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Long-term efficacy and respective potencies of botulinum toxin A and B: a randomized, double-blind study

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Conflicts of interest
G.K., T.S., E.A. and B.V. have received speaker honoraria from Allergan and Eisai Pharma. G.K., T.S. and B.V. have received speaker honoraria from Ipsen Pharma and Merz Pharma. E.A. has received speaker honoraria from Boehringer-Ingelheim, Ipsen Pharma, Merz Pharma, GlaxoSmithKline, Lundbeck Pharma and UCB. He has received personal compensation for serving on scientific advisory boards from Boehringer-Ingelheim, Merck and UCB. M.P. consults for the pharmaceutical industry (Novartis). The other authors report no conflicts of interest.

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Summary

Background Mouse units (mU) are used for quantification of the biological activity of botulinum A and B toxin preparations. However, in human tissue, mU values between preparations are not equivalent and lack of clarity concerning efficacy and safety remains with regard to their respective potencies, duration of drug effect and diffusion qualities.

Objectives To compare short-term and long-term effects of Botox® (BOT; Allergan Inc., Irvine, CA, U.S.A.) and Neurobloc®/Myobloc® (NBC; Solstice Neurosciences Inc., Malvern, PA, U.S.A.) in different doses and dilutions in a human skin model.

Methods In this prospective randomized double-blind study, 18 healthy volunteers (eight women and 10 men; mean ± SD age 28·4 ± 5·7 years) were injected intradermally with pure saline, BOT and NBC at 10 points in the abdomen in random order, using the BOT/NBC conversion ratio 1 : 75 and different dilution schemes. For an objective outcome, the ninhydrin sweat test was used to compare the anhidrotic areas (action halos). Ten measurements were taken during a time period of 54 weeks.

Results Both preparations showed a peak effect at week 3, with significantly larger anhidrotic areas for NBC. Thereafter, however, the rate of decline was lower in BOT and after week 24, mean BOT areas were larger. The effect of dilution was higher in NBC and the mean dose equivalence conversion ratio (BOT/NBC) was 1 : 29 (area under the curve). Gender effects were seen in both products, with smaller action halos in women.

Conclusions These results have important implications in clinical routine, especially for autonomic indications.

Botulinum neurotoxin (BTX) has been well studied in dermatology, neurology and other disciplines. In addition, BTX treatments are the most common aesthetic procedures in Europe and the U.S.A. If applied parenterally, the human nervous system is affected by all seven BTX subtypes (A–G).[1] Due to their prior availability and immunological aspects, type A preparations are most commonly used for intramuscular injection.[2] BTX-B is often the only effective BTX available when patients develop antibodies to type A. In other indications like drooling or hyperhidrosis, BTX-B has been well documented to be effective and safe and is often used there primarily.[3] In Europe, among others, Botox® (BOT; Allergan Inc., Irvine, CA, U.S.A.) and Neurobloc® (identical to Myobloc®) (NBC; both Solstice Neurosciences Inc., Malvern, PA, U.S.A.) are widely used as BTX-A and BTX-B preparations, but controversy remains about their respective potencies, diffusion characteristics and long-term efficacy.[4] Rates of equivalency for BOT/NBC reported in the medical literature range between 1 : 20 and more than 1 : 125.[5–7]

As BTX-A and BTX-B do not have the same affinities to neuroglandular and neuromuscular endplates, objective and reproducible methods are needed for both targets to establish reliable conversion ratios between the two toxins. Physicians need precise conversion factors when switching from one product to the other as overdosing or underdosing may result in severe clinical consequences.[8] A precise conversion factor is also necessary to interpret dosages given in the literature and can have economic implications.

In several recent studies, the ninhydrin sweat test (NST) methodology was adapted to detect BTX-A antibodies and to compare dose ratios of different BTX-A preparations.[9,10] The aim of this study was to find an appropriate conversion factor between BOT and NBC and to study the effect of dilution and long-term efficacy in both products. Therefore, we employed the NST and studied the autonomic effects of BOT and NBC in a reproducible human skin model in a double-blind fashion.

Materials and methods

Test individuals

According to our sample size calculation, we recruited 18 healthy volunteers (eight women and 10 men; mean ± SD age 28·4 ± 5·7 years) not previously exposed to BTX for at least 1 year and with the following characteristics: no dermatological 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.

Interventions

Test individuals were injected in our botulinum toxin outpatient clinic intradermally (G.K.) with BOT and NBC at 10 points in the abdomen using 0·3-mL syringes (Becton Dickinson, Le Pont de Claix, France) with 8-mm 30 gauge needles. One injection was performed with pure saline (0·03 mL). All injections were applied midintradermally in an identical manner, raising a small elevation of the skin. Based on the literature[7,11] and on clinical routine, we chose a BOT/NBC conversion ratio of 1 : 75. With this ratio, three different doses of each product were administered. Additionally, BOT was administered with three different dilution schemes (low, medium and high dilution) and NBC was administered with two dilution schemes (medium and high; a lower dilution was not feasible, as the product comes in a prediluted state) (Table 1). Test individuals 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.

Assessments and clinical outcome measure

To measure potencies using an objective outcome measure, the NST was used to compare the anhidrotic effect of BOT and NBC (see earlier work for details).[9–13] In short, the toxin acts on cholinergic innervated sweat glands, producing an action halo (anhidrotic area surrounded by a hypohidrotic rim). The NST visualizes the amino acids in sweat. In the present study, the NST was carried out on abdomen sweat prints after induction of sweating by drinking 500 mL of hot tea, performing standardized physical activity (running steps), and using a heated blanket. Sweating was induced in all test individuals in the same manner. The anhidrotic and hypohidrotic areas were outlined by an investigator (B.V.) who was blinded to the treatment received. Size and area were then calculated by computer: the ninhydrin-stained sheets were scanned and the size of the action halos was determined automatically by home-written software in Interactive Data Language (Research Systems Inc./ITT Visual Information Solutions, Boulder, CO, U.S.A.), based on a two-dimensional region-growing algorithm, and were normalized to yield mm^{-2} values using a 10 × 10 mm reference region. The measurements were taken at 2 days and 1, 3, 7, 12, 17, 24, 31, 41 and 54 weeks after injection. One test individual was also assessed at 82 weeks after injection.

Table 1 Doses and dilutions of Botox® (BOT) and Neurobloc® (NBC) injections

Injection volume (mL)	BOT units (dilution)	NBC units (dilution)
0·12	8 (100 U/1·5 mL)	600 (10 000 U/2 mL)
0·06	4 (100 U/1·5 mL)	300 (10 000 U/2 mL)
0·03	2 (100 U/1·5 mL)	150 (10 000 U/2 mL)
0·03	4 (100 U/0·75 mL)	–
0·12	4 (100 U/3 mL)	300 (10 000 U/4 mL)

Injections were randomly allocated according to a computer-generated randomization list. For control condition one injection was performed with pure saline (0·03 mL), not shown in this table.

Before inclusion in the study, written informed consent was obtained from all healthy volunteers. The study was approved by the local Ethics Committee.

Statistics

The relative potency was estimated for anhidrosis and hypohidrosis based on the week 3 measurement and the area under the curve (AUC). For each of these four endpoints, a separate mixed-effects model with the fixed factors log dose, treatment (BOT/NBC) and the random factor test individual was applied. For each treatment the three dose groups with equal dilution ratio were used in the analysis. To obtain normally distributed residuals, the square roots of the anhidrosis and hypohidrosis measurements were used as dependent variables. Heteroscedasticity across treatment groups was accounted for by allowing the variances to differ between treatment groups. The relative potency was calculated as $r = \exp(-b0/b1)$ where b0 is the coefficient of the fixed factor treatment (implemented with a dummy variable) and b1 the coefficient of the fixed factor log dose. The corresponding 95% confidence intervals were computed using Fieller's theorem. Missing values were estimated by interpolation provided that a measurement before and after the missing value was available. Additionally, for the computation of the AUC for three patients the week 54 measurements were imputed with the week 41 measurements according to the last value carried forward principle. The AUC was computed using measurements up to week 54. To assess the impact of volume, NBC measurements at dose

300 mouse units (mU) and BOT measurements at dose 4 mU at different volumes were used. A mixed model with the fixed factors log(volume), treatment and the random factor test individual was applied for each treatment group and anhidrosis/hypohidrosis separately. All analyses were performed with the statistics package R 2.10.1 (R Foundation, Vienna, Austria). The two-sided significance level was set to 5%. To assess the impact of gender, a mixed-effects model with the fixed factors log dose, gender and the random factor test individual was applied for each treatment group and anhidrosis/hypo-hypohidrosis separately.

Results

Time course

The comparison of the time courses of anhidrosis and hypohidrosis of BOT and NBC is shown in Figure 1. In all considered doses NBC showed a sharp peak at week 3. Similarly for BOT, the maximum was reached at week 3 in all considered doses, but the peak was less sharp. After week 3, however, the rate of decline was much higher for NBC: we found a 65% effect reduction for NBC compared with a 29% reduction for BOT at week 17 and a > 99% reduction for NBC at week 54 compared with a 75% reduction for BOT, based on a mean of hypohidrotic and anhidrotic areas.

Relative potency

At week 3 the observed efficacy in the investigated doses of NBC was larger than for the investigated doses of BOT. Similar results were found in the AUC for anhidrosis. Therefore, the estimates of relative potency are derived by extrapolation and depend critically on model assumptions (linearity of the dose/response curves). Thus the estimates are not fully reliable. Only for the AUC of hypohidrosis could the relative potencies be calculated without extrapolation and showed a BOT/NBC ratio of 1 : 29 (95% confidence interval 1 : 21·7–1 : 37·0).

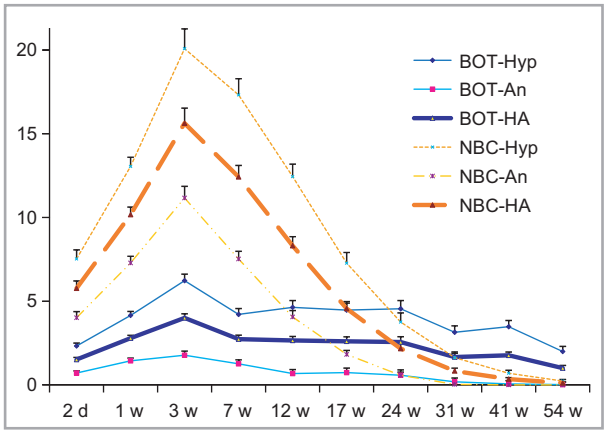

Fig 1. Time course of both products. BOT, Botox®; NBC, Neurobloc®/Myobloc®; Hyp, hypohidrosis; An, anhidrosis; HA, mean of hypohidrotic and anhidrotic area; *x*-axis, time (d, day; w, week); *y*-axis, action halo of hypohidrotic and anhidrotic areas (cm^2); error bars represent SEM for the estimated least-squares means.

Impact of dilution

At week 3 there was a significant positive effect of the volume on hypohidrosis for BOT and NBC (both $P < 0·01$). For anhidrosis only a marginal significant effect was observed for NBC ($P = 0·048$) but not for BOT ($P = 0·29$) (Fig. 2). For the AUC no significant effect of volume was detected for BOT (anhidrosis $P = 0·77$, hypohidrosis $P = 0·32$) but for NBC a significant positive effect on anhidrosis ($P = 0·028$) and hypohidrosis ($P < 0·01$) was observed.

Impact of gender

At week 3 for BOT there was significantly less hypohidrosis and anhidrosis in women compared with men ($P = 0·042$ and $P < 0·001$). Similarly for NBC, less anhidrosis was found in women ($P < 0·01$) and less hypohidrosis (borderline significance, $P = 0·058$). For BOT the AUC also showed significantly less anhidrosis in women ($P < 0·01$) but no significant difference in hypohidrosis ($P = 0·12$). For NBC the AUC for anhidrosis ($P < 0·001$) and hypohidrosis ($P < 0·01$) was significantly less in women (Fig. 3).

Discussion

A strong controversy remains about the respective potencies, long-term efficacy and diffusion characteristics of the two

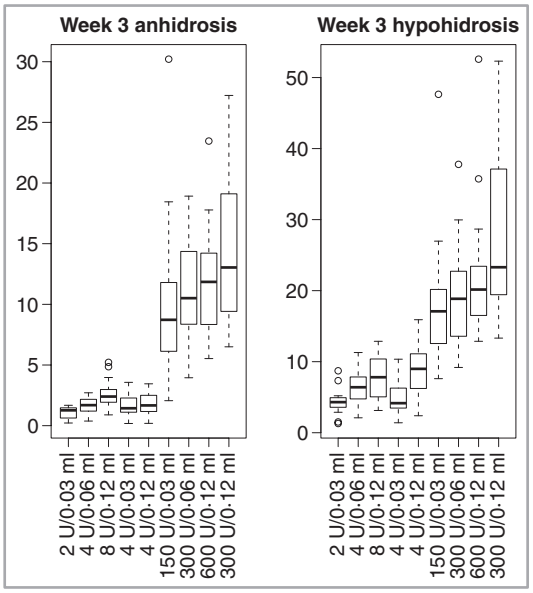

Fig 2. Box plots showing effects on anhidrosis (left) and hypohidrosis (right) at week 3 for all injected doses and dilutions. *x*-axis, injected doses and dilutions (Botox®: 2, 4 and 8 mU; Neurobloc®/Myobloc®: 150, 300 and 600 mU); *y*-axis, area (cm^2) of hypohidrosis and anhidrosis.

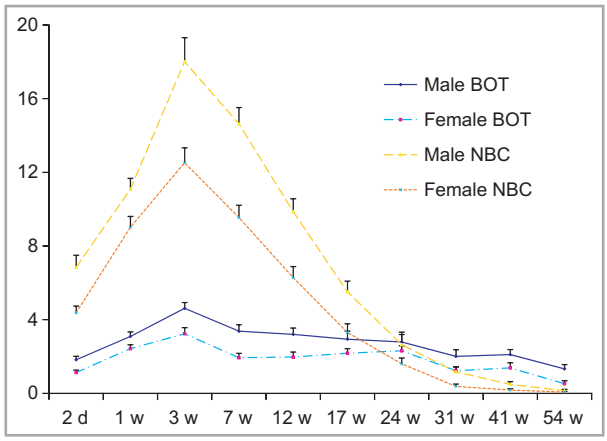

Fig 3. Effect of gender. BOT, Botox®; NBC, Neurobloc®/Myobloc®; *x*-axis, time (d, day; w, week); *y*-axis, action halo of hypohidrotic and anhidrotic areas (cm^2); error bars represent SEM for the estimated least-squares means.

types of clinically used botulinum neurotoxin preparations, BTX-A and BTX-B. 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.[9,10,13]

It has been suggested that BTX preparations in human tissue reach their maximal effect 2–3 weeks after injection, then maintaining this state for about 2·5 months before the effect gradually declines.[4] Time–effect responses of different BTX preparations have not been investigated systematically and only few data exist examining long-lasting effects.[14] We found particular time–effect curves for each product with a peak for all doses and dilutions 3 weeks after the injection. For all doses and volumes, peak effects were considerably higher for NBC compared with BOT and the effect of volume was higher in NBC; however, the effect reduction after the third week was stronger in NBC. Long-term effects for BTX preparations have been suggested to last 2–4 months in the muscle and 3–9 months in autonomic indications.[4,14–16] Unexpectedly, we found long-term effects exceeding 12 months in most of the test individuals, predominantly for BTX-A. One test individual was assessed > 1½ years (82 weeks) after injection and still showed hypohidrotic areas, although considerably larger areas for BOT (mean 3·8 cm^2) compared with NBC (mean 0·2 cm^2). However, as we studied healthy volunteers, shorter-duration effects should be expected in patients with hyperhidrosis.

It was demonstrated in a mouse model that sprouting at paralysed cholinergic endplates is a temporary recovery process. The sprouts retract ultimately, as the original synapses regenerate through a long-term remodelling process.[17] From our data we suggest that SNAP-25 transporter proteins, which are the target for BTX-A, require more time to regenerate than VAMP proteins, the target for BTX-B. Recently, apart from the presynaptic target, effects of BTX-A on the postsynaptic aquaporin 5 water channel proteins were suggested, implying functional inhibition of sweat glands by inhibiting water flux.[18] This effect might be a distinct effect for BTX-A, as SNAP-25, a SNARE protein, was found colocalized to vesicles containing aquaporin proteins in rat kidney.[19] This circumstance could account for the long-lasting effects of BTX-A; however, it does not explain the shorter-lasting peak effects of BTX-B.

Based on the experience from the literature[6,7] and clinical routine, we injected the toxins with a BOT/NBC ratio of 1 : 75 which resulted in significantly larger hypohidrotic and anhidrotic areas for NBC 3 weeks after injection and a higher AUC for anhidrosis. Therefore, respective potencies could be calculated only for the AUC in hypohidrosis. Using this method, we found a BOT/NBC ratio of 1 : 29, which is a little higher than the estimated conversion ratio (BOT/NBC of 1 : 20) in an earlier study in axillary hyperhidrosis.[5] However, as each product has its particular effect characteristics over time, the respective outcome parameter (peak dose or AUC) affects the conversion ratio. Additionally, the chosen dilution would also influence the ratio, as higher dilutions seem to have a stronger impact on NBC compared with BOT.

The biological activity of all therapeutic BTX preparations is assayed on the same standardized mouse model, 1 mU being defined as the LD_{50} in this model. However, in humans, the activity labelling of the different therapeutic preparations cannot be compared directly. Suggested conversion ratios between BOT and NBC generally range between 1 : 20 and 1 : 125,[5–7] and for autonomic indications 1 : 20–1 : 40.[5,16] Meanwhile, comparing dose ratios between BTX-A and BTX-B, the method used to measure biological activity is particularly important. Soon after BTX-B was introduced in clinical routine, higher autonomic adverse events, including dryness of mouth and eyes with corneal, nasal and genital mucosa irritation and accommodation difficulties, were reported for BTX-B, indicating a higher neuroglandular affinity.[20] Therefore, separate conversion ratios for muscular and autonomic applications should be established. The conversion ratio we found in our skin model is considerably lower than most suggested ratios in the literature. Certainly, reliable conclusions on the conversion ratio can be made only for cutaneous injections, and conversion ratios in the muscle might be higher.

BTX-A (BOT) and BTX-B (NBC) are pharmaceutical preparations that belong to the same family of *Clostridium botulinum* exotoxins but have different biochemical characteristics and biological effects: they come from different *Clostridium* strains (BOT: Hall A; NBC: Bean B), target different SNARE proteins (BOT: SNAP-25; NBC: VAMP), are differently stabilized (BOT: vacuum drying; NBC: pH reduction) and contain different amounts of protein (BOT: 5 ng; NBC: 100 ng) with a different specific biological activity (BOT: 60 mU ng^{-1} BTX; NBC: 5 mU ng^{-1} BTX, where mU ng^{-1} BTX equates to the amount of biologically active toxin).[4,21] Whereas the low pH value in NBC is most likely to be responsible for the significantly higher pain during injection compared with BTX-A,[22] the lower specific biological activity in NBC accounts for its high antigenicity.[2]

Apart from the controversy about the respective potencies, there is also considerable discussion on the diffusion characteristics of different BTX products.[1,21] In our study, both products showed larger hypohidrotic areas with higher dilutions, demonstrating the importance of volume for diffusion. However, in NBC anhidrotic areas were also increased with higher volumes, which might indicate a higher diffusion rate for BTX-B. Although, as the two toxins were not injected with a correlative conversion factor, the higher dilution effect of NBC could be influenced by the conversion rate. This has repercussions in clinical practice when planning the number of injections in the target area. On the other hand, high diffusion may affect bordering tissues and thereby cause side-effects.

Similarly, for both products we consistently found gender differences, with smaller effects in women compared with men. Whereas it is often stated that BTX injections should be performed in an individualized dose and pattern, the gender difference was never described and investigated systematically in earlier studies. This has, however, important clinical implications for the treatment of hyperhidrosis and potentially for indirect BTX antibody detection tests.[9] Gender differences in perspiration could account for this effect, as men produce more sweat through fewer sweat glands per unit area.[23] Theoretically, more sweat would make anhidrotic areas smaller; however, the higher density of sweat glands in women with more neuroglandular endplates most likely results in faster binding and consumption of the available toxin and thereby leads to less diffusion.

We chose the NST skin model because the abdominal skin provides homogeneous research conditions and the NST is an objective and highly reproducible tool to test BTX activities in humans. Based on this double-blind, randomized controlled trial with highly reproducible objective results, we suggest that a conversion ratio of about 1 : 29 can safely be used for autonomic indications. Both products have their specific time–effect curves with high peak dose effects in NBC and long-term effects in BOT. When treating hyperhidrosis, differences in gender should be considered.

What's already known about this topic?

- Botulinum toxin (BTX) A and B are safe and effective in the treatment of hyperhidrosis. In human tissue, mU values between preparations are not equivalent.

What does this study add?

- The results indicate that both products have particular time courses with strong short-term effects for BTX-B and long-lasting effects (> 1 year) for BTX-A, a higher effect of dilution for BTX-B and respective potencies (Botox®/Neurobloc®) of 1 : 29 (area under the curve). Both toxins have stronger anhidrotic effects in men compared with women.

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