DERMATOLOGIC SURGERY

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SPECIAL ISSUE

Introducing A Novel Botulinum Toxin Preparation

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Introduction

There are at least six different botulinum toxin presentations available somewhere in the world, BOTOX, Dysport, Myobloc, Chinese BTXA, Xeomin and Neuronox although they may be known by different names in different areas. Myobloc which is the B serotype, is quite different from the others which are all the A serotype. BOTOX was the first to be approved (by the US FDA in 1989), Dysport was second (in the EU) in 1991. Xeomin was approved in Germany in 2005 and Neuronox was also approved in Korea in 2006. Neuronox (also marketed as Meditoxin in Korea as well as Siax, Botulift and Cunox in various countries) was developed by Korean scientists beginning during the last century and this led to the foundation of the company of development and formulation - Medytox in 2000.

The aim of the project was to produce a product very similar to Allergan's BOTOX. Neuronox is a complexed BTX-A produced by the same Hall strain of bacteria as BOTOX. It is purified to produce a very homogeneous 900 kD toxin complex and is packaged as a 100 U vial which also contains 0.9 mg of saline and 0.5 mg of human serum albumin.(A 200 U vial was produced in 2009 and a 50 U vial in 2010.) It could therefore be expected to behave in a similar manner to the Allergan product and this is confirmed by Korean studies[1–3] and others included in this special issue. So far Medytox has received approval from the Korean FDA for the use of Neuronox to treat benign essential blepharospasm, frown lines, cerebral palsy-induced spasticity and upper limb post-stroke spasticity. They have also received approval in a number of countries including Thailand, India and Brazil.

Medytox has not so far faced the more difficult challenges of approval in Europe and North America. This special issue is a fascinating look at an interesting new product. We await evidence which will be produced in an attempt to widen the geographic extent of Neuronox clinical approval.

References

1. Yoon JS, Kim JC, Lee SY. Double-blind, randomized, comparative study of meditoxin versus botox in the treatment of essential blepharospasm. Korean J Ohthalmol 2009;23(3):137–41.
2. Kim K, Shin H-I, Kwon BS, Kim SJ, et al. Neuronox versus BOTOX for spastic equinus gait in children with cerebral palsy: a randomized, double-blinded, controlled multicentre clinical trial. Dev Med Child Neurol 2011;53:239–44.
3. Chung ME, Song DH, Park HJ. A comparative study of biological activity between four botulinum toxin type A preparations in mice. Dermatol Surg 2013;39:155–164.

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ORIGINAL ARTICLE

A Pharmacodynamic Comparison Study of Different Botulinum Toxin Type A Preparations

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BACKGROUND Because more botulinum toxin (BoNT) preparations have become available worldwide, there is a clinical need to compare the pharmacologic profiles of these products.

OBJECTIVE We compared three different preparations: onabotulinumtoxinA (ona-BoNT/A), abobotulinumtoxinA (abo-BoNT/A), and Neuronox (neu-BoNT/A), in a mouse model using a digit abduction scoring (DAS) assay.

METHODS The efficacy, duration of effect, and safety margin of each preparation was determined after delivering a single injection to the right gastrocnemius (0–240 U/kg body weight of neu-BoNT/A or ona-BoNT/A; 0–600 Speywood Units/kg body weight of abo-BoNT/A).

RESULTS Neu-BoNT/A (intramuscular (IM) median effective dose (ED_{50}) 11.2 ± 2.7 U/kg) and ona-BoNT/A (IM ED_{50} 11.9 ± 2.4 U/kg) had similar effects in terms of muscle weakness at significantly lower doses than abo-BoNT/A (IM ED_{50} 41.2 ± 2.4 U/kg; $p < .001$). The safety margin (ratio between IM ED_{50} and IM median lethal dose (LD_{50})) of neu-BoNT/A (10.7 ± 2.6 U/kg) was also similar to that of ona-BoNT/A (10.3 ± 1.3 U/kg) but significantly higher than that of abo-BoNT/A (5.9 ± 0.4 U/kg; $p < .02$). Neu-BoNT/A and ona-BoNT/A also produced comparable patterns of DAS response and body weight recovery by day 29.

CONCLUSION Neu-BoNT/A and ona-BoNT/A may be interchangeable based on a simple dose ratio.

All authors are employees of Medytox Inc., Korea.

The botulinum toxins (BoNTs) that *Clostridium botulinum* produce are the most potent toxins known for inducing paralysis by inhibiting acetylcholine release at the neuromuscular junction.[1] There are seven serotypes (types A–G), and type A toxin has been used successfully in a wide range of clinical applications such as strabismus, hemifacial spasm, and spasticity.[2,3]

There are five commercially available preparations of BoNT type A (BoNT/A): onabotulinumtoxinA (ona-BoNT/A; BOTOX, Allergan Inc., Irvine, CA), abobotulinumtoxinA (abo-BoNT/A; Dysport, Ipsen Limited., Wrexham, UK), BTXA (Lanzhou Institute, China), incobotulinumtoxinA (inco-BoNT/A; Xeomin, Merz Pharmaceuticals, Frankfurt am Main, Germany), and Neuronox (nue-BoNT/A; Medytox Inc., Cheonwon-gun, South Korea). Despite widespread clinical use, comparative dose ratios or conversion factors of BoNT formulations remain controversial because they have been shown to have differences in efficacy and systemic effects.[4,5] Although the ona-BoNT/A to abo-BoNT/A conversion ratio for clinical use varies, even for the same indication such as for cervical dystonia (1:3–1:5),[6,7] a one-to-one dose ratio of inco-BoNT/A to ona-BoNT/A does not produce any significant difference in efficacy and side effects for treatment of cervical dystonia.[8] Nevertheless, whether a simple conversion ratio exists between BoNT preparations remains controversial.[9] Therefore, a more profound understanding of comparative dose ratios is necessary.

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Neu-BoNT/A was approved for blepharospasm in South Korea in 2006, and it has recently become popular in Asia and Latin America. We performed intramuscular (IM) injections in mice to compare the efficacy, duration of effect, and safety margin of neu-BoNT/A with those of ona-BoNT/A and abo-BoNT/A. The results of this pharmacodynamic animal study may prove helpful in the clinical setting and for basic research on this newly manufactured BoNT/A product.

Materials and Methods

Animals

Female, ICR CD1 mice (18–23 g; Orient Bio, Inc., received from Charles River, Sung-nam, Gyeonggi-do, Korea) were housed in groups of 10 with a 12-hour light–dark cycle and allowed ad libitum access to food and water. The Animal Research Committee of Medytox Inc. approved this study based on the Animal Protection Act.

Toxin Preparation and Administration

Each vial was diluted and administered as previously described.[4] Three preparations of BoNT/A (neu-BoNT/A, ona-BoNT/A, and abo-BoNT/A) were reconstituted in the same volume of saline for each study. For the sake of convenience, 500 s.U (Speywood Units) of abo-BoNT/A was regarded as 100 U, so abo-BoNT/A data was converted to values five times as high. After reconstitution, the preparations were serially diluted using saline to nine doses (0.0, 0.5, 1.0, 5.0, 10.0, 30.0, 60.0, 120.0, 240.0 U/kg of body weight). One U of neu-BoNT/A was calculated by measuring the intraperitoneal (IP) median lethal dose (LD_{50}) in female ICR CD1 mice.

Each mouse received a single IM injection of 5 μL of neurotoxin or vehicle (saline) into the head of the right gastrocnemius using a 28-G needle attached to a 25-μL Hamilton syringe. For each experiment, 10 mice were injected with each dose, and the injections were performed sequentially from low to high concentrations. The experiments were repeated three to six times.

Digit Abduction Score Assay

We used a modified digit abduction score (DAS) assay.[4,10] the injection administrator and an observer blinded to the treatment separately scored startle response, in which the animal extends its hind limbs and abducts its hind digits. The degree of digit abduction was assessed based on a 5-point scale (0 = normal to 4 = maximal reduction in digit abduction and leg extension). IM median effective dose (ED_{50}) and IM median lethal dose (LD_{50}) were calculated at the peak DAS response for each dose and at the number of dead mice, respectively.

Intramuscular ED_{50}, LD_{50}, and Safety Margin

Doses that produced half-maximal weakness (IM ED_{50}) values were derived from the logarithmic regression equations of the peak DAS response for each preparation. Half-maximal weakness was defined as a DAS value of 2. The peak DAS response was usually observed on day 2.

The dose at which half of the mice in each group died after injection was defined as the IM LD_{50}. Mice were followed for up to 4 days postinjection because most mice with severe symptoms died within 4 days.

The safety margin was calculated by dividing IM LD_{50} by IM ED_{50}.[4] This ratio reflects the safety range in which the product can be effectively injected, as well as the relationship between the half-lethal dose and half-weakening dose.[5]

Statistical Analysis

IM ED_{50} and IM LD_{50} values and safety margins were compared using one-way analysis of variance (ANOVA) followed by post hoc *t*-tests. The duration of action was compared using a two-way ANOVA followed by one-way ANOVA and *t*-tests to compare each point in time. $p < .05$ was considered significant.

Results

Dose Response in DAS Values

All preparations caused a dose-dependent increase in DAS values, as shown by plotting each dose and its maximal DAS response (Figure 1). The units of toxins for neu-BoNT/A and ona-BoNT/A that resulted in a DAS score of 4 at day 2 were similar (120 U/kg of body weight), whereas more units were required for abo-BoNT/A to achieve the same effect (300 s.U/kg of body weight). The mean equations for the best-fit logarithmic regression line were $y = 0.8454\text{Ln}(x) - 0.0679$ for six vials of neu-BoNT/A, $y = 0.8371\text{Ln}(x) - 0.1122$ for six vials of ona-BoNT/A, and $y = 1.1109\text{Ln}(x) - 1.9939$ for three vials of abo-BoNT/A. The DAS curves for neu-BoNT/A and ona-BoNT/A were nearly identical (Figure 1).

Duration of Effect and Body Weight

Although 60 U/kg of body weight of neu-BoNT/A and ona-BoNT/A and 150 s.U/kg of body weight of abo-BoNT/A produced comparable DAS scores, abo-BoNT/A demonstrated higher efficacy (day 3 and 10, $p < .05$). All preparations had comparable recovery patterns until day 29 after injection. DAS values increased until day 2 after injection, rapidly recovered until day 7, and then slowly continued to recover (Figure 2A). At these doses, there was no significant difference in duration of effect between neu-BoNT/A and ona-BoNT/A by day 29 (Figure 2A). Moreover, mice injected with the same amount (60 U/kg of body weight) of neu-BoNT/A or ona-BoNT/A recovered their body weight in a similar pattern (Figure 2B).

IM ED_{50}, LD_{50}, and Safety Margins

IM ED_{50} and LD_{50} of neu-BoNT/A and ona-BoNT/A were comparable but lower than those of abo-BoNT/A (Table 1), indicating that a similar degree of weakness can be achieved with the same dose of

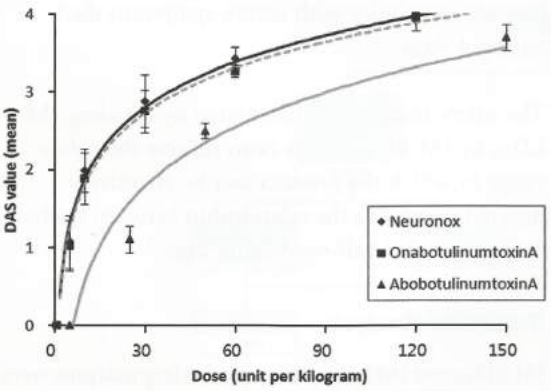

Figure 1. Dose-maximal digit abduction scoring (DAS) response curve. Dose is shown in U/kg of body weight. All lines represent best-fit logarithmic regression lines and mean values of three to six experiments. Median effective doses are measured from this graph as doses to DAS value 2.

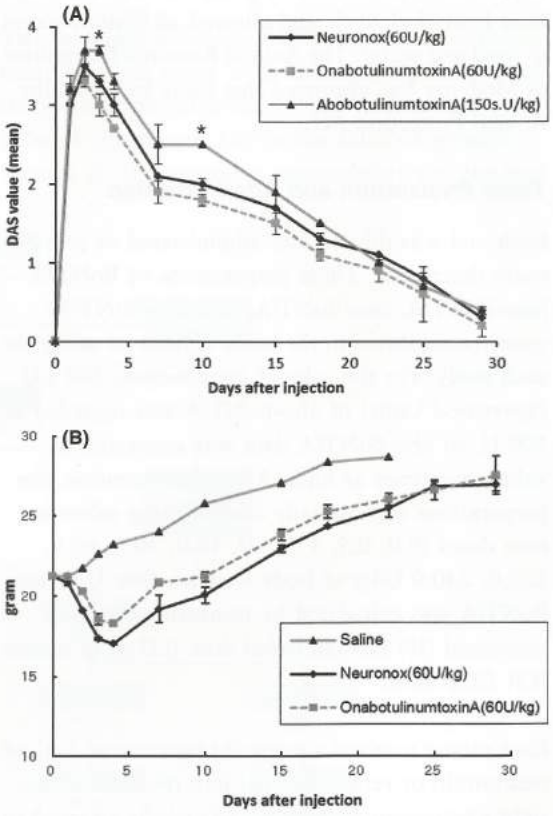

Figure 2. Duration of action (A) and body weight recovery (B). All lines represent mean values of three to six experiments. Asterisks indicate significant differences between Neuronox and abobotulinumtoxinA ($p < .01$).

TABLE 1. Comparison of Digit Abduction Scoring (DAS) Intramuscular (IM) Median Effective Dose (ED_{50}) and IM Medial Lethal Dose (LD_{50}) and Safety Margins for Three Preparations of Botulinum Toxin Type A

Preparation	*IM ED_{50}, U/kg of Body weight*	*IM LD_{50}, U/kg of Body weight*	*Safety margin, (IM LD_{50}/IM ED_{50})*
neu-BoNT/A, mean ± SD (n = 6)	11.2 ± 2.7	114.1 ± 11.5	10.7 ± 2.6
ona-BoNT/A, mean ± SD (n = 6)	11.9 ± 2.4	120.9 ± 8.5	10.3 ± 1.3
abo-BoNT/A, mean ± SD (n = 3)	41.2 ± 2.4†	242.0 ± 20.9†	5.9 ± 0.4*
neu-BoNT/A:abo-BoNT/A	1:3.7	1:2.1	1.8:1

Data represent the mean of three to six experiments with 10 mice per dose.
Significant differences between Neuronox (neu-BoNT/A) and abobotulinumtoxinA (abo-BoNT/A): *$p < .02$; †$p < .001$.

neu-BoNT/A and ona-BoNT/A and a higher dose of abo-BoNT/A. As seen in Table 1, the safety margin for abo-BoNT/A was significantly lower than for neu-BoNT/A and abo-BoNT/A, indicating that its ratio of IM LD_{50}–IM ED_{50} was lower than for the other two preparations.

Discussion

Differences in the purity and formulation of currently available BoNT/A preparations can affect their pharmacodynamics. This study clarified the dose-response relationship, duration of effect, and safety margin of neu-BoNT/A in mice and compared the results with those of ona-BoNT/A and abo-BoNT/A.

The dose-response relationship between neu-BoNT/A and ona-BoNT/A did not vary in terms of weakening muscles (Figure 1), although the response of abo-BoNT/A was lower than for the other two preparations, and the conversion ratio from abo-BoNT/A to the other two differed depending on the dose. Thus, a one-to-one ratio between neu-BoNT/A and ona-BoNT/A may be feasible, but we could not determine a fixed independent dose ratio between neu-BoNT/A and abo-BoNT/A.

Higher safety margins mean greater separation between ED_{50} and LD_{50}.[4] We found that abo-BoNT/A had the lowest safety margin—approximately half those of the other two (Table 1), although neu-BoNT/A and ona-BoNT/A had similar safety margins, indicating that the ratio between IM LD_{50} and IM ED_{50} for neu-BoNT/A does not differ from that for ona-BoNT/A. IM ED_{50} and IM LD_{50} were different from those reported previously using Swiss Webster mice.[4] Different animal care systems and species can result in different IM ED_{50} and IM LD_{50}, although we found a safety margin ratio between ona-BoNT/A and abo-BoNT/A similar to that previously reported (1.8).[4]

IM LD_{50} reflects the amount of toxin that escapes from the muscle (target site) and is used to calculate the safety margin. Theoretically, toxin diffusing from an injection site can affect the lethality rate, so IM LD_{50} doses should not be confused with IP LD_{50} doses, which are defined as toxin units.[4] The safety margin that we found for neu-BoNT/A suggests that it may diffuse away from the target site into systemic circulation in a pattern similar to that of ona-BoNT/A. Other pharmacodynamic properties of neu-BoNT/A are also similar to those of ona-BoNT/A, including the duration of effect and recovery of body weight (Figure 2).

The differences in safety margin between neu-BoNT/A and abo-BoNT/A might be due to differences in molecular mass (900 kDa and 500–900 kDa, respectively) and formulation (500 μg of albumin per vial and 125 μg of albumin plus 2.5 mg of lactose per vial).[4,5] These differences may affect stability and permeability in the injected site.

Although there are limitations in applying these data directly to humans,[10] and further studies are needed,

our results reveal that a single dose conversion at a 1:1 ratio is possible because the dose-response curves between neu-BoNT/A and ona-BoNT/A are similar. Our findings are in accordance with those of two previous clinical studies in which neu-BoNT/A demonstrated efficacy and safety profiles comparable with those of ona-BoNT/A in the treatment of essential blepharospasm and spasticity in cerebral palsy.[11,12]

References

1. Simpson LL. The origin, structure, and pharmacological activity of botulinum toxin. Pharmacol Rev 1981;33(3):155–88.
2. Grazko MA, Polo KB, Jabbari B. Botulinum toxin A for spasticity, muscle spasms, and rigidity. Neurology 1995;45 (4):712–7.
3. Jankovic J, Schwartz K, Donovan DT. Botulinum toxin treatment of cranial-cervical dystonia, spasmodic dysphonia, other focal dystonias and hemifacial spasm. J Neurol Neurosurg Psychiatr 1990;53(8):633–9.
4. Aoki KR. A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice. Toxicon 2001;39 (12):1815–20.
5. Aoki KR, Ranoux D, Wissel J. Using translational medicine to understand clinical differences between botulinum toxin formulations. Eur J Neurol 2006;13(Suppl 4):10–9.
6. Bhaumik S, Behari M. Botulinum toxin A-injection for cervical dystonia. J Assoc Physicians India 1999;47(3):267–70.
7. Ranoux D, Gury C, Fondarai J, Mas JL, et al. Respective potencies of Botox and Dysport: a double blind, randomized, crossover study in cervical dystonia. J Neurol Neurosurg Psychiatry 2002;72(4): 459–62.
8. Benecke R, Jost WH, Kanovsky P, Ruzicka E, et al. A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. Neurology 2005;64(11):1949–51.
9. Rosales RL, Bigalke H, Dressler D. Pharmacology of botulinum toxin: differences between type A preparations. Eur J Neurol 2006;13(Suppl 1):2–10.
10. Aoki KR. Botulinum neurotoxin serotypes A and B preparations have different safety margins in preclinical models of muscle weakening efficacy and systemic safety. Toxicon 2002;40(7): 923–8.
11. Kim K, Shin HI, Kwon BS, Kim SJ, et al. Neuronox versus BOTOX for spastic equinus gait in children with cerebral palsy: a randomized, double-blinded, controlled multicentre clinical trial. Dev Med Child Neurol 2011;53(3):239–44.
12. Yoon JS, Kim JC, Lee SY. Double-blind, randomized, comparative study of Meditoxin versus Botox in the treatment of essential blepharospasm. Korean J Ophthalmol 2009;23(3): 137–41.

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ORIGINAL ARTICLE

Comparative Study of Biological Activity of Four Botulinum Toxin Type A Preparations in Mice

MYUNG EUN CHUNG, MD, PHD,* DAE HEON SONG, MD, PHD,* AND JOO HYUN PARK, MD, PHD†

BACKGROUND Units of available botulinum toxin preparations are not interchangeable, and the dose-conversion ratios between such preparations remain controversial.

OBJECTIVE To compare the efficacy and safety of four botulinum toxin type A preparations.

MATERIALS AND METHODS Murine gastrocnemius compound muscle action potentials (CMAPs) were recorded before and after injecting the four botulinum toxin preparations (onabotulinumtoxinA, abobotulinumtoxinA, new botulinum toxin, and incobotulinumtoxinA).

RESULTS In all preparations, CMAP amplitudes decreased until 4 days after receiving the injection and then gradually recovered. On postinjection day 84, the amplitudes returned to baseline in all groups except the high-dose groups. CMAP amplitude in the contralateral limb also decreased up to postinjection days 4 to 7 and then gradually returned to baseline by postinjection day 28.

CONCLUSION The dose-conversion ratio between onabotulinumtoxinA and abobotulinumtoxinA was determined to be 1:2.6; previous reports of 1:3 were considered too high. A dose-conversion ratio between onabotulinumtoxinA and new botulinum toxin of 1:1 was deemed appropriate. OnabotulinumtoxinA and incobotulinumtoxinA demonstrated a dose-conversion ratio of 1:1.07. The efficacy of incobotulinumtoxinA was slightly lower than that of onabotulinumtoxinA. These dose-conversion ratios are applicable solely from an efficacy standpoint and not for safety. This study was conducted in mice, so it may not translate perfectly to human applications.

This study was sponsored by Medybox Inc., Korea.

Botulinum toxin type A is a toxin produced by anaerobic *Clostridium botulinum* bacteria and is a 900-kd protein complex in its native state. The complex consists of 150-kd neurotoxic components, nontoxic proteins, and hemagglutinin.[1] Its clinical usage began in the 1970s, and its application has since expanded to include dystonia (blepharospasm and cervical dystonia), spasticity, axillary hyperhidrosis, migraine, and anesthetic enhancement.[2,3]

Commercially available botulinum toxin type A preparations include onabotulinumtoxinA (OboNT) (BOTOX, Allergan Inc., Irvine, CA), abobotulinumtoxinA (ABoNT) (Dysport, Ipsen Limited., Wrexham, UK), incobotulinumtoxinA (IBoNT) (Xeomin, Merz Pharmaceuticals, Frankfurt am Main, Germany), and Neuronox (NBoNT) (Medytox Inc., Cheonwon-gun, South Korea). OBoNT has molecular weight of 900 kd as a result of combining 150 kd neurotoxic components and complexing proteins. ABoNT is 500–900 kd, and NBoNT is 900 kd. In contrast to other botulinum toxin type A preparations, IBoNT has a molecular weight of 150 kd because it consists of only neurotoxic components.[4] These preparations have different efficacies and safety because of their unique biologic

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nature, and there is controversy regarding the dose conversion ratio between the preparations.[5,6]

The dose-conversion ratios between OBoNT to ABoNT have been reported to vary between 1:1 and 1:6, but recent literature has not supported ratios of 1:4 or greater.[5–8] Although only a few studies have compared NBoNT and OBoNT, Stone and colleagues reported that NBoNT and OBoNT had equivalent efficacy.[9] Of the studies comparing IBoNT and OBoNT, some have reported equivalent efficacy and stability, whereas others have reported IBoNT to be less potent than OBoNT.[3,4,10,11] It is difficult to apply a consistent conversion factor between the preparations, so unexpected adverse effects have arisen with the use of different botulinum toxin type A preparations during treatment. For example, when a ratio of 1:3 OBoNT to ABoNT was used in a cervical dystonia trial, the occurrence of dysphagia in the OBoNT group was higher than in the ABoNT group.[12]

Various methods are used to evaluate the biologic activity of botulinum toxin type A. The mouse intraperitoneal (IP) median lethal dose (LD_{50}) method is a quantitative method that is used after injecting toxin into the peritoneal cavity of mice. In vitro measurement of endopeptidase activity through enzyme-linked immunosorbent assay (ELISA) and in vivo quantitative tests consisting of the digit abduction scoring (DAS) assay are additional quantitative methods.[13,14] There are also ex vivo quantitative test methods such as using the mouse phrenic nerve–hemidiaphragm. Of these, the IP LD_{50} method is less ideal in that it does not evaluate the toxin's efficacy of neuromuscular transmission inhibition directly, but measures lethality based on respiratory muscle paralysis.[15,16] In addition, large numbers are required for accurate assessment.[17] The ELISA method, which measures endopeptidase activity, does not use animals (and is hence more cost-effective), but it is less sensitive than the mouse bioassay.[14] The method using the phrenic nerve–hemidiaphragm of mice is sensitive, but it requires skillful technique and lacks reproducibility.[18,19] The DAS assay uses a scoring system, but it can be difficult to distinguish between two given scores, and the results are discrete non-continuous data.[15,16]

The biologic activity of botulinum toxin can be assessed by measuring the compound muscle action potential (CMAP), which evaluates the inhibition of neuromuscular transmission.[20] CMAP, which is an amplified and recorded microcurrent that occurs during contraction of muscle, can numerically express the degree of inhibition of neuromuscular transmission. Many clinical trials have analyzed the efficacy of botulinum toxin by obtaining CMAP in the extensor digitorum brevis of humans. A number of studies have used experimental animals to evaluate the efficacy of botulinum toxin using CMAP.[3,5,7,11,15,16,20,21]

Therefore, we measured and compared the efficacy and then calculated dose-conversion ratios of the four commercially available botulinum toxin type A preparations by measuring CMAP.

Materials and Methods

Animals

Female ICR/CD-1 mice (8 weeks old, weighing approximately 30 g each) were purchased from Orient Bio, Inc. (Sandaewon-dong, Seongnam-si, South Korea), housed in groups of seven in a 12-hour light–dark cycle, and given free access to food and water. The Animal Subjects Committee of College of Medicine, Catholic University of Korea approved the protocol.

Toxin Preparation and Administration

A blinded experimenter who did not participate in CMAP measurement or DAS scoring prepared four commercially available botulinum toxin type A preparations. Each toxin was reconstituted and diluted in 5 μL of physiologic saline. Three hundred U of ABoNT was regarded as equivalent to 100 U of other toxin preparations to simplify comparison. The mice were anesthetized using an IP injection of

ketamine hydrochloride 0.25 mg.The hind leg was shaved and 5 μL of the diluted toxin was injected into the right gastrocnemius after anesthesia using a 26-G Hamilton syringe (Hamilton Company, Reno, NV) with the following dosages: 0.0, 0.3, 1.0, 3.3, 10.0, 33.3, 100.0 U/kg for the OBoNT, NBoNT, and IBoNT groups, 0.0, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 U/kg for the ABoNT group. Each dosage group consisted of seven mice.

CMAP Recordings

CMAP was measured using Nicolet Viking Quest (Viasys Healthcare, Madison, WI). Mice were anesthetized and fixed in the prone position. Before measuring CMAP, the site of electrode insertion was shaved. Skin surface temperature was maintained at 32–36°C using a heat lamp and heat plate. Skin surface temperature was measured using a noncontact infrared thermometer (PT-3S, OPTEX, Tokyo, Japan) at the recording electrode insertion site. For stimulation, 1-cm stainless steel needle electrodes were placed subdermally for the entire length of the needle. The anode was inserted just lateral to the midline of the mouse, and the tip of the needle electrode was inserted at the site 1 mm caudal to the tail base. The cathode was inserted 3 mm lateral to the anode. A 1-cm subdermal needle was inserted into the proximal thigh as a ground electrode and a 6-mm-long gold cup ear clip electrode was used for the recording electrode. The recording electrode was placed on the belly of the gastrocnemius, and the reference was placed on the tendon of the gastrocnemius. The intensity was gradually increased until the highest CMAP amplitude without artifact was consistently obtained. Most of the electric stimuli were delivered within 10–40 mA and at a duration of 0.1 ms. Filter settings were 2–10,000 Hz. The CMAP of mice was measured before (0) and 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 70, and 84 days after the botulinum toxin A injections.

DAS Assay

The four botulinum toxin type A preparations were compared using the DAS assay to determine efficacy. After botulinum toxin injections, an observer masked to type of preparation used scored varying degrees of digit abduction on a 5-point scale (0 = resting foot, digits spread to the same degree as the noninjected leg; 1 = resting foot, less digit abduction than in the noninjected leg or two digits touching each other and the rest spread completely; 2 = resting foot, slight space open at tips of all digits or three digits touching each other; 3 = five digits touching each other if foot is at rest or four digits touching each other if foot is flexed; 4 = flexed foot, all five digits touching each other).[12,21] This was scored on days 0, 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, and 56 after injection.

Statistical Analysis

The means and standard deviations of CMAP amplitudes and the degree of change (in percentage) from baseline were obtained. We performed a one-way analysis of variance for each dose to compare the efficacies of the four different preparations and used Duncan's test as a post hoc test. We used the CMAP obtained on the day each preparation would show maximum effect to calculate CMAP median effective dose (ED_{50}) and CMAP toxic dose (TD_{20}). CMAP ED_{50} was defined as the dose producing a 50% reduction of CMAP amplitude to that of the baseline value on the injection site. CMAP TD_{20} dose was defined as a 20% decrease in CMAP amplitude value to that of the baseline value obtained on the opposite site of injection. CMAP ED_{50} and CMAP TD_{20} were estimated using probit analysis. SAS 9.1 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses, and $p < .05$ was considered statistically significant.

Results

Changes in CMAP Amplitude over Time in the Injected Gastrocnemius

CMAP amplitude was measured to electrophysiologically quantify the chemodenervative effects of botulinum toxin of the injected muscle. In all preparations, CMAP amplitude decreased in a

dose-dependent manner. Maximum decreases were observed at approximately day 4, and CMAP amplitude began to recover gradually. By postinjection day 84, the amplitude returned to baseline for all groups except the 33.3-U/kg groups for OBoNT, NboNT, or IBoNT and the 100-U/kg group for ABoNT (Figure 1). In the largest-dose groups (OBoNT, NBoNT, IBoNT: 100 U/kg U, ABoNT: 300 U/kg), all animals died 2–4 days after injection, with the exception of the IBoNT 100-U/kg group, in which four of seven mice survived until the end of the study.

Changes in CMAP Amplitude over Time in the Noninjected Contralateral Gastrocnemius

CMAP amplitude was measured in the left gastrocnemius to detect effects of diffusion to the contralateral side. A dose-dependent reduction in CMAP amplitude was noted in all botulinum toxin type A preparations. No decrease in CMAP amplitude was observed at a dose of 3.3 U/kg or less for OBoNT, NBoNT or IBoNT or for 10 U/kg or less for ABoNT. For doses of 10 U/kg or above for OBoNT, NBoNT or IBoNT and 30 U/kg or above for ABoNT, CMAP amplitude decreased until postinjection days 4 to 7 and then gradually recovered. On postinjection day 28, the amplitude returned to baseline for all toxin preparations (Figure 2).

Comparing Efficacy Using CMAP Amplitudes

To compare efficacies of the four preparations, we obtained decrease in CMAP amplitude as a percentage of baseline CMAP amplitude on postinjection day 4 (Table 1). At a dose of 3.3 U/kg of OBoNT, NboNT, and IBoNT and 10 U/kg of ABoNT, CMAP was 11.3 ± 4.6% in OBoNT, 4.0 ± 1.8% in ABoNT, 4.8 ± 1.5% in NBoNT, and 17.3 ± 6.6% in IBoNT. There was a significantly larger decrease in the CMAP amplitude for NBoNT and ABoNT than for OBoNT and a significantly larger decrease in CMAP amplitude for OBoNT than IBoNT ($p < .001$). At a 10-U/kg dose of OBoNT, NBoNT, and IBoNT and 30 U/kg of ABoNT, CMAP was 1.4 ± 0.8% for OBoNT, 0.7 ± 0.1% for ABoNT, 1.4 ± 0.6% for NBoNT, and 3.6 ± 3.1% for IBoNT. The decrease in CMAP amplitude was not different between OBoNT, AboNT, and NBoNT, but all three toxin preparations had a significantly larger decrease in CMAP amplitude than IBoNT ($p = .02$). In addition, CMAP ED_{50}, which was defined as the dose of toxin preparation that decreases CMAP amplitude 50% after injection, was calculated for the peak effect at each dose (Table 2). CMAP ED_{50} was 0.50 U/kg for OBoNT and NBoNT but somewhat higher for IBoNT (0.53 U/kg) and ABoNT (1.30 U/kg). The efficacy of the preparations derived from CMAP ED_{50} was similar for OBoNT and NBoNT. IBoNT was slightly lower than the other two preparations, and ABoNT was slightly higher than OboNT, at a ratio of 1:3. The dose-conversion ratio of OBoNT to NBoNT was 1:1, that of OBoNT to ABoNT was 1:2.6, and that of OBoNT to IBoNT was 1:1.07.

CMAP TD_{20} and Safety Margins for Botulinum Toxin Type A Preparations

To compare the effects of toxin diffusion with the contralateral side for each botulinum toxin type A preparation, we calculated CMAP TD_{20}, which is the dose showing a 20% decrease in CMAP amplitude in the left gastrocnemius (Table 2). CMAP TD_{20} was 99.0 U/kg for ABoNT, 65.2 U/kg for IBoNT, 54.9 U/kg for NBoNT, and 45.0 U/kg for OBoNT. The safety margin, which was obtained by dividing CMAP TD_{20} by CMAP ED_{50}, was 122.0 for IBoNT, 110.33 for NBoNT, 88.6 for OBoNT, and 75.5 for ABoNT (Table 2).

Comparing Efficacy by Using the DAS Assay

All preparations produced dose-related increases in the magnitude and duration of muscle weakness in the DAS assay (Figure 3). We calculated the DAS ED_{50}, which was defined as the dose resulting in a DAS of 2 after injection from the peak effect at each dose (Table 3). To determine the systemic effect of botulinum toxins, we calculated DAS IM LD_{50}, defined as the dose that kills 50% of mice after toxin injection (Table 3). NBoNT had the lowest DAS

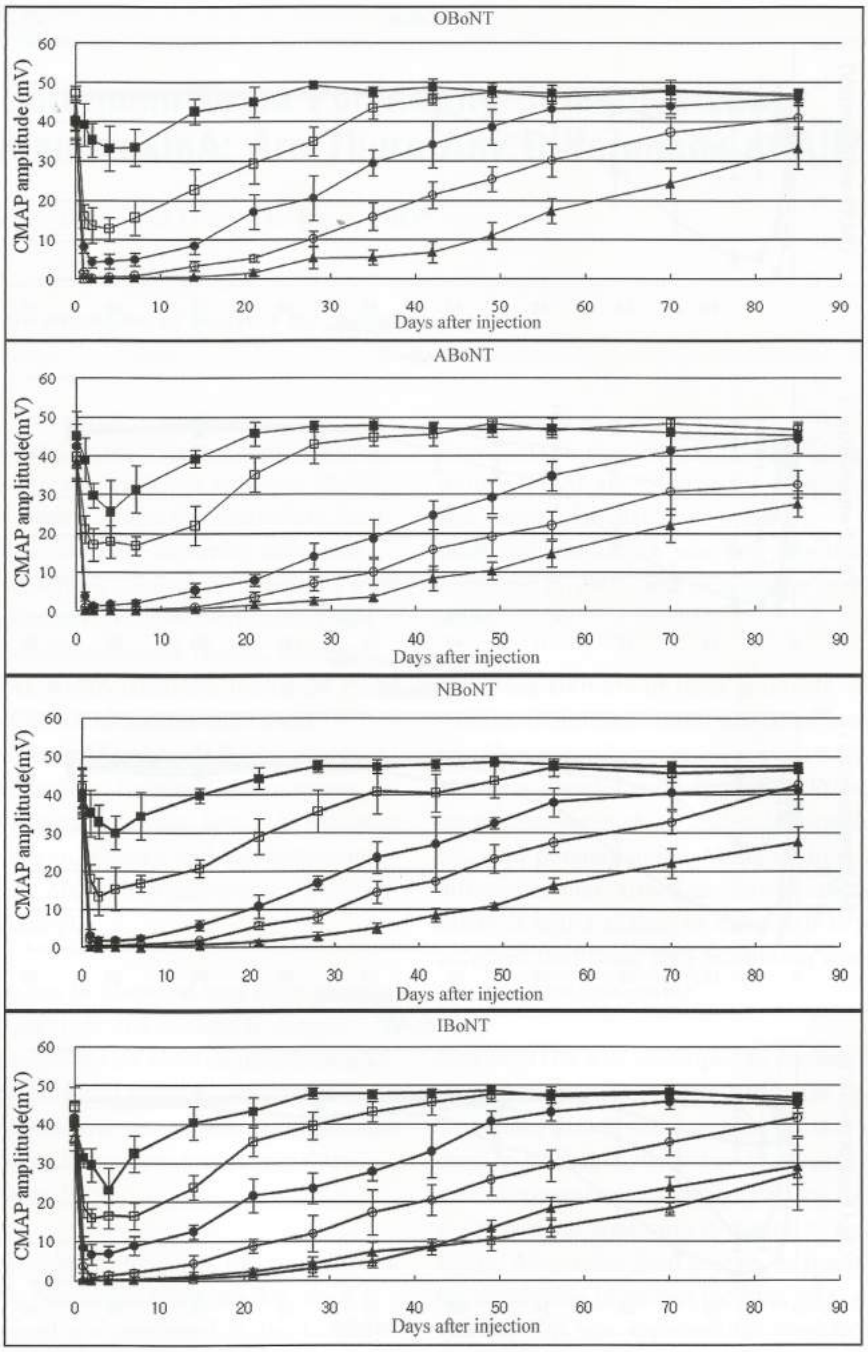

Figure 1. Changes in compound muscle action potential (CMAP) amplitude over time with different botulinum toxin type A preparations, recorded from the injected limb. CMAP amplitude was measured from baseline to peak from the right gastrocnemius of each mouse before and 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 70, and 84 days after injection. Each point was the mean ± standard deviation, $n = 7$. ■: 0.3 U/kg (1.0 U/kg for abobotulinumtoxinA (ABoNT)), □: 1.0 U/kg (3.0 U/kg for ABoNT), ●: 3.3 U/kg (10.0 U/kg for ABoNT), ○: 10.0 U/kg (30.0 U/kg for ABoNT), ▲: 33.3 U/kg (100.0 U/kg for ABoNT), △: 100.0 U/kg (300.0 U/kg for ABoNT).

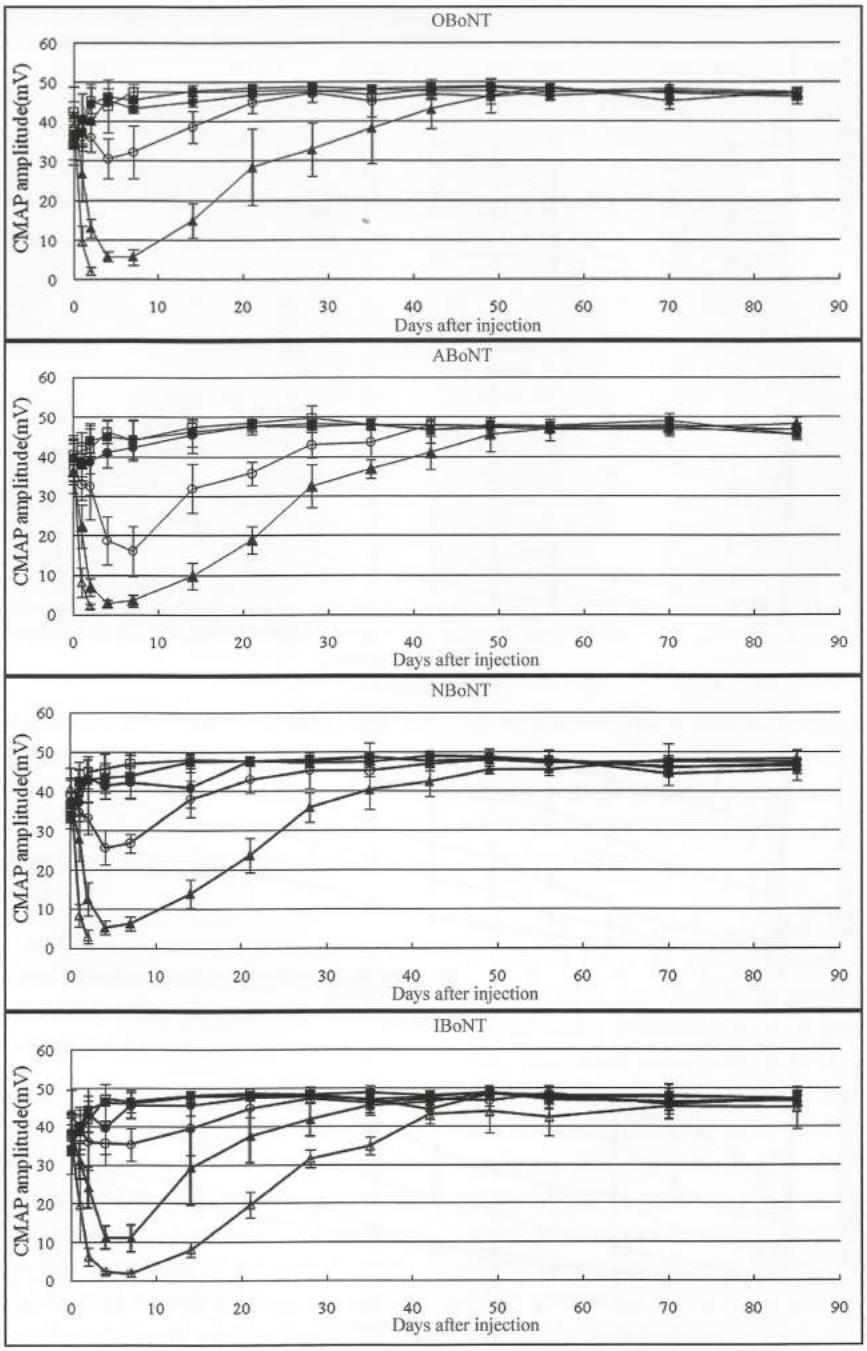

Figure 2. Changes in compound muscle action potential (CMAP) amplitude over time with different botulinum toxin type A preparations, recorded from the contralateral, noninjected limb. CMAP amplitude was measured from baseline to peak from the left gastrocnemius of each mouse before and 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 70, and 84 days after injection. Each point was the mean ± standard deviation, $n = 7$. ■: 0.3 U/kg (1.0 U/kg for abobotulinumtoxinA (ABoNT)), □: 1.0 U/kg (3.0 U/kg for ABoNT), ●: 3.3 U/kg (10.0 U/kg for ABoNT), ○: 10.0 U/kg (30.0 U/kg for ABoNT), ▲: 33.3 U/kg (100.0 U/kg for ABoNT), △: 100.0 U/kg (300.0 U/kg for ABoNT).

TABLE 1. Percentage of Compound Muscle Action Potential in Target Muscle 4 Days After Injection of Botulinum Toxin Type A Preparations

IBoNT, OBoNT, NBoNT (ABoNT) Dose, U/kg	*OBoNT*	*ABoNT*	*NBoNT*	*IBoNT*	
	Mean ± standard deviation				*p-Value*
0.3 (1.0)	76.5 ± 15.8	62.2 ± 29.9	76.2 ± 14.7	58.3 ± 16.3	.25
1.0 (3.0)	27.4 ± 6.8	44.9 ± 8.2	38.1 ± 16.7	38.2 ± 10.8	.06
3.3 (10.0)	11.3 ± 4.6	4.0 ± 1.8	4.8 ± 1.5	17.3 ± 6.6	<.001*
10.0 (30.0)	1.42 ± 0.79	0.70 ± 0.12	1.36 ± 0.63	3.6 ± 3.1	.02†
33.3 (100.0)	0.17 ± 0.34	0.17 ± 0.29	0.56 ± 0.70	0.29 ± 0.37	.35
100.0 (300.0)	–	–	–	0.27 ± 0.27	–

*IncobotulinumtoxinA (IBoNT) > onabotulinumtoxinA (OBoNT) > Neuronox (NboNT) = abobotulinumtoxinA ABoNT ($p < .001$).
†IBoNT > OBoNT = NBoNT = ABoNT ($p = .02$).

TABLE 2. Comparison of Compound Muscle Action Potential (CMAP) Median Effective Dose (ED_{50}), CMAP Toxic Dose (TD_{20}), and Safety Margin

Toxin	*CMAP ED_{50}, U/kg*	*CMAP TD_{20}, U/kg*	*Safety margin (CMAP TD_{20}/CMAP ED_{50})*
OnabotulinumtoxinA	0.50	45.0	88.6
AbobotulinumtoxinA	1.30	99.0	75.5
Neuronox	0.50	54.9	110.3
IncobotulinumtoxinA	0.53	65.2	122.0

ED_{50} (7.1 U/kg), which was not significantly different from that of OBoNT (8.2 U/kg). The DAS ED_{50} of IBoNT was 11.6 U/kg, which was slightly less effective than NBoNT and OBoNT. ABoNT had the lowest efficacy (20.9 U/kg), but at a conversion ratio of 1:3, its efficacy was slightly higher than that of OBoNT. We could not measure DAS IM LD_{50} for IBoNT because it had a mortality of < 50% at the highest dose of 100 U/kg. DAS IM LD_{50} was 59.7 U/kg for OBoNT and NBoNT and 179.1 U/kg for ABoNT. There were no significant differences in safety margin between the preparations (7.2 for OBoNT, 8.4 for NBoNT, and 8.6 for ABoNT).

Discussion

Currently available botulinum toxin type A preparations differ in purification methods and formulations, which raises concern regarding differences in efficacy and properties.[6,22] We discovered similarities in and differences between efficacy of the preparations depending on dose and duration of the postinjection period.

When OBoNT and ABoNT were compared using percentage of CMAP in the injection site at postinjection day 4, ABoNT had significantly higher efficacy when converted at a ratio of 1:3; (3.3 U/kg of OBoNT vs 10U/kg of ABoNT and 10U/kg of OBoNT vs 30 U/kg of AboNT). When CMAP ED_{50} of the two was compared, we determined the dose-conversion ratio to be 1:2.6, which was consistent with reports indicating that a ratio of 1:3 was too high. Recent studies using DAS assay on rats, electromyography of the frontalis, and anhydrotic action suggest that the dose-conversion ratio of OBoNT to ABoNT should be lower than 1:3 and that the desirable ratio was 1:2.5, which was previously noted,[5,6,8,23,24] but when the conversion ratio of OBoNT to ABoNT was derived from CMAP TD_{20}, it was 1:2.2, which was even lower than 1:2.6. This explains why the safety margin of ABoNT is narrower. This also suggests that, as Sampaio and colleagues reported, the dose-conversion ratio that pertained to efficacy alone could not be used to predict adverse effects.[25]

When OBoNT and NBoNT were compared using decrease in CMAP amplitude as a percentage of baseline on postinjection day 4, NBoNT showed significantly higher efficacy only at the dose of

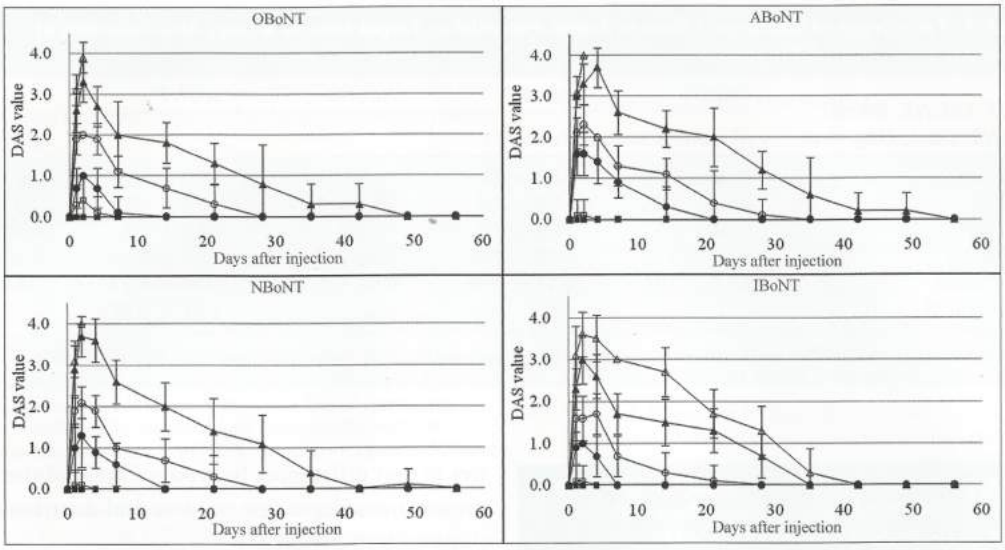

Figure 3. Changes in digit abduction scoring (DAS) over time with different botulinum toxin type A preparations, obtained from the injection limb. DAS score was measured from the right gastrocnemius of each mouse before and 1, 2, 4, 7, 14, 21, 28, 35, 42, 49, and 56 days after injection. Each point was the mean ± standard deviation, $n = 7$. ■: 0.3 U/kg (1.0 U/kg for abobotulinumtoxinA (ABoNT)), □: 1.0 U/kg (3.0 U/kg for ABoNT), ●: 3.3 U/kg (10.0 U/kg for ABoNT), ○: 10.0 U/kg (30.0 U/kg for ABoNT), ▲: 33.3 U/kg (100.0 U/kg for ABoNT), △: 100.0 U/kg (300.0 U/kg for ABoNT).

TABLE 3. Comparison of Digit Abduction Scoring (DAS) Median Effective Dose (ED_{50}), DAS Intramuscular (IM) Median Lethal Dose (LD_{50}), and Safety Margin

Toxin	*DAS ED_{50}, U/kg*	*DAS IM LD_{50}, U/kg*	*Safety margin (DAS IM LD_{50}/DAS ED_{50})*
OnabotulinumtoxinA	8.2	59.7	7.2
AbobotulinumtoxinA	20.9	179.1	8.6
Neuronox	7.1	59.7	8.4
IncobotulinumtoxinA	11.6	–	–

3.3 U/kg; no difference was observed at other doses. CMAP ED_{50} was 0.50 U/kg for both preparations. The safety margin derived from the percentile changes of CMAP was 110.3 for NBoNT, which was higher than that of OBoNT (88.6). The safety margin calculated using the DAS assay was 8.4 for NBoNT, which was slightly higher than that of OBoNT (7.2). Although few studies have compared these toxins, one study assessing muscle power in mice reported similar efficacy.[9] It may be too hasty to conclude that the dose-conversion ratio is 1:1 because of a lack of adequate and well-designed clinical comparison studies, but the efficacy of OBoNT and NBoNT appears to be equal in mice.

In terms of the percentile change in CMAP at postinjection day 4, IBoNT was significantly less efficacious than OBoNT, NBoNT, and triple-dose ABoNT at doses of 3.3 U/kg and 10 U/kg. CMAP ED_{50} was 0.53 U/kg, which was slightly higher than 0.50 U/kg for OBoNT and NBoNT. This leads to a dose-conversion ratio between OBoNT and IBoNT of 1:1.07. The safety margin for IBoNT derived from the percentile change in CMAP was 122.0, which is the highest of the toxin preparations. Four of seven mice in the highest-dose (100 U/kg) group of IBoNT survived until the end of the study. Thus, although IBoNT is considered to be less efficacious than the others, it is also considered to have the least adverse effects. Comparative studies based on the extensor digitorum brevis muscle of healthy volunteers reported comparable efficacy and duration of action of OBoNT and IBoNT, although potency

bioassays with mice demonstrated that the potency of IBoNT was less than that of OBoNT.[3,4] Our results suggest that the dose-conversion ratio of OBoNT to IBoNT may be slightly higher than 1:1, and that IBoNT may slightly less potent than OBoNT.

Although there were significantly different efficacies between the toxins at doses of 3.3 and 10.0 U/kg, there was no significant difference in percentile change of CMAP at low doses (0.3 and 1.0 U/kg). Therefore, effects at low doses may differ during clinical usage, inasmuch as the results were different at those doses.

In previous murine studies, the DAS ED_{50} of OBoNT was reported to be 3.5, 6, or 2 U/kg.[13,26] We determined CMAP ED_{50} to be 0.50 U/kg and concluded that different definitions for CMAP ED_{50} and DAS ED_{50} caused the gap between CMAP ED_{50} and DAS ED_{50}. We defined the DAS ED_{50} as the dose effecting a score of 2 on the DAS assay (resting foot, slight space open at tips of all digits or three digits touching each other) during the peak effect period after toxin injection and CMAP ED_{50} as the dose at which the CMAP amplitude decreased by 50% during the peak effect period. The DAS assay measures the degree of digit abduction after the injection of botulinum toxin into the gastrocnemius. The DAS assay is known to be reproducible and has high interrater reliability,[13] but because it measures paralysis of the digit abductor muscle adjacent to the gastrocnemius rather than the injected gastrocnemius itself, it may include the effects of local diffusion, which may be misinterpreted as efficacy. In contrast, recording CMAP of the injected muscle may be a more direct method of estimating toxin efficacy. We discovered that the peak effect was observed at postinjection days 2 to 4 obtained according to the DAS assay or recording CMAP amplitudes, although based on the DAS assay, all mice recovered before postinjection day 56. Nevertheless, based on CMAP amplitudes, effects seemed to continue until postinjection day 84 in the high-dose groups. Furthermore, although the DAS assay failed to demonstrate a paretic effect in the lowest-dose group, such effects were clearly evident even in this group by recording CMAP amplitudes, suggesting that changes in CMAP amplitude are more sensitive than the DAS assay in quantifying the effects of botulinum toxin. Because few studies have reported CMAP amplitude decrements for various doses showing meaningful clinical efficacy, there are limitations in determining efficacy using CMAP ED_{50}. Nevertheless, changes in CMAP amplitude could be a finer indicator of chemodenervation than the DAS assay in that it evaluates the injected muscle directly, expresses results numerically, and is more sensitive.[15,16]

In this study, there was a difference in values of safety margin derived from the CMAPs and from the DAS assay: 88.6 from CMAP and 7.2 from DAS for OBoNT and 75.49 from CMAP and 8.6 from DAS for ABoNT. The safety margin was higher in OBoNT than in ABoNT when derived from the CMAP test, but the DAS assay lead to higher safety margins in ABoNT. Different definitions for CMAP TD_{20} and DAS IM LD_{50} used to calculate the safety margin may also have led to these results. When comparing the safety margins of preparations, it may be more desirable to apply the safety margin obtained using overweakening of the muscle rather than the lethal dose.

This study was conducted in mice to compare the efficacy and toxicity of four different botulinum toxin preparations, but caution is required when such results are applied clinically. Because murine myoanatomy differs from that of humans, this can lead to differences in the uptake and spread of botulinum toxin.[8] Furthermore, this study used CMAP ED_{50}, CMAP TD_{20}, DAS ED_{50}, and DAS IM LD_{50} derived from a single study, which could limit any form of generalization.

In terms of efficacy, OBoNT and ABoNT had a dose-conversion ratio of 1:2.6, which was slightly lower than previous reports of 1:3. OBoNT and NBoNT had a dose-conversion ratio of 1:1, and OBoNT and

IboNT had a ratio of 1:1.07, which was slightly higher than 1:1, but these ratios were based on efficacy alone without considering adverse effects and should not be misinterpreted as bioequivalence ratios.

References

1. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. N Engl J Med 1991;324:1186–94.
2. Brin MF. Development of future indications for BOTOX. Toxicon 2009;54:668–74.
3. Jost WH, Kohl A, Brinkmann S, Comes G. Efficacy and tolerability of a botulinum toxin type A free of complexing proteins (NT 201) compared with commercially available botulinum toxin type A (BOTOX) in healthy volunteers. J Neural Transm 2005;112:905–13.
4. Hunt T, Clarke K. Potency evaluation of a formulated drug product containing 150-kd botulinum neurotoxin type A. Clin Neuropharmacol 2009;32:28–31.
5. Wohlfarth K, Schwandt I, Wegner F, Jrgens T, 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.
6. Wohlfarth K, Sycha T, Ranoux D, Naver H, et al. Dose equivalence of two commercial preparations of botulinum neurotoxin type A: time for a reassessment? Curr Med Res Opin 2009;25:1573–84.
7. Wohlfarth K, Kampe K, Bigalke H. Pharmacokinetic properties of different formulations of botulinum neurotoxin type A. Mov Disord 2004;19(Suppl 8):S65–7.
8. Rosales RL, Bigalke H, Dressler D. Pharmacology of botulinum toxin: differences between type A preparations. Eur J Neurol 2006;13(Suppl 1):S2–10.
9. Stone AV, Ma J, Whitlock PW, Koman LA, et al. Effects of Botox and Neuronox on muscle force generation in mice. J Orthop Res 2007;25:1658–64.
10. Aoki KR. Botulinum neurotoxin serotypes A and B preparations have different safety margins in preclinical models of muscle weakening efficacy and systemic safety. Toxicon 2002;40:923–8.
11. Wohlfarth K, Mller C, Sassin I, Comes G, et al. Neurophysiological double-blind trial of a botulinum neurotoxin type A free of complexing proteins. Clin Neuropharmacol 2007;30:86–94.
12. Ranoux D, Gury C, Fondarai J, Mas JL, et al. Respective potencies of Botox and Dysport: a double blind, randomized, crossover study in cervical dystonia. J Neurol Neurosurg Psychiatry 2002;72:459–62.
13. Aoki KR. A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice. Toxicon 2001;39:1815–20.
14. Hallis B, James BA, Shone CC. Development of novel assays for botulinum type A and B neurotoxins based on their endopeptidase activities. J Clin Microbiol 1996;34:1934–8.
15. Torii Y, Goto Y, Takahashi M, Ishida S, et al. Quantitative determination of biological activity of botulinum toxins utilizing compound muscle action potentials (CMAP), and comparison of neuromuscular transmission blockage and muscle flaccidity among toxins. Toxicon 2010;55:407–14.
16. Sakamoto T, Torii Y, Takahashi M, Ishida S, et al. Quantitative determination of the biological activity of botulinum toxin type A by measuring the compound muscle action potential (CMAP) in rats. Toxicon 2009;54:857–61.
17. Pearce LB, Borodic GE, First ER, MacCallum RD. Measurement of botulinum toxin activity: evaluation of the lethality assay. Toxicol Appl Pharmacol 1994;128:69–77.
18. Bigalke H, Wohlfarth K, Irmer A, Dengler R. Botulinum A toxin: Dysport improvement of biological availability. Exp Neurol 2001;168:162–70.
19. Yoneda S, Shimazawa M, Kato M, Nonoyama A, et al. Comparison of the therapeutic indexes of different molecular forms of botulinum toxin type A. Eur J Pharmacol 2005;508: 223–9.
20. Cichon JV, McCaffrey TV, Litchy WJ, Knops JL. The effect of botulinum toxin type A injection on compound muscle action potential in an in vivo rat model. Laryngoscope 1995;105:144–8.
21. Osuchowski MF, Teener J, Remick D. Noninvasive model of sciatic nerve conduction in healthy and septic mice: reliability and normative data. Muscle Nerve 2009;40:610–6.
22. Aoki KR, Ranoux D, Wissel J. Using translational medicine to understand clinical differences between botulinum toxin formulations. Eur J Neurol 2006;13(Suppl 4):S10–9.
23. Karsai S, Adrian R, Hammes S, Thimm J, 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.
24. Hexsel D, Dal'Forno T, Hexsel C, Do Prado DZ, et al. A randomized pilot study comparing the action halos of two commercial preparations of botulinum toxin type A. Dermatol Surg 2008;34:52–9.
25. Sampaio C, Costa J, Ferreira JJ. Clinical comparability of marketed formulations of botulinum toxin. Mov Disord 2004;19 (Suppl 8):S129–36.
26. Aoki KR. Preclinical update on BOTOX® (botulinum toxin type A)-purified neurotoxin complex relative to other botulinum neurotoxin preparations. Eur J Neurol 1999;6(Suppl 4):S3–10.

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MINI REVIEW

A New Botulinum Toxin Potentially Bioequivalent to OnabotulinumtoxinA: Are There Any Differences at All?

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Botulinum toxins refer to a group of proteins classified into seven distinct serotypes (A–G) produced by different strains of *Clostridium botulinum*, which is a naturally occurring endospore-forming strict anaerobic eubacteria.[1] There are more than six commercially available botulinum toxin products: onabotulinumtoxinA (Botox, Allergan Inc., Irvine, CA), BTXA (Lanzhou Biological Products Institute, China), abobotulinumtoxinA (Dysport, Ipsen Ltd., Wrexham, UK), rimabotulinumtoxinB (Neurobloc, Solstice Neurosciences, Louisville, KY), Neuronox (neu-BoNT/A, Medytox Inc., Cheonwon-gun, Korea), and incobotulinumtoxinA (Xeomin, Merz Pharmaceuticals, Frankfurt am Main, Germany).

These products can be classified into three groups based on their serotype and molecular nature: serotype A complex toxins (onabotulinumtoxinA, abobotulinumtoxinA, BTXA, and neu-BoNT/A), serotype A complex–free toxins (incobotulinumtoxinA), and serotype B complex toxins (rimabotulinumtoxinB; Table 1).[2–7]

Of these, onabotulinumtoxinA is the best known, dominating the botulinum toxin market since it was first approved and marketed in the United States in 1989. AbobotulinumtoxinA has ranked second in the toxin market and has been available in Europe, Asia, and Latin America and in the United States since the Food and Drug Administration (FDA) approved it for cervical dystonia and severe glabellar lines in 2009. IncobotulinumtoxinA was first introduced in Germany in 2005 and is available in the United Sates.

The comparability of these products, especially of onabotulinumtoxinA and abobotulinumtoxinA, has been the subject of controversy for the past two decades, mainly due to different characteristics such as molecular weight, excipient, and potency units. Although how those differences may affect its clinical efficacy and safety is still a matter of debate, it is widely accepted that these two botulinum toxin products are not interchangeable.

Neu-BoNT/A was developed to provide features close to onabotulinumtoxinA from the beginning. As with many follow-on biologics, the aim was to develop a reliable alternative that could reduce the cost of treatment and that physicians could use easily without confusion in dosing or concerns with safety. After more than 6 years of research, including developing programs focusing on comparability, neu-BoNT/A was approved for blepharospasm in South Korea in 2006. It has become one of the best-received botulinum toxin products available in Asian and Latin American countries such as South Korea,

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TABLE 1. Characteristics of Botulinum Toxin Preparations

	Botox	*BTXA*	*Dysport*	*Myobloc*	*Neuronox*	*Xeomin*
Manufacturer	Allergan, Inc.	Lanzou Institute	Ipsen, Ltd..	Solstice Neurosciences	Medytox Inc	Merz Pharmaceuticals
Pharmaceutical form	Powder	Powder	Powder	Solution	Powder	Powder
Clostridium botulinum strain	Hall A	n/a	Ipsen strain	Bean B	Hall A	Hall A
Toxin serotype	A	A	A	B	A	A
Active substance	900 kDa complex	900 kDa complex	500–900 kDa complex	700 kDa complex	900 kDa complex	150 kDa complex-free
U/vial	100	100	500	5000	100	100
Excipients/vial	HSA (0.5 mg) NaCl (0.9 mg)	Gelatin (5 mg) Sucrose (25 mg) Dextran (25 mg)	HSA (0.125 mg) Lactose (2.5 mg)	HSA (0.5 mg) NaCl (5.844 mg) Sodium succinate (1.621 mg)	HSA (0.5 mg) NaCl (0.9 mg)	HSA (1.0 mg) Sucrose (5 mg)
Storage	2–8°C or <–5°C	<–5°C	2–8°C	2–8°C	2–8°C or <–5°C	<25°C

HSA, human serum albumin; NaCl, sodium chloride.

Japan, Thailand, and Brazil for therapeutic and cosmetic indications.

We review the scientific evidence with regard to the comparability of onabotulinumtoxinA and neu-BoNT/A.

Comparison of Microbiologic, Physicochemical, and Biochemical Profiles: OnabotulinumtoxinA Versus neu-BoNT/A

Considering that botulinum toxin is a biologic agent produced in living cells, the origin of bacterial strains should be carefully considered. Medytox Inc. uses *Clostridium botulinum* type A Hall strain, which is the same bacterial strain that Allergan uses. The complete DNA sequence of the type A toxin complex was analyzed including all nontoxic components, as well as the toxin itself (GeneBank accession number DQ409059). The deduced amino acid sequence of neu-BoNT/A was aligned with Allergan's data published in 2003,[8] and there were no differences in terms of amino acid sequence between the two products (data not shown). Thus *C. botulinum* type A Hall in Allergan (AF488749, 488748, 488747, AF488746, AF488745) and Medytox (DQ409059) are essentially from the same strain used for the production of botulinum toxin type A complex.

Botulinum toxin produced using the *C. botulinum* is purified from the culture solution. Because each manufacturer has treated the methods used for culture and purification as confidential information, they have never been published or presented in public. Therefore, it is impossible to conduct any direct comparison with regard to the manufacturing process itself, but the molecular weight of the toxin complex of the final bulk product, which such processes would affect, has been at the center of the debate over the last two decades when comparing different products. Some claim that onabotulinumtoxinA is a homogeneous 900-kDa toxin complex, whereas abobotulinumtoxinA is a mixture of 500- and 900-kDa complexes.[9] Such differences in molecular weight may cause differences in patterns of diffusion or spread around the injection site.[10,11]

Although the effect of the difference in molecular weight is controversial, and the clinical significance of molecular weight is unclear, we decided to produce a highly homogeneous 900-kDa toxin complex. Because a single vial of commercial botulinum toxin product contains only a few nanograms of toxin complex (with an approximately 10,000 times greater amount of human serum albumin), it is not possible to extract the toxin complex from the vials. Thus, the structure of these products cannot be directly compared. Instead, size-exclusion high-performance liquid chromatography (SE-HPLC) analysis of neu-BoNT/A was performed with a mixture of molecular weight standards and confirmed a single peak in the chromatogram that reflects its homogeneity (Figure 1). The molecular weight of the toxin complex was determined by comparison with a calibration curve generated from referential molecular standards. The average molecular weight of the 10 bulk batches was 904 ± 7 kDa, which is close to the molecular weight of bulk toxin complex that Allergan's research group has reported: 880 kDa according to SE-HPLC analysis and 925 ± 45 kDa according to light scattering analysis.[12]

It is still controversial whether the molecular mass of the toxin complex strongly correlates with unwanted diffusion or spread of botulinum toxin products. Nevertheless, from a theoretical viewpoint, one could maintain that, because onabotulinumtoxinA and neu-BoNT/A are close in molecular size, their diffusion patterns would be similar.

Although formulations vary between botulinum toxin preparations, human serum albumin (HSA) is used as an excipient in all of the preparations except for Chinese BTXA, in which gelatin is used instead (Table 1). The neu-BoNT/A 100-U vial, like the onabotulinumtoxinA 100-U vial, contains 100 U of the purified toxin complex, 0.9 mg of sodium chloride, and 0.5 mg of HSA. AbobotulinumtoxinA has less HSA (0.125 mg) and contains 2.5 mg of lactose in a 500-U vial (Dysport package insert). (In the United States, abobotulinumtoxinA is available in a 300-U vial.) IncobotulinumtoxinA contains 1.0 mg of HSA and 5 mg of sucrose in a 100-U vial. The composition of rimabotulinumtoxinB is also different from the others: 0.5 mg of HSA, 5.844 mg of sodium chloride, and 1.621 mg of sodium succinate[13] (Neurobloc package insert). Although it is not clear whether different excipients can affect clinical results, there have been some reports suggesting possible effects of human serum albumin on the biologic availability of botulinum toxin.[14] Because neu-BoNT/A has the same composition as

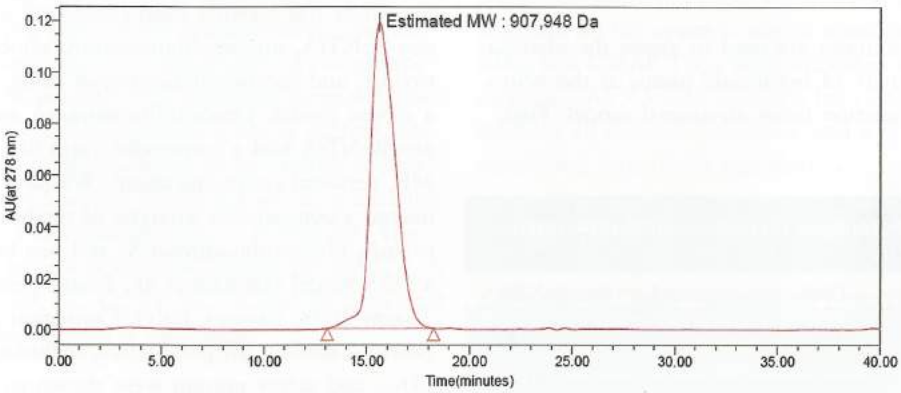

Figure 1. Size-exclusion high-performance liquid chromatography chromatogram of the purified botulinum toxin type A complex performed using a Shodex Protein KW-804 size exclusion column (8.0 mm inner diameter × 300 mm length, particle size 7 μm). The eluted sample was monitored with ultraviolet absorbance at 278 nm.

onabotulinumtoxinA, there would be no difference in the effect of excipients between them.

From the above evidence, neu-BoNT/A could be expected to behave clinically in a similar manner to onabotulinumtoxinA because it shares the same strain of *C. botulinum*, a similar size toxin complex, and the same ingredients in the vial.

Preclinical Comparability Based on Pharmacodynamic Models

Other questions need to be addressed before discussing the clinical use of neu-BoNT/A. A major question is the potency of neu-BoNT/A units. One unit of botulinum toxin usually corresponds to the calculated intraperitoneal median lethal dose (LD_{50}) in mice. Head-to-head comparisons of several lots of onabotulinumtoxinA and reference batches of neu-BoNT/A using the same in vivo potency assay suggest that the in vivo potencies of the two products are practically the same (Table 2), but because methods of performing the assay are specific to each manufacturer (type of vehicle, dilution scheme, laboratory protocols), it is difficult to compare units of biological activity, let alone convert one into the other.[15,16] Thus several pharmacodynamic studies have been conducted to compare the biologic activity of the two products.

Several techniques are used to assess the pharmacologic activity of botulinum toxins at the neuromuscular junction using an animal model. First, compound muscle action potential (CMAP) is an electro-physiological measure of muscle function. The microcurrents generated during muscle contraction are amplified and recorded and thus can quantify the degree of acetylcholine release blockage at the neuromuscular junction.[17,18] Second, a digit abduction scoring (DAS) assay, modified by Aoki and colleagues,[19,20] is a descriptive scale used to assess the degree of focal weakness as a result of chemodenervation caused by injecting the toxin into the hind limb. In this preclinical model, safety margins can be calculated according to the ratio between the median effective dose (ED_{50}; the degree of focal weakness) and the LD_{50} (systemic diffusion after intramuscular injection of the toxin). Third, a muscle force generation model incorporating a rat hind limb could be used for the assessment of multiple parameters, including reduction in muscle force generation and potential toxin diffusion into the contralateral limb. In all three models described above, the duration of efficacy and the time course of recovery could also be assessed.

TABLE 2. Potency of onabotulinumtoxinA versus neu-BoNT/A

	OnabotulinumtoxinA	*Neu-BoNT/A*
Calculated potency, U*	105 ± 6.45	110 ± 3.95

Seven independent experiments using three different batches of Botox and neu-BoNT/A were performed.
*Medytox's in-house potency assay based on murine lethality was applied to measure the biologic potency of these products. (1 U = 1 mouse median lethal dose).

The dose-response relationship of onabotulinumtoxinA and neu-BoNT/A for inhibiting muscle force was first reported in a murine model, and the time course of recovery was derived from the effects of toxin-induced muscle paralysis; these preparations resulted in bioequivalent responses.[21] A comparative study has recently been completed of neu-BoNT/A, onabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA using CMAPs in a mouse model. OnabotulinumtoxinA and neu-BoNT/A had a conversion ratio of 1:1 (Chung ME, personal communication). We have also conducted a comparative analysis of onabotulinumtoxinA, abobotulinumtoxinA, and neu-BoNT/A in a DAS model (SB Kim et al., Poster presented in Toxins 2008, Baveno, Italy). Consistent with previous studies, all parameters, including ED_{50}, LD_{50}, and safety margin were shown to be comparable, indicating that onabotulinumtoxinA and neu-BoNT/A are similar, at least at the preclinical, animal model stage.

Comparative Clinical Studies

Based on the current literature, it is likely that 1 U of onabotulinumtoxinA corresponds to 2–5 U of abobotulinumtoxinA.[22,23] Even though there have been studies reporting that incobotulinumtoxinA is interchangeable with onabotulinumtoxinA,[24,25] others maintain that incobotulinumtoxinA is less potent than onabotulinumtoxinA.[20,26]

Because neu-BoNT/A was only recently introduced to the market, there is little literature available at this point. A double-blind, randomized, comparative study of Neuronox (a domestic brand name of neu-BoNT/A) and onabotulinumtoxinA in treating essential blepharospasm[27] concluded that there were no significant differences between the two treatment groups in efficacy (severity of spasms, changes in eyelid closing force, functional visual status) or safety at a 1:1 dose ratio. Another randomized, double-blind, placebo-controlled multicenter clinical trial comparing onabotulinumtoxinA and neu-BoNT/A reported that neu-BoNT/A is as effective and safe as onabotulinumtoxinA for the treatment of spasticity in children with cerebral palsy.[28] A double-blind, randomized, comparative study for treating glabellar lines also demonstrated comparable efficacy and safety of neu-BoNT/A and onabotulinumtoxinA at a 1:1 dose ratio, consistent with the results obtained from animal studies (CH Won. unpublished data).

Conclusions

More than five botulinum toxin type A preparations are available worldwide. OnabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA have received FDA approval for therapeutic and cosmetic indications. Although more extensive research with regard to long-term safety and additional comparative clinical studies for various indications are required, neu-BoNT/A seems to be similar to onabotulinumtoxinA based on preliminary studies in several different areas (ophthalmology, neurology, and aesthetics) and can be expected to be a competitor in markets where the two products compete.

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References

1. Oguma K, Fujinaga Y, Inoue K. Clostridium botulinum toxin. Toxin Rev 1997;16(4):253–66.
2. Jankovic J, Vuong KD, Ahsan J. Comparison of efficacy and immunogenicity of original versus current botulinum toxin in cervical dystonia. Neurology 2003;60(7):1186–8.
3. Setler P. The biochemistry of botulinum toxin type B. Neurology 2000;55(12 Suppl 5):S22–8.
4. Dressler D, Benecke R. Xeomin®: perspektiven einer neuen therapeutischen Botulinumtoxinpräparation. Aktuelle Neurologie 2006;33(3):138–41.
5. Dressler D. Complete secondary botulinum toxin therapy failure in blepharospasm. J Neurol 2000;247(10):809–10.
6. Carruthers A, Carruthers J. Botulinum toxin products overview. Skin Therapy Lett 2008;13(6):1–4.
7. Pickett A, Panjwani N, O'Keeffe R. Potency of type A botulinum toxin preparaions in clinical use. 40th annual meeting of the interagency botulinum research coordinating committee (IBRCC). Atlanta, USA; 2003.
8. Zhang L, Lin W-J, Li S, Aoki KR. Complete DNA sequences of the botulinum neurotoxin complex of Clostridium botulinum type A-Hall (Allergan) strain. Gene 2003;315:21–32.
9. Wenzel R, Jones D, Borrego JA. Comparing two botulinum toxin type A formulations using manufacturers' product summaries. J Clin Pharm Ther 2007;32(4):387–402.
10. Frevert J, Dressler D. Complexing proteins in botulinum toxin type A drugs: a help or a hindrance? Biologics 2010;4:325–32.
11. Trindade de Almeida AR, Marques E, De Almeida J, Cunha T, et al. Pilot study comparing the diffusion of two formulations of botulinum toxin type A in patients with forehead hyperhidrosis. Dermatol Surg 2007 ;33(1 Spec No.):S37–43.
12. Lietzow MA, Gielow ET, Le D, Zhang J, et al. Subunit stoichiometry of the Clostridium botulinum type A neurotoxin complex determined using denaturing capillary electrophoresis. Protein J 2008;27(7–8):420–5.
13. Xeomin (SPC) Drugs.com [Internet]. Available from: http://www.drugs.com/uk/xeomin-spc-8243.html cited June 28, 2011.
14. Bigalke H, Wohlfarth K, Irmer A, Dengler R. Botulinum A toxin: Dysport improvement of biological availability. Exp Neurol 2001;168(1):162–70.
15. Sesardic D, Leung T, Gaines Das R. Role for standards in assays of botulinum toxins: international collaborative study of three

preparations of botulinum type A toxin. Biologicals 2003;31 (4):265–76.

16. Pearce LB, Borodic GE, First ER, MacCallum RD. Measurement of botulinum toxin activity: evaluation of the lethality assay. Toxicol Appl Pharmacol 1994;128(1):69–77.
17. Sakamoto T, Torii Y, Takahashi M, Ishida S, et al. Quantitative determination of the biological activity of botulinum toxin type A by measuring the compound muscle action potential (CMAP) in rats. Toxicon 2009;54(6):857–61.
18. Torii Y, Goto Y, Takahashi M, Ishida S, et al. Quantitative determination of biological activity of botulinum toxins utilizing compound muscle action potentials (CMAP), and comparison of neuromuscular transmission blockage and muscle flaccidity among toxins. Toxicon 2010;3:407–14.
19. Aoki KR. A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice. Toxicon 2001;39 (12):1815–20.
20. Aoki KR. Botulinum neurotoxin serotypes A and B preparations have different safety margins in preclinical models of muscle weakening efficacy and systemic safety. Toxicon 2002;40(7): 923–928.
21. Stone AV, Ma J, Whitlock PW, Koman LA, et al. Effects of Botox and Neuronox on muscle force generation in mice. J Orthop Res 2007;25(12):1658–64.
22. Brashear A. Clinical comparisons of botulinum neurotoxin formulations. Neurologist 2008;14(5):289–98.
23. Sampaio C, Costa J, Ferreira JJ. Clinical comparability of marketed formulations of botulinum toxin. Mov Disord 2004;19 (Suppl 8):S129–36.
24. Jost WH, Kohl A, Brinkmann S, Comes G. Efficacy and tolerability of a botulinum toxin type A free of complexing proteins (NT 201) compared with commercially available botulinum toxin type A (BOTOX) in healthy volunteers. J Neural Transm 2005;112(7):905–13.
25. Hunt T, Clarke K. Potency evaluation of a formulated drug product containing 150-kd botulinum neurotoxin type A. Clin Neuropharmacol 2009;32(1):28–31.
26. Wohlfarth K, Müller C, Sassin I, Comes G, et al. Neurophysiological double-blind trial of a botulinum neurotoxin type a free of complexing proteins. Clin Neuropharmacol 2007;30(2):86–94.
27. Yoon JS, Kim JC, Lee SY. Double-blind, randomized, comparative study of Meditoxin versus Botox in the treatment of essential blepharospasm. Korean J Ophthalmol 2009;23(3):137–41.
28. Kim K, Shin H-I, Kwon BS, Kim SJ, et al. Neuronox versus BOTOX for spastic equinus gait in children with cerebral palsy: a randomized, double-blinded, controlled multicentre clinical trial. Dev Med Child Neurol 2011;53(3):239–44.

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ORIGINAL ARTICLE

Efficacy and Safety of a Novel Botulinum Toxin Type A Product for the Treatment of Moderate to Severe Glabellar Lines: A Randomized, Double-Blind, Active-Controlled Multicenter Study

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BACKGROUND A new botulinum toxin type A (NBoNT) produced from the same strain of *Clostridium botulinum* as onabotulinumtoxinA (OBoNT) is widely used in Asia.

OBJECTIVES To compare the efficacy and safety of NBoNT and OBoNT for moderate to severe glabellar wrinkles.

METHODS A double-blind, randomized, active-controlled, phase III study was performed. Three hundred fourteen patients were randomized at a 1:1 ratio to receive 20 U of toxin. The primary end point was the responder rate according to investigator live assessment at maximum frown at week 4. Secondary end points were responder rates according to investigator live assessment with frowning and at rest at weeks 8, 12, and 16, with additional photographic assessment by a panel of blinded raters 4 weeks after injection. Subjective satisfaction scores were also evaluated.

RESULTS Four weeks after treatment, responder rates for maximum frown were 93.7% (133/142) in the NBoNT group and 94.5% (138/146) in the OBoNT group. For secondary end points, there was no significant difference between the two groups for any variable at any time point. Noninferiority of NBoNT was confirmed. There were no serious adverse effects with either toxin.

CONCLUSION NBoNT is equally as effective as OBoNT for the treatment of glabellar frown lines. Both toxins were well tolerated.

This study was sponsored by Medytox Inc. Dr. Woo Shun Lee was an employee of Medytox Inc., Korea.

The cosmetic use of botulinum toxin type A (BTX-A) for the treatment of dynamic facial wrinkles has increased dramatically over the past 2 decades, particularly in the upper face.[1–3] Five botulinum toxin type A (onabotulinumtoxinA (OBoNT), Botox, Allergan Inc., Irvine, CA; abobotulinumtoxinA (ABoNT), Dysport, Ipsen Inc./Medicis Inc., Basking Ridge, NJ; Xeomin, Merz Pharmaceuticals, Frankfurt am Main, Germany; Neuronox (NBoNT), Medytox Inc., Ochang, Korea; BTXA, Lanzhou Biological Product Institute, Hong Kong, China) and one botulinum toxin type B (Myobloc, Solstice, Louisville, KY) preparation are being marketed worldwide, with several others being developed.[4,5] There has been controversy regarding interchangeability between OBoNT and ABoNT, the prototypical BTX-A products, because they have different characteristics.[6,7]

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NBoNT (Neuronox Botulift/Siax/Meditoxin; Medytox Inc.) is a novel product that is produced from the same strain of *Clostridium botulinum* as OBoNT. Both are composed of 100 U of botulinum toxin, 0.5 mg of human serum albumin, and 0.9 mg of sodium chloride, which allows physicians to use both products in a similar manner. NBoNT was first approved in Korea for blepharospasm in 2006 and has since been approved in 22 countries and become popular in Asia. The noninferiority of NBoNT to OBoNT at a 1:1 dose ratio has been proven in phase III clinical studies for essential blepharospasm and focal spasticity in cerebral palsy.[8,9]

We sought to demonstrate the noninferiority of NBoNT to OBoNT at a 1:1 dose ratio for the treatment of moderate to severe glabellar lines.

Methods

This was a prospective, multicenter, randomized, double-blind, parallel, active-controlled, local phase III clinical trial of the efficacy and safety of NBoNT performed in six centers in South Korea. We adhered to the guidelines of 1975 Declaration of Helsinki, and the Institutional Review Board and Ethnics Committee of each center approved the study (http://www.clinicaltrials.gov number NCT01237977). Informed consent was obtained from each patient before all procedures.

Subjects

Eligible subjects were men and women aged 20 to 65 with moderate to severe glabellar frown lines at maximum frown (severity score of 2 or 3 on the Facial Wrinkle Scale (FWS), Table 1). Exclusion criteria included any medical condition (e.g.,

TABLE 1. Clinical Outcome Measures: Rating Scales and Definitions

Measure	*Scale*	*Definition*
Facial Wrinkle Scale, maximal frown	3	Severe; lines appear clearly formed. The bottoms of the deepest lines are not visible from the surface
	2	Moderate; lines appear clearly formed. The bottoms of the deepest lines are visible from the surface
	1	Mild; lines noted
	0	None; lines not noted
Facial Wrinkle Scale, rest	3	Severe; lines readily apparent
	2	Moderate; lines noticeable
	1	Mild; lines somewhat noticeable
	0	None; lines not noticeable
Subject improvement assessment	+4	Complete improvement (~100% improvement)
	+3	Marked improvement (~ 75% improvement)
	+2	Moderate improvement (~50% improvement)
	+1	Slight improvement (some improvement, 25% improvement)
	0	Unchanged
	−1	Slight worsening (~25% worse)
	−2	Moderate worsening (~50% worse)
	−3	Marked worsening (~75% worse)
	−4	Very marked worsening (~100% worse)
Subject satisfaction	7	Very satisfied
	6	Satisfied
	5	Somewhat satisfied
	4	Indifferent
	3	Somewhat dissatisfied
	2	Dissatisfied
	1	Very dissatisfied

myasthenia gravis, Lambert-Eaton syndrome, amyotrophic lateral sclerosis) that might have put the patient at risk with botulinum toxin, prior use of medications that might affect the neuromuscular junction (e.g., muscle relaxants, spectinomycin hydrochloric acid, aminoglycosides, polypeptide antibiotics, anticholinergics, benzodiazepines), any allergies or hypersensitivity to the investigational drugs or their components, previous treatment with botulinum toxin within 3 months, other procedures that might affect glabellar and forehead lines within 6 months, or any history of glabellar treatment (including forehead) such as a face lift and/or permanent implants or scars that might affect the treatment results. Patients whose glabellar lines could not be satisfactorily improved with manual pressure were also excluded. Patients were not eligible if they had dermatologic disorders or infection at potential injection sites or a history of facial nerve paralysis or ptosis. Pregnant or lactating women were excluded

Study Procedures and Treatment

After confirmation of eligibility, patients were randomized into two groups at a 1:1 ratio and treated at visit 1 (week 0, baseline). Each patient received a total dose of 20 U (4 U/0.1 mL) of NBoNT or OBoNT in a double-blind manner. The 0.5-mL total injection volume was divided into five injections: 0.1 mL (4 U) in the procerus, 0.1 mL (4 U) in each medial corrugator, and 0.1 mL (4 U) in the middle of each corrugator (Figure 1).

During the 16-week observation period, patients were assessed every 4 weeks. At each visit, the investigator and the patient assessed efficacy and safety, and standardized digital photographs of the treated facial area were taken using the same setting and equipment (EOS-350D; Canon Inc., Tokyo, Japan) to ensure reproducibility. Three blinded raters assessed the photographs according to the FWS.

Efficacy Measures

Physicians assessed the glabellar line severity using the FWS. Subjects assessed the change in line severity on a 9-point scale and rated their degree of satisfaction with the treatment on a 7-point scale (Table 1). The primary end point was the responder rate at maximum frown at week 4 based on investigator live assessment (face-to-face observation). Secondary end points were responder rate at maximum frown at weeks 8, 12, and 16; responder rate of glabellar lines at rest based on investigator live assessment at weeks 4, 8, 12, and 16; and responder rate at maximum frown and at rest based on photographic assessment at week 4. In accordance with previous studies of OBoNT, responders were defined as having a post-treatment score of 0 or 1 and a pretreatment score of 2 or 3.[10,13–15] This means an improvement of at least 1 point in patients with moderate wrinkles and at least 2 points in those with severe wrinkles. In addition, we included the glabellar line improvement rates determined according to subjects' own

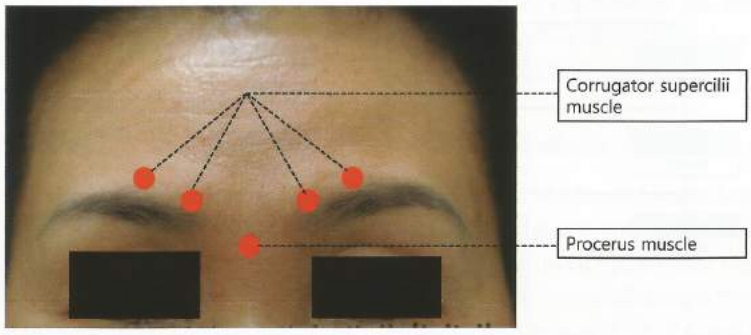

Figure 1. Treatment injection sites.

assessment and satisfaction rates as secondary end points. Scores more than 2 points higher (moderately improved) were considered to be improvement, and scores more than 6 points higher (satisfied) were considered to be satisfaction.

Safety Measures

Adverse events (AEs) were documented based on investigator- and subject-reported signs and symptoms, physical examination, and laboratory tests. BTX-A antibody testing was performed in 100 subjects in two of six study centers (Asan Medical Center and Seoul National University Bundang Hospital) at weeks 0 (visit 1) and 16 (visit 5) using a mouse bioassay.

Statistical Methods

All randomized and treated subjects with data for primary end points were included in the full analysis set (FAS). The per protocol (PP) set was the subset of patients of the FAS that did not commit any major protocol violations.

For the primary end-point parameter, we calculated the lower limit of the 97.5% one-sided confidential interval (CI) for the difference in responder rates between two groups. The interpretation of the CI was based on the null hypothesis that the expected difference in responder rates between the treatment groups was lower than the noninferiority margin of −15%. If the lower bound of the estimated CI exceeded the limit of −15%, one could conclude that the NBoNT was not inferior to OBoNT. This confirmatory analysis was based on the PP analysis. For secondary end points, paired *t*-tests, Pearson chi-square tests, or Fisher exact tests were performed. Safety analysis was based on a safety evaluation set that included all patients who received a study drug.

Results

Two hundred ninety-one of 314 patients enrolled completed the study without major deviation and therefore constituted the PP set: 142 in the NBoNT group and 146 in the OBoNT group (Figure 2). Demographic characteristics of the two

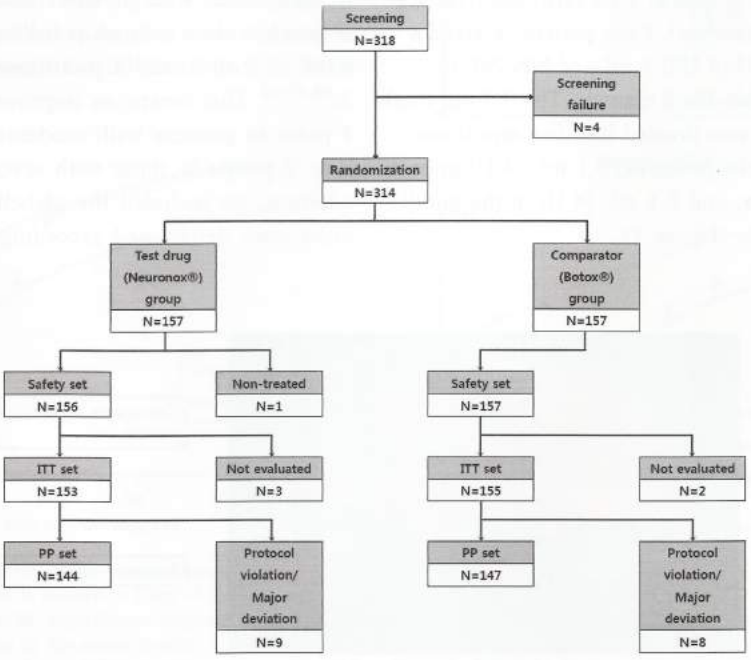

Figure 2. Disposition of patients.

groups were comparable, and the groups did not differ in their pretreatment line severity at rest or maximum frown. The majority of patients had moderate to severe glabellar frown lines at rest (54.9% NBoNT group, 56.1% OBoNT group) and severe glabellar frown lines at maximum frown (53.5% NBoNT group; 54.8% OBoNT group) (Table 2).

Investigator Assessment

Both groups had significant improvement of glabellar lines (Figure 3). Four weeks after injection, the responder rate at maximum frown for the PP set was 93.7% (133/142) in the NBoNT group and 94.5% (138/146) in the OBoNT group. In addition, the responder rate of the FAS (94.1%) was similar to that of the PP set (94.8%). The 95% CIs for the difference in responder rates between the two treatment groups (−6.3 to 4.6% for PP; −5.8 to 4.4% for FAS) clearly supported the hypothesis that NBoNT was not inferior to OBoNT, because the lower limit of the 97.5% one-sided CI (−6.3 for PP; −5.8 for FAS) exceeded the predefined noninferiority margin of −15%. There was no statistically significant difference between responder rates of the two groups at week 4 in the PP set or the FAS ($p = .77$). Responder rates remained high in both groups at weeks 8 and 12 (85.1% and 75.0% NBoNT group, 86.9% and 70.8% OBoNT group) and decreased to 46.0% for the NBoNT group and 48.3% for the OBoNT group at week 16. There was no statistically significant difference in the responder rate between the groups at any time point ($p = .42$) (Figure 4A).

TABLE 2. Patient Demographic and Baseline Characteristics (Per Protocol Set)

Characteristic	*New Botulinum Toxin Type A*	*Onabotuli-numtoxinA*
Demographic	$n = 157$	$n = 157$
Age		
Mean ± standard deviation, median (range)	48 ± 8.8, 49 (25–64)	47 ± 8.8, 48 (27–64)
<50, *n* (%)	79 (50.3)	85 (54.1)
≥50, *n* (%)	78 (49.7)	72 (45.9)
Sex, *n* (%)		
Male	22 (14.0)	33 (21.0)
Female	135 (86.0)	124 (79.0)
Previous botulinum toxin exposure, *n* (%)		
Naïve	146 (93.0)	142 (90.4)
Not naïve	11 (7.0)	15 (9.6)
Baseline	$n = 142$	$n = 146$
Facial Wrinkle Scale score, *n* (%)*		
At rest		
None	8 (5.5)	16 (10.9)
Mild	58 (40.3)	48 (32.6)
Moderate	38 (26.4)	46 (31.3)
Severe	40 (27.8)	37 (25.2)
At maximum frown		
None	0 (0.0)	0 (0.0)
Mild	0 (0.0)	0 (0.0)
Moderate	66 (47.5)	66 (45.2)
Severe	76 (53.5)	80 (54.8)

*Glabellar frown lines according to investigator live assessment (per protocol set).

The responder rates at rest based on investigator live assessment were lower than those at maximum frown at all time points, with no intergroup differences (41.6%, 44.0%, 42.9%, and 40.3% for the NBoNT group; 45.2%, 45.5%, 43.8%, and 38.6% for the OBoNT group at weeks 4, 8, 12, and 16, respectively, $p = .53$). Because of the larger proportion of subjects with baseline resting scores of 0 or 1, a subgroup analysis of subjects with baseline scores of 2 or 3 was performed. This comprised 78 subjects in the NBoNT group and 82 in the OBoNT group. In this analysis, responder rates were lower than those at maximum frown at weeks 4 and 8 but higher at weeks 12 and 16 (Figure 4B).

In the blinded rater photographic assessment, similar results were observed for both groups (Table 3). Responder rates at maximum frown were higher than in a resting state, and there was no significant difference between two groups ($p = .39$). The investigators observed higher responder rates during direct encounters than during indirect photographic assessment for both treatment groups at rest and at maximum frown (Figure 4, Table 3).

Figure 3. Representative clinical photographs showing patients at maximum frown: (A) patient treated with new botulinum toxin type A, (B) patient treated with onabotulinumtoxinA. Standardized photographs of two patients at maximal frown, at baseline (left) and 4 (center) and 16 (right) weeks after treatment. The dramatic lessening of glabellar lines for all patients at week 4 should be noted. By week 16, the glabellar lines have begun to reappear but are still less severe than at baseline.

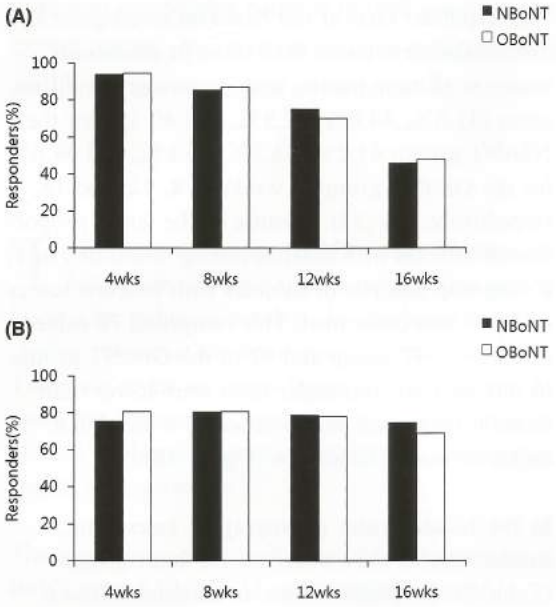

Figure 4. Percentage of responder based on physician's live assessment for the per protocol set. (A) At maximum frown, responders were 93.7%, 85.1%, 75.0%, and 46% for the new botulinum toxin type A (NBoNT) group and 94.5%, 86.9%, 70.8%, and 48.3% for the onabotulinumtoxinA (OBoNT) group, at weeks 4, 8, 12, and 16, respectively; (B) At rest, responders were 75.6%, 80.5%, 79.0%, and 74.7% for the NBoNT group and 80.5%, 80.5%, 77.8%, and 68.3% for OBoNT group at weeks 4, 8, 12, and 16, respectively.

TABLE 3. Investigator Photograph Assessment at Week 4 for Per Protocol Set

	NBoNT n = 142	*OBoNT* n = 146	p-*Value**
Rest			
Responder	42 (29.2)	36 (24.7)	.56
Nonresponder	102 (70.8)	110 (75.3)	
Maximum frown			
Responder	103 (73.6)	113 (77.9)	.39
Nonresponder	37 (26.4)	32 (22.1)	

Number of missing values: 1 in onabotulinumtoxinA (OBoNT) group at rest, 2 in new botulinum toxin type A (NBoNT) group, and 1 in OBoNT group at maximum frown.
*Pearson chi-square test.

Subject Assessment

Subject assessment of improvement of glabellar lines and satisfaction (Table 1) yielded comparable results for both groups. Peak improvement rate, defined as the proportion of patients who scored more than 2 points more (moderately improved) were 86.5% (122/142 patients) for the NBoNT group and 90.3% (131/146) for the OBoNT group at week 8 (Figure 5A) and afterward gradually decreased to 71.2% and 66.9% by week 16. Subjective satisfaction reached its peak at week 4

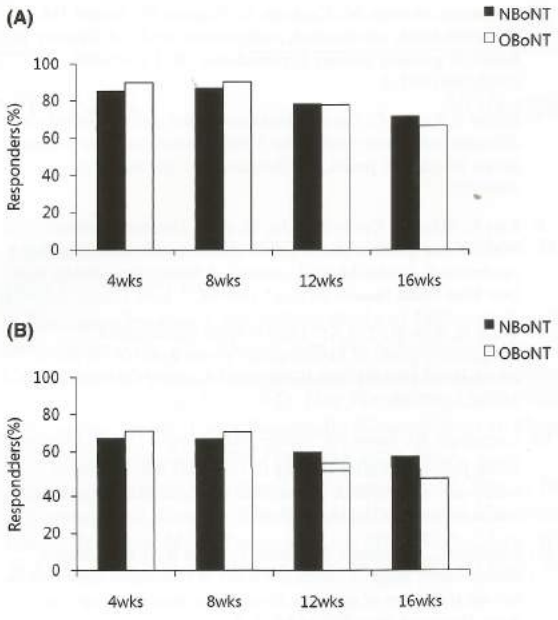

Figure 5. Subject improvement assessment and satisfaction for per protocol set. (A) According to subject assessment, improvement rates were 85.2%, 86.5%, 79.3%, and 71.2% for the new botulinum toxin type A (NBoNT) group and 90.4%, 90.3%, 77.8%, and 66.9% for the onabotulinumtoxinA (OBoNT) group at weeks 4, 8, 12, and 16, respectively. (B) Subject satisfaction rates were 67.6%, 67.4%, 60.0%, and 56.8% for the NBoNT group and 70.6%, 70.3%, 53.5%, and 49.7% for the OBoNT group at weeks 4, 8, 12, and 16, respectively.

and gradually decreased over weeks 8, 12, and 16 (Figure 5B). There was no statistically significant difference between the two groups for any secondary end point at any point in time.

Safety

Three hundred thirteen subjects were evaluated: 156 for the NBoNT group and 157 for the OBoNT group. Overall incidence of AEs was 26.9% in the NBoNT group and 22.3% in the OBoNT group. There were two cases of serious AEs (1.3%) in the NBoNT group; one was a case of gastroenteritis, and the other was of acute pyelonephritis. Both cases were considered not to be related to the treatment. The incidence of AEs from which the causal relationship with treatment could not be excluded was 10.9% (17/156) in the NBoNT group and 7.6% (12/157) in the OBoNT group. All cases were mild. The common (>1%) treatment-related AEs were eyelid ptosis (5/156, 3.2% NBoNT group; 3/157, 1.9% OBoNT group) and extraocular muscle disorder (1/156, 0.6% NBoNT group; 4/157, 2.6% OBoNT group). All other related AEs had a total incidence of <1%. There were no treatment-related AEs resulting in discontinuation of the study in either group and no statistically significant difference in the incidence and severity of AEs between the two groups. No patient from two selected centers developed neutralizing BTX-A antibodies during the course of study.

Discussion

This study was designed to compare the efficacy and safety of NBoNT with that of OBoNT, which has the largest world market share. Both agents led to clinical improvement, and there was no significant difference between the two groups in any variable at any point. The noninferiority of NBoNT to OBoNT was confirmed in the responder rate at 4 weeks at maximum frown. These results suggest that NBoNT and OBoNT are equally efficacious, as shown in previous clinical trials using a 1:1 dose ratio.[8,9]

OBoNT has been studied in large worldwide clinical trials since the Food and Drug Administration approved it for glabellar lines in 2002. Previous studies using 20 U of OBoNT have suggested the possibility of differences in response between ethnic groups. The responder rates for maximum frown at week 4 were 76.7% and 83.7% in pivotal studies for OBoNT conducted in the United States, whereas responder rates were 88.6%, 95.1%, and 94.1% in similar studies in China and Japan.[2,10–15] Our results of 93.8% and 94.6% are comparable with those of studies conducted in Asia, which may reflect the difference in muscle mass or frowning habits between Asian and Western individuals. The mean age of our cohort was comparable with those of studies done in the United States (48.2 and 47.5 in our study vs 44.7 and 47.7 in the United States), with more severe baseline wrinkles (53.5% and 55.8% vs 33.5% and 46.0%).

The primary end point was responder rate at week 4 based on face-to-face direct assessment for maxi-

mum frown, and we added photograph-based evaluations to provide additional objectivity. The investigators observed higher responder rates during direct encounters than indirect photographic assessment, which has been demonstrated in other studies.[2,12–14] Direct face-to-face assessment has advantages over photographic assessment in that the assessor is able to evaluate how much effort the participant makes in attempting to frown; at times, photographs failed to capture maximum frown.

Limitations of our study include that the majority of the patients were female and that all subjects were Korean, which may not represent a broader patient population. The total duration of the study was 16 weeks, without any long-term data.

In terms of safety, ptosis was the most common treatment-related AE. In recent large-scale clinical studies, the incidence of ptosis with BTX-A was reported to range from 0.7% to 3%.[16] According to the recent meta-analysis of the safety of OBoNT, eyelid ptosis was reported in 3.6% of OBoNT-treated patients.[17] Our study result is in accordance with previous literature.

In conclusion, NBoNT is as effective and safe as OBoNT in the treatment of moderate to severe glabellar lines over a period of at least 16 weeks.

References

1. Carruthers A, Carruthers J. Botulinum toxin type A: history and current cosmetic use in the upper face. Semin Cutan Med Surg 2001;20:71–84.
2. Carruthers JA, Lowe NJ, Menter MA, Gibson J, et al. A multicenter, double-blind, randomized, placebo-controlled study of the efficacy and safety of botulinum toxin type A in the treatment of glabellar lines. J Am Acad Dermatol 2002;46:840–9.
3. Lowe NJ, Ascher B, Heckmann M, Kumar C, et al. Double-blind, randomized, placebo-controlled, dose-response study of the safety and efficacy of botulinum toxin type A in subjects with crow's feet. Dermatol Surg 2005;31:257–62.
4. Pickett A, Perrow K. Formulation composition of botulinum toxins in clinical use. J Drugs Dermatol 2010;9:1085–91.
5. Klein AW, Carruthers A, Fagien S, Lowe NJ. Comparisons among botulinum toxins: an evidence-based review. Plast Reconstr Surg 2008;121:413e–22e.
6. Simonetta Moreau M, Cauhepe C, Magues JP, Senard JM. A double-blind, randomized, comparative study of Dysport vs. Botox in primary palmar hyperhidrosis. Br J Dermatol 2003;149:1041–5.
7. 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. Kim K, Shin HI, Kwon BS, Kim SJ, et al. Neuronox versus BOTOX for spastic equinus gait in children with cerebral palsy: a randomized, double-blinded, controlled multicentre clinical trial. Dev Med Child Neurol 2011;53:239–44.
9. Yoon JS, Kim JC, Lee SY. Double-blind, randomized, comparative study of Meditoxin(R) versus Botox(R) in the treatment of essential blepharospasm. Korean J Ophthalmol 2009;23:137–41.
10. Carruthers JD, Lowe NJ, Menter MA, Gibson J, et al. Double-blind, placebo-controlled study of the safety and efficacy of botulinum toxin type A for patients with glabellar lines. Plast Reconstr Surg 2003;112:1089–98.
11. Monheit G, Carruthers A, Brandt F, Rand R. A randomized, double-blind, placebo-controlled study of botulinum toxin type A for the treatment of glabellar lines: determination of optimal dose. Dermatol Surg 2007;33:S51–9.
12. Ascher B, Zakine B, Kestemont P, Baspeyras M, et al. 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.
13. Harii K, Kawashima M. A double-blind, randomized, placebo-controlled, two-dose comparative study of botulinum toxin type A for treating glabellar lines in Japanese subjects. Aesthetic Plast Surg 2008;32:724–30.
14. Wu Y, Zhao G, Li H, Zheng Z, et al. Botulinum toxin type A for the treatment of glabellar lines in Chinese: a double-blind, randomized, placebo-controlled study. Dermatol Surg 2010;36:102–8.
15. Kawashima M, Harii K. An open-label, randomized, 64-week study repeating 10- and 20-U doses of botulinum toxin type A for treatment of glabellar lines in Japanese subjects. Int J Dermatol 2009;48:768–76.
16. Rzany B, Ascher B, Monheit GD. Treatment of glabellar lines with botulinum toxin type A (Speywood Unit): a clinical overview. J Eur Acad Dermatol Venereol 2010;24:1–14.
17. Brin MF, Boodhoo TI, Pogoda JM, James LM, et al. Safety and tolerability of onabotulinumtoxinA in the treatment of facial lines: a meta-analysis of individual patient data from global clinical registration studies in 1678 participants. J Am Acad Dermatol 2009;61:961–70.

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4 Articles about Neuronox® in Dermatologic Surgery

Introducing A Novel Botulinum Toxin Preparation

Volume 39, Number 1 Part II, January, 2013

Article I. A Pharmacodynamic Comparison Study of Different Botulinum Toxin Type A Preparations

• AUTHORS

*SUNG-BEOM KIM, MS, BHUPAL BAN, PHD, KEUN-SUK JUNG, BA, AND GI-HYEOK YANG, PHD**

CONCLUSION

Neu-BoNT/A(Neuronox®) and ona-BoNT/A may be interchangeable based on a simple dose ratio.

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Article II. Comparative Study of Biological Activity of Four Botulinum Toxin Type A Preparations in Mice

• AUTHORS

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CONCLUSION

The dose-conversion ratio between onabotulinumtoxinA and abobotulinumtoxinA was determined to be 1:2.6; previous reports of 1:3 were considered too high. A dose-conversion ratio between onabotulinumtoxinA and new botulinum toxin (Neuronox®) of 1:1 was deemed appropriate. OnabotulinumtoxinA and incobotulinumtoxinA demonstrated a dose-conversion ratio of 1:1.07. The efficacy of incobotulinumtoxinA was slightly lower than that of onabotulinumtoxinA. These dose-conversion ratios are applicable solely from an efficacy standpoint and not for safety. This study was conducted in mice, so it may not translate perfectly to human applications.

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Article III. Efficacy and Safety of a Novel Botulinum Toxin Type A Product for the Treatment of Moderate to Severe Glabellar Lines: A Randomized, Double-Blind, Active-Controlled Multicenter Study

• AUTHORS

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CONCLUSION

NBoNT(Neuronox®) is equally as effective as OBoNT for the treatment of glabellar frown lines. Both toxins were well tolerated.

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Article IV. A New Botulinum Toxin Potentially Bioequivalent to OnabotulinumtoxinA: Are There Any Differences at All?

• AUTHORS

*GI-HYEOK YANG, PHD, AND HYUN HO JUNG, PHD**

CONCLUSION

More than five botulinum toxin type A preparations are available worldwide. OnabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA have received FDA approval for therapeutic and cosmetic indications. Although more extensive research with regard to long-term safety and additional comparative clinical studies for various indications are required, neu-BoNT/A(Neuronox®) seems to be similar to onabotulinumtoxinA based on preliminary studies in several different areas (ophthalmology, neurology, and aesthetics) and can be expected to be a competitor in markets where the two products compete.

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