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Lecture Objectives

- Review the history of botulinum toxins (BoNT)
- Describe the mechanism of action of BoNT at the motor neuron
- Discuss pharmacologic differences observed with the currently available BoNT preparations
- Introduce the clinical utility of botulinum toxin
BoNTs

- Biological product of *Clostridium botulinum bacterium*\(^1\)
- Therapeutic agents for a variety of conditions including
  - Ocular disorders: strabismus, blepharospasm
  - Muscle over-activity: CD, UMN \(^2\):
  - Pain: Chronic Migraine
  - GU: OAB
  - Aesthetic: wrinkles
  - Secretory: hyperhydrosis
  - Other conditions
- 7 BoNT serotypes (A-G)\(^1\). Only types A & B are available for clinical use\(^3\)

History of Botulinum Toxins

1700’s-1800’s

1920  Sommer
Identified, purified Botulinum toxin

1944
E. Schantz
Identified BoNT
Site of Action

1949  Burgan
Identified BoNT
Site of Action

1968

1970’s-80’s

Upper Limb Spasticity
2010

1° Axillary HH
2004

BOTOX® Cosmetic
2002

Allergan
BOTOX®
1989

Myobloc® / NeuroBloc®
2000

Xeomin/Merz
US Approval 2011

Dysport US
2009 (CD)

Dysport®
1991
Normal Innervation
Synaptic vesicle

Acetylcholine

SNARE Protein Complex

Neuronal membrane

Ca^{2+}
Botulinum Toxins
Mechanism of Action

- **All BoNTs**
  - Work presynaptically
  - Block the release of acetylcholine
    - Leading to graded/dose dependent weakness or release of glandular products
  - Different serotypes have different
  - Intracellular targets
  - Duration of effect
  - “Potency”
Structure of Botulinum Toxin

Light Chain (50kDa)

Heavy Chain (100kDa)

Total complex size: 500-900kD
Neurotoxin Component: 150kD

Associated (Accessory) Proteins

HA

NTNH
BoNT: Binding/Endocytosis
Light Chain Endocytosis
Cleavage/Translocation/Blocking
Light Chain Clevage
Light Chain Cleavage
Light Chain Exits to Cytosol
BoNT:SNAP Cleavage Targets

BoNT B: Cleaves VAMP

BoNT A: Cleaves SNAP25
Denervation/Reinnervation
Botulinum Toxin: Worldwide Available Preparations

- **Botulinum toxin type A Preparations: World Wide**
  - BOTOX®; Allergan, Inc.
  - Dysport®; Ipsen Pharmaceuticals
  - Xeomin®; Merz
  - Puretox, Mentor
  - Reloxin, Europe
  - BTXA, China

- **Botulinum toxin type B Serotype**
  - Myobloc®/NeuroBloc®*; Solstice Neurosciences
  - Licensed for distribution in the US, Europe, and Japan

- **Preparations are unique and units are not interchangeable**:1,2
  - Different strains of C. botulinum used in manufacturing process
  - Purification and formulation methods differ
  - Differences in accessory proteins

*Brand name in Europe.
Reported Uses Clinical Applications of BoNTs

• Abnormal Muscular Contractions
  – Strabismus\(^1\)
  – Cerebral palsy
  – Multiple sclerosis
  – Spasticity Upper limb\(^1\)
  – Spastic bladder (OAB, detrusor)\(^1\)
  – Achalasia (esophageal)
  – Chronic anal fissures,
  – Bladder: detrusor overactivity\(^1\)

• Other Applications
  – Hyperhidrosis\(^1\)
  – Migraine & tension-type HA\(^1\)
  – Myofascial pain
  – Sialorrhea
  – Obesity
  – GU: OAB, BPH, sphincter dyssnergia

• Focal Dystonias
  – Blepharospasm\(^1\)
  – Cervical dystonia\(^1,2\)
  – Oromandibular facial-lingual
  – Spasmodic dysphonia
  – Task-specific (Writer’s Cramp)
  – Musician’s cramp

• Other Involuntary Movements
  – Voice, head, and limb tremor
  – VII nerve facial spasm disorder\(^1\)
  – Hemifacial spasm
  – Palatal myoclonus
  – Tics

1. FDA-approved use for BOTOX®
2. FDA-approved for Myobloc®
2009 FDA Alert/Revised Prescribing Information for BoNTs

- **A Boxed Warning was added to all BoNTs**
  - Highlighting the possibility potentially life-threatening distant spread of toxin effect following local injection.
  - A Risk Evaluation and Mitigation Strategy (REMS) was added to include a *Medication Guide* for patients understand detailing risks/benefits of BoNT.

- To reinforce the differences in toxin potency and reduce potential for dosing errors the FDA established *unique generic drug names* for each toxin.

- The new established names reinforce these differences and the lack of interchangeability among products.

- **Units/dosing are specific to each BoNT product**
  - The practice of using conversion tables between toxins is not recommended.
  - Dose or units of biological activity cannot be compared or converted between products.
BoNTs: U.S. Preparations: FDA Unique Generic Names & Indications (2013)

- **OnabotulinumtoxinA (BOTOX®; Allergan, Inc.)**
  - Licensed for distribution worldwide
  - US Indications: Cervical dystonia, strabismus, blepharospasm, hemifacial spasm, hyperhidrosis, post stroke spasticity, detrusor/ overactive bladder, improved appearance of glabellar lines, migraine

- **AbobotulinumtoxinA (Dysport®; Ipsen Pharmaceuticals)**
  - Licensed for distribution in the USA, UK and Europe
  - US Indication: Cervical dystonia. In clinical trials in USA for other conditions

- **IncobotulinumtoxinA (Xeomin®; Merz)**
  - Licensed for distribution in Europe, US
  - US Indications: Cervical dystonia, blepharospasm, glabellar Lines

- **RimabotulinumB (Myobloc®/NeuroBloc®*; Solsticel Neurosciences)**
  - Licensed for distribution in the US, Europe, and Japan
  - US Indication: Cervical dystonia

*Brand name in Europe.
# Botulinum Neurotoxins: Clinical Differentiation

<table>
<thead>
<tr>
<th>Complex Size</th>
<th>Target Protein</th>
<th>Amount of Protein</th>
<th>Reconstituted pH</th>
<th>Final Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BoNT-A&lt;sup&gt;*&lt;/sup&gt;</strong> (Botox®)</td>
<td>SNAP-25</td>
<td>5 ng/100U</td>
<td>Neutral</td>
<td>Vacuum Extraction</td>
</tr>
<tr>
<td><strong>BoNT-A&lt;sup&gt;‡&lt;/sup&gt;</strong> (Dysport®)</td>
<td>SNAP-25</td>
<td>4.25 ng/500U</td>
<td>Neutral</td>
<td>Lyophilization</td>
</tr>
<tr>
<td><strong>BoNT-A&lt;sup&gt;‡&lt;/sup&gt;</strong> (Xeomin)</td>
<td>SNAP-25</td>
<td>0.6 ng/100U</td>
<td>Neutral</td>
<td>Lyophilization</td>
</tr>
<tr>
<td><strong>BoNT-B&lt;sup&gt;†&lt;/sup&gt;</strong> (Myobloc&lt;sup&gt;©&lt;/sup&gt;)</td>
<td>VAMP</td>
<td>50 ng/5000U</td>
<td>5.6</td>
<td>Solution</td>
</tr>
</tbody>
</table>

- **Complex Size**: The size of the complex formed by the neurotoxin and its receptor.
- **Target Protein**: The specific protein that the neurotoxin binds to.
- **Amount of protein**: The amount of protein per unit of neurotoxin.
- **Reconstituted pH**: The pH of the solution after reconstitution.
- **Final Formulation**: The final form of the product.

<sup>*</sup>Allergan, Inc-Botox® Package Insert. <sup>†</sup>Solstice Neurosciences-Myobloc™ Package Insert. <sup>‡</sup>Ipsen Ltd-Dysport® Package Insert.
Differences in Serotype Pharmacology: Clinical Considerations

- Toxin Subtype
- Differences
- Complex size
- Protein load
- Diffusion characteristics
- Intracellular target
- Activation level

Different Therapeutic Profile
- Dose
- Duration
- Migration
- Safety
- Antigenicity
Onabotulinumtoxin A/Botox® is available in 100 unit vials. 1 vial is diluted with 1, 2, 4, or 8 mL of preservative-free 0.9% saline, yielding preparations of 10.0, 5.0, 2.5, or 1.25 units / 0.1 mL, respectively. Reconstituted Botox® should be used within 24 hours and stored at 2º - 8º C (200 unit vials available).

AbobotulinumtoxinA/Dysport™ is available in 300 or 500 unit vials. For CD, one 500-unit vial is diluted with 1 mL preservative-free 0.9% saline, yielding a preparation of 500 units/mL. Reconstituted Dysport™ should be used within 4 hours and should be stored at 2º to 8º C.

IncobotulinumtoxinA/Xeomin BoNT-A® is available in 50 and 100 unit vials and reconstituted with 0.9% saline. Reconstituted Xeomin™ should be used within 24 hours and should be stored at 2º to 8º C. Unopened vials can be stored at room temperature, refrigerated or frozen.

RimabotulinumtoxinB- BoNT-B formulated as Myobloc® is supplied as a sterile injectable solution at a concentration of 5,000 units/mL. Vials contain 0.5 mL (2500 units), 1.0 mL (5000 units), or 2.0 mL (10,000 units).
BoNT: Clinical Effects on Muscle Overactivity

- Onset usually within 3 to 5 days; maximum effect at approximately 4 weeks
- Clinical benefit usually >12 weeks; may be extended with adjunctive therapy\(^1\)
- Can be used in conjunction with phenol, surgery, oral medications, intrathecal baclofen,\(^2\) and other rehabilitation modalities

The Basis for Botulinum Toxin Use in Pain

- Improved pain was noted in initial cervical dystonia and spasticity studies
- Brin 1986 series: cervical dystonia:
  - 64% motor improvement
  - 74% pain improvement


http://www.library.ucla.edu/libraries/biomed/his/PainExhibit/panel3.htm
Regulated Exocytosis Multiple Neurotransmitters/Neuropeptides Released From Vesicle

Adapted from *Trends in Cell Biology*, July 1997.
Goals/Clinical Benefits of BoNT Treatment

- Improved passive and active function: better mobility, activity, daily function, and independence
- Increased patient comfort: less pain, better limb positioning for sitting and sleeping
- Reduced disfigurement
- Prevention or delay of musculoskeletal complications
- Improved quality of life and increased well-being
- Reduced burden of care

Guidelines for BoNT Injection: Muscle/Dose Selection

- Determine which muscles need to be injected
- Determine the appropriate dosage and the number and volume of injections per session
- Use the smallest effective total dose and volume
- Use appropriate techniques to achieve precise injection and reduce the risk of complications
  - For limb muscles, use of electromyography (EMG) guidance or electrical stimulation may be helpful in identifying specific muscles (e.g., smaller muscles such as flexor digitorum sublimis)
BoNT Technical Considerations

- **Dose calculation**: It may be more relevant to consider muscle mass, degree of spasticity, and patient body weight than the disease for dose calculation.

- **Administration**: BoNT is administered by IM injection with avid binding to endplates of the motor neuron/muscle spindle and relatively contained diffusion.

- **Injection technique**: Methods of target or muscle localization include the anatomical methods, EMG guidance, electrical stimulation, and ultrasound.

## Techniques for BoNT Injections

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Anatomic approach | • No equipment needed  
                    • Some muscles are accurately, quickly, and easily isolated              | • Accuracy may be a problem  
                    – Examiner inexperience  
                    – Anatomic variation  
                    – Anatomic rearrangement  
                    • Due to spasticity, contractures, deformity, surgery  
                    • Difficulty isolating deep or overlapping muscles  
                    • Requires patient cooperation  
                    • Impaired motor control may cause difficulties |
| EMG               | • EMG is widely available  
                    • Amplifier boxes are inexpensive  
                    • Clinician familiarity with EMG  
                    • Provides auditory feedback for needle localization and muscle activity | • EMG signal falsely attributed to target muscle  
                    – Co-contraction, mass synergy  
                    – Impaired selective motor control may cause difficulties  
                    – Anatomic variations/rearrangements due to spasticity, surgery, deformities  
                    • Requires patient cooperation  
                    • Impaired motor control  
                    • May require sedation in children  
                    • Uses larger needle/more painful |

## Techniques for BoNT Injections

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<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Electrical stimulation</td>
<td>• Equipment is inexpensive, widely accessible</td>
<td>• Requires stimulator or EMG machine</td>
</tr>
<tr>
<td></td>
<td>• May be more accurate than EMG</td>
<td>• Time-consuming/cost</td>
</tr>
<tr>
<td></td>
<td>• Some muscles are quickly and easily isolated</td>
<td>• Pain</td>
</tr>
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<td></td>
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<td>• Requires patient cooperation</td>
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<td></td>
<td>• Difficult to isolate deep or overlapping muscles</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>• Improved accuracy</td>
<td>• Equipment availability</td>
</tr>
<tr>
<td></td>
<td>• Involuntary muscle activity limits muscle localization with recruitment</td>
<td>• Cost</td>
</tr>
<tr>
<td></td>
<td>– Visualize target muscle with/without AROM*</td>
<td>• Steep learning curve for clinicians</td>
</tr>
</tbody>
</table>

Side Effects Associated with BoNT Therapy

- **Weakness**
  - Local: most important is dysphagia
  - Systemic: minimal weakness, malaise

- **Autonomic effects**
  - Dry mouth, constipation

- **Local effects**
  - Pain, hematoma, infection, rash

- **Antibodies** (subsequent resistance)
## Equipment for Specific Muscles

<table>
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<tr>
<th>Target Muscle</th>
<th>Needle Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniofacial muscles, Salivary Gland</td>
<td>30-gauge, 0.5-1 inch</td>
</tr>
<tr>
<td>Superficial muscles, upper back</td>
<td>25- to 27-gauge, 1- to 1.5-inch</td>
</tr>
<tr>
<td>Scalenes</td>
<td>1-1.5-inch, 26- or 27-gauge, injectable, monopolar electrode or hypodermic</td>
</tr>
<tr>
<td>Deep compartment muscles of the lumbosacral region</td>
<td>Longer injectable monopolar electrode needles, 75-120 mm or longer, spinal needles with or without use of alligator clip for EMG/Estim</td>
</tr>
</tbody>
</table>
BoNT Injection

- Muscle/target specific IM injection\(^1\)
- Smaller muscles require only 1 injection site within the belly of the muscle\(^2\)
- For larger, longer, or wider muscles several injection sites are suggested (2-4)\(^2\)
  - Clinical Pearl: Consider using a 4 quadrant injection technique at each skin penetration site
    - Similar to the 4 quadrant needle technique used with EMG

Nonresponse to BoNT Therapy

- **Primary non-responder**: no response to initial injection
- **Secondary non-responder**: relative or complete loss of efficacy at subsequent injections

**Reasons for non-response**
- Inadequate muscle injection technique / improper targeting
- Inappropriate muscle selection
- Dose may be too low
- Change in pattern of muscle involvement
- Soft tissue contracture
- Neutralizing antibodies may be present (but in spasticity, rare)

**Tests for non-response**: frontalis test, ADQ CMAP stimulation test, antibody assays (limited sensitivity, specificity)

BoNT Resistance: Neutralizing Antibody Formation

- Avoiding resistance
  - Extend treatment interval as long as possible, minimum 3 months between treatments
  - Avoid “booster” or “touch up” injections

- Factors influencing antibody formation
  - Toxin dose
  - Duration/frequency of treatment/injections
  - Prior immunoresistance to other BoNT serotype
  - Protein load
  - Antigen quality and quantity

Why Are Neutralizing Antibodies Important?

- Loss of or limited therapeutic effect
  - Not always due to antibodies
    - Incorrect dose/targeting, contracture, poorly defined goals
  - Patients must seek alternatives that are less effective or are associated with more adverse events

- It is important to minimize risk of antibody formation
  - Use lowest dose in units
    - Less neurotoxin protein load (ng)$^{1,2}$
  - Use longest interval between injections$^{1,2}$
    - This will be determined by the duration of effect

Management Post BoNT Injections: Patient Instructions

• Explain that effect of BoNT will be evident in 3 to 7 days, that BoNT alone may not improve function

• Post Injection therapy program
  – Initiate aggressive stretching of injected muscles, may include splinting and bracing
  – Initiate strengthening of opposing muscles
  – Functional retraining with therapist

• Avoid re-injection of BoNT for at 90 days
Summary: Botulinum Toxin Therapy

• BoNT may be useful for treating a wide variety of conditions by inducing toxin mediated reduction in neurotransmitter release.

• This includes blocking of acetylcholine at the
  – NMJ of muscles
  – Muscle spindles
  – Neurglandular junction

• Antinocioceptive effects of BoNTs may be due to
  – Direct effects of the toxin i.e. reduced release of pain neurotransmitters
  – Indirect effects of reduced muscle contraction/spasm

• Commercially available BoNTs are non-interchangeable

• Accurate targeting is important for efficacy, safety and reduction of adverse events