Field effect of two commercial preparations of botulinum toxin type A: A prospective, double-blind, randomized clinical trial

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Background: The dose equivalence of commonly used commercial preparations of botulinum toxin type A, Dysport (abotulinumtoxinA [ABO] 500 U, Ipsen Biopharm Limited, Wrexham, United Kingdom) and Botox (onabotulinumtoxinA [ONA] 100 U, Allergan, Irvine, CA), remains unclear.

Objective: We sought to evaluate the field effect for ABO and ONA at dose equivalences of 2.5:1.0 U and 2.0:1.0 U, in both muscular and sweat gland activity.

Methods: In all, 59 female patients with forehead wrinkles were enrolled. Patients were randomized for dose equivalence between ABO and ONA, group A (2.0:1.0 U, ABO:ONA) or group B (2.5:1.0 U, ABO:ONA) administered in the frontalis muscles. Clinical assessment, Minor test, and electromyography evaluations were performed at baseline, 28 days, and 112 days.

Results: In group B, the field of anhidrotic effect of ABO showed a greater area and larger horizontal diameter than ONA at 28 and 112 days. At maximum frontalis muscle activity (day 112) patients receiving ABO demonstrated greater improvement based on the Wrinkle Severity Scale. No differences were found in frontalis muscle activity at rest between groups A and B based on results of Wrinkle Severity Scale, electromyography, and interindividual variability data at 28 and 112 days.

Limitations: Currently, there are no objective measurements other than electromyography to evaluate the field effect of botulinum toxin type A in muscles.

Conclusion: At a dose equivalence of 2.0:1.0 U (ABO:ONA), similar field effects were found for both muscle and sweat gland activity. At a higher dose equivalence of 2.5:1.0 U (ABO:ONA), injections of ABO showed greater area and larger horizontal diameter in field of anhidrotic effect at 28 and 112 days than ONA. (J Am Acad Dermatol 2012;67:226-32.)

Key words: anhidrotic action halos; botulinum toxin type A; dose equivalence; field effect.

There is consensus among clinicians that the cosmetic use of botulinum toxin type A (BoNTA) is safe and effective when correctly administered. After local injection, BoNTA evokes a highly specific, local muscle paralysis and sweat gland activity cessation that results in a clinically delimited area, usually round or oval, demonstrating absence of voluntary muscular contraction and sweat gland activity.

The two most commonly used commercial preparations of BoNTA authorized for cosmetic use worldwide are Dysport (abotulinumtoxinA [ABO]),

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manufactured by Ipsen Biopharm Limited, Wrexham, United Kingdom, and Botox (onabotulinumtoxinA [ONA]), manufactured by Allergan, Irvine, CA. ABO is currently available for cosmetic use in 500 U vials in Europe and South America, whereas the 300 U vial is approved for use in the United States. ONA is available in 100 U vials. Although these are distinct products, with different formulations and potencies, questions remain regarding the dose equivalence between these two products. In addition, varying ratios have been reported in the literature with regard to the number of equivalent units of ABO to ONA.[1-5]

This prospective, double-blind, randomized study compared the field effects of these two products using two dosing ratios, 2.5:1.0 U and 2.0:1.0 U (ABO:ONA), for both muscular and sweat gland activity. Subjective and objective measurements at 28 and 112 days after standardized injections included: the area and horizontal diameter of the field of anhidrotic effect (FAE) as a primary outcome measurement; forehead Wrinkle Severity Scale (WSS) scores,[6] and evoked compound muscle action potentials (ECMAP)[7] in the frontalis muscle as secondary outcome measures.

CAPSULE SUMMARY

- This study evaluated the field effects of abotulinumtoxinA (ABO) and onabotulinumtoxinA (ONA) at dose equivalences of 2.5:1.0 U and 2.0:1.0 U, on both muscular and sweat gland activity.
- The dose equivalence of 2.0:1.0 U (ABO:ONA) showed similar field effects for muscle and sweat gland activity.
- Injections of ABO at dose equivalence of 2.5:1.0 U (ABO:ONA) showed greater area and larger horizontal diameter in field of anhidrotic effect than ONA at 28 and 112 days postinjection.
- These results support the hypothesis that the extension of the field effect is mainly dose dependent.

METHODS

Study design and patients

This prospective, single-center, randomized, double-blind study was approved by the ethics committee of Hospital de Clinicas of Porto Alegre. All participants were patients from a research center in Porto Alegre, Brazil. All of them provided written consent. The main inclusion criteria were: presence of moderate to severe forehead wrinkles on both sides at maximum contraction of frontalis muscle according to WSS score; were naïve to BoNTA injections; positive sweating by Minor test, and scores of II to V on the Sweat Intensity Visual Scale.[8]

Methods

At baseline, demographic data, current smoking habits, Glogau classification,[9] WSS scores, and initial Minor test[10] were assessed. A standard set of 3 photographs of the forehead muscles (relaxed, contracted, and relaxed under Minor test) was taken. Measurement of ECMAP was performed using surface electrodes on the forehead and electrical stimulation of the facial nerve according to standard neurophysiological procedures.

The products were reconstituted using 0.9% sterile saline solution without preservative, immediately before injection (5-20 minutes before administration), as follows:

- ABO 500 U per vial—reconstituted with 2 mL of the solution, resulting in a concentration of 250 U/mL of the reconstituted product;
- ABO 500 U per vial—reconstituted with 2.5 mL of 0.9% of the solution, resulting in a concentration of 200 U/mL of the reconstituted product;
- ONA 100 U per vial—reconstituted with 1 mL of 0.9% of the solution, resulting in a concentration of 100 U/mL of the reconstituted product.

Two injection points were marked using a template created from an x-ray sheet to ensure location of injection points were consistently marked from patient to patient. Standard isovolumetric injections, using ultrafine II 0.3-mL syringes, with a 29G needle, 0.5 cm in length, short needle (Becton Dickinson Labware, Franklin Lakes, NJ) were administered using cut caps of the needles with the template positioned on the patient's forehead. Caps of needles were cut and used to ensure that the products were injected at a standardized depth of 3 mm.

Patients were randomized to dose equivalence groups between ABO and ONA (2.0:1.0 U or 2.5:1.0 U) and to the side of the forehead in which the injection (left or right) was to be administered. The randomization list was generated by a statistician and blinding was maintained using sealed envelopes each containing the patient number, corresponding dilution, and the side of the forehead that each product was injected.

Isovolumetric (0.02 mL) doses of 5 U or 4 U of ABO were injected on one side of the forehead and 2 U of ONA were injected on the contralateral side of the forehead of the same subject, as follows:

- Group A (2.0:1.0 U ABO:ONA): patients received 4 U/0.02 mL of reconstituted ABO on one side of the forehead and 2 U/0.02 mL of reconstituted ONA on the contralateral side.

Abbreviations used:

ABO:	abotulinumtoxinA (Dysport)
BoNTA:	botulinum toxin type A
ECMAP:	evoked compound muscle action potentials
EMG:	electromyography
FAE:	field of anhidrotic effect
FME:	field of muscular effect
ONA:	onabotulinumtoxinA (Botox)
WSS:	Wrinkle Severity Scale

- Group B (2.5:1.0 U ABO:ONA): patients received 5 U/0.02 mL of reconstituted ABO on one side of the forehead and 2 U/0.02 mL of reconstituted ONA on the contralateral side.

Clinical and photographic assessments, Minor test, and electromyography (EMG) evaluations were performed at 28 and 112 days postinjection. Clinical assessments included WSS score at rest and at maximum frontalis muscle activity using a validated 4-point scale. Photographic assessments at rest and at maximum activity of the frontalis muscle and under Minor test were recorded. The amplitude of the ECMAP in the frontalis muscle on stimulation of the facial nerve was performed by an experienced neurologist (F. T. R.). The ECMAP was accessed by an EMG device (TECA Sapphire, TECA Corp, Pleasantville, NY). The horizontal diameter and area of FAE at days 28 and 112 were expressed in centimeters and quantified by software (Mirror, Canfield Scientific Inc, Fairfield, NJ). Calculation of the area was performed considering the perimeter of the field effect, assuming that they were not all perfect circles.

After the last visit, two blinded independent dermatologists evaluated patients' photographs at 28 and 112 days postinjection, using the standard 4-point scale (WSS).

Statistical methods

A Student *t* test was used for continuous variables and the χ^2 test was used for categories variables. Mann-Whitney U and Wilcoxon tests were used for nonparametric variables.

Assuming a SD of 0.276 for the diameter and area, an equivalence limit of Δ of 0.2 cm, and no difference between the two treatment groups, a sample size of 23 patients per treatment group was deemed sufficient to perform the test with type I error probability of α of 0.025 and power of 90%.

The interindividual variability data among diameters and areas of FAE for ABO and ONA were also evaluated; these values were expressed as average change from day 28 to day 112 in area/diameter. Other data were expressed by mean ± SD, median and range, or percent. A *P* value less than .05 was considered to be significant. SSPS 16.0 (SPSS Inc, Chicago, IL) was the statistical program used to analyze the data.

RESULTS

A total of 59 female patients aged between 18 and 60 years were included in this study. One patient withdrew consent before randomization. In all, 58 patients were randomized to groups A and B and 54 completed the study: 26 in group A and 28 in group B. Four patients did not complete the study.

At baseline, there were no significant differences between groups A and B regarding age (44 ± 9 vs 42 ± 12 years, $P = .46$), current smoking habits (26% vs 11%, $P = .84$), ethnicity (89% vs 86% Caucasian, $P = .74$), Glogau scale (81.5% vs 82% Glogau grade II, $P = .53$), and WSS (92.5% vs 78.5% for moderate scores).

Differences in WSS score at maximum frontalis muscle activity were not statistically significant at days 28 and 112 for either group, with the exception of WSS score at maximum frontalis muscle activity at day 112 in group B that favored ABO (more patients had greater improvement at day 112 according to the WSS score) (Table I).

There were no differences between the groups at day 28 and 112, according to the ECMAP results (Table II).

Table III describes the diameters and areas of FAE for group A, ONA and ABO, at days 28 and 112. No statistical significance was found between the FAE of the two tested doses and products (Figs 1 and 2). The FAE at days 28 and 112 for group B demonstrated statistical difference with regard to a larger horizontal diameter and area for ONA when compared with ABO (Figs 3 and 4, and Table IV). This statistically significant difference in group B was 0.16 ± 0.16 cm in diameter and 0.46 ± 0.4 cm^2 for the area, favoring ABO.

No statistical significance was found in the interindividual variability data among diameters and areas of FAE for ABO and ONA, which varied from 0 to 1 cm for both groups and products.

According to evaluations performed by two independent dermatologists, the WSS score at rest and maximum frontalis muscle activity were similar in group A. However, WSS score at maximum frontalis muscle activity on day 28 and at rest on day 112 favored ABO (more patients presented greater improvement according to the WSS score) ($P = .02$ and $P = .04$, respectively) in group B. There were no differences in WSS score at rest on day 28 and at maximum frontalis muscle activity on day 112 ($P = .56$ and $P = .36$, respectively).

Table I. Wrinkle Severity Scale scores according to investigator evaluations at baseline, 28 days, and 112 days, either at rest or maximum contraction stratified by group

	Investigators evaluation					
	Group A			Group B		
	ABO	ONA	*P*	ABO	ONA	*P*
WSS score at rest at baseline None/mild/moderate/severe (n)	3/22/2/—	2/22/3/—	.32	2/20/6/—	2/19/7/—	.32
WSS score at maximum frontalis muscle activity at baseline None/mild/moderate/severe (n)	—/—/18/9	—/—/18/9	1.00	—/—/17/11	—/—16/12	.32
WSS score at rest at 28 d None/mild/moderate/severe (n)	6/20/1/—	5/21/1/—	.56	9/19/—/—	9/17/2/—	.16
WSS score at maximum frontalis muscle activity at 28 d None/mild/moderate/severe (n)	—/12/13/2	—/12/13/2	1.0	2/16/9/1	2/12/11/3	.06
WSS score at rest at 112 d None/mild/moderate/severe (n)	8/17/1/—	9/16/1/—	.31	8/19/1/—	7/20/1/—	.56
WSS score at maximum frontalis muscle activity at 112 d None/mild/moderate/severe (n)	2/7/13/4	2/7/13/4	1.0	2/15/8/3	—/12/13/3	.008

WSS, Wrinkle Severity Scale.

Table II. Results of amplitude of evoked compound muscle action potentials for groups A and B at 28 and 112 days

EMG results, μV	ABO	ONA	*P*
ECMAP at 28 d (group A)	475.67 ± 414.00	546.78 ± 505.43	.70
ECMAP at 112 d (group A)	578.47 ± 365.20	666.00 ± 479.43	.16
ECMAP at 28 d (group B)	492.53 ± 290.70	468.27 ± 263.31	.61
ECMAP at 112 d (group B)	482.63 ± 279.58	539.16 ± 387.72	.36

ECMAP, Evoked compound muscle action potentials; *EMG*, electromyography.

Table III. Horizontal diameter and area of field of anhidrotic effect at 28 and 112 days at dose equivalence of 2.0:1.0 ABO:ONA (group A)

	ABO	ONA	*P*
Diameter at 28 d, cm	1.05 ± 0.36	1.06 ± 0.22	.83
Area at 28 d, cm^2	1.46 ± 0.65	1.39 ± 0.43	.50
Diameter at 112 d, cm	0.87 ± 0.33	0.78 ± 0.25	.18
Area at 112 d, cm^2	0.96 ± 0.61	0.79 ± 0.43	.08

There were no serious adverse events. Drug-related adverse events were mild and transitory, and included minimal bleeding at the injection sites immediately after administration in 17.2% (5 of 29) and 13.7% (4 of 29) of the patients in group A and B, respectively.

DISCUSSION

This study evaluated the field effect for two commercial preparations of BoNTA (ABO and ONA) at two dose equivalences, 2.0:1.0 U and 2.5:1.0 U, administered on contralateral sides of the forehead. The field of muscular effect (FME) of the tested products was evaluated using the validated 4-point WSS[6] and ECMAP[7] by EMG. The FAE of the tested products was evaluated by measurement of the anhidrotic areas surrounding the injection points under Minor test, according to their shape, horizontal diameter, and area (Figs 1 to 4). These were later analyzed using the Mirror system (Canfield Scientific Inc).

Evaluations of WSS score and ECMAP values showed no differences in the FME in the two study groups, except in group B at day 112 at maximum frontalis muscle activity, which favored ABO. According to the independent expert panel, a significant difference in WSS scores was demonstrated in group B at days 28 and 112, also favoring ABO.

Patients included in group A showed similar results regarding the diameter and FAE for both ABO and ONA. Group B showed a greater diameter and FAE for ABO when compared with ONA. Although the difference between days 28 and 112 in average diameter for both products was only 0.16 cm in group B, the SD between patients was 3 times greater (ranging from 0.26-0.31 cm), meaning that standard isovolumetric injections of the same dose

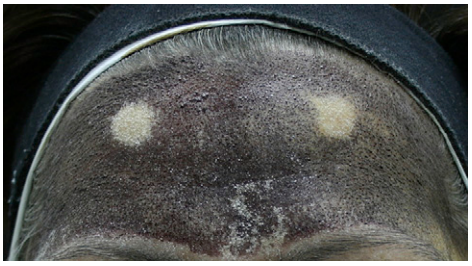

Fig 1. Area of anhidrotic effect 28 days after injections of 2 U of onabotulinumtoxinA on left side of forehead, and 4 U of abotulinumtoxinA on right side of forehead.

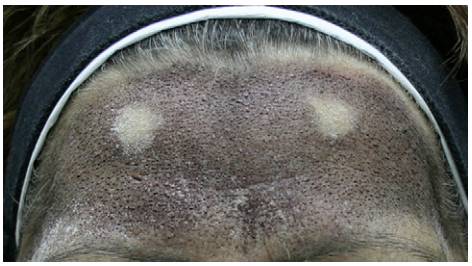

Fig 2. Area of anhidrotic effect 112 days after injections of 2 U of onabotulinumtoxinA on left side of forehead, and 4 U of abotulinumtoxinA on right side of forehead.

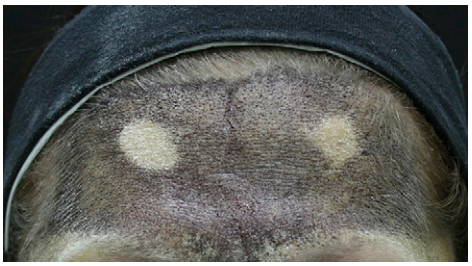

Fig 3. Area of anhidrotic effect 28 days after injections of 2 U of onabotulinumtoxinA on left side of forehead, and 5 U of abotulinumtoxinA on right side of forehead.

Fig 4. Area of anhidrotic effect 112 days after injections of 2 U of onabotulinumtoxinA on left side of forehead, and 5 U of abotulinumtoxinA on right side of forehead.

Table IV. Horizontal diameters and areas of field of anhidrotic effect at 28 and 112 days at dose equivalence of 2.5:1.0 ABO:ONA (group B)

	ABO	ONA	*P*
Diameter at 28 d, cm	1.28 ± 0.26	1.12 ± 0.31	<.001
Area at 28 d, cm^2	1.88 ± 0.56	1.45 ± 0.57	<.001
Diameter at 112 d, cm	1.12 ± 0.26	0.96 ± 0.29	.003
Area at 112 d, cm^2	1.30 ± 0.47	0.98 ± 0.45	<.001

administered to different individuals produced significantly different sizes of FAE. This suggests that variability between patients may play a more important role in impacting the size of the FAE than the products or doses tested in this study.

Hexsel et al[11] studied the FAE of ABO on compensatory hyperhidrosis, showing that small doses of 5 U of ABO significantly reduced sweating around injection points. ABO produces similar FAE, even when reconstituted in 3 times more volume. These findings suggested that the type of skin, location, and amount of previous sweating may play a more important role in the size of the FAE versus depth and concentration of the product.[11]

Cliff et al[12] found that the area of anhidrosis was greater for ABO when compared with ONA by a mean of 77%, when a dose equivalence of 3:1 was used. Other articles indicated higher "diffusion" of ABO[13,14] when using a higher dose equivalence of 3:1. Karsai et al[15,16] suggested that this is simply a dose effect as opposed to the claim that one product "diffuses" more than the other. The current study also supports the assertion that higher doses of BoNTA are relevant in producing a greater FAE and FME.

Other authors refer to the FAE and FME as "diffusion," "spread," and "migration" of BoNTA.[13,17-21] Some of these terms are misleading and incorrectly used, as described by Pickett.[22] "Spread" is the physical movement caused by the injection of the toxin and "diffusion" is the passive movement of the product away from the injection site, taking several days to fully conclude.[22] de Almeida and De Boulle[18] discussed preclinical and clinical data in an effort to differentiate BoNTA preparations with respect to their diffusion characteristics. Pickett et al[23] subsequently reported important points about the mechanism of action and effect of BoNTA, including the lack of role the neurotoxin-complex size has regarding the diffusion of BoNTA, and the comparison of inaccurate dose equivalences of different preparations leading to unsound conclusions regarding the diffusion characteristics of a product.

The effects of BoNTA in adjacent muscles have also been studied.[24-26] Carli et al[24] showed no significant difference among ABO, ONA, and incobotulinumtoxinA injections in the FME of BoNTA in adjacent muscles. The effects of BoNTA

in adjacent muscles depend on clinical, temporal, and EMG factors[26] and on physical aspects such as injection technique and muscle targeting. However, the clinical expression of BoNTA field effect is diverse and their mechanism remains unknown.[26]

The primary reason for the difference between the products' potency is the assay method used during manufacturing. The assay method used to test ABO is more sensitive than the assay used for ONA, resulting in different potencies per unit in both formulations.[12,15,27] Hambleton and Pickett[27] measured different samples of ABO and ONA using the two different assay methods used by the companies at that time. Their results showed that there was an approximate 3:1 ratio; that is, 1 U of ONA was equivalent to approximately 3 U of ABO. This was the origin of comparative ratios of the products, which still persists today.

Based on clinical evidence, other authors suggest that the equivalence between ABO and ONA is around 2.5:1.0 U or even 2.0:1:0 U.[7,28-30] In a previous pilot study, the current authors found similar results for ABO and ONA in muscles and sweat glands in a small sample of 18 patients using the dose equivalence of 2.5:1.0 U.[31] In that study, only the differences in diameters of FME and FAE over 3 mm were considered clinically relevant.[31] Recently, Karsai and Raulin[32] have carried out a systematic review in the literature and found that dose equivalences less than 3:1 (2.5:1.0 U or even 2.0:1.0 U) for ABO and ONA should be used.

Heckmann et al[33] studied the effects of different doses of ABO on axillary sweat glands. They found an efficacious dose of 100 U of ABO per axilla whereas the majority of studies suggested 50 U of ONA per axilla to treat the same condition. The current study, therefore, also supports a dose equivalence of 2.0:1:0 U between the two products.

CONCLUSION

This study evaluated the field effect of BoNTA on muscles and sweat glands in a number of patients appropriately powered to determine differences, if they existed.

The tested products, ABO and ONA, at the dosing ratios of 2.0:1.0 U showed similar field effects in both muscles and sweat glands. However, injections using the dose ratios of 2.5:1.0 U showed greater diameter and area of the FAE for ABO when compared with ONA. Both doses and dose equivalences tested in this study were effective and safe, and clinically very similar.

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REFERENCES

1. Durif F. Clinical bioequivalence of the current commercial preparations of botulinum toxin. Eur J Neurol 1996;2:17-8.
2. Matarasso SL. Comparison of botulinum toxin types A and B: a bilateral and double-blind randomized evaluation in the treatment of canthal rhytides. Dermatol Surg 2003;29: 7-13.
3. Lowe NJ. Botulinum toxin type A for facial rejuvenation. Dermatol Surg 1998;24:1216-8.
4. Moore P, Naumann M. General and clinical aspects of treatment with botulinum toxin. In: Moore P, Naumann M, editors. Handbook of botulinum toxin treatment. 2nd ed Oxford: Blackwell Science; 2003. pp. 28-75.
5. Sposito MMM. New indications for botulinum toxin type A in cosmetics: mouth and neck. Plast Reconstr Surg 2002;110: 601-11.
6. Hexsel D, Rodrigues TC, Zechmeister-Prado D, Gamboa ML. A phase IV controlled randomized double-blinded study on the anhydrotic action halos and muscle activity of two commercial preparations type A botulinum toxin administered to the upper third of the face. Poster presented at: 67th American Academy of Dermatology Meeting; March 6-11, 2009; San Francisco, CA.
7. Wohlfarth K, Schwandt I, Wegner F, Jürgens T, Gelbrich G, Wagner A, et al. Biological activity of two botulinum toxin types A complexes (Dysport and Botox) in volunteers: a double-blind, randomized, dose-ranging study. J Neurol 2008;255:1932-9.
8. Hexsel D, Rodrigues TC, Soirefmann M, Zechmeister-Prado D. Recommendations for performing and evaluating the results of minor's test according to a sweating intensity visual scale. Dermatol Surg 2010;36:120-2.
9. Glogau RG. Aesthetic and anatomic analysis of the aging skin. Semin Cutan Med Surg 1996;15:134-8.
10. Minor V. Ein Neues Verfahren zu der klinischen Untersuchung der Schweissabsonderung. Z Neurol 1927;101:302-8.
11. Hexsel DM, Soirefmann M, Rodrigues TC, do Prado DZ. Increasing the field effects of similar doses of *Clostridium* botulinum type A toxin-hemagglutinin complex in the treatment of compensatory hyperhidrosis. Arch Dermatol 2009; 145:837-40.
12. Cliff SH, Judodihardjo H, Eltringham E. Different formulations of botulinum toxin type A have different migration characteristics: a double-blind, randomized study. J Cosmet Dermatol 2008;7:50-4.
13. Trindade de Almeida AR, Marques E, Almeida J, Cunha T, Boraso R. Pilot study comparing the diffusion of two formulations of botulinum toxin type A in patients with forehead hyperhidrosis. Dermatol Surg 2007;33:S37-43.
14. Karsai S, Raulin C. Do different formulations of botulinum toxin type A really have different migration characteristics [letter]? J Cosmet Dermatol 2008;7:230.
15. Karsai S, Raulin C. Botox and Dysport: is there a dose conversion ratio in dermatology and aesthetic medicine? J Am Acad Dermatol 2010;62:346-7.
16. Karsai S, Adrian R, Hammes S, Thimm J, Raulin C. 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.
17. Carruthers A, Bogle M, Carruthers JD, Dover JS, Arndt KA, Hsu TS, et al. A randomized, evaluator-blinded, two-center study of

the safety and effect of volume on the diffusion and efficacy of botulinum toxin type A in the treatment of lateral orbital rhytides. Dermatol Surg 2007;33:567-71.
18. de Almeida AT, De Boulle KJ. Diffusion characteristics of botulinum neurotoxin products and their clinical significance in cosmetic applications. J Cosmet Laser Ther 2007;9(Suppl): 17-22.
19. Lim EC, Seet RC. Botulinum toxin: description of injection techniques and examination of controversies surrounding toxin diffusion. Acta Neurol Scand 2008;117:73-84.
20. de Sa Earp AP, Marmur ES. The five D's of botulinum toxin: doses, dilution, diffusion, duration and dogma. J Cosmet Laser Ther 2008;10:93-102.
21. Eleopra R, Tugnoli V, Caniatti L, De Grandis D. Botulinum toxin treatment in the facial muscles of humans: evidence of an action in untreated near muscles by peripheral local diffusion. Neurology 1996;46:1158-60.
22. Pickett A. Dysport: pharmacological properties and factors that influence toxin action. Toxicon 2009;54:683-9.
23. Pickett A, Dodd S, Rzany B. Confusion about diffusion and the art of misinterpreting data when comparing different botulinum toxins used in aesthetic applications. J Cosmet Laser Ther 2008;10:181-3.
24. Carli L, Montecucco C, Rossetto O. Assay of diffusion of different botulinum neurotoxin type A formulations injected in the mouse leg. Muscle Nerve 2009;40:374-80.
25. Yaraskavitch M, Leonard T, Herzog W. Botox produces functional weakness in non-injected muscles adjacent to the target muscle. J Biomech 2008;41:897-902.
26. Roche N, Schnitzler A, Genêt FF, Durand MC, Bensmail D. Undesirable distant effects following botulinum toxin type a injection. Clin Neuropharmacol 2008;31:272-80.
27. Hambleton P, Pickett AM. Potency equivalence of botulinum toxin preparations. J R Soc Med 1994;87:719.
28. Schnider P, Moraru E, Kittler H, Voller B, Kranz G, Auff E. Botulinum toxin in the treatment of focal hyperhidrosis. Wien Klin Wochenschr 2001;113:36-41.
29. Ascher B, Zakine B, Kestemont P, Baspeyras M, Bougara A, Santini J. A multicenter, randomized, double-blind, placebo-controlled study of efficacy and safety of 3 doses of botulinum toxin A in the treatment of glabellar lines. J Am Acad Dermatol 2004;51:223-33.
30. Wohlfarth K, Sycha T, Ranoux D, Naver H, Caird D. Dose equivalence of two commercial preparations of botulinum neurotoxin type A: time for a reassessment? Curr Med Res Opin 2009;25:1573-84.
31. Hexsel D, Dal'Forno T, Hexsel C, Zechmeister-Prado D, Lima MM. A randomized pilot study comparing the action halos of two commercial preparations of botulinum toxin type A. Dermatol Surg 2008;34:52-9.
32. Karsai S, Raulin C. Current evidence on the unit equivalence of different botulinum neurotoxin A formulations and recommendations for clinical practice in dermatology. Dermatol Surg 2009;35:1-8.
33. Heckmann M, Plewig G. Hyperhidrosis Study Group. Low-dose efficacy of botulinum toxin A for axillary hyperhidrosis: a randomized, side-by-side, open-label study. Arch Dermatol 2005;141:1255-9.