Chapter 5

What Practical Tips Can I Suggest during Botulinum Toxin Injection?

Woong-Woo Lee and Beomseok Jeon

Additional information is available at the end of the chapter

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Abstract

Botulinum toxin injections are effective for hyperactivities of muscles and glands that are mediated by acetylcholine (Ach) release in neuronal terminal. The effects of botulinum toxin are reversible because the involved nerves build new sprouts and synapses with time. Thus, botulinum toxin is relatively safe and repeated applications are needed. To maximize effects of botulinum toxin without side effects, understanding the action mechanism of botulinum toxin and determining appropriate target sites are very important. Many guidelines have already been published and provided useful information for these. Therefore, in this chapter, we concentrate more on practical tips for botulinum toxin injections.

Keywords: botulinum toxin, acetylcholine, dystonia, spasticity, hemifacial spasm

1. Introduction

The natural Botulinum neurotoxin (BoNT) is produced by *Clostridium botulinum*, a type of spore-producing gram-positive bacilli. Sources of botulism poisoning are soil, honey, canned foods, etc., because *C. botulinum* thrives in anaerobic conditions. BoNT can be denatured at over 80°C (176°F). BoNT consists of a heavy chain and light chain, which are polypeptides linked by a disulfide bond [1]. Initially, the heavy chain binds to the surface of a nerve cell. The light chain, which acts as a Zn-dependent protease, is internalized into the nerve cell, and then, it cleaves specific soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins: synaptosome-associated protein (SNAP-25), syntaxin, and vesicle-associated membrane protein (VAMP). There are several serotypes of BoNT. Type A, C1, and E break down SNAP-25; type C hydrolyzes syntaxin, and type B, D, F, and G block the function of

VAMP. As a result, acetylcholine (Ach) remains inside the vesicles and cannot be released into the synaptic cleft. The duration of the BoNT activity is generally limited to several weeks or several months because the involved nerves form new sprouts and synapses that can release Ach [2].

The first agent, which was approved by the Food and Drug Administration in 1989, is Botox® (Allergan Inc, Irvine, CA, USA). Botulinum toxin type A (BoNT/A) is most commonly used these days: Botox®, OnabotulinumtoxinA; Dysport®, AbobotulinumtoxinA; Xeomin®, IncobotulinumtoxinA; Hengli/CBTX-A®, Chinese botulinumtoxin A; Neuronox/Meditoxin®, South Korea botulinumtoxin A, etc. Botulinum toxin type B is also frequently used: Myobloc®/Neurobloc®, RimabotulinumtoxinB.

The practitioners of BoNT injection need to remember that BoNT is a protein. This means that BoNT can denature easily and contribute to the generation of neutralizing antibodies. BoNT has to be stored at 2–8 °C (36–46 °F) and administrated only for a limited time [3–5]. Freezing, light exposure, and shaking should be avoided. To reduce the incidence of antibody generation, the following are recommended: maintain at least a 3-month interval between injections; use the smallest possible dose, and avoid booster injections. Detailed prescription information for widely used BoNTs is summarized in **Table 1** [3–5].

	Botox® [3]	**Dysport® [4]**	**Myobloc® [5]**
Contents	OnabotulinumtoxinA	AbobotulinumtoxinA	RimabotulinumtoxinB
	Human albumin	Human albumin	Human albumin
	Sodium chloride	Lactose	Sodium succinate
		Cow's milk proteins	Sodium chloride
Target SNARE protein	SNAP-25	SNAP-25	VAMP
Storage	Store at 2–8°C (36–46°F)	Store at 2–8°C (36–46 °F)	Store at 2–8°C (36–46°F)
	Inject BoNT within 24 hours	Inject BoNT within 24 hours	Inject BoNT within 4 hours, once diluted
		Do not freeze	Do not freeze
		Protect from light	Do not shake
			Protect from light
Reconstitution	Sterile, preservative-free 0.9% sodium chloride		Provided as solution (pH 5.6)
			Can be diluted with normal saline
Indications and usage	Cervical dystonia	Cervical dystonia	Cervical dystonia
	Blepharospasm	Glabella lines	
	Spasticity in adult	Upper limb spasticity in adult	

	Botox® [3]	**Dysport® [4]**	**Myobloc® [5]**
	Chronic migraine		
	Strabismus		
	Axillary hyperhidrosis		
	Overactive bladder		
Contraindications	Hypersensitivity to any BoNT	Hypersensitivity to any BoNT	Hypersensitivity to any BoNT
	Infection	Infection	Infection
		Allergy to cow's milk protein	
Limit of a total dose	400 Units	1000 Units	NA
Minimal interval	3 months	12 weeks	NA
Side effects	Generalized weakness, diplopia, ptosis, dysphagia, dysphonia, dysarthria, urinary incontinence, breathing difficulties		
	Pain, inflammation, bleeding		
	Flu, rhinitis, pharyngitis		
Drug interactions	Aminoglycosides and other agents interfering with neuromuscular transmission (eg. Curare-like agents)		
	Anticholinergics		
	Muscle relaxants		
	Other BoNT		
Cautions	Pregnancy (Category C)		
	Nursing mothers		
	Pediatric/geriatric use		
Immunogenicity	Positive antibodies: 0.0–1.2%	Positive antibodies: 0.0–3.6%	Neutralizing activity
	Risk factors: frequent intervals, higher doses		- 1 years: 10% (for 36% ELISA-positive cases)
			- 18 months: 18% (for 50% ELISA-positive cases)

SNARE, soluble N-ethlymaleimide sensitive factor attachment protein receptor; SNAP-25, synaptosome-associated protein; VAMP, vesicle-associated membrane protein; BoNT, botulinum neurotoxin; NA, not applicable.
*The incidence of antibodies are highly dependent on the methodology of the assay. Therefore, the simple comparisons between different BoNTs are illogical.

Table 1. Summary of prescribing information.

Early BoNTs did not overcome the following problems: short-lasting effects, necrotizing problem, and systematic toxicity [6]. However, there have been remarkable advances since Scott et al. [7] successfully improved strabismus with purified BoNT injections. Now BoNT is widely used to reduce the hyperactivity of muscles or glands mediated by Ach release [1]. For

neurological disorders, BoNT can be effectively used for hyperkinetic movement disorders (dystonia, hemifacial spasm, myoclonus, myokymia, tremor, etc.), spasticity, drooling, and chronic migraines. Applications for strabismus, spasms of the gastrointestinal tract or genito-urinary tract, and cosmetic work are possible.

In this chapter, although there have been many guidelines published, we focus more on the practical tips for BoNT injections in the treatment of several neurological symptoms.

2. Before botulinum toxin injection

2.1. Selection of appropriate patients for botulinum toxin injections

The principle action mechanism of BoNT is to block the release of Ach from presynaptic nerve terminals. Therefore, the Ach-mediated hyperactivities of muscles and glands could be good targets for BoNT therapy. The following list shows representative indications for BoNT injection.

- Hyperkinetic movement disorders
  - o Dystonia (cervical dystonia, blepharospasm, oro-mandibular dystonia, limb dystonia, laryngeal dystonia, etc.)
  - o Hemifacial spasm
  - o Myoclonus, myokymia, tremor, dyskinesia, tics, etc.
- Other neurological disorders
  - o Spasticity
  - o Headache (chronic tension type headache, chronic migraine)
  - o Drooling, etc.
- Other disorders
  - o Strabismus
  - o Hyperhidrosis
  - o Spasm or spasticity of the gastro-intestinal and genito-urinary tracts
  - o Cosmestic applications, etc.

The cautions and contraindications are as important as the proper indications. Patients with neurological disorders involving anterior horn cells, peripheral nerves, neuro-muscular junctions, and muscles require special care when injecting BoNT. Concomitant use of drugs, which could affect neuro-muscular transmission or cause muscle weakness, is also not recommended (**Table 1**) [3–5]. In addition, most BoNTs do not guarantee the safety of pregnant women, nursing mothers, and pediatric patients. Hypersensitivities to any of the BoNTs or

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their components and the infection of target sites are important contraindications. Especially, Dysport® is contraindicated in patients with allergies to cow's milk protein [4]. Anti-platelet or anti-coagulation agents could cause a hematoma.

2.2. Selection of botulinum toxin

Despite past studies using different types of BoNT, it is unclear which one is the most effective BoNT. Furthermore, it is generally accepted that the doses between different BoNT products are not interchangeable [3–5, 8–10]. However, in real practice, patients who have been treated with different BoNT products could visit your clinic any time. In this regard, a simple conversion ratio between Botox® and Dysport® was made [11]. Based on the average recommended dose of Dysport®(500 Units) and Botox® (200 Units) for patients with cervical dystonia, a conversion ratio of 2.5:1 was assumed and it was found that Dysport® showed no inferiority to Botox® at this ratio. The ratio of 2.5:1 has the practical advantage by simplifying the interchanging process. This issue will be addressed in detail later.

3. Preparing botulinum toxin injection

3.1. Reconstitution

The widely used solvent for reconstituting BoNT is 0.9% normal saline. Hypotonic saline or distilled water is not suitable as a solvent because it could cause pain. Recently one preliminary study suggested that reconstituting BoNT with Ringer acetate could reduce the injection site pain rather than with normal saline by normalizing the pH values of the solution [12]. Myobloc® is provided as a solution (pH 5.6) that can be used as is or diluted with normal saline [5].

Regardless of the BoNT subtype, one unit of BoNT was defined as the calculated median intraperitoneal lethal dose (LD_{50}) in mice. However, the biological activity varies between BoNTs. Although there is no consensus for the conversion ratio, we use the ratio of 2.5:1 for Dysport® and Botox® because the non-inferiority of Dysport® has already been proven [11]. On the assumption that the ratio of 2.5:1 is bioequivalent, the practitioner can make two kinds of solutions (**Table 2**). To make a solution with a high concentration, 1 ml of 0.9% normal saline is needed for 1 ample (100 Units) of Botox® and 2 ml of 0.9% normal saline for 1 ample (500 Units) of Dysport®. For a solution with a low concentration, 2 ml and 4 ml are mixed with 1 ample of Botox® and Dysport®, respectively. Then, the same volume of each solution indicates the same potency (**Table 2**). It means that physicians do not need complicated calculations. Because the same conversion ratio (2.5:1) is also applied to the limitations for the total doses of Dysport® (1000 Units) and Botox® (400 Units), we believe that it is very simple and practical.

After injecting normal saline for reconstitution, the next step is to mix gently to minimize unnecessary destruction of the toxin. Because the available time is short after reconstitution

(within 24 hours for Botox® and Dysport®) [3, 4], it is more efficient to inject BoNT after gathering sufficient numbers of patients who need BoNT treatment.

	Botox®, OnabotulinumtoxinA	**Dysport®, AbobotulinumtoxinA**
Units per 1 ample	100 Units	500 Units
Method #1 (high concentration)	100 Units (1 ample): 1 ml of 0.9% N/S	500 Units (1 ample): 2 ml of 0.9% N/S
	→ 0.1 ml solution = 10 Units of Ona-BoNT/A	→ 0.1 ml solution = 25 Units of Abo-BoNT/A
	→ 0.01 ml solution = 1 Unit of Ona-BoNT/A	→ 0.01 ml solution = 2.5 Units of Abo-BoNT/A
Method #2 (low concentration)	100 Units (1 ample): 2 ml of 0.9% N/S	500 Units (1 ample): 4 ml of 0.9% N/S
	→ 0.1 ml solution = 5 Units of Ona-BoNT/A	→ 0.1 ml solution = 12.5 Units of Abo-BoNT/A
	→ 0.01 ml solution = 0.5 Units of Ona-BoNT/A	→ 0.01 ml solution = 1.25 Units of Abo-BoNT/A

BoNT/A, botulinum neurotoxin type A.

Table 2. Practical tips of conversion between Botox® and Dysport®.

3.2. Preparation of the syringe and needle

Generally, a 1-ml syringe, which can control the volume by 0.01 ml, is preferred. The practitioner needs 0.03–0.05 ml more volume than the target volume, taking into consideration the dead space inside of the needle. After the syringe is filled properly with the BoNT solution, the practitioner should remove the air. The following are tips for removing air bubbles and aligning the needle.

- Draw down the syringe quickly while holding the cap of syringe. It is based on the inertia effect, and just one time is enough to collect bubbles on top. Tapping the body is also available.
- Pull slightly back, before pushing forward, the plunger of the syringe. It will be helpful in saving the BoNT in the dead space of the needle.
- Rotate the bevel of the needle in the same direction with the scale marks of the syringe.

The selection of the injection needle depends on the target sites. Because most facial muscles are thin and close to the skin, a thin and short needle such as a 23- or 24-gauge needle is suitable for the delivery of BoNT solution. In contrast, neck and limb muscles are thick, large, and located relatively deep from skin. Thus, a thick and long needle such as a 21-gauge needle is necessary.

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4. Injecting botulinum toxin

4.1. Determining target sites

BoNT injection is recommended for use in only limited areas. The reasons for the limited use are because of the total dose and cost of BoNT. Therefore, determining the target is the most important procedure in treatment with BoNT.

Determining target sites has the same meaning as finding symptomatic muscles. It is not difficult to locate target sites for disorders with static muscular hypertonia (such as fixed dystonia or spasticity). However, mobile hyperkinetic movement disorders are another matter. Especially for mobile dystonia, finding target muscles is not simple because the direction of abnormal movement seems to be irregular or changes every moment. Ultrasonography, computed tomography, magnetic resonance imaging, and electromyography (EMG) are supportive tools to help ascertain the target [13]. In particular, EMG can provide dynamic information and help in the precise injection of BoNT to target muscles in real time. However it should be kept in mind that EMG is not a substitute for knowledge of anatomy and is a mere supportive tool. Another important thing is to avoid BoNT injections near sites where side effects occur frequently. Particularly the procedure for head and neck requires special care.

Practical tips for examining patients and determining target sites are introduced below.

4.1.1. Cervical dystonia

The final posture of cervical dystonia (CD) patients consists of a combination of dystonic muscle contractions, compensatory movements, and secondary musculo-skeletal changes. Therefore, examination in various situations is helpful in differentiating target dystonic muscles from the others.

Before the examination, enough exposure of the neck and adjacent muscles is very important. This procedure is essential for precise inspection. In addition, sensory trick by scarf or clothes could be eliminated. Then, the physicians should see the rotation and deviation of neck and adjacent muscles on a neutral position, and describe how they are seen. It can be summarized as the combination of turning to one side (torticollis), tilting or shifting to one side (laterocollis), bending forward (antecollis) or backward (retrocollis). Shoulder elevation is frequently accompanied.

Here are several methods to distinguish compensatory movements. The physicians have to tell the patients "let the neck and shoulder relax as they move", and make them walk repeatedly. Palpation of the candidate muscles is helpful in assessing whether they are hypertrophic or contain bands. The features of dystonia such as task-specificity, overflow, and null-point could also be important clues. Especially for tremulous CD, if a tremor diminishes at a specific position, the muscles inducing that position would be affected by dystonia. Short-term follow-up can reveal critical information. The effect of BoNT for CD is generally maximized within 2 or 3 weeks after injection. Therefore, early visits can provide information whether previous

target sites were appropriate as well as whether the patient is a BoNT non-responder. The dynamic progression of dystonia is another reason why follow-up is important.

4.1.2. Facial involuntary movements

Determining target sites in facial involuntary movements is relatively simple compared to CD. Therefore, when BoNT is injected into the face, avoiding side effects is given much weight. The face contains other important anatomical structures such as the eye balls, lacrimal ducts, salivary glands, nerves, and vessels. The direction of the needle always must head away from the eye balls. Two canaliculi per eye exist in the medial canthus. BoNT injection into the lower canaliculus generally is avoided because it is believed to have a main role in transporting tears to the lacrimal sac and nasolacrimal duct. When injecting into the masseter muscle, it is better to preserve the lower posterior portion where the parotid gland is placed.

Asymmetry and ptosis should be considered. To prevent asymmetry, injections are often given also on the contralateral side. To prevent ptosis, BoNT injections to the mid-portions of the upper eyelid and frontalis area are avoided. Especially, when the orbicularis oculi is the target, injection into the pretarsal area has stronger effects and less frequent ptosis than injections into the preseptal area [14, 15].

4.1.3. Limb dystonia

Using overflow phenomenon and mirror movement is a good way to differentiate main symptomatic spasms from compensatory ones [16]. If hand dystonia is too complex to determine a target, an EMG-guided approach will be useful. However, it is not easy to inject only into the real target site because the hands are comprised of small muscles for delicate movements. And paralysis of specific muscle rather can cause great inconvenience in most situations except when that specific task is performed.

4.2. Determining target volume

Once the target muscles for BoNT injection have been chosen, the next step is determining how much BoNT and how many injection sites are required. The recommended volumes for several target muscles are provided in the prescription information [3–5]. However, the response varies from person to person and information for every possible indication is not included. Thus, the injection volume and site should be individualized taking into consideration race, sex, age, medical condition, etc.

The followings are additional helpful tips in determining the injection volume.

- For larger target muscles, a larger volume of BoNT is required.
- It is safer to apply a low dose first.
- Imagining the diffusion pattern of BoNT is important.

Borodic et al. [17] reported several important features for the BoNT/A diffusion pattern in the longissimus dorsi of rabbits: the diffusion of BoNT/A occurred in a dose-dependent manner;

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the spread pattern in the injected muscle was more linear than in a remote muscle of the same distance; lower doses did not affect a different muscle located 45 mm from the injection site while higher doses diffused even into that muscle. This study supports the concept that injection into multiple sites with lower doses could reduce side effects by preventing the diffusion of BoNT, in other words its biological effect, to other muscles beyond the injection site [18]. Therefore, Rosales et al. [19] recommended a "high potency, low dilution" of BoNT/A for oromandibular, lingual, cranial, cervical, and distal limb dystonias, which are small and localized targets. In contrast, A "low potency, high dilution" of BoNT/A is more useful for big muscles.

4.3. Methods of botulinum toxin injection

The method of injection mainly depends on the anatomical features of target sites.

Superficial fat compartments are distributed throughout the entire face excluding the upper eyelids, nose, and mouth [20]. Most facial muscles are located just beneath the skin and subcutaneous fat, whereas peri-ocular and peri-oral areas have little fatty tissue. Facial skin is relatively thin compared to the other parts. Especially, the skin thickness of the palpebral areas is only around 0.5 mm. Therefore, when the orbicularis oculi or orbicularis oris muscle is the target, the needle should be inserted horizontally to skin as much as possible although more pain at the injection site is inevitable. Smoothing out skin wrinkles is important for easy needling. Vesicle formation is also recommended in these areas because it seems to be helpful in localizing the extent of chemo-denervation. For other facial muscles, the angle between needle and skin needs to be set a little bit higher so that the needle can be inserted into a deeper location. Another important consideration in facial muscle anatomy is that the facial muscles have few muscle spindles except in jaw muscles. It refers that BoNT injections directly impact on extrafusal muscle fibers in face.

On the other hand, neck and limb muscles are large and located more deeply. For effective delivery of BoNT, a long needle inserted perpendicularly to the skin is necessary. This direction of insertion is also good for reducing pain. Grasping and fixing muscles are essential for precise targeting. Postures of activating relevant muscles and imaging- or EMG-guided methods may be of great help to approach deep muscles. Before administering the BoNT solution, pulling back is required to ascertain whether the needle reaches the target muscle or adjunctive vessel.

5. Conclusion

To provide the appropriate BoNT treatment, the practitioner should be well informed of the etio-pathogenesis of disorders and the anatomy of the target area, as well as the action mechanism of BoNT and methods of storage and injection. Patients must also understand that BoNT treatment is just a way to relieve symptoms, and not treatment for underlying causes. Repeated applications of BoNT are required because the effects last for 3 months on average. Paradoxically, BoNT is a reversible and very safe toxin in terms of side effects. We hope that

the guides of this chapter are very helpful in understanding and using BoNT in a clinical setting.

Author details

Woong-Woo Lee[1] and Beomseok Jeon[2*]

*Address all correspondence to: brain@snu.ac.kr

1 Department of Neurology, Eulji General Hospital, Seoul, Korea

2 Department of Neurology, Seoul National University Hospital, Seoul, Korea

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