar sites (Fig. 1.1). A total of 264 patients were enrolled (203 treated with BNT-A, 61 with placebo). There was a significantly greater reduction in glabellar line severity with BTX-A than with placebo (all measures, every follow-up visit; $P < 0.022$). The effect was maintained for many patients throughout 120 days (Carruthers et al. 2002).

The same authors investigated in a double-blind, randomized clinical trial the efficacy, safety and duration of the effect of four dosages of BNT type A in the treatment of glabellar

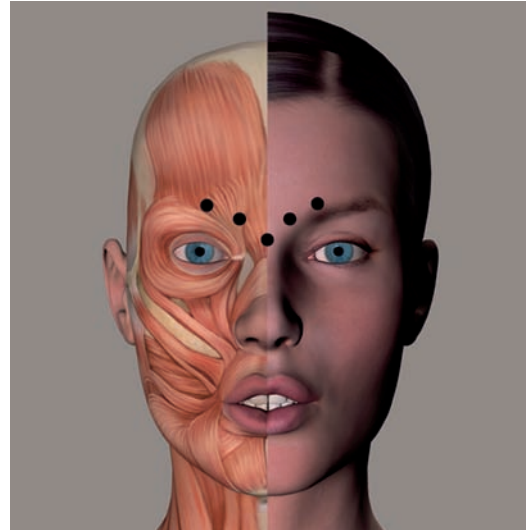


Fig. 1.1. Injection points as in the early Botox-Glabella studies (based on Carruthers et al. 2002)

rhytids in females. Eighty female subjects with moderate to severe wrinkles at maximum frown entered the study. Patients were randomly administered 10, 20, 30 or 40 Botox U in seven injection points (Fig. 1.2). Objectively, 10 U of BNT type A was significantly less effective than 20, 30 or 40 U. The relapse rate at 4 months was significantly higher in the 10-U group (83%) versus 40, 30 or 20 U (28%, 30% and 33% respectively). The authors concluded that 20–40 Botox U was significantly more effective at reducing glabellar lines than 10 U (Carruthers et al. 2005).

A similar study in male patients was published the same year. In this comparable study, 80 men were randomized to receive a total dose of either 20, 40, 60 or 80 U of Botox distributed in seven points in the glabellar and lower forehead area. The 40, 60 and 80 U dosages of BNT type A were consistently more effective in reducing glabellar lines than the 20-U dose (duration, peak response rate, improvement from baseline). There was a dose-dependent increase in both the response rate at maximum frown and the duration of effect assessed by the trained observer.

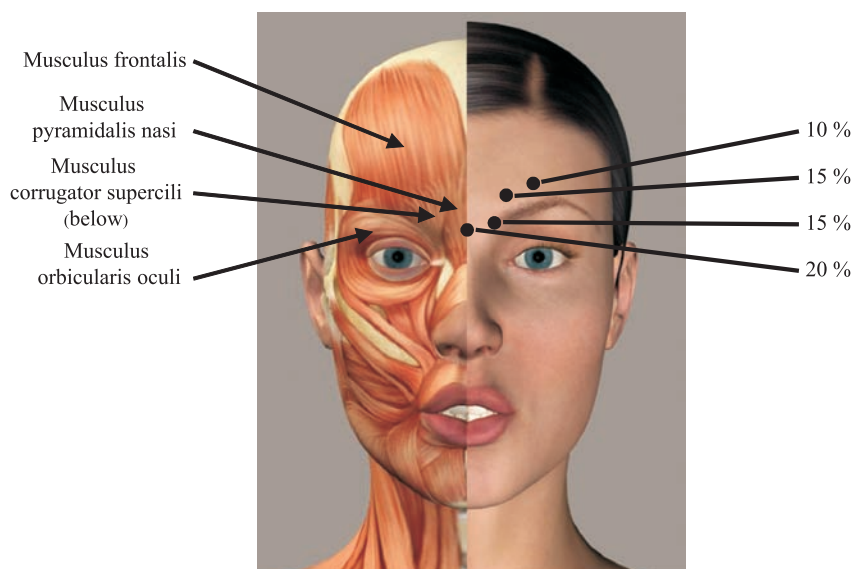


Fig. 1.2. Injection points as in the recent Botox-Glabella studies (based on Carruthers et al. 2005)

The authors conclude that male participants with glabellar rhytids benefit from starting dosages of at least 40 U of Botox (Carruthers et al. 2005).

Based on these studies, the recommended Botox dosage for the glabella should be at least 20 Botox U. Men might benefit from even higher dosages starting with 40 Botox U.

1.10.2 Dysport

So far there have been three trials published focusing on the optimal dosage for the glabella (Ascher et al. 2004, Ascher et al. 2005, Rzany et al. 2006). The first study from Asher et al. (2004) is a dose-ranging study comparing 25, 50 and 75 Dysport U with placebo. A total of 119 patients with moderate to severe glabellar lines at rest were treated. The dosage was distributed over five intramuscular glabellar sites forming a bird-shaped pattern (Fig. 1.1). Outcome measures included evaluations of glabellar lines by independent experts from blinded standardized photographs at rest 1 month after treatment, physician evaluations and patient assessments

during a 6-month period. A significant efficacy was reported for the three BNT-A groups for at least 3 months after injection (at least $P < .015$). Investigator and patient evaluations suggested that 50 U was the optimal dosage (Ascher et al. 2004).

Answer to key question 1: The initial doses focused on 20 Botox U for the glabella. In two subsequent studies higher doses were recommended. However, different injection points were used. The latter studies included two additional points targeting not only the corrugator but also parts of the frontalis muscle. For Dysport the recommended dose for the glabella is 50 Dysport U. Based on these studies, a ratio for Botox and Dysport of 1:2.5 seems reasonable.

1.11 Effectiveness: Dosages and Repeated Treatments

Key question 2: *How often do patients come back and does the required dosage change after frequent visits?*

This is an important question. The frequency of re-injection visits depends on several factors: the regaining of muscular movement (which depends on the strength of muscles and the initial dose), the consequently increased visibility of mimic wrinkles, and other factors such as costs.

1.11.1 Botox

So far there has been no data published. There is, however, information from a poster that was presented during the EADV 2004 (Carruthers A and Carruthers J, 2004). In this study, data from a 50-patient cohort was investigated. Patients needed to have at least ten treatments. The glabella was the most frequently treated area. No specific dosage for the glabella is given. The mean dosage for all areas treated was 40 Botox U. The median interval between treatments was 17.1 weeks with a range from 0.43 to 155.3 weeks.

1.11.2 Dysport

In the German-Austrian retrospective study, 945 patients were followed for at least three consecutive injections. The median interval between BNT-A treatment cycles was 5.9–6.5 months (25th–75th percentile: 4.4–8.9 months) and changed little with repeated treatments (Fig. 2.2).

For the glabella the median BNT-A dosage over all treatment cycles in those who received injections in the glabella was 50–60 Dysport U (25th–75th percentile: 40–70 U); for those who received injections in the glabella only, the median BNT-A dosage was 50–70 Dysport U (25th–75th percentile: 50–100 U). The dosage did not change over the different study periods. (Rzany et al. 2007).

Answer to key question 2: There are two patient cohorts. Based on these data, patients treated with Botox returned three times a year,

patients treated with Dysport twice a year for re-injection.

1.12 Safety

Here it is important not only to consider short-term safety but also long-term safety. Short-term safety is affected by the proportion of patients in whom muscles adjacent to the treated areas are influenced. For the glabella area this means the number of patients who will develop eyelid ptosis after injection with BNT-A. Again, it is the clinical trials that count.

1.13 Short-term Safety: Eyelid Ptosis

Short-term safety will be measured by clinical trials.

Key question 3: *How many patients developed eyelid ptosis after treatment of the glabella?*

1.13.1 Botox

Using Botox in the glabellar area, Carruthers et al. reported a lid ptosis rate of 5.4% in their first large placebo-controlled study (6 out of 203 patients; Carruthers et al. 2002), declining to 1.0% (2 out of 202 patients) in a subsequent study (Carruthers et al. 2003). In the most recent studies no lid ptosis occurred in a study of 160 patients (Carruthers et al. 2005; Carruthers and Carruthers 2005).

1.13.2 Dysport

When using Dysport, Ascher et al. reported no ptosis in his 102 patients treated with 25, 50 and 75 U (Ascher et al. 2004). In the German study, only one case of eyelid ptosis was reported among

127 patients treated with 50 Dysport U (Rzany et al. 2006).

Answer to key question 3: The risk for eyelid ptosis is present. However, it is small and temporary.

1.14 Long-term Safety: Eyelid Ptosis

Long-term safety will usually not be investigated by clinical trials. Here patient cohorts will be able to answer the questions. Fortunately, we have data from two large cohorts for the two major brands.

Key question 4: *What is the risk for eyelid ptosis after repeated treatments?*

1.14.1 Botox

In the Carruthers study (Carruthers and Carruthers 2004), adverse events were documented in 5 (0.6%) of 853 treatments. Eyelid ptosis was reported three times.

1.14.2 Dysport

In the German/Austrian retrospective study, adverse events (AE) were, in general, uncommon. Of the 945 patients, 90.6% ($n = 856$) did not experience any AE over any treatment cycle. The total AE rate per treatment cycle was 4.1% ($n = 39/945$) in cycle one, decreasing to 2.0% ($n = 11/553$) in cycle five, giving an overall mean incidence of 2.5% per treatment cycle. Importantly, most AEs were mild and resolved without further intervention. There were no serious or unexpected AEs.

Local hematoma was the most frequently reported AE (1.25% per treatment cycle; range: 1.8–0.7%). Lid or brow ptosis was uncommon (0.46% of treatment cycles; range: 0.85–0.1%) and

generally mild. All patients who experienced lid or brow ptosis ($n = 16$) received injections to the glabella or frontalis. A total of 3698 treatments in the glabella or frontalis were given to 907 patients. Therefore, the incidence of lid or brow ptosis in patients who received injections to the glabella and/or frontalis regions was 0.51% per treatment cycle or 1.8% per patient (Rzany et al. 2007).

Answer to key question 4: The risk for eyelid ptosis after repeated treatments is very small.

Please note that further information on safety is available in Chapter 7.

1.15 Marketing and Evidence

The market for BNT-A in aesthetic medicine is still growing. However, as in every market, there is close competition between companies. Therefore, it is important to keep a clear mind when a company claims superiority in efficacy and safety for their product. The following questions might come in handy when being approached by a representative of the company with new data claiming to show either better efficacy or safety.

What dosages and dilutions were used? This is very important: if you compare two products, one with a higher and one with a lower dosage, it might not be a surprise that the product with a relatively higher dose has more side effects.

How good is the clinical trial? It is not necessary to be a specialist of evidence based medicine (EBM). Just keep the following questions in mind when looking at a clinical trial.

Was the trial randomized? i.e. were the treatment groups distributed by chance? If not, just disregard it.

Was the trial blinded? Good clinical trials should always be blinded. A good example of a possibly absolute blinding is an expert committee who grades efficacy based on photographs.

Were the treatment groups equal after randomization? Sometimes randomization might fail. If there are differences in gender or age between the

study groups, be extremely cautious when looking at the data. In such a case, the analysis should be at least multivariate to try to account for the failure of randomization. Do not worry about the statistical test! Just look to see whether the analysis was uni- or multivariate. If the analysis was univariate (e.g. comparing only one factor at a time) it could be prone to more biases than a multivariate analysis.

How big was the trial? If you have a trial which assesses the superiority or inferiority of two BNT preparations, the number of patients should be high since only small differences are likely. So if a head-to-head trial has less than 100 patients it might be better to disregard it.

What should a good clinical trial be?
Randomized, blinded, large enough to answer the question!

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<http://www.springer.com/978-3-540-34094-2>

Botulinum Toxin in Aesthetic Medicine

de Maio, M.; Rzany, B.

2007, XIX, 141 p., Hardcover

ISBN: 978-3-540-34094-2