Dose equivalence of two commercial preparations of botulinum neurotoxin type A: Time for a reassessment?

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COMMENTARY

Dose equivalence of two commercial preparations of botulinum neurotoxin type A: time for a reassessment?

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ABSTRACT

Background: The units of different preparations of botulinum neurotoxin type A (BoNT-A) have different potencies, and dosing recommendations for each product are not interchangeable. Historically, there has been debate concerning the dose-equivalence ratio that should be used in clinical practice.

Methods: Published evidence was considered to establish an appropriate dose-conversion ratio for the two main commercially available preparations of BoNT-A – Dysport (Dp) and Botox (Bx).

Results: Four key areas of evidence were identified: nonclinical and preclinical studies; studies exploring the diffusion characteristics and effects of complexing proteins; comparative experimental data from human studies; and clinical studies. Nonclinical data indicate that the principal reasons for differences in unit potency between the two products are dilution artefacts in the mouse assay. Use of saline as a diluent, at high dilutions, results in significant loss of potency in the Bx assay, whereas use of gelatin phosphate buffer in the Dp assay procedure protects the toxin during dilution. The published data on mouse assays show a Dp : Bx unit ratio range of 2.3–2.5 : 1 in saline and 1.8–3.2 : 1 in gelatin phosphate buffer. Data indicate that complexing proteins or size of the complex, which is highly pH sensitive, play no role in toxin diffusion and that Dp and Bx have similar diffusion characteristics when used at comparable doses. Randomized, controlled clinical studies indicate that 3 : 1 is more appropriate than 4 : 1, but the two products are not equivalent at this ratio. Comparative human experimental studies using the extensor digitorum brevis test, facial lines and anhidrotic action halo tests support dose-conversion ratios less than 3 : 1.

Limitations: Data comparing dose equivalence ratios from the non-clinical setting should be extrapolated into the clinical setting with some caution.

Conclusions: Dose-conversion ratios between Dp and Bx of 4 : 1 and greater are not supported by the recent literature.

Introduction

Botulinum neurotoxin type A (BoNT-A) is a potent inhibitor of acetylcholine release at the neuromuscular junction[1]. BoNT-A has been extensively studied in numerous clinical conditions and is widely used to treat a range of neuromuscular disorders, including cervical dystonia, focal spasticity, blepharospasm and hemifacial spasm[2–7]. Furthermore, BoNT-A can be effective in patients who suffer from smooth muscle

dysfunction in the gastrointestinal or urogenital tract[8,9], from hypersalivation or hyperhidrosis. BoNT-A is also administered cosmetically for the treatment of facial lines and is now one of the most common aesthetic procedures performed[10].

BoNT-A is a protein complex that is derived from the bacterium *Clostridium botulinum*. As such, it is a biological agent that is produced commercially using different methods and processes. There are currently three commercially available preparations of type A toxins: Dysport (Dp), Botox (Bx) and Xeomin*, all of which are supplied as lyophilized powder for reconstitution prior to injection. For each indication, the number of units required and the number of injection sites varies. Importantly, due to their biological nature, each preparation has different properties; the trade names should not be used generically to describe the toxin and dosing recommendations for each product as they are not interchangeable with other BoNT-A preparations. This means that the number of units recommended for treatment is specific for each of these BoNT-A preparations. For example, the number of units recommended for treatment with Dp is not interchangeable with that of Bx[11].

In the past, the lack of direct comparability between commercially available BoNT-A preparations has led to confusion over which conversion factor to use when switching patients from one product to the other or interpreting doses published in the literature[12]. Historically, physicians tended to use a Dp : Bx conversion ratio of between 4 : 1 and 5 : 1 based on initial recommendations which were originally assumed before comparative evidence was available[13]. However, the range of Dp : Bx ratios reported in the literature has varied from 2 : 1 to 11 : 1, further adding to the confusion surrounding the issue of dose equivalence[12].

Although there has historically been considerable debate concerning the dose-equivalence ratio that should be used in clinical practice evidence now shows that the Dp : Bx equivalence ratio is lower than previously thought; the use of high dose-conversion ratios, as recommended in the older literature, is no longer justified. In particular, when converting Bx dosages from literature or clinical experience to Dp doses, using too high a dose-conversion ratio may lead to a tendency to overdose with Dp. In clinical practice, doses, regardless of the preparation, should always be adjusted for each individual patient.

This commentary article discusses preclinical and clinical evidence in order to help to clarify which Dp : Bx dose-conversion ratio is, in the authors' opinion, most appropriate in the context of day-to-day clinical practice. In addition to the authors' personal clinical and experimental work, a literature search was conducted for publications directly comparing dosages of two commercially available preparations – Dp and Bx. Xeomin, the third commercial BoNT-A preparation, has only recently become available and there remains insufficient evidence on which to base dose-conversion ratios for this and the other preparations. All comparative papers on the mouse assay methodology used by the two manufacturers were identified by the authors. Comparative study data were identified using a PubMed search using the search terms Botox AND Dysport. Only controlled studies directly comparing the two products are included and only studies in healthy volunteers or in the following clinical indications were considered: blepharospasm, hemifacial spasm, cervical dystonia (spasmodic torticollis) hyperhidrosis, focal spasticity. The search period was between 2002 and 2008; clinical studies published before 2002 were captured in the systematic review by Sampaio *et al.*[14] – a summary of which is included here.

The evidence and studies considered in this commentary can be broadly divided into four key areas and is presented as such: nonclinical studies that consider the role of excipients in dose conversion, as well as evidence from animal assays; the diffusion characteristics of the preparations and the effects of complexing proteins on diffusion; comparative experimental data from human studies using either the extensor digitorum brevis (EDB) test, facial wrinkles and muscle electromyography (EMG) or the anhydrotic action halo test; and, finally, comparative clinical studies providing evidence from randomized, controlled clinical trials.

Examining the nonclinical perspective

The potency of both Dp and Bx is described in terms of mouse LD_{50} units, in which one LD_{50} unit is the median lethal dose in a pre-specified mouse population using a detailed assay procedure. However, it is important to understand that, despite the same definition, one Dp unit is not equivalent to one Bx unit, as the potency of each unit is defined for each preparation using a different assay methodology. As detailed below, the difference in unit potency is an effect of assay methodology and not a characteristic of the BoNT-A formulation tested. Ultimately, differences in external excipients (gelatin) added to the assay

*Dysport is a registered trade name of Ipsen Limited; Botox is a registered trade name of Allergan Incorporated; Xeomin is a registered trade name of Merz Pharmaceuticals GmbH, Germany

diluent in routine Quality Control (QC) testing, and the concentration of the excipient Human Serum Albumin (HSA) in the final product vials, both impact on the final measured potency.

Several studies have demonstrated the importance of assay methodologies in the resulting differences between units of Dp and units of Bx. In the original reports on the effect of assay diluent on unit potency, Hambleton and Pickett measured the potency of Dp and Bx using each assay method for each product[15,16]. These studies showed that a Bx unit was 3.15 times more potent when measured using the Dp assay methodology (gelatin-containing phosphate buffer; GPB, as the diluent), whereas a Dp unit was 2.5 times less potent when measured using the Bx assay methodology (saline diluent). Subsequent studies have confirmed these findings and observed similar changes in the potency of Dp and Bx units when different assay diluents are used[17,18]. More recently, a multicentre international collaborative study was conducted to test the effect of assay conditions on defining the activity of BoNT-A preparations in an attempt to standardize the measurement of BoNT-A across laboratories[19]. The mean results from tests on both Dp and Bx using both diluents are shown in Table 1, showing that the dilution artifacts in the NaCl assays result in a significant loss of potency for both preparations.

Table 1. *Illustration of the impact of diluent on toxin potency (mean assay data from Table 3, Sesardic* et al. *2003*[19]*)*

Batch	A (Dp)	C (Bx)
Labelled LD_{50} units/vial	2000	100
Manufacturer's tested LD_{50} units/vial	1787	122
Measured in GPB diluent		
Mean LD_{50} units/vial	1818	219
n	13	12
95% CI	1663–1987	198–242
Measured in saline diluent		
Mean LD_{50} units/vial	1009	154
n	3	3
95% CI	773–1316	131–182
Potency saline/GPB	55.5%	70.3%

The study was carried out in ten laboratories in five countries. Sample A was bulk active substance used for Dp, with a specific activity of 1.3×10^8 units/mg but formulated into a 2000-unit vial with 500 μg HSA for this study. Sample C was formulated from batch 79-11 with a specific activity of 2×10^7 units/mg
GPB, gelatin phosphate buffer; CI, confidence interval; *n*, number of assays performed
Dp : Bx unit ratio can be calculated from $(C_{measured}/C_{manufacturer})/(A_{measured}/A_{manufacturer})$ and were 2.3 : 1 in saline and 1.8 : 1 in GPB

These nonclinical studies illustrate that using saline as a diluent is associated with a loss in potency at the high dilutions used during the assay procedures (by definition, down to 1 LD_{50} and lower), although it is important to note that these high dilutions are only used in nonclinical and quality control testing[20]. HSA is an excipient added to both commercial products to prevent inactivation of the toxin during dilution. At the high dilutions used in the saline assay, HSA concentrations become inadequate to prevent toxin loss. By comparison, gelatin protein contained in the GPB assay diluent compensates for reduction in HSA concentrations, preventing potency losses. In addition, the effect of saline is different for the two products. In the assay with a saline diluent, the apparent potency of Dp declined to 55% of the value in GPB, whereas the apparent potency of Bx declined less, to 70% (Table 1)[19]. It is probable that HSA concentrations in the commercial products account for this apparent difference as there is 75% less HSA in Dp (125 μg per vial) than in Bx (500 μg per vial), making Dp more sensitive to these dilution artifacts than Bx. Thus, when using saline as a diluent, not only does loss of potency occur at high dilutions, but the potency loss varies based on the amount of HSA present in the commercial product, indicating that dose-equivalence ratios calculated using saline assays will be sensitive to the degree of dilution and not be the same as those in clinical use.

The observations described in the above studies imply that dose ratios between the products differ when highly dilute solutions in saline are made for QC purposes, compared with when the more concentrated solutions are used in the clinic. Using data from the most comprehensive assessment of diluent effects – the multicentre study cited above – the Dp : Bx unit ratio was 2.3 : 1 in saline and 1.8 : 1 in GPB[19].

The effects of diluents on the mouse LD_{50} assay results are also seen in another animal-based assay, the mouse hemidiaphragm test. In this test, a hemidiaphragm/phrenic nerve preparation is immersed in a bath of Ringer's solution, the nerve stimulated and the muscle contractions measured with a strain gauge. Toxin is then added and the time taken for 50% paralysis is noted. As in the mouse assay, the actual concentrations used are much lower than those used in the clinic. Concentration/effect curves for Dp and Bx measured using this method showed that the curve for Dp was steeper (Figure 1) – that is, the loss of efficacy with dilution was higher for Dp than for Bx, as would be expected given the different HSA concentrations in the two formulations[21]. The role of excipients as a toxin stabilizer at these high dilutions was confirmed in a series of experiments using the same

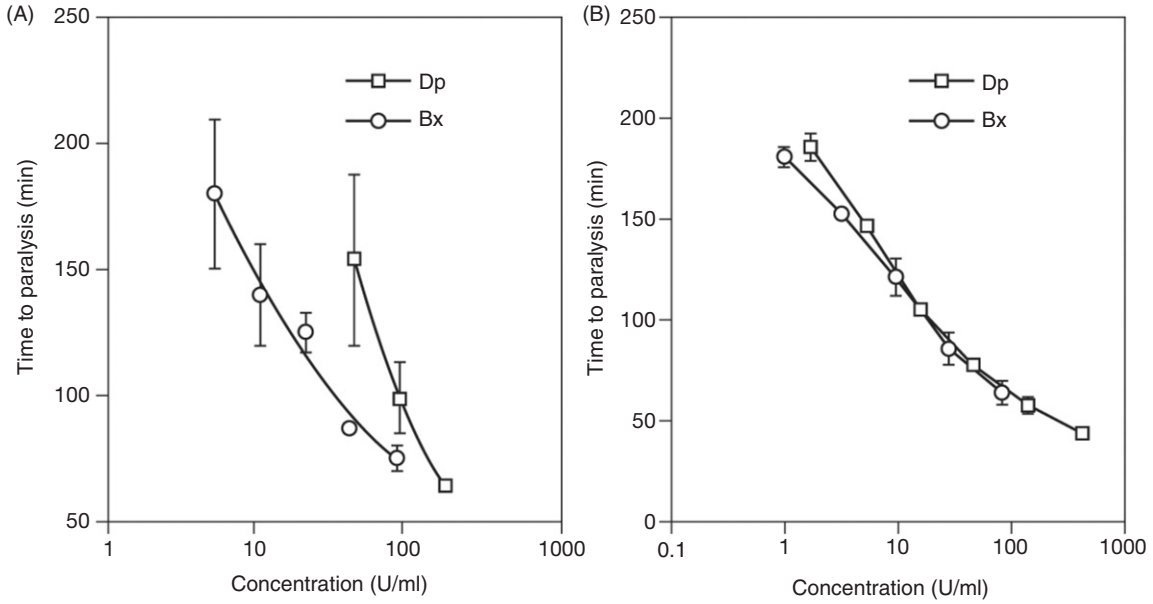

Figure 1. *Concentration-response curves for Dp and Bx, as determined by the mouse hem idiaphragm test in saline (A) (from reference[21]) and in saline with 0.1% serum albumin added (B) (from reference[20]). The parameter tested is time to paralysis for increasing dilutions of the test toxin. For comparison, the recommended dilutions for clinical use are 200–500 units/mL for Dp and 12.5–200 units/mL for Bx. Figure 1A reproduced by kind permission of the publishers, Bigalke H. Botulinumtoxine: Wirksamkeit und Antigenizitaet.* Klin Neurophysiologie *2001;32:210–12. ©2001 Thieme. Figure 1B reproduced with permission from Wohlfarth* et al. *Botulinum A toxins: units versus units. Naunyn Schmiedebergs Arch Pharmacol 1997;355:335–40. ©1997 Springer-Verlag.*

Table 2. *Summary of digit abduction scoring data*

		Bx	Dp	Unit ratio
Mice				
Aoki, 2001[22]	ED_{50} U/kg	6.2	22.9	3.7
Aoki, 1999[23]	ED_{50} U/kg	3.5	12.7	3.6
Aoki, 1999[23]	ED_{50} U/kg	3.5	15.2	4.3
Aoki, 2001[22]	LD_{50} U/kg	81.4	160.8	2.0
Rats				
Rosales *et al.*, 2006[24]	ED_{50} U/kg	3.4	8.5	2.5
Rosales *et al.*, 2006[24]	IM_{50} U/kg	4.2	9.7	2.3

ED_{50}, median effective dose; LD_{50}, median lethal dose producing a lethal response in 50% of the population
IM_{50} = threshold of diffusion effect seen as weakness in thigh muscles (after injection in the gastrocnemius) for 50% of the population

hemidiaphragm test but with Ringer's solution containing 0.1% bovine serum albumin[20]. With this extra excipient, the concentration response curves of the two products were very similar (Figure 1).

The Digit Abduction Scoring (DAS) test in mice and rats has also been used in an attempt to establish a dose-conversion ratio between Dp and Bx. Although the results of these studies (summarized in Table 2) have produced contradictory results, these can be explained by differences in methodology. For example, experiments in mice in which higher unit ratios (3.6 : 1–4.3 : 1) were established used higher dilutions for efficacy than experiments that established lower unit ratios for toxicity (1.7 : 1–2.0 : 1) using a more concentrated solution[22,23]. Although the author of these studies suggests that this shows that Bx has a larger safety margin than Dp[22,23], independent DAS experiments in rats showed no appreciable difference in unit ratios for efficacy (2.5 : 1) or spread to adjacent muscles (2.3 : 1), using the same dilution for both measures (Table 2)[24].

Nonclinical studies thus show that the diluent (presence or absence of stabilizing proteins such as gelatin) is a key consideration when conducting animal model

experiments to establish a dose-conversion ratio. It is our opinion that studies using gelatin-containing phosphate buffer in their assays potentially provide a more accurate assessment of dose ratios, with respect to the more concentrated solutions used in the clinical setting, than assay studies using saline. Considering studies using GPB gives unit ratios between 1.8 : 1 and 3.2 : 1.

Effects of complexing proteins

The active ingredient of both Dp and Bx is *Clostridium botulinum* type A neurotoxin in a complex with hemagglutinin and non-hemagglutinin proteins that protect the toxin molecule from degradation at low pH – for example, against the acid environment of the stomach, as would be the case in botulism food poisoning. However, the complex is not covalently linked and is therefore pH sensitive; it dissociates at alkaline and physiological pH values[25,26]. In both products, the toxin dissociates from its complexing proteins at physiological pH[27], after which it diffuses to surrounding tissues[28]. *In vitro* experiments have shown that this dissociation is both rapid (<1 minute) and complete[29]. As such, the complexing proteins play no role in the diffusion of the toxin itself; consideration of the complexing proteins within the clinical setting is therefore unnecessary.

Although complexing proteins have no role in the diffusion of toxins *per se*, toxin diffusion is a key consideration in clinical practice. It is important that toxins should not diffuse beyond the target muscle following injection as this may cause unwanted side effects as a result of toxin activity in those secondary, nearby muscles. Injecting a specific amount of toxin in a larger volume of diluent will increase the area into which the toxin is initially spread by the injection process, hence leading to an initial larger zone from which the toxin may then diffuse further. For example, measuring the compound muscle action potential (CMAP) in neighboring muscles, following injection of different toxin dilutions into the EDB muscle in the foot, showed that higher dilutions of both Dp and Bx had a great effect on neighboring muscle contraction, implying more diffusion from this small muscle[30]. Importantly, there was no significant difference on the effect of dilution between the two products when compared with each other at the same dilutions, indicating no difference in diffusion characteristics between preparations[30]. In a similar series of experiments, the diffusion-induced reduction of muscle activity in neighboring muscles for Bx and a complex-free BoNT-A preparation were found to be similar, confirming the irrelevance of the complexing proteins for diffusion[31].

The above data indicate that complexing proteins play no role in toxin diffusion and that Dp and Bx have similar diffusion characteristics when used at comparable concentrations. Instead, it is more likely that differences in diffusion that may have been observed between preparations are the result of different toxin doses, as this clearly impacts on diffusion. In turn, this can be explained by the use of inappropriately high dose-conversion ratios, resulting in unequal doses of toxin and relative overdosing of Dp.

Dp : Bx dose-conversion ratio – new human experimental approaches

Comparative experimental data in humans have several advantages over clinical study data. For example, the nonclinical experimental set-up allows dose ratios to be quantified in a standardized setting where potentially confounding effects (such as toxin concentration) can be controlled. Nonclinical models, however, must consider experimental factors that can lead to artifactual differences in responses between different toxin products.

Studies in healthy volunteers

One human experimental method of objectively assessing the dose/response of different preparations is the EDB model in which toxin is injected into the extensor digitorum brevis foot muscle in healthy volunteers and the CMAP is measured with surface electrodes after supramaximal stimulation of the peroneus nerve[32]. With this methodology, Wohlfarth *et al.* conducted a dose-ranging study that was designed to investigate the dose equivalence, diffusion characteristics and safety of Dp and Bx in 79 healthy volunteers[30]. In this study, dose effects were measured in the steep section of the dose–response curve, where the effect changes rapidly with dose, producing the most accurate results.[30]. This is an important consideration as clinical studies conducted at, or near, maximum dose may observe little or no change with increasing dose. Both products were associated with similar significant reductions in CMAP amplitude in the EDB from 2–12 weeks after injection. Dose–response curves for each product at each concentration are shown in Figure 2. As would be expected, increasing the dose (toxin concentration) increases the effect on CMAP for both Dp and Bx[30]. Statistical modeling using

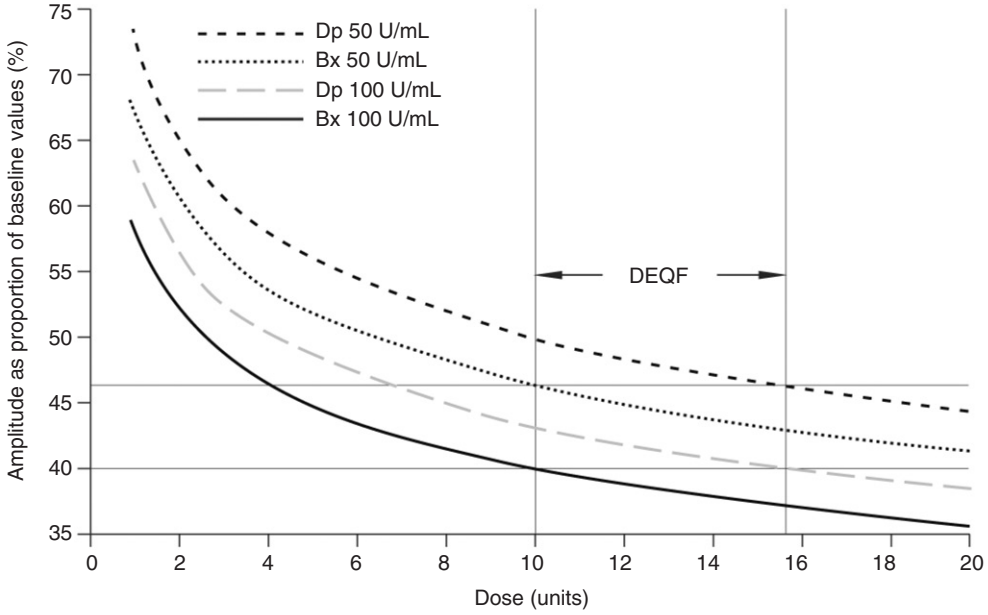

Figure 2. *Calculated dose–response curves for Dp and Bx at each of two concentrations (from reference[30]). The curves are derived from data for the amplitude of the compound muscle action potential in the human extensor digitorum brevis muscle 2 weeks after treatment. The horizontal lines are the equal response levels at these doses for high (lower line) and low (upper line) concentrations. 10 units of Bx in this figure is shown to equal 16 units of Dp (Dp : Bx ratio 1.6 : 1) at both dilutions. Reproduced with permission of the author and publisher from Wohlfarth K* et al. *Biological activity of two botulinum toxin type A complexes (Dysport® and Botox®) in volunteers: A double-blind, randomized, dose-ranging study. J Neurol 2008;255:1932-9. ©2008 Springer.*

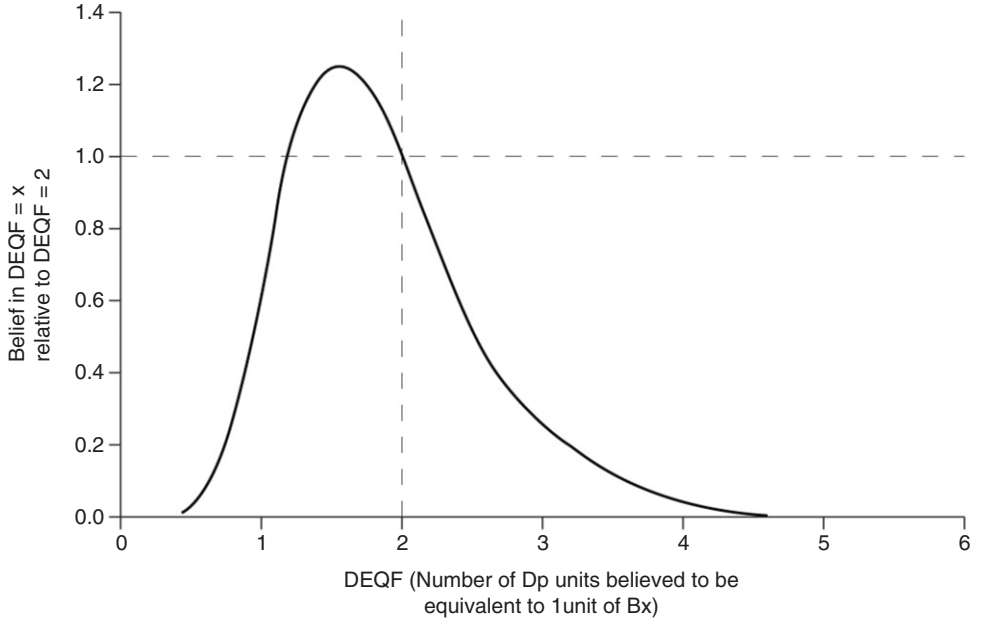

Figure 3. *Likelihood ratios for various DEQFs relative to the belief that DEQF = 2 (2 units of Dp : 1 unit of Bx) (from reference[30]). DEF, dose-equivalence factor. Reproduced with permission of the author and publisher from Wohlfarth* et al. *Biological activity of two botulinum toxin type A complexes (Dysport® and Botox®) in volunteers: a double-blind, randomized, dose-ranging study. J Neurol 2008;255:1932-9. ©2008 Springer.*

CMAP amplitude data from the EDB tests produced a Dp : Bx ratio of 1.57 : 1 (95% confidence interval [CI]: 0.77–3.20 units). Likelihood ratios for various dose-conversion factors suggested that a dose-equivalence ratio of 3 : 1 was just within statistical error limits; ratios over 3 : 1 were too high (Figure 3)[30]. This study currently represents the largest quantitative dataset relating to the comparative

activity of Dp and Bx reported in human muscle and supports dose-conversion ratios reported from previous EDB studies[20].

The use of botulinum toxin for dermatological indications, such as facial lines and hyperhidrosis, has led to further, very visual methodologies that can be used to generate measurable objective data regarding the effects of toxin dose on treatment response. These studies also have the advantage of allowing quantitative comparative data to be easily collected in a clinical treatment situation.

The anhidrotic action halo test allows visualization of the spread of toxin following injection; action of toxin on the cholinergic sweat glands[33] produces an anhidrotic area that can be visualized with the iodine–starch test or the Ninhydrin sweat test. In an attempt to establish an appropriate conversion factor between Dp and Bx, the effects of injecting different doses and dilutions of each product into the skin of the abdomen in healthy volunteers was measured[34]. Anhidrotic areas were objectively measured using the Ninhydrin sweat test after sweating was induced by exercise and hot drinks. Results from this objective study yielded dose-equivalence conversion ratios (Dp : Bx) of 1.3 : 1 for anhidrosis and 1.6 : 1 for hypohidrosis, supporting a dose ratio of less than 2 : 1.

Another study used similar methodology to compare the effects of Dp and Bx on the halo of weakness and anhidrosis in the frontalis muscles following injection of 2.5 : 1, respectively, at the same volume and at a controlled depth in volunteers[35]. No statistically significant differences were observed between the sizes of the halos produced corresponding to an equivalence ratio of 2.5 : 1. The authors also report use of this ratio in their clinical practice and state that they obtain similar and comparable results with the two products.

A further study has investigated the anhidrotic effects of Dp and Bx at different concentrations following single injections into the back of healthy subjects[36]. Anhidrotic areas, identified using the iodine-starch test after 3 weeks, suggested a dose-equivalence conversion ratios (Dp : Bx) of 1.2 : 1, in agreement with previous findings.

Studies in patients

In an independent study, Karsai *et al.* measured facial line severity and underlying EMG activity in patients treated with Dp or Bx on either side of their forehead, at a dose ratio of 3 : 1[37]. At this conversion ratio, maximum effect was the same for both compounds; however, Dp had a significantly longer lasting effect on wrinkle scoring and on EMG activity, suggesting that the conversion ratio for bioequivalent effects may be less than 3 : 1[37]. It is important to note that the dose–response curve when measured in the human EDB muscle shows a classic parabolic form and appears to level off at a maximal effect of 85–90% decrement from

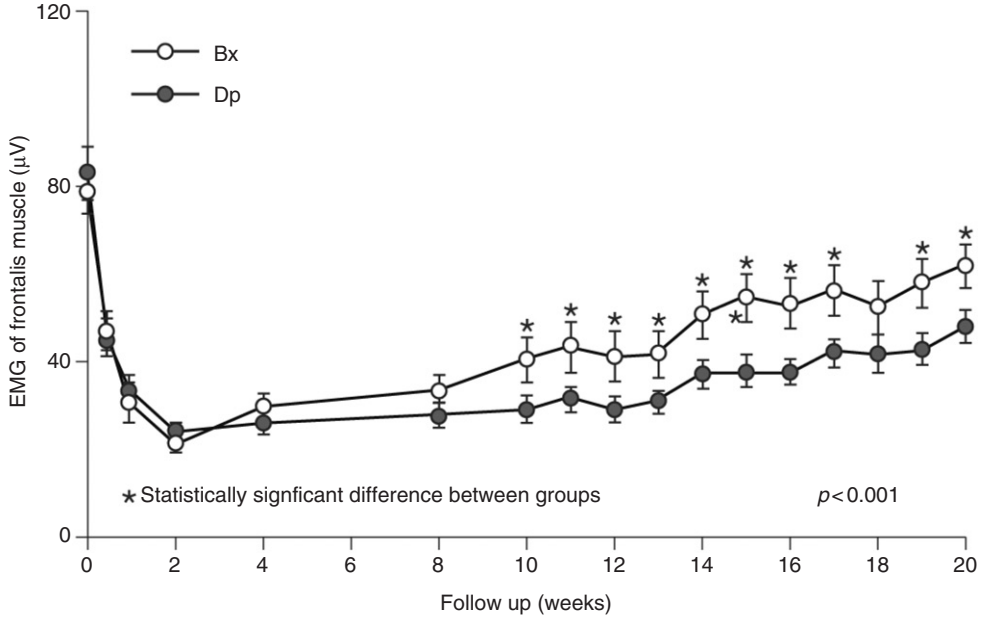

Figure 4. *EMG activity in the human frontalis muscle prior to injection of 12 units of Bx or 36 units of Dp and during the 20-week follow-up period (mean ± SEM). At maximum effect (weeks 2–4), no difference is seen. Only when the effect is no longer at a maximum can a consistent difference be seen (from reference[37]). SEM, standard error of the mean. *Statistically significant difference between groups. Reproduced with permission from Karsai* et al. *A randomized double-blind study of the effect of Botox and Dysport/Reloxin on forehead wrinkles and electromyographic activity. Arch Dermatol 2007;143:1447–9.*
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baseline (85–90% "paralysis")[32]. This effect is clearly visible in the data from Karsai *et al*. For both EMG and wrinkle score, the maximum effect around week 2 is the same for both products; only as the effect declines at later stages (from week 4) do differences become apparent (Figure 4)[37]. This has important implications as it means that measurement of conversion ratios at or near maximum effect is not optimal; measurement at maximal effect will be relatively dose-insensitive, giving equivalent effects over a wide range of dose ratios.

The anhidrotic action halo tests described earlier have also been used in patients. In a study in patients with forehead hyperhidrosis, the effects of Dp and Bx were compared, at dose ratios of 2.5 : 1, 3 : 1 or 4 : 1[38]. During the 6 months after treatment, the area of anhidrosis was larger with Dp in 93% of medial-medial or lateral-lateral comparisons. This also suggests that the effective Dp : Bx ratio is below 2.5 : 1[38].

Finally, a double-blind study has investigated the dose-equivalence conversion ratio of Dp to Bx by measuring the anhidrotic effect and muscular effect of multiple intradermal injections of toxin into the palmer skin of patients with hyperhydrosis, using the iodine-starch test 4 weeks after treatment and reduction in CMAP in three hand muscles, respectively[39]. Using these two methodologies the overall dose conversion ratio seemed to be between 1 : 1 and 1.5 : 1.

Clinical study data

There remains a paucity of head-to-head randomized, controlled trials comparing Dp and Bx. Sampaio *et al.* conducted a systematic review of head-to-head randomized trials comparing Dp and Bx in neurological indications (cervical dystonia, blepharospasm, hemifacial spasm, Meige syndrome, spasmodic dysphonia, and laryngeal dystonia) in 2004[14]. No randomized, controlled studies directly comparing Dp and Bx in these neurological indications have been published since this review. As such, this paper remains the 'gold standard' on dose-conversion ratios in clinical practice. In this important review, the authors identified only four large-scale head-to-head studies that compared Dp and Bx using the Cochrane criteria employed[40] (for full details see Sampaio *et al.*). The results of these studies are summarized in Table 3[14]. In these studies, patients were treated for either cervical dystonia, blepharospasm or hemifacial spasm using dose ratios of 3 : 1 and 4 : 1[41–44].

In the first study, 91 patients with blepharospasm or hemifacial spasm were treated with Dp and Bx at a dose ratio of 4 : 1. Although there was a trend for greater duration of efficacy and more side-effects with Dp, no significant differences were observed between groups[44]. This study therefore suggests that the dose ratio of 4 : 1 is too high[14].

In a second, larger double-blind crossover study, 212 patients with blepharospasm received one injection of each product at different treatment sessions, according to a Dp : Bx ratio of 4 : 1. There was no significant difference between Dp and Bx in treatment duration, although significantly more side effects were observed with Dp[43]. Although lower dose ratios were not tested, the results suggest that lower dose ratios may result in equivalent efficacy and achieve a better tolerability profile.

In a third study, 73 patients with cervical dystonia who were receiving Bx were randomly assigned to one of two treatment groups: one group was switched from Bx to Dp using a 3 : 1 Dp : Bx ratio and the other continued receiving a previously established dose of Bx. No significant difference between treatment groups was noted in responder rates, duration of effect or side effects, and the authors concluded that the two products were equivalent when administered at this ratio[41].

In the final study identified in the review[14], Dp was compared with Bx at ratios of 4 : 1 and 3 : 1 in 54 patients with cervical dystonia previously treated with Bx using a double-blind, randomized, double-crossover design. Data from this study showed that Dp was significantly more effective at relieving muscle spasm and pain at both dose ratios, although side effects were higher with Dp[42]. This is the only such study to compare two dose ratios in the same patients. This study suggests that the dose-conversion ratio could be lower than 3 : 1 for routine clinical use[42,45].

From these four papers, Sampaio and colleagues concluded that a dose ratio of 3 : 1 is more appropriate than 4 : 1 but that the two products are still not equivalent at this ratio[14]. In fact, these studies revealed that Dp consistently shows a more marked and/or longer-lasting clinical effect and increased side-effect profile than Bx, not only when using a dose-conversion factor of 4 : 1, but also at 3 : 1, suggesting that the dose-conversion ratio should actually lie below 3 : 1[14]. This is also in agreement with the data from animal model studies, more recent human experimental data (discussed above) and a recently published study comparing Dp and Bx in patients with primary axillary hyperhidrosis. In this latter randomized, double-blind study using a dose conversion ratio of 3 : 1, both products showed similar efficacy, although Dp was associated with a non-significant increase in duration of benefit compared with Bx[46]. This would again suggest that a ratio of 3 : 1 is too high to demonstrate dose equivalency.

Table 3. *Summary of data from randomized, controlled clinical trials*

Reference	Indication/design	Patients	Unit ratio	Parameters	Effect, Dp/Bx	Mean total dose, Dp/Bx, units	AE, Dp/Bx
Sampaio *et al.*, 1997[44]	BP/HF, parallel group	91	4 : 1	Duration of effect	13.3 ± 5.9/11.2 ± 5.8 weeks (p = ns)	100 BP, 45 ± 7.1 HF/ 25 BP, 7.5 HF	50/47% of patients (p = ns)
Nussgens *et al.*, 1997[43]	BP, crossover	212	4 : 1	Duration of effect	8.03 ± 4.6/7.98 ± 3.8 weeks (p = ns)	182.1 ± 55.1/45.4 ± 13.3	All AE: 24.1/17.0% of treatment sessions ($p < 0.05$) Ptosis: 6.6/1.4% ($p < 0.01$)
Odergren *et al.*, 1998[41]	Cervical dystonia, parallel group	73	3 : 1	Responder rate Tsui score (adjusted) Duration of effect	76/66% 4.8/5.0 84 ± 13.6/81 ± 14.4 days (all p = ns)	477 ± 131/152 ± 45	58/69% of patients (p = ns)
Ranoux *et al.*, 2002[42]	Cervical dystonia, double crossover	54	4 : 1	Tsui score	4.92 ± 2.86/3.25 ± 2.96 (p = 0.01)	Dose for each patient based on two preceding successful treatments with Bx	All AE: 36%/17.6% of patients (p = 0.03) Dysphagia: 17.3/3% of patients
				TWSTRS pain score	5.37 ± 6.49/2.59 ± 5.43 (p = 0.02)		
				Duration of effect	114 ± 69.3/89.3 ± 39 days (p = 0.02)		
			3 : 1	Tsui score	4.27 ± 2.91/3.52 ± 2.96 (p = 0.02)		All AE: 33/17.6% of patients (p = 0.06) Dysphagia: 15.6/3% of patients
				TWSTRS pain score	4.41 ± 5.76/2.59 ± 5.43 (p = 0.04)		
				Duration of effect	96.9 ± 39.3/89.3 ± 39 days (p = ns)		

AE, adverse event; BP, blepharospasm; HF, hemifacial spasm; TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale

A 4 : 1 dose conversion ratio has also been evaluated in a double-blind, randomized study in eight patients with severe primary palmar hyperhidrosis using the Minor's iodine starch test to quantify sweat production after treatment[47]. This study showed a trend towards larger improvement after treatment with Dp and a higher incidence of adverse effects, further supporting the conclusions of Sampaio that the two products are still not equivalent at this ratio.

Although these clinical studies point towards a dose ratio lower than 3 : 1, it should be noted that only 4 : 1 and 3 : 1 dose ratios have been adequately tested in head-to-head trials of adequate quality in neurological patients. Thus, it cannot be ruled out that direct head-to-head studies using lower dose ratios would also indicate equivalence. We have identified only one such study in aesthetic medicine, on glabellar line severity, using a conversion ratio of 2.5 : 1[48]. However, the methodology used in this study has been criticized[49], precluding firm conclusion regarding this conversion ratio.

There is clearly a justified need for further well-designed clinical studies at lower ratios, especially given that evidence from retrospective studies suggests that there is a need for a lower dose-conversion factor in order to maintain clinical efficacy and patient well-being in clinical practice. This is illustrated by a retrospective study in 207 patients with cervical dystonia who received either Dp or Bx for up to 13 years which suggests a lower dose conversion[50]. Similar efficacy between both products was observed at a relatively low mean dose of 389 U Dp and 145 U Bx, producing a dose ratio of 2.7 : 1[50].

Limitations

The discussions in this commentary should be considered in light of several limitations. While data comparing dose equivalence ratios from the non-clinical setting can provide advantages over studies in the clinical setting, this data should be extrapolated into the clinical setting with some caution. Furthermore, we have not considered indirect comparisons between BoNT-As; although there are numerous published randomized, controlled trials comparing either Dp or Bx to placebo, measurements and evaluations differ between trials making comparisons across studies misleading. Also, there may be limitations to our search strategy; however, we are confident that we have discussed all controlled trials directly comparing Dp to Bx in this commentary that met our inclusion criteria. It should be noted that we have not included data from direct comparative trials that may have been conducted in medical conditions currently under investigation such as overactive bladder and anal fissures and we did not review the controlled-trial literature in neurological indications prior to 2002, to avoid duplication of the work of Sampaio *et al*; we refer interested readers to this publication for further information. As a general limitation, studies testing single dose-conversion ratios at maximum effect are limited in their ability to accurately assess the most appropriate dose-conversion ratio.

Conclusions

Historically, there has been considerable debate concerning the dose equivalence ratio between Dp and Bx that should be used in clinical practice, originating from excessive unit differences that were proposed before the availability of quantitative data. As we have discussed, action halo, EMG and EDB studies suggest that much lower dose-conversion ratios may be appropriate. In the opinion of the authors, the evidence we have presented suggests that the effective clinical dose-conversion ratio is lower than previously assumed, with 1 unit of Bx being approximately equivalent to 2–2.5 units of Dp. In our opinion, there is a considerable body of evidence to indicate that dose-conversion ratios of 4 : 1 or more are contraindicated and could lead to a tendency to overdose with Dp. We recommend that physicians using both products should be aware of this and consider using a lower conversion factor as a guide, adjusting as necessary based on patient response to ensure that each patient receives treatment that is adequately tailored to their individual needs.

Transparency

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