

INVESTIGATIVE REPORT

Anhidrotic Effect of Intradermal Injections of Botulinum Toxin: A Comparison of Different Products and Concentrations

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Botulinum toxin is used in various fields of medicine, including in the treatment of hyperhidrosis. Three products containing botulinum toxin are commercially available in Sweden; Botox[®], Dysport[®] and Neurobloc[®]. In the literature dose-response has varied with respect to these 3 products. We hypothesized that the dilution level of botulinum toxin is of importance for the effect and we therefore investigated anhidrosis after intradermal injections of each product in 3 different concentrations. Nine healthy subjects received 0.1 ml injections in the back. The anhidrotic areas were identified by an iodine-starch test after 3 weeks. When the 3 products were diluted to 100 U/ml level the achieved mean anhidrotic areas were approximately the same. This is in strong contrast with the large dose conversion factors suggested for intramuscular injections of the products. Furthermore, the lowest used concentrations for Botox[®] (25 U/ml) and Neurobloc[®] (100 U/ml) led to the largest anhidrotic mean area per unit, respectively. The optimal concentration in this study was 25 U/ml for Botox[®], 100 U/ml for Dysport[®] and 100 U/ml for Neurobloc[®], but for Botox[®] and Neurobloc[®] the optimal concentrations may be even lower. **Key words;** botulinum toxin; hyperhidrosis; dilution; Botox; Dysport; Neurobloc.

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Botulinum toxin (BTX) is a potent neurotoxin with many therapeutic applications, e.g. with respect to both neuromuscular disorders and primary focal hyperhidrosis.

BTX cleaves, and thereby demolishes, enzymes necessary for the release of acetylcholine in peripheral cholinergic nerve endings. There are 7 serotypes of BTX (A–G), which are structurally similar but diverge in their acceptor binding, enzymatic activity, antigenicity and species sensitivities. Two type A BTXs are produced commercially, Botox[®] (Allergan Inc., Irvine, California, USA) and Dysport[®] (Ipsen, Slough, UK), and 1 type B BTX, Myobloc[™] in the USA and Neurobloc[®] in Europe (Elan Pharma International, Shannon, Ireland). These

products are differently formulated and have different characteristics (see Table I) (1–4).

The biological activity of all these products is measured in units, where 1 unit is the lethal dose for 50% of mice (LD50) of a given weight after injection in the peritoneal cavity (5). According to the literature 1 unit of each commercial product is, however, not equal when it comes to the treatment of humans (1, 5–11).

Botox[®] has been found to be 3–6 times more potent than Dysport[®] in various studies of muscular disorders (6–9) and 50–100 times more potent than Neurobloc[®] (1, 10–11). However, Wohlfart et al. (5) did not observe any difference in potency between Botox[®] and Dysport[®] when the products were diluted to the same concentration. In this study, however, albumin was added to obtain the same albumin content.

Our observations showed that Dysport[®] (500 U/ml) diluted with physiological, unpreserved saline to the same concentration as Botox[®] (100 U/ml) had the same effect in a dose only 1.5 times higher than Botox[®] (Naver, unpublished data).

The recommended initial doses from the respective manufacturers when treating axillary hyperhidrosis is 50 U per axilla for Botox[®] and 100 U per axilla for Dysport[®], which results in the conversion factor 1:2 (Botox[®]: Dysport[®]). In a study on patients with axillary hyperhidrosis, Heckmann & Plewig (12) showed that 100 units of Dysport[®] was as effective as 200 units of Dysport[®].

The differences in potency between the BTX products therefore seem to be smaller when affecting sudomotor cholinergic nerves. Furthermore, autonomic side-effects, such as dryness of the mouth, have been observed more frequently when using Neurobloc[®], which may point to the potential superior value of Neurobloc[®] for treatment of autonomic dysfunctions such as hyperhidrosis (13).

Successful treatment of axillary hyperhidrosis has been made with 2000 U of Neurobloc[®] compared with 100 U of Botox[®] (14) and in another study a very low dose (250 U) of Neurobloc[®] was used with good results (15).

We hypothesize that the concentration of the injected solution is of importance to achieve the optimal effect. The aim of this study was therefore systematically to study the anhidrotic effect after intradermal injections

Table I. Vial contents and characteristics for Botox®, Dysport® and Neurobloc®

	Botox®	Dysport®	Neurobloc®/Myobloc™
Serotype	A	A	B
Form	Vacuum dried powder	Lyophilized powder	Solution
pH	7.3	6.0–7.0	5.6
Complex size	900 kDa	900 kDa	700 kDa
Units/vial	100 U	500 U	2500 U (0.5 ml) 5000 U (1.0 ml) 10000 U (2.0 ml)
Neurotoxin protein/vial	5 ng/100 U	4.35 ng/500 U	25 ng/2500 U 50 ng/5000 U 100 ng/10000 U
Remaining contents	500 µg albumin 0.9 mg sodium chloride	125 µg albumin 2.5 mg lactose	0.05% (500 µg/ml) albumin 0.1 M sodium chloride 0.01 M disodium succinate hydrochloric acid ^a water for injections ^a
Enzyme to cleave	SNAP-25	SNAP-25	VAMP

^aNo information about the amount of additive is found in the summary of product characteristics.

SNAP: synaptosomal-associated protein; VAMP: vesicle-associated membrane protein

of Botox®, Dysport® and Neurobloc® diluted to different concentrations.

MATERIALS AND METHODS

Nine fully informed and consenting doctors at the department of neurology, participated in this pilot study. The local ethics committee previously approved a similar study, which has been performed at the university hospital in Uppsala. All subjects were male, age range 36–65 years (mean age 50 years). They received injections of Botox®, Dysport® and Neurobloc® diluted with physiological, unpreserved saline. Botox® and Dysport® were diluted to the concentrations 100 U/ml, 50 U/ml and 25 U/ml, respectively, and Neurobloc® to 500 U/ml, 250 U/ml and 100 U/ml. Physiological saline was injected as a control. A total of ten 0.1 ml injections were given to every participant, except for one person who received less than 0.1 ml of Dysport® 100 U/ml. That anhidrotic area is therefore not included in the results.

The injections were given in the back in 3 vertical rows. To check that the solution was injected intradermally it was observed that a bulge formed as a consequence. The first row was placed 19 cm (10 cm for 2 persons) in the left lateral direction from the spinal column and the second row 9 cm (5 cm for 2 persons) in the left lateral direction. The third row was placed 9 cm (5 cm for 2 persons) in the right lateral direction from the spinal column. In the first row Botox® (100 U/ml, 50 U/ml and 25 U/ml) were injected in the backs of 5 participants, the other 4 participants were given injections of Dysport® in the same 3 concentrations. In the second row the 2 products were switched, so the 5 participants who were given Botox® in the first row were given Dysport® in the second row and vice versa. This was in order to exclude differences in effect depending on the position in the back. All 9 participants received injections of Neurobloc® (500 U/ml, 250 U/ml and 100 U/ml) in the third row. The top injection in each row was given at the same level as the seventh cervical vertebra (vertebra prominens) and the injections beneath were given at 5–7 cm intervals.

Iodine-starch test

To identify the area of anhidrotic skin an iodine-starch test (Minor's test) (16) was performed 21 days after the injections (28 days for one person). 5% iodine alcohol solution (iodium

5 g, potassium iodide 3.5 g, spiritus fortis 83 g, aqua sterilisata ad 100 g) was applied on the back of every participant and then each participant stayed in a sauna until a small amount of sweat could be seen on the skin. When the sweat was visible a white sheet of paper (45 g/m²) was pressed against the back. The paper stained black when pressed on the hidrotic back. Anhidrotic areas exhibited themselves via white circles on the paper (Fig. 1). For one participant the imprint from where Botox® 100 U/ml had been injected was indistinct due to a small crease on the paper; that anhidrotic area is therefore not included in the results.

Area measuring

The papers documenting the anhidrotic areas were placed under a microscope (Olympus SZX12), with a magnification of 3.5. The microscope was connected with a camera (Olympus DP10) and a computer (Dell, precision 340). Each anhidrotic area was photographed and thereafter measured in the program Olympus DP-soft (version 3.2).

Anhidrotic mean area and respective anhidrotic mean area per unit

The anhidrotic mean area was calculated for each concentration studied, using all the participants' individual results.

Since all injections in the study had a volume of 0.1 ml, the dose differed between the concentrations used. The anhidrotic mean area for 1 unit of each concentration was therefore calculated in order to compare the effect of the different injections.

Statistical analyses

The statistical significance of differences was calculated using a non-parametric test (Mann-Whitney *U* test).

RESULTS

The results of the study are based on the anhidrotic areas documented 21 days (28 days for one person) after the injections.

The largest anhidrotic mean area appeared where Neurobloc® 500 U/ml had been injected (7.2 cm²)

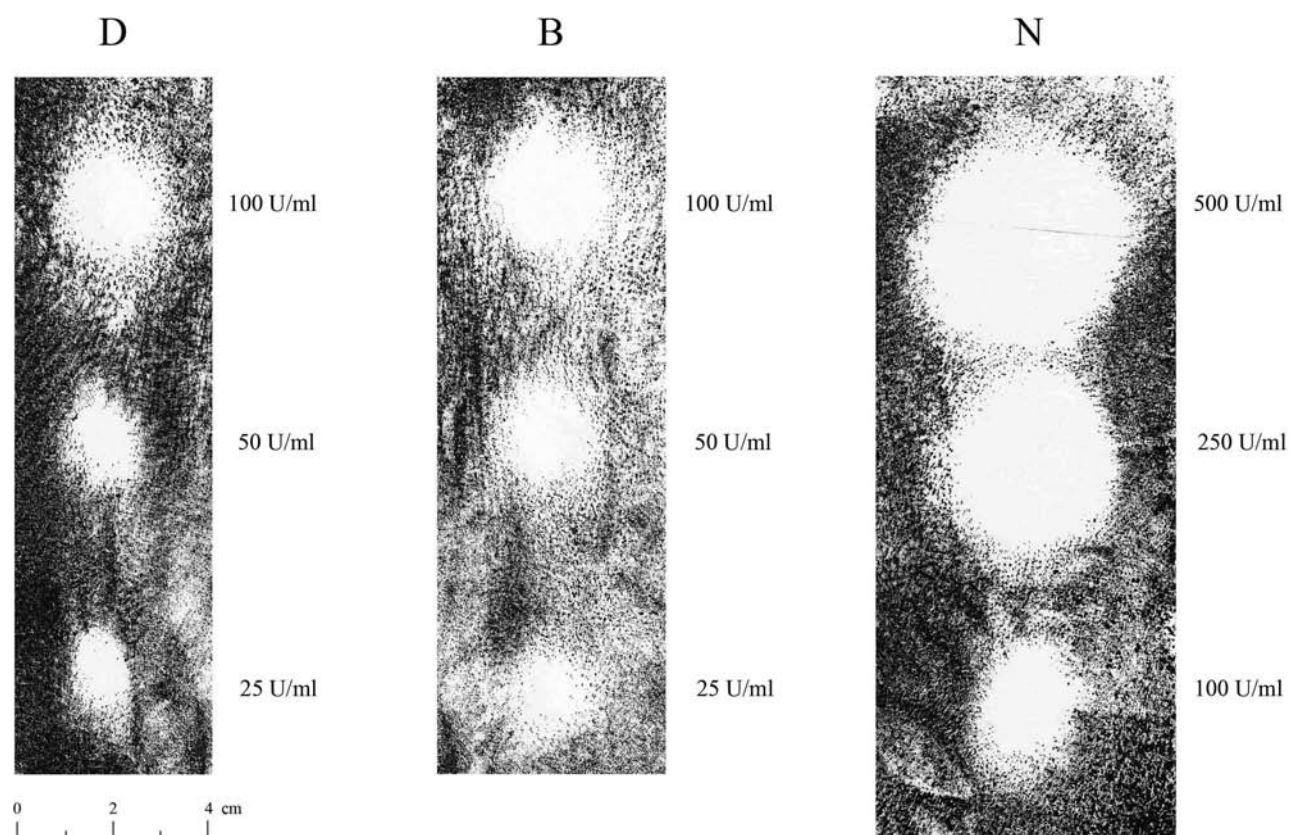


Fig. 1. Imprints (iodine-starch) of the anhidrotic areas after injection of Dysport® (D), Botox® (B) and Neurobloc® (N) from one of the participants. The respective concentrations used at each injection are shown next to the imprints.

followed by Neurobloc® 250 U/ml (6.4 cm²). These 2 mean areas were significantly larger than the mean areas appearing after injections of the 3 concentrations of Botox®, the 3 concentrations of Dysport® and Neurobloc® 100 U/ml ($p < 0.01$). However, there was no significant difference between Neurobloc® 500 U/ml and Neurobloc® 250 U/ml. Anhidrotic mean areas with standard deviations for each concentration are shown in Fig. 2(A). Two of the participants manifested, compared with the other 7, very small anhidrotic areas where Neurobloc® had been injected, which explains the spread around the mean values.

The anhidrotic mean area for 1 unit of each concentration was calculated to enable a comparison of the effect of the different injections (see Materials and Methods). These mean areas with standard deviations are shown in Fig. 2(B).

The largest anhidrotic mean area per unit appeared after injection with Botox® 25 U/ml (0.69 cm²) followed by Botox® 50 U/ml (0.52 cm²) and thereafter Botox® 100 U/ml (0.35 cm²). A statistically significant difference manifested itself between Botox® 25 U/ml and 100 U/ml ($p < 0.01$) and between Botox® 50 U/ml and 100 U/ml ($p = 0.02$). There was no difference in anhidrotic mean area per unit between the 3 concentrations of Dysport® (0.30 cm²). When comparing the

different concentrations of Neurobloc® it was seen that injections of Neurobloc® 100 U/ml resulted in the largest anhidrotic mean area per unit (0.31 cm²) followed by Neurobloc® 250 U/ml (0.25 cm²) and thereafter Neurobloc® 500 U/ml (0.14 cm²). A statistically significant difference was noted between Neurobloc® 100 U/ml and 500 U/ml ($p = 0.04$) and between Neurobloc® 250 U/ml and 500 U/ml ($p < 0.01$).

To investigate the difference in effect on sudomotor cholinergic nerves between the 3 products the mean values of the anhidrotic skin areas after injections with 10 U (100 U/ml) of each product were compared. The calculated conversion factors were 1:1.2 (Botox®:Dysport®) and 1:1.1 (Botox®:Neurobloc®).

To exclude differences in effect depending on the position of the injections in the back, Botox® was injected in the lateral side of the back of 5 persons and in the medial side of the back of 4 persons (vice versa for Dysport®). No such differences were observed.

DISCUSSION

This study, which focuses on sudomotor cholinergic nerves, clearly shows that lower concentrations of Botox® and Neurobloc® (in the chosen concentration interval) lead to a relatively enhanced anhidrotic effect.

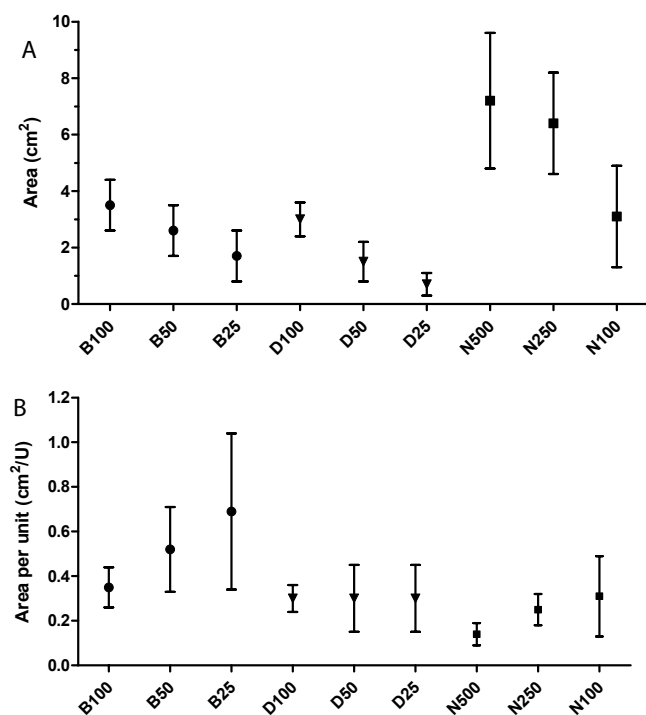


Fig. 2. (A) Size of the anhidrotic area of skin after injections of various doses of Botox®, Dysport® and Neurobloc®. Mean values and standard deviations are shown for each concentration ($n=9$, $n=8$ for B100 and D100). B=Botox®, D=Dysport® and N=Neurobloc®. B100 represents Botox® 100 U/ml, B50 represents Botox® 50 U/ml and so on. (B) The same results expressed as anhidrotic area per unit of Botox®, Dysport® and Neurobloc®.

The lowest concentrations studied in relation to Botox® and Neurobloc® were 25 U/ml and 100 U/ml, respectively, and these dilutions gave the best relative anhidrotic effect for each product. This was measured as the anhidrotic area per unit (see Materials and Methods). The optimal concentrations for Botox® and Neurobloc® may, however, be even lower, and this should be studied further.

There was no difference between the anhidrotic mean areas per unit comparing the 3 different concentrations of Dysport®. Dilution at the lower concentrations used in this study would probably have no advantage. On the other hand, our earlier studies showed that dilution of Dysport® from a concentration of 500 U/ml to 100 U/ml is favourable (Naver, unpublished data). Dysport® has probably reached the maximum of effective dilution at a concentration of 100 U/ml.

Most studies on the 3 products, performed on muscles that are innervated by α -motorneurons, have shown considerably large conversion factors, 1:3–6 (Botox®: Dysport®) (6–9) and 1:50–100 (Botox®:Neurobloc®) (1, 10–11). However, in these studies, the concentrations of the products have been various. In our study, when the differences in effect between the 3 products at a concentration of 100 U/ml were calculated, the conversion factors became 1:1.2:1.1 (Botox®:Dysport®: Neurobloc®). In addition, Wohlfarth et al. (5) discovered

that when Botox® and Dysport® were diluted to the same concentration there was no difference in effect between them, but they also had the same albumin content.

Albumin is an important component of the products, because it decreases the aggregation of the BTX molecules and the adsorption of BTX in the vial and syringe. The addition of saline to the BTX product leads to a lower albumin concentration, which can limit the positive effect of dilution. The amount of albumin is much lower in Dysport® (29 μ g albumin/ng neurotoxin) compared with Botox® (100 μ g albumin/ng neurotoxin). In this study, no further benefits were seen when diluting Dysport® to concentrations lower than 100 U/ml, which can perhaps be explained by the low albumin content. However, if albumin is added to the Dysport® solution it may be possible to achieve an enhanced effect even at concentrations lower than 100 U/ml. Neurobloc® contains 10 μ g albumin/ng neurotoxin, but cannot be compared in this respect with the BTX type A products, since it is formulated as a solution and contains other additives that prevent aggregation (2).

Only a few studies have been made on sudomotor cholinergic nerve function after injection of BTX. A comparison of Botox® with Dysport® and Neurobloc®, using linear regression, showed similar reductions in sweating using iodine-starch and quantitative sudomotor axon reflex test (QSART) methods (17). However, while we compared the products in equal concentrations, the calculated dose conversion factors in the study by Schlereth et al. (17) were based on doses not diluted to the same concentration. Even though the concentrations are important to achieve optimal effect, the study showed important dose-response results that are in congruence with ours. It also showed a serotype effect in which Neurobloc® was more autonomic nerve specific. Other studies, including ours, confirm this observation (13–14).

The participants themselves functioned as a control when the differences were studied, resulting in a correct relationship between the products. However, there were differences between individuals.

Since Botox® and Dysport® are serotype A toxins, while Neurobloc® is a serotype B toxin, it is not possible fully to compare Neurobloc® with the 2 other products. BTX A and BTX B have dissimilar acceptor bindings on the neurons and affect different enzymes (synaptosomal-associated protein-25 and vesicle-associated membrane protein, respectively).

The serotype A and serotype B BTX-complexes also have different sizes, which can affect diffusion through the tissue. However, the slightly alkaline pH in tissue promotes the complex to dissociate, releasing the native toxin, which is 150 kDa for both serotypes (18).

For this technique to be applicable in the treatment of hyperhidrosis, both the effect and the duration of effect require further study, as do the optimal concentrations

and injection volumes. The value of this study includes possible lower treatment costs, fewer side-effects and a reduction in the risks of immunization.

REFERENCES

1. 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.
2. Summary of product characteristics [cited 2007 May 28]. Available from: <http://www.emea.europa.eu/humandocs/Humans/EPAR/neurobloc/neurobloc.htm>
3. Carruthers A, Carruthers J. Toxins 99, new information about the botulinum neurotoxins. *Dermatol Surg* 2000; 26: 174–176.
4. Pickett A, O’Keeffe R, Panjwani N. The protein load of therapeutic botulinum toxins. *Eur J Neurol* 2007; 14: e11.
5. Wohlfarth K, Göschel H, Frevert J, Dengler R, Bigalke H. Botulinum A toxins: units versus units. *Naunyn Schmiedeberg Arch Pharmacol* 1997; 355: 335–340.
6. Ranoux D, Gury C, Fondarai J, Mas JL, Zuber M. Respective potencies of Botox and Dysport: a double blind, randomised, crossover study in cervical dystonia. *J Neurol Neurosurg Psychiatry* 2002; 72: 459–462.
7. Odergren T, Hjaltason H, Kaakkola S, Solders G, Hanko J, Fehling C, et al. A double blind, randomised, parallel group study to investigate the dose equivalence of Dysport® and Botox® in the treatment of cervical dystonia. *J Neurol Neurosurg Psychiatry* 1998; 64: 6–12.
8. Sampaio C, Ferreira JJ, Simoes F, Rosas MJ, Magalhaes M, Correia AP, et al. DYSBOT: a single-blind, randomized parallel study to determine whether any differences can be detected in the efficacy and tolerability of two formulations of botulinum toxin type A – Dysport and Botox – assuming a ratio of 4:1. *Mov Disord* 1997; 12: 1013–1018.
9. Durif F. Clinical bioequivalence of the current commercial preparations of botulinum toxin. *Eur J Neurol* 1995; 2: 17–18.
10. Brashear A, Lew MF, Dykstra DD, Comella CL, Factor SA, Rodnitzky RL, et al. Safety and efficacy of Neurobloc (botulinum toxin type B) in type A-responsive cervical dystonia. *Neurology* 1999; 53: 1439–1446.
11. Brin MF, Lew MF, Adler CH, Comella CL, Factor SA, Jankovic J, et al. Safety and efficacy of Neurobloc (botulinum toxin type B) in type A-resistant cervical dystonia. *Neurology* 1999; 53: 1431–1438.
12. Heckmann M, Plewig G. Low-dose efficacy of botulinum toxin A for axillary hyperhidrosis: a randomized, side-by-side, open-label study. *Arch Dermatol* 2005; 141: 1255–1259.
13. Dressler D, Benecke R. Autonomic side effects of botulinum toxin type B treatment of cervical dystonia and hyperhidrosis. *Eur Neurol* 2003; 49: 34–38.
14. Dressler D, Saberi FA, Benecke R. Botulinum toxin type B for treatment of axillar hyperhidrosis. *J Neurol* 2002; 249: 1729–1732.
15. Hecht MJ, Birklein F, Winterholler M. Successful treatment of axillary hyperhidrosis with very low doses of botulinum toxin B: a pilot study. *Arch Dermatol Res* 2004; 295: 318–319.
16. Minor V. Ein neues verfahren zu der klinischen untersuchung der schweissabsonderung. *Deutsche Z Fur Nervenheilkunde* 1927; 101: 302–306.
17. Schlereth T, Mouka I, Eisenbarth G, Winterholler M, Birklein F. Botulinum toxin A (Botox®) and sweating-dose efficacy and comparison to other BoNT preparations. *Auton Neurosci* 2005; 117: 120–126.
18. Friday D, Bigalke H, Frevert J. In vitro stability of botulinum toxin complex preparations at physiological pH and temperature. International conference on basic and therapeutic aspects of botulinum and tetanus toxins, Hannover, Germany, June 08–12, 2002. *Naunyn Schmiedeberg Arch Pharmacol* 2002; 365 Suppl 2 R20.