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Respective Potencies of Botox® and Dysport® in a Human Skin Model: A Randomized, Double-blind Study

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Abstract: Mouse units used to quantify the activity of botulinum A toxin preparations are not equivalent and issues concerning efficacy and safety remain with regard to their respective potencies and diffusion qualities in human tissue. We compared the effects of Botox® (BOT) and Dysport® (DYS) in different doses and dilutions in a human skin model. Eighteen (8 women, 10 men) healthy volunteers, aged 28.4 years ± 5.7 years were injected intradermally with pure saline, BOT and DYS at 16 points in the abdomen in random order and in a double-blind condition, using two conversion ratios (1:3 and 1:4) and three different dilution schemes. For an objective outcome, the Ninhydrin sweat test was used to compare the anhidrotic areas. Both preparations showed a linear dose and dilution relationship with similar variances of responses for anhidrosis and hypohidrosis, indicating the same reliability of response. The dose equivalence conversion ratios (BOT: DYS) were 1:1.3 for anhidrosis and 1:1.6 for hypohidrosis (1:1.1-1.5 and 1:1.4-1.8 95% confidence intervals). The diffusion characteristics of both products were similar. A dose equivalence factor of more than 1:2 (BOT:DYS) is not supported by these objective and reproducible data. © 2008 Movement Disorder Society

Key words: botulinum toxin A; dose unit ratio; ninhydrin sweat test; respective potencies

Botulinum toxin (BoNT) has been well studied in neurology as well as other disciplines and can be considered the treatment of choice in many of its indications. Although the human nervous system is affected by all seven subtypes (A–F) if applied parenterally,[1] type A preparations are most commonly used in clinical practice for various reasons, such as its prior availability, immunologic aspects, safety, and efficacy.[2] Among the type A preparations, the efficacy and safety of Botox® (BOT) (Allergan, Irvine, CA) and Dysport® (DYS) (Ipsen, Maidenhead, UK) have been well established in various disorders.[3] In Europe, both preparations are widely used but controversy remains about their respective potencies and their diffusion characteristics.[4] Different experimental paradigms have been used to find an appropriate conversion factor, but the results are conflicting, indicating a ratio BOT:DYS (r.B:D) between 1:2 and 1:11.[5–9]

Studies comparing BOT and DYS in humans have mainly focused on the clinical outcome, e.g., in cervical dystonia, blepharospasm, and hemifacial spasm.[7,8] These trials, even if performed in a prospective randomized double-blind design, are susceptible to high variability such as fluctuations of the disease, injection techniques, and muscle activity during injection.[10] Moreover, assessing therapeutic success depends partly on the patients' subjective reports, which may be distorted if the treatment outcome does not meet expectations.

Determining a more precise conversion factor would help physicians to find the appropriate dosage when switching from one product to another since overdosing or underdosing may result in severe clinical

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TABLE 1. *Doses and dilutions of Botox® (BOT) and Dysport® (DYS) injections*

Injection volume (ml)	DYS units (Dilution)	BOT units 1:4 (Dilution)	BOT units 1:3 (Dilution)
0.12	24 (500 U/2.5 ml)	6 (100 U/2 ml)	8 (100 U/1.5 ml)
0.06	12 (500 U/2.5 ml)	3 (100 U/2 ml)	4 (100 U/1.5 ml)
0.03	6 (500 U/2.5 ml)	1.5 (100 U/2 ml)	2 (100 U/1.5 ml)
0.03	12 (500 U/1.25 ml)	3 (100 U/ 1 ml)	4 (100 U/0.75 ml)
0.12	12 (500 U/5ml)	3 (100 U/4 ml)	4 (100 U/3 ml)

Injections were randomly allocated according to a computer-generated randomization list. One injection was performed with pure saline (0.03 ml), not shown in this table.

consequences.[11] A more precise conversion factor would also be useful to better interpret dosages given in the literature. And finally, there are certain economic implications.

In two recent studies, the ninhydrin sweat test (NST) methodology was adapted to develop a simple and clinically useful test for indirectly detecting antibodies against BoNT.[12,13] A significant correlation between the anhidrotic area and the well-established mouse diaphragm test was found in dystonic patients with and without suspected antibody formation against BoNT-A. This indicates that the NST may serve as a surrogate model for muscular effects of the toxin.

The aim of this study was to find an appropriate conversion factor between BOT and DYS in a reproducible human skin model and to study the effect of dilution in both products in double-blind conditions.

MATERIALS AND METHODS

Eighteen (8 women, 10 men) healthy volunteers, aged 28.4 years ± 5.7 years, not previously exposed to BoNT for at least 1 year and with the following characteristics: no dermatologic alterations on the abdomen in the area of measurement; body mass index between the 15th and 85th percentiles; drug-free; and no history of abnormal sweating, were injected intradermally with BOT and DYS at 15 points in the abdomen using 0.3 mL syringes (Becton Dickinson, France) with 8-mm 30-gauge needles. One injection was performed with pure saline (0.03 ml). All injections were applied midintradermal in the identical manner raising a small elevation of the skin. On the basis of the literature[4] and on clinical routine, we have chosen two conversion ratios, (r.B:D) 1:3 and 1:4. Additionally, the preparations were administered with three different dilution schemes as shown in Table 1. Subjects were allocated to four different injection schemes according to a computer-generated randomization list. Both the investigators and volunteers were blinded to treatment allocation to maintain double-blind conditions.

To measure potencies using an objective outcome measure, the NST was used to compare the anhidrotic effect of BOT and DYS.[14] The toxin acts on cholinergic innervated sweat glands, producing an anhidrotic area (action halo). The NST visualizes the amino acids in sweat. An imprint of the sweat production is produced on standard photocopying paper, dyed, and fixed with a solution of 1% ninhydrin dissolved in acetone. Application of heat (30 min at 50°C) accelerates the ninhydrin reaction.[15] In the present study, the NST of abdomen sweat prints was carried out after induction of sweating by drinking 500 ml of hot tea, performing standardized physical activity, and using a heated blanket. All subjects were induced to sweat by the same manner. The anhidrotic and hypohidrotic areas were outlined by an investigator who was blinded to the treatment received. Size and area were then calculated by computer. The measurements were carried out 2 days, 1 week, 3 weeks, and 7 weeks after injection. Before inclusion in the study, written informed consent was obtained from all subjects. The study was approved by the local Ethics Committee.

Statistics

Relative potency was estimated separately for anhidrosis and hypohidrosis. For both endpoints, a mixed effects analysis of variance with fixed factors log dose, treatment (BOT/DYS), time (four time points, modeled as categorical variable), sex and random factor proband was used. To obtain normally distributed residuals, the square root of the measurements was used as dependent variable. The relative potency was calculated from the coefficients of the mixed effects model. A relative potency "r" has the interpretation that in the mean (across probands) a dose "d" of DYS is as effective as a dose "r· d" of BOT. To compare the variance of the responses of DYS and BOT an enriched mixed model incorporating separate variance estimates for the DYS and BOT groups was compared with a likelihood ratio test to the original model. To assess the impact of volume, DYS measurements at dose 12 and BOT

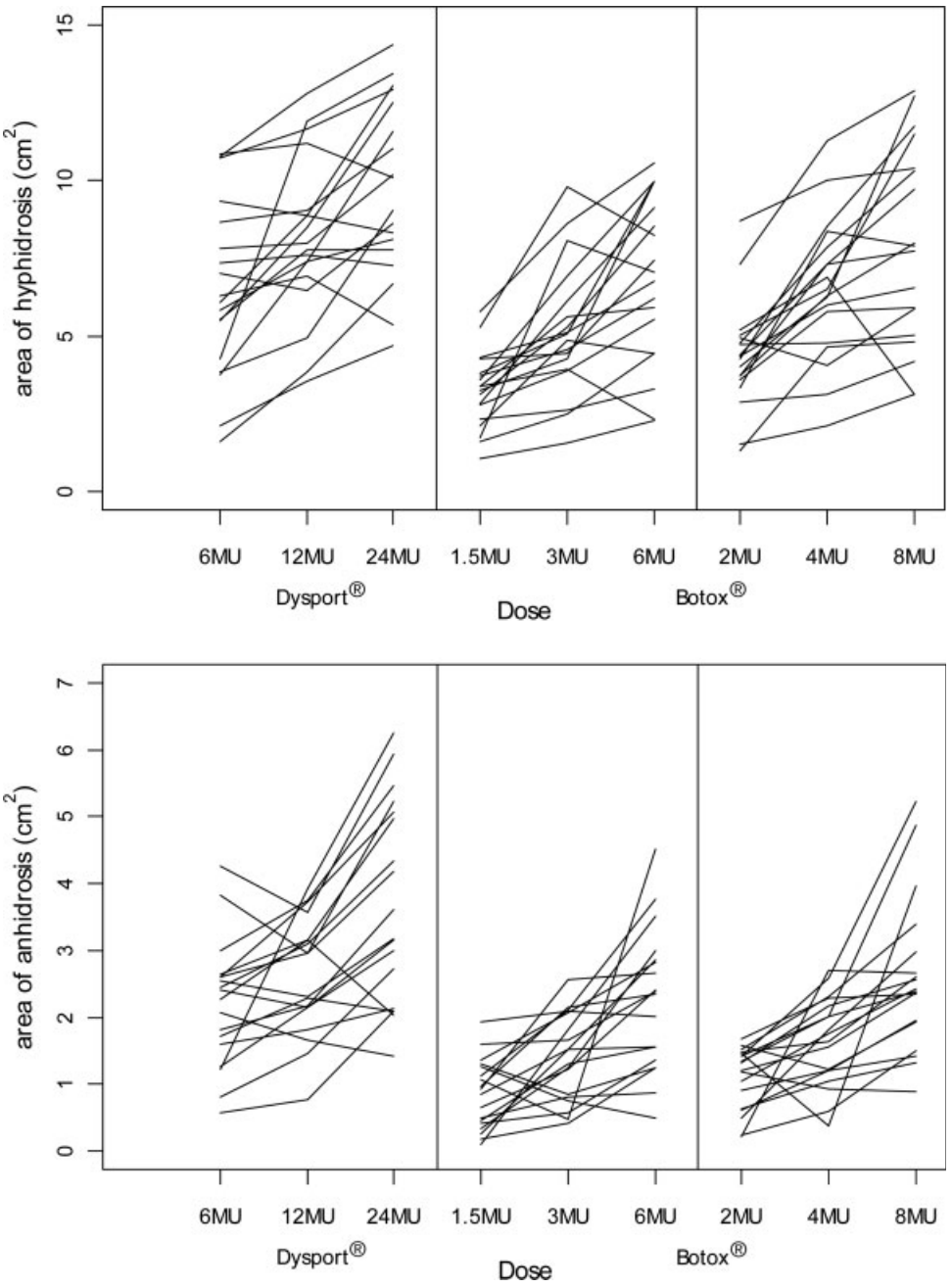

FIG. 1. Areas of hypohidrosis and anhidrosis in each subject three weeks after injection. Each line represents the hyphidrotic and anhidrotic response of a single subject at different doses of DYS and BOT. For both preparations, within each subject, a clear dose response relationship can be observed. I.g. in the case of anhidrosis in DYS, all but two of the 18 subjects experienced a stronger response at dose 24 than at dose 6. For the change in anhidrosis between dose 6 and 24, the standard deviation was 1.4 cm^2. Similarly, for Botox at the highest dose 8 MU the standard deviation for anhidrosis was 1.2 cm^2.

measurements at dose 4 both at volumes 3, 6, and 12 were used. A mixed model with fixed factors log (Volume), treatment (DYS/BOT), time (four time points, modeled as categorical variable) and random factor proband was calculated. All analyses were performed with the statistics package R: A Language and Environment for Statistical Computing, Version 2.3.0 (R Foundation for Statistical Computing, Vienna, Austria).

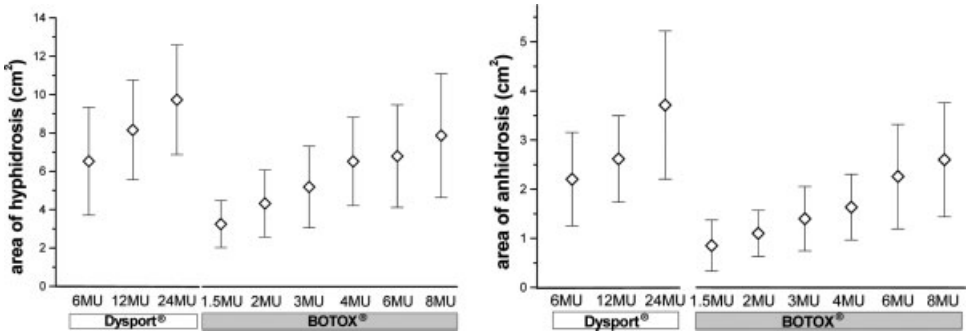

FIG. 2. Areas of anhidrosis and hypohidrosis three weeks after injection. Graphs indicate the mean and standard deviations in all subjects. The action halos show a linear dose effect relationship in both products. The estimated coefficient (+-standard error) for the factor log(dose) in a mixed model [with fixed factors treatment (BOT/DYS), log(dose) and random factor proband] for the area of anhidrosis (resp. hypohidrosis) at week 3 is 1.06 ± 0.09 (resp. 2.54 ± 0.20).

RESULTS

Comparing the variances of responses to BOT and DYS injections, no significant differences were found for either anhidrosis or hypohidrosis ($P = 0.25$ and 0.33, respectively), indicating the same reliability of response for both preparations. The data are highly reproducible; a low degree of scatter was seen (see Fig. 1).

The results show a linear dose effect relationship for both products (see Fig. 2).

Because the action halos of DYS were much larger than those of BOT even at the lower conversion ratio used for this study (r.B:D 1:3), a best fit for the dose conversion ratio was calculated for both anhidrotic and hypohidrotic areas. The dose conversion ratios obtained, (r.B:D) 1:1.3 to 1.6, are shown in Table 2.

A significant dependence of the responses to injection volume was found for hypohidrosis ($P < 0.001$) but not for anhidrosis ($P = 0.2$) for both products. With higher volumes, hypohidrotic areas were clearly increased for both preparations, demonstrating the importance of volume. The difference was maximal at 3 weeks after injection, but by 7 weeks, the hypohidrotic areas were similar, regardless of volume (see Fig. 3).

DISCUSSION

A strong controversy remains about the respective potencies and diffusion characteristics of the two most commonly used BoNT preparations, BOT and DYS. In a human skin model in 18 healthy adults, we used the NST, a well-established objective and highly reproducible outcome measure to compare the two products.[12,13] We found a linear dose relationship and similar diffusion characteristics in both products. Respective potencies were found at a r.B:D between 1:1.3 and 1:1.6. These results are in contrast to earlier studies that claimed to show considerably higher conversion ratios and different diffusion characteristics between the two products.[9,10,16]

All botulinum neurotoxins are assayed on the same standardized mouse model, a mouse unit being defined as the LD50 in this model. The prime reason for the difference in DYS and BOT potency units is due to the different methods used for the mouse potency assays. To standardize each batch of DYS, the toxin is diluted over the range of potencies tested in a phosphate buffer containing gelatin (GPB). For the "BOT assay", saline is used as the diluent. Saline compared to GPB leads to significant loss in potency in the BOT assay at the high dilution ranges required for the assay.[17] In an independent multicenter comparison of laboratory assays, the two batches were tested with both diluents.[17] The loss of mouse unit potency associated with the saline assay was confirmed in both batches. 1.9 units of DYS were equipotent to one unit of BOT in the saline diluent assay and 1.8 to 3.1 units of DYS were equivalent to one unit of BOT in the GPB diluent assay.[18,19]

Other mouse models including the digit abduction scoring test showed conflicting results with conversion ratios (B:D) of 1:3.7, 1:4.3.[20,21] and (B:D) 1:2.5.[10] Studies comparing BOT and DYS in humans focused on the clinical outcome and showed even more conflicting results than the animal studies from controlled

TABLE 2. *Action halo dose conversion ratios*

	BOT:DYS dose equivalence ratio	95% Confidence intervals
Anhidrosis	1:1.3	1.1–1.5
Hypohidrosis	1:1.6	1.4–1.8

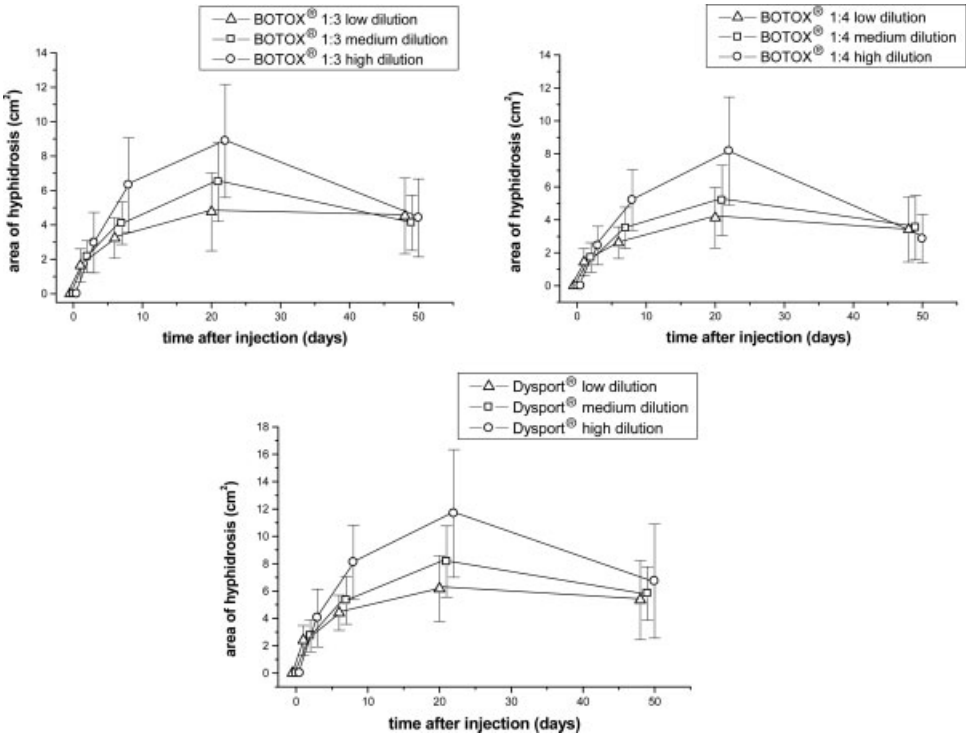

FIG. 3. Time course of the size of the action halo (area of hyphidrosis) for different dilutions (men/SD). BOT 1:3 (4 mU), low dilution (100 mU/0.75 ml); medium dilution (100 mU/1.5 ml); high dilution (100 mU/3 ml). BOT 1:4 (3mU), low dilution (100 mU/1 ml); medium dilution (100 mU/2 ml); high dilution (100 mU/4 ml). DYS (12 mU) low dilution (500 mU/1.25 ml); medium dilution (500 mU/2.5 ml); high dilution (500 mU/5 ml). Data points are staggered at each injection time to visually separate the different dilutions. All confidence intervals are standard deviations.

laboratory environments.[7,8,16] Data ranged between conversion r.B:D of 1:2.2 and 1:11.[5,9,22] In a recent systematic review carried out to search for controlled studies on dose equivalence, the authors could identify only four studies[7,8,23,24] that fulfilled the Cochrane criteria for evidence-based medicine.[4] In these studies, conversion ratios lower than (B:D) 1:3 were not tested. The authors concluded that a r.B:D of 1:3 supported equivalence of degree and duration of effect, although bioequivalence of the two products could not be assumed. There was a consistent trend for all four studies for DYS treatment to have more adverse events and longer-lasting effects for the tested ratios (B:D) 1:3 and 1:4. In both studies that tested the r.B:D of 1:3 in cervical dystonia, the clinical effect of DYS was higher than that of BOT.[7,24] These findings suggest that clinically, the best conversion ratio is less than 1:3. Despite that evidence, recent commercially sponsored studies claimed considerably higher conversion ratios.[9,16]

Apart from the controversy about the respective potencies, there is also considerable discussion on the diffusion characteristics of the two products.[21,25] It has been stated that DYS has more side effects than BOT due to a higher diffusion rate.[10,21] Different pharmaceutical properties of the two products as complexing proteins or excipients have been suggested to cause these diffusion differences.[21] However, our study in human tissue indicates similar diffusion rates for the two products. It has been shown that the toxin molecule separates promptly from the complexing proteins at physiological pH, and this might be one reason why the spread is similar.[2] With the three different volumes at constant doses, we found increased hypohidrotic (but not anhidrotic) areas with higher volumes in both products in a linear manner. These findings are in line with two experimental studies on rats[10,25] and were recently confirmed in humans in the extensor digitorum brevis test.[26]

We chose the NST skin model because the abdominal skin provides homogenous research conditions and the NST is an objective and highly reproducible tool to test BoNT activities in humans. We also consider our findings as having implications for the muscle situation, since earlier studies indicated that the NST may serve as a surrogate model for muscular effects of the

toxin.[12,13] Furthermore, it does not seem being conceivable that different brands of the same active ingredient should have different affinities to neuroglandular and neuromuscular synapses.

Certainly, reliable conclusions on the conversion ratio and diffusion characteristics found in our human skin model, can only be made for cutaneous injections. Furthermore, we found a linear dose-response relationship for both products only for small doses, which might differ with higher doses. However, reviewing the literature, more recent and independent studies and randomized controlled trials even in muscular indications suggest lower conversion ratios and similar diffusion characteristics between the two products, as found in our study. We want to emphasize that statements that DYS tends to have higher efficacy, longer duration and higher frequency of adverse events than BOT, as recently published,[10] indicate that most head-to-head trials comparing the two products used too high conversion ratios.

On the basis of this double-blind, randomized controlled trial with highly reproducible objective results, we suggest that lower conversion ratios are to be used to prevent the diffusion of unbound toxin. Taking into account a tolerated toxin variability of $\pm 20\%$ in the commercially available products, we conservatively conclude that a dose equivalence factor of more than (BOT: DYS) 1:2 is not supported by our data.

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