IncobotulinumtoxinA: A Highly Purified and Precisely Manufactured Botulinum Neurotoxin Type A*

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INTRODUCTION

- Since the first botulinum neurotoxin (BoNT) formulation was approved by the US Food and Drug Administration (FDA) in 1989, the number of approved indications, and BoNT products commercially available worldwide, has increased.
- Aesthetic dermatologic applications of BoNT were the most common non-surgical cosmetic procedures in the US in 2017, and have high levels of patient satisfaction.
- The three most widely used and commercially available BoNT type A (BoNT-A) formulations are: abobotulinumtoxinA (Dysport®/Azzalure®, Ipsen Biopharm); incobotulinumtoxinA (Xeomin®/Bocouture®, Merz Pharmaceuticals GmbH); and onabotulinumtoxinA (Botox®/Vistabel®, Allergan Inc.)
- Several new BoNT-A formulations have recently been introduced in different countries; however, incobotulinumtoxinA currently remains the only BoNT formulation approved in commercial markets worldwide that was intentionally designed to contain only the required therapeutic component, the pure BoNT, free from unnecessary clostridial proteins.
- Here we discuss the role of these unnecessary proteins in BoNT-A, and the unique manufacturing and purification process for incobotulinumtoxinA, with a focus on the implications for use in aesthetic medicine.

METHODS

Role of Unnecessary Clostridial Proteins

- IncobotulinumtoxinA contains only the 150 kDa BoNT-A therapeutic component purified from the unnecessary clostridial proteins.
  - In contrast, abobotulinumtoxinA, onabotulinumtoxinA, and, the newer addition, prabotulinumtoxinA (Nabota®, Daewong Therapeutics, Korea/Evolus®, Evolus Inc., Europe, USA/Nuceiva®, Evolus Inc., Canada) all contain the HA and NTNHA proteins complexed with the 150 kDa BoNT.
  - Investigational drug candidate daxibotulinumtoxinA (RT002, Revance Therapeutics Inc.) is composed of the active 150 kDa BoNT-A with a peptide excipient, RTP-004, derived from the human immunodeficiency virus type 1 (HIV-1) transactivator of transcription (TAT) protein.
- Contrary to their role as mediator for the oral toxicity of naturally occurring BoNT, complexing proteins have no role in clinical applications.
- IncobotulinumtoxinA is the only currently approved BoNT-A formulation that can be stored unreconstituted at ambient temperatures, which may be a benefit in everyday clinical practice.

Immunogenicity

- Although unnecessary clostridial proteins play no role in clinical applications when injected,or in stabilizing the molecule they may act as adjuvants, stimulating an immune response, thus potentially altering the response to BoNT therapy.
- Two types of antibodies may be produced in response to injected BoNT:
  - Neutralizing (leading to reduced efficacy or non-response even at low doses)
  - Non-neutralizing (do not affect biological activity, but may act as adjuvants)
- As BoNT treatment effects are temporary, and repeat injections are required to maintain efficacy,clinicians should consider both the temporal extent of exposure and BoNT protein load to reduce the risk of neutralizing antibody formation and treatment non-response.
  - Previous exposure for use in aesthetics may lead to non-response if BoNT-A were required for essential therapeutic treatment (eg, of post-stroke spasticity) later in life.
- In contrast to abobotulinumtoxinA and onabotulinumtoxinA, repeated incobotulinumtoxinA treatment in rabbits did not result in the formation of neutralizing antibodies, suggesting immunogenicity is lower with incobotulinumtoxinA.
- A recent analysis of the US FDA adverse event (AE) reporting system database found the incidence of AEs involving decreased therapeutic effect was 2.2% (15/689) for incobotulinumtoxinA; 9.2% (79/858) for abobotulinumtoxinA; and 11.6% (1247/10,733) for onabotulinumtoxinA.

IncobotulinumtoxinA: Advanced Manufacturing and Purification

- IncobotulinumtoxinA is purified and precisely manufactured in a world-class German facility using advanced technology under Good Manufacturing Practice.
- The unnecessary clostridial proteins are removed in a refined process using step-wise chromatography to precisely isolate the therapeutic component (**Figure 1**), followed by lyophilization.
- The unique and precise purification process of incobotulinumtoxinA ensures that only the active 150 kDa neurotoxin, needed to achieve the clinical effect, is included.
- The specific activity of abobotulinumtoxinA, incobotulinumtoxinA, and onabotulinumtoxinA is reported as 154 U/ng, 227 U/ng, and 137 U/ng, respectively, based on the mean BoNT concentration in 100 U (0.65 ng, 0.44 ng, and 0.73 ng of the 150 kDa BoNT, respectively; **Figure 2**).
- The high specific activity of incobotulinumtoxinA is consistent with no inactivation of the BoNT during purification.
  - In comparison, as onabotulinumtoxinA is reported to contain 0.73 ng of neurotoxin protein, the low specific activity of onabotulinumtoxinA suggests that a proportion of BoNT protein is inactive.

Implications for Use in Aesthetic Medicine

- IncobotulinumtoxinA is proven to effectively reduce upper facial lines for up to 4 months post-treatment, with a high level of subject satisfaction and no treatment-related serious AEs in a Phase III trial leading to upper facial lines approval in Europe.
- The safety profile of incobotulinumtoxinA was further confirmed in a pooled analysis of 13 prospective multicenter studies in aesthetic indications of crow's feet, glabellar lines, and upper facial lines.
  - Overall, the frequency of treatment-related AEs was low and analysis of repeat-dose studies suggested the incidence of AEs may decrease with repeated treatments over time.
- In incobotulinumtoxinA clinical studies, no previously BoNT-naive subject developed neutralizing antibodies or demonstrated secondary lack of treatment response, even with doses up to 800 U in the treatment of upper-limb spasticity, consistent with the low immunogenicity of incobotulinumtoxinA.
- In the published literature, all subjects who developed neutralizing antibodies and secondary non-response after incobotulinumtoxinA treatment had received treatment with another BoNT formulation.

New Entrants to the BoNT Commercial Market

- Two new BoNT-A entrants are currently under review or planning to file FDA Biologics License Applications.
- The specific potency of prabotulinumtoxinA was recorded as 133 U/ng compared with 240 U/ng for incobotulinumtoxinA, with a high percentage of inactive neurotoxin.
  - PrabotulinumtoxinA is a similar version of onabotulinumtoxinA in terms of pharmacological development and manufacturing compared with currently approved formulations.
- DaxibotulinumtoxinA is a new BoNT-A formulation currently in clinical development for aesthetic (glabellar lines) and therapeutic (cervical dystonia and plantar fasciitis) indications.
  - DaxibotulinumtoxinA includes an excipient peptide, RTP-004, consisting of the protein transduction domain sequence from the HIV-1 TAT protein at each end.

Figure 1. Protein content of incobotulinumtoxinA.

(a) Representative SDS-polyacrylamide gel electrophoresis of the active pharmaceutical ingredient of incobotulinumtoxinA showing the heavy chain (Hc, ≈ 100kDa) and the light chain chain (Lc, ≈ 50kDa). In the native form of the neurotoxin Hc and Lc are linked by a disulfide bond, which is cleaved in this analysis. (b) Representative size exclusion chromatography of the active pharmaceutical ingredient of incobotulinumtoxinA. The neurotoxin is eluted in a volume corresponding to a molecular weight of ≈ 150kDa.

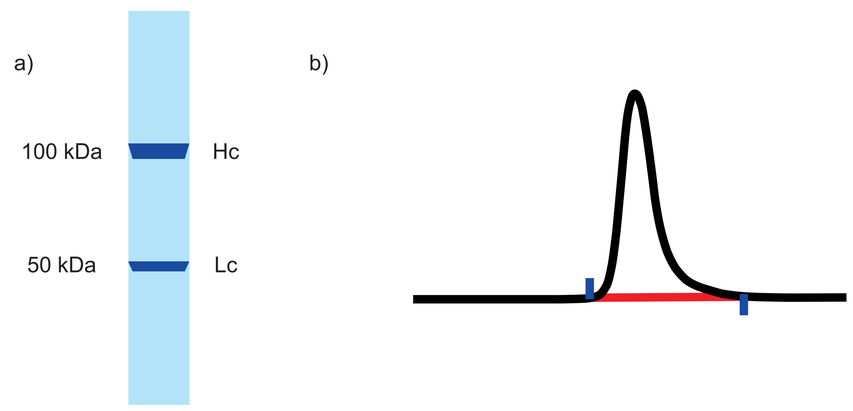
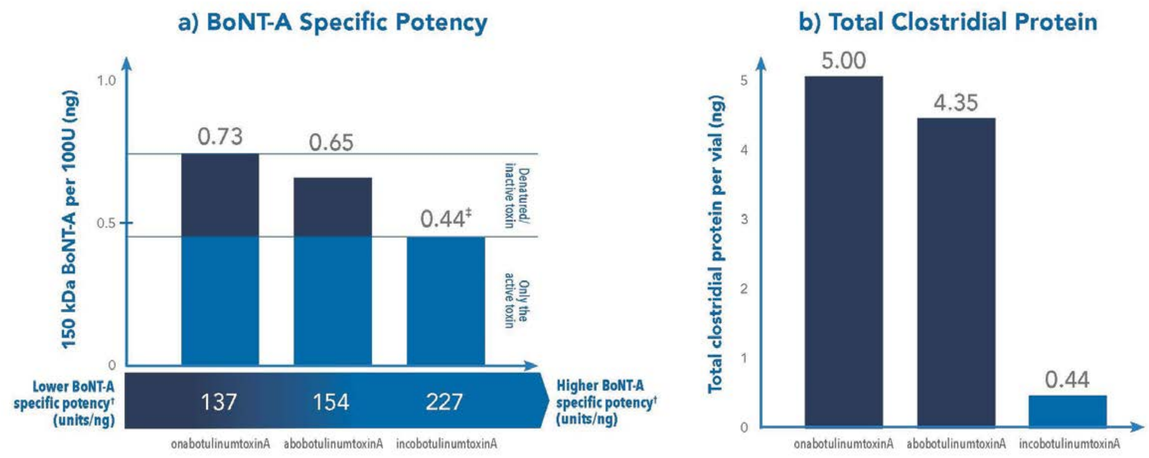


Figure 2. Specific potency and clostridial protein content of BoNT formulations.



†Unique manufacturing technology of incobotulinumtoxinA isolates the therapeutic component and has allowed for the highest specific BoNT-A potency: 100 U / 0.44 ng = 227 U/ng; ‡Lower neurotoxin protein in incobotulinumtoxinA than previously reported may be due to higher sensitivity in the ELISA assay used for analysis.
Abbreviations: BoNT-A, botulinum neurotoxin type A; ELISA, enzyme-linked immunosorbent assay; ng, nanograms; pg, picograms; U, units

CONCLUSIONS

- **The least immunogenic BoNT-A formulation to meet treatment requirements should be considered, thus minimizing potential for lack of efficacy due to neutralizing antibodies**
  - **Both neurotoxin protein load and protein load over time can increase the risk of diminished efficacy or non-response due to neutralizing antibodies over a lifetime**
  - **May also impact essential treatment for therapeutic indications later in life**
- **IncobotulinumtoxinA currently remains the only approved BoNT formulation that was intentionally designed to contain only the required therapeutic BoNT component.**
  - **The unique and precise purification of incobotulinumtoxinA represents innovative advances in BoNT manufacturing.**
- **These data suggest incobotulinumtoxinA offers an advantage over other BoNT-A formulations, due to its lower potential to provoke an immune response when used clinically.**

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Disclosure: M. Kerscher has conducted clinical trials for Galderma/Q-Med, Ipsen, and Merz Pharmaceuticals GmbH (as Head of the Division of Cosmetic Sciences, University of Hamburg, Germany), and has acted as a speaker for Galderma/Q-Med and Merz Pharmaceuticals; R. Wanitphakdeedecha has no conflicts of interest to disclose.; A. Trindade de Almeida is an advisor for Allergan, Galderma/QMed, Lupin, Mantecorp, Merz Pharmaceuticals, and Sinclair,has participated in clinical trials for Allergan, has received research support from Merz Pharmaceuticals, and is a speaker for Allergan, Merz Pharmaceuticals, and Theraskin.; C. Maas is an investigator for Merz Pharmaceuticals GmbH and Allergan, and has acted an advisor to Merz.; J. Frevert is a former employee of, and current consultant for, Merz Pharmaceuticals GmbH.

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