Comparison of botulinum neurotoxin type A formulations in Asia

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BACKGROUND

- All protein-based products, such as botulinum neurotoxin type A, are potentially immunogenic and can lead to diminished or complete absence of efficacy, especially if administered repeatedly.
- As such, the protein content in botulinum neurotoxin formulations is an important consideration when selecting a product for treatment.
- Because formulation data are not always widely accessible, this study analyzed the neurotoxin composition of new toxin formulations that have recently emerged in Asia relative to incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH).

METHODS

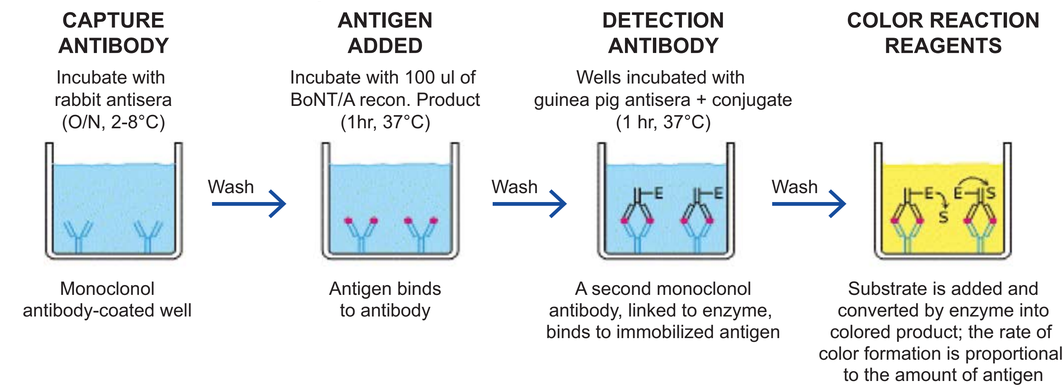
- The products analyzed and their properties are described in **Table 1**.
- The different protein and neurotoxin contents of the products were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) with antisera (**Figure 1**).
- Because the manufacturing process for a biologic therapeutic should be consistent and every batch representative, the batches were selected arbitrarily.
- Care was taken to transport and store the samples at 2°C–8°C, except samples of incobotulinumtoxinA which can be stored at room temperature.
  - The composition of the neurotoxin elements of each product compared to those of incobotulinumtoxinA was then analyzed in duplicate to determine the mean amount of neurotoxin protein.
- All analyses were carried out with an ELISA approved by the FDA (Food and Drug Administration, Silver Spring, MD, USA).
- For incubation, phosphate buffered saline + 0.1% bovine serum albumin (solution 1) and PBS + 6% gelafusal (Serumwerke Bernburg, Bernburg, Germany; solution 2) (Merck, Darmstadt, Germany, or Riedel-de-Haen, Seelze, Germany) were used. - Additional reagents included O-phenylenediamine dihydrochloride (Sigma-Aldrich Corp, St. Louis, MO, USA) and horse anti-serum reacting with the neurotoxin complex of BoNT/A (UK National Institute for Biological Standards and Control, NIBSC).
- Following a modified protocol, the 150 kDa neurotoxin purified from the "Hall Strain", *C. botulinum* type A, was confirmed by western blot as complexing protein-free and detoxified by 0.4% formaldehyde treatment to produce the nontoxic antigen for antibody preparation.
  - Complexing proteins (excluding the botulinum neurotoxin protein) were prepared as previously published.
- The purified toxin was dialyzed against 50 mM TRIS (tris[hydroxymethyl] aminomethane)/HCl pH = 7.9, Q-sepharose column chromatography-purified (GE Healthcare, Munich, Germany) and column-bound complexing proteins were eluted.
  - Antibodies against BoNT/A were immobilized on a CNBr sepharose matrix (GE Healthcare).
  - BoNT/A was removed through affinity chromatography, eluted, and its composition checked for integrity.

Table 1. Properties of botulinum neurotoxin type A products analyzed in this study

Product name	Innotox®	Botulax®	Meditoxin®/ Neuronox®	Nabota®	Relatox®	Xeomin®
Manufacturer	Medytox	Hugel Inc.	Medytox Inc.	Daewong	Microgen	Merz
Dosage (U)	25	100	100	100	100	100
Composition	Complex	Complex	Complex	Complex (900 kDa)	Complex (900 kDa)	Purified toxin (150 kDa)
Appearance	Liquid	Lyophilizate	Lyophilizate	Lyophilizate	Lyophilizate	Lyophilizate
Formulation	Polysorbate (no HSA)	0.5 mg HSA; 0.9 mg NaCl	0.5 mg HSA; 0.9 mg NaCl	0.5 mg HSA; 0.9 mg NaCl	6 mg gelatine; 12 mg maltose	4.7 mg sucrose; 1 mg HSA
Storage	2ºC–8ºC	2ºC–8ºC	2ºC–8ºC	2ºC–8ºC	2ºC–8ºC	Room temperature (20ºC–25ºC)
Clostridial protein per 100 U (pg)	N/A	5,000[12]	N/A	N/A	N/A	416

Notes: In other countries, Meditoxin is sold as Neuronox. As polysorbate prevents accurate ELISA readings, Innotox was not reported further in our work.
Abbreviations: HSA, human serum albumin; N/A, information not publicly available.

Figure 1. Sandwich ELISA Technique



RESULTS

- Highly sensitive sandwich ELISA was used to quantify the amount of BoNT/A protein in Botulax, Meditoxin, Nabota, and Relatox (**Table 2**).
- IncobotulinumtoxinA was independently analyzed in parallel as a control and found to have a mean toxin content of 416 pg/vial, comparable to reports from another batch.
  - It should be noted that this variation from published values is due to these batches of toxin being no longer available for the present analysis and the use of a different batch of incobotulinumtoxinA, as well as a 5% inter-vial variability during the manufacturing process (unpublished data 2018, Merz Pharmaceuticals GmbH).

Table 2. Determination of content of botulinum neurotoxin type A protein in products by ELISA

Product name	Batch name	Dosage (lyophilized)	Amount of neurotoxin protein per 100 units (pg)	Specific potency (U/pg neurotoxin)	Calculated proportion (%) of inactive neurotoxin
Botulax®	HUA 15133	100 U/vial	844 ± 43‡	0.118	103
Meditoxin®/Neuronox®	FAA 1587	100 U/vial	575 ± 6	0.174	38
Nabota®	084962	100 U/vial	754 ± 11‡	0.133	81
Relatox®	0615	100 U/vial	578 ± 48	0.173	33
Xeomin®	31149	100 U/vial	416 ± 6	0.240	No inactive neurotoxin found

Notes: Innotox® (not reported in this table) contains the surfactant polysorbate,25 which can interfere with antibody–antigen binding during ELISA and lead to inaccurate and low concentrations. Innotox's toxin content, therefore, could not be accurately measured using standard ELISA, which is validated for experimental conditions without polysorbate. *Calculation based on claim that Xeomin contains only the active neurotoxin (=100%); ‡Value above standard curve.

- Botulax and Nabota contained 844 and 754 pg of neurotoxin protein, respectively; and the percentage of inactive neurotoxin was calculated to be 103 and 81.
- The potency per pg of neurotoxin in Botulax and Nabota was found to be 0.118 and 0.133 U, respectively. This was less potent than Xeomin's 0.240 U per pg of neurotoxin.
- Meditoxin and Relatox contained 575 and 578 pg of neurotoxin protein, respectively, which were slightly higher than that of the Xeomin, as was the calculated percentage of inactive neurotoxins at 38 and 33, respectively.
  - The potency per pg of neurotoxin protein in Meditoxin and Relatox was found to be 0.174 and 0.173U, respectively, which were also lower than Xeomin's 0.240 U per pg of neurotoxin.
- By comparison, Xeomin with no inactive neurotoxin contained 416 pg/vial of purified neurotoxin and 0.240 U of efficacy per pg of neurotoxin, yielding the lowest neurotoxin protein content and consequently the highest specific potency compared to the four Asian botulinum neurotoxin type A formulations.

DISCUSSION

- All BoNT/A formulations contain the 150 kDa neurotoxin (active molecule). Xeomin, however, consists solely of the 150 kDa neurotoxin.
  - All products are based on the botulinum toxin complex with about sixfold more additional bacterial proteins, assuming a molecular weight of ≈900 kDa for the complex.
- Some of the complexing proteins are hemagglutinins, which are glycoprotein binding proteins.
  - In contrast to the 150 kDa neurotoxin, these complexing proteins have the potential to bind to dendritic cells, the sentinel cells of the immune system.
  - These cells must be activated as the first step of the initiation of an immune response.
- Complexing protein-containing products have a higher potential to cause an immune response.
  - The formation of antibodies in patients treated with complexing protein-containing products in aesthetic medicine has been reported.
  - In contrast, antibody formation was not observed in patients treated with Xeomin free of complexing proteins.
- A further important factor determining the potential for immunogenicity of BoNT/A formulations is the amount of neurotoxin protein present. This is associated with increased antigen levels and, consequently, a greater risk of antibody production.
  - It was demonstrated in cervical dystonia patients treated with BoNT products that the specific potency (U per pg neurotoxin) is correlated with the antibody-induced therapy failure.
  - It is, therefore, helpful for the clinician to receive information about the specific potency of different botulinum toxin products.
- As Xeomin's manufacturing process isolates only the active 150 kDa neurotoxin, Xeomin is entirely free of complexing protein.
  - Xeomin remains the only BoNT/A product marketed as containing "purified neurotoxin" that has been registered with regulatory authorities in the USA and Europe.
- One can conclude that the lower specific potency of Botulax, Nabota, Meditoxin, and Relatox may actually indicate the presence of significant amounts of inactive, rather than active, neurotoxin.
  - High neurotoxin protein levels detected in this study were not due to biologically efficacious neurotoxin, but due to inactive toxin provided that all products were equipotent in containing 100 U per vial.
  - This inactive neurotoxin cannot be taken up by neurons but might represent an immunogenic impurity. These inactive components, which have no clinical efficacy, per se, may stimulate antibody production.

CONCLUSIONS

- **Although Botulax, Nabota, Meditoxin and Relatox had more neurotoxin than Xeomin in an equivalent volume, they contained greater amounts of inactive neurotoxin.**
  - **However, this inactive neurotoxin cannot be taken taken up by neurons, and hence has no clinical efficacy but might represent an immunogenic impurity leading to immunogenic stimulation and antibody production.**
- **In the future, comparative studies on the efficacy, effective duration, and safety profile of all neurotoxin products, particularly on the incidence of secondary treatment failures due to neutralizing antibody formation in patients undergoing long-term treatment with BoNT/A, will be necessary.**

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