Department of Microbiology
Joint Graduate Seminar

Modern Application of Botulinum Toxin - A Neurotoxin from *Clostridium botulinum*

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Outline

1. Introduction to *Clostridium botulinum*
2. Disease caused by botulinum neurotoxin
3. Characteristics of botulinum neurotoxin
4. Mechanisms leading to muscle paralysis
5. Treatments of diseases caused by botulinum neurotoxin
6. Modern applications of botulinum neurotoxin
7. Conclusion

Clostridium botulinum

- Gram positive
- Sporulating anaerobic bacillus
- Produce exotoxin, botulinum neurotoxin (BoNT)
- BoNTs are one of the most deadly toxins, approximately 10 million times more deadly than cyanide (1)

Botulism

- Food poisoning, occurs in improper handling of meat
- Botulism from Latin "*botulus*" meaning "sausage"
- Botulinum toxin (BoNT), first described as "sauage poison"
- A protein produced by *Clostridium botulinum*

Risks of acquiring BoNTs

- Oral poisoning
  - Primary intoxication
    - Ingestion of contaminated food with preformed toxin
  - Primary infection with secondary intoxication
    - Ingestion of food contaminated with the microorganism and produce toxin *in situ*
    - wound infection
- Inhalation poisoning
  - Inhalation of the spores

Botulinum Neurotoxin

- Seven serotypes, denoted A-G
- Similar functional and structural characteristics, but with distinct pharmacologic profiles
- All are potent blockers of synaptic transmission in peripheral cholinergic nervous system synapses
- Denervation of striated muscle and eccrine glands
- Four serotypes A, B, E and F causes human botulism, a fatal neuroparalytic disease
- Serotype A (denoted BoNT/A or BTX-A) are the ingredient of most BoNT drugs

Botulinum Neurotoxin

- Inactive precursor protein: each of the serotypes is synthesized as a single polypeptide chain with molecular mass about 150 kDa.
- Cleaved into 50kDa light chain (LC) and a 100 kDa heavy chain (HC)
- Linked by a disulfide bridge and by a belt, a loop from the HC that wraps around the LC.

Botulinum Neurotoxin

- Activated mature toxin consists of three modules:
  - An N-terminal LC Zn^{2+}-metalloprotease and
  - the HC that encompasses the N-terminal~50-kDa translocation domain (H_N),
  - and the C-terminal ~50-kDa receptor-binding domain (H_C)
- The H_C comprises two subdomains—a β-sheet jelly roll fold, denoted H_{CN}, and a β-tree foil fold carboxy subdomain, known as H_{CC}

The crystal structures of BoNT/A [Protein Data Bank number (PDB) 3BTA]

Extracted from M. Montal. 2010. Botulinum Neurotoxin: A Marvel of Protein Design. Annu. Rev. Biochem. 79:591-617.

Mechanisms of BoNTs in Muscle Paralysis

- The receptor-binding domain (Hc) of the BoNTs interacts with a protein receptor in the epithelial cells and a coreceptor in ganglioside
- BoNTs enter the target cells (the junctional region of cholinergic nerve endings) via receptor mediated endocytosis
- The acidic environment in endosomes induces conformational change in HC
- Insertion of HC into the endosomal membrane
- Forming a transmembrane protein-conducting channel
- The tanslocation domain (Hn) translocates the LC to the cytosol

Mechanisms of BoNTs in Muscle Paralysis

- LCs are endoproteases that cleave a specific component of the synaptic vesicle docking-fusion complex called SNARE (soluble N-ethylmaleimide-sensitive factor attachment prtein receptor) complex (3,4)
- SNARE complex is a catalyst of membrane fusion and is essential for neurotransmitter release
- Resulting inhibition of acetylcholine release at neuromuscular junctions
- the toxin interferes with nerve impulses and causes flaccid paralysis of muscles in botulism

Treatment

- Death usually occurs due to respiratory failure or cardiac arrest
- Recovery depends on the capacity of new motor axons to reinnervate paralysed muscle fibres
- Weeks or months according to the quantity and type of toxin
- Full functioning of the muscle fibre after the toxin is metabolized
- Intensive care is crucial, especially artificial ventilation

Treatment

- passive immunotherapy provides immediate protective immunity in the case of emergency
- Immunotherapies against botulism have reduced botulism mortality rates from approximately 60% to less than 10% (11)
- most frequent antitoxin preparations are equine products such as the bi- or trivalent antitoxin (type AB or ABE) introduced by the FDA in the 1970s (12)
- The other type of antitoxin is the human Botulism Immune Globulin (BabyBIG) approved by the FDA in 2003
  - BIG-IV to treat infant botulism caused by type A or B toxins (13)

Symptomatic relief of BoNTs

- Symptoms can be reduced or reversed by enhancing phasic Ca2+ influx
- by blocking voltage-dependent K+ channels in nerve terminals
- allowing acetylcholine release within the synaptic cleft
- Compounds shown to act as reversible K channel blockers with an ability to allow synaptic response recovery from serotype A BoNT poisoning (5-9)
  - TEA (tetraethylammonium),
  - 4-aminopyridine (4-AP) and
  - 3,4- diaminopyridine (3,4-DAP)

Bioterrorism

- BoNTs are classified by the US CDC as one of the six highest-risk threat bioterrorism agents
  - Ease of production
  - Extreme lethel potency (1 ng kg-1) (2)
  - Duration of their paralytic activity
  - No approved pharmacological treatments for BoNT intoxication

Medical Applications

- BoNTs have been used to in various medical areas for therapeutic purposes
  - Blepharospasm and strabismus
  - Cosmetic
  - Sweating
  - Migraine
- Common brand names
  - Botox
  - Dysport

Blepharospasm and strabismus

- In late 1960s, Alan Scott and Edward Schantz were the first to prepare botulinum toxin for therapeutic purposes.
- In 1980s, BoNT/A was used for the first time in human to treat strabismus and blepharospasm. (10)
- In 1989, Botox was approved by U.S. FDA for the treatment of strabismus and blepharospasm
- The effects of each injection can last for 4-6 months
- Patients are required to re-inject 2-3 times a year

Facial Aesthetics

- BoNT/A revolutionized the aesthetic treatment of the aging face since it's first application

- Two U.S. FDA approved BoNT/A formulations:
  - onabotulinumtoxinA (Botox Cosmetic)
  - abobotulinumtoxinA (Dysport™ and Azzalure)

- It has been shown that BoNT/A has a cosmetic indication for
  - the treatment of glabellar lines
  - can reduce wrinkles and lines in the forehead, crow's feet area, mid and lower face and neck,
  - and can be used to correct asymmetries and for muscle contouring

Treatment Recommendation

- Optimal dose is 50U
- Divided equally into five injection points
- Depends on genders, size and strength of the muscles in the glabellar complex
- BoNTs inhibit the release of acetycholine in neuromuscular junctions in the glabellar complex
- resulting temporary muscle paralysis
- Effect can last three to six months

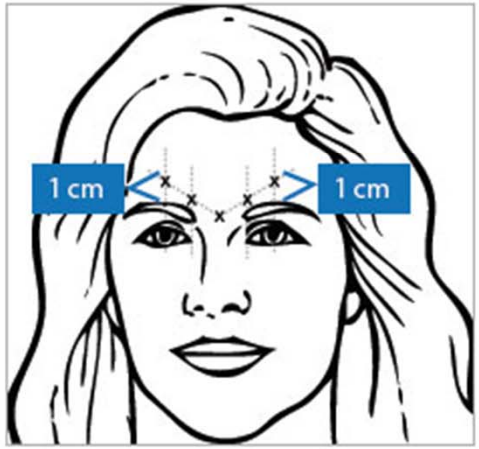
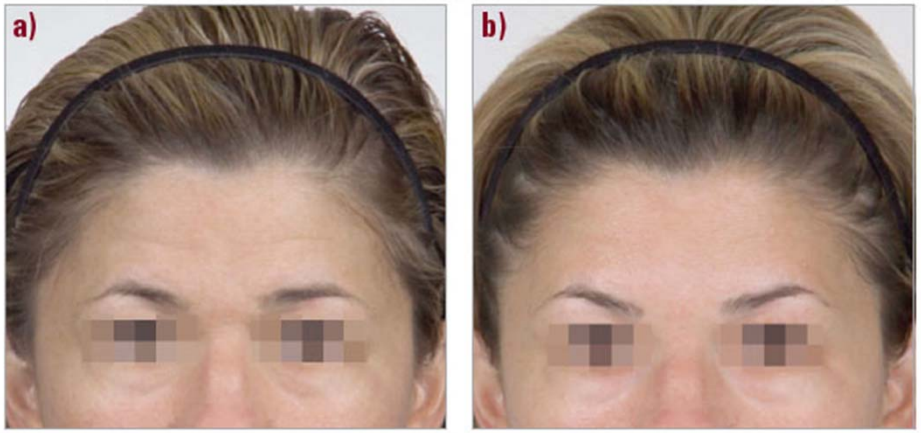


FIGURE 1. Injection points for treating the glabellar complex with Dysport. Reprinted, with permission, from Moy et al. *Archives of Facial Plastic Surgery* 2009;11:77-83.[23] Copyright © 2009 American Medical Association. All rights reserved.

Extracted from M. Kane, L. Donofrio, B. Ascher, D Hexsel, G. Monheit, B. Rzany and R. Weiss. 2010. Expanding the use of Neurotoxin in facial aesthetics: A consensus panel's assessment and recommendations. Journal of Drugs in Dermatology. 9(supp. 1)s1-22.

FIGURE 1. Before and after treatment of glabellar frown lines with BTX-A: **a)** before procedure (at rest); **b)** one month after procedure (at rest). Reproduced with permission of Merz Pharmaceuticals.



Extracted from G. Sattler. 2010. Current and Future Botulinum Neurotoxintype A Preparations in Aesthetics: A Literature Review. Journal of Drugs in Dermatology. 9(9)1065-71.

Sweating

- Hyperhidrosis is caused by over activity of the sympathetic nervous system
- BoNT/A has been shown to be effective to treat focal hyperhidrosis at palms and axillae
- 100 U of BoNT/A is usually used in each palm to treat palmar hyperhidrosis

Migraine

- In October 2010, U.S. FDA approved Botox injection to prevent headaches
  - in adult patients with chronic migraine
- Multiple injections around the head and neck
  - given every 12 weeks
  - to dull future headache symptoms
- The exact mechanism of BoNTs on migraine is unknown

Limitations

- Diffusion of BoNTs to adjecent muscles at the site of injection
  - Ptosis after facial injection (14)
- Spread of BoNT to distant areas
  - Incread jitter and changes in the single fiber EMG (15)
- Development of resistance to BoNTs
  - Treatment in cervical dystonia has been about 6.5% (16)

Conclusion

- BoNT brings great impact to the aesthetic industry
- BoNT is a double-edged sword
- Possibility of bio-engineering therapeutic agents with higher efficiency and with less side effects

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