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RECONSTITUTING NEUROMODULATORS IN CLINICAL PRACTICE

Neuromodulators have been used in clinical practice for decades, for both therapeutic and cosmetic applications. They are neuromuscular blocking agents that inhibit acetylcholine (ACh) release. In dermatology, they are typically used for temporary improvement in the appearance of dynamic, and to an extent, static lines. Facial aging is a result of a host of both extrinsic and intrinsic factors, including ultraviolet radiation, gravity, atrophy and remodeling of adipose, osseous, and cartilaginous structures, and changes in the muscle activity of facial musculature.¹ Neuromodulators can be used to treat muscle hyperactivity, unwanted muscle movement contributing to wrinkle formation, asymmetries, and can also be used to treat hyperhidrosis.

Botulinum neurotoxin (BoNT) works by binding cholinergic receptors selectively and irreversibly in the nerve terminals, thereby blocking ACh release through enzymatic cleavage of the SNAP-25 protein.¹ This prevents muscular contraction of the treated muscles by blocking the nervous impulses that cause the depolarization of the muscle membrane and sweat production by blocking ACh release in the autonomic cholinergic fibers from sympathetic fibers of the sweat glands.¹ There are seven different serotypes, denominated A – G.¹ Botulinum neurotoxin-A (BoNT-A) and botulinum neurotoxin-B (BoNT-B) are approved for use in humans. Different BoNT-A products are commercially available in Canada for cosmetic use.

Onabotulinumtoxin-A (ONA) (BOTOX COSMETIC®, Allergan Inc, Irvine, CA), branded as Botox®, was the first neuromodulator approved for anti-aging. It was the only therapeutic agent of its kind available for many years. It has become a leading cosmetic treatment for improving the appearance of unwanted wrinkles and other manufacturers have since entered the market. Abobotulinumtoxin-A (ABO) (DYSPORE AESTHETIC™, Ipsen Ltd, Maidenhead, UK), and incobotulinumtoxin-A (INCO) (XEOMIN COSMETIC™, Merz Pharmaceuticals, Frankfurt, Germany) are two commonly used competitors, which have also had great success in clinical practice for their cosmetic applications.

Although they are all BoNT-A neuromuscular paralytic agents, there are differences in the manufacturing processes of these commercially available neuromodulators that give them unique properties. Differences in the purification methods, and the added inactive ingredients, can play a role in determining the characteristics of each product, such as the affinity for their target, effects on muscle paralysis, antigenicity, and the duration of efficacy.

The dose of each BoNT-A product is measured in units (U) of biological activity. The biological activity is determined in animals. One unit is calculated as the median lethal dose (LD50) of reconstituted product in mice injected intraperitoneally.² The potency of each BoNT-A product is different, thus the labelled units of one product cannot be converted directly into another and are not interchangeable between products.³⁻⁵

The products are all packaged in a lyophilized powdered state and must be reconstituted before use.³⁻⁵ INCO is a visible white powder, whereas ONA and ABO are more colourless. ONA should be kept frozen (<5°C), ABO in the fridge (2 – 8°C) and INCO can be kept at room temperature (<25°C) until reconstituted.³⁻⁵ The product monographs of all three products recommend reconstitution with 0.9% preservative-free sterile saline (NaCl).³⁻⁵ In clinical practice, NaCl with preservatives is often used off-label however, as preservatives tend to significantly decrease the pain, discomfort and burning of the injections for the patient. Studies have demonstrated that preservatives do not compromise the efficacy of these agents and that they increase the duration of the

storage period after reconstitution and reduce the risk of bacterial contamination.⁶

A 20-27 gauge beveled needle can be used to draw up the NaCl in a syringe for reconstitution before injection.⁴⁻⁵ The exposed portion of the rubber stopper on the vial should be cleaned with alcohol before the needle is inserted vertically into the vial through the rubber stopper. Once the needle of the syringe containing the diluent is put into the vial, it should pull the diluent into the vial. The syringe is then removed from the vial, and the neuromodulator and NaCl is mixed by carefully rotating the vial for ONA and ABO, and by swirling and inverting the vial to ensure all of the white powder is dissolved for INCO.³⁻⁵ If the diluent is not easily pulled into the vial and the seal is broken, the vial should be discarded.³⁻⁵ Neurotoxins can be denatured with strong agitation, foaming, or bubbling, thus the NaCl diluent should be introduced gently into the vial. The reconstituted product should be a clear, colourless solution, and free of particulate matter. The date and time can be recorded on the label, or in practice, on the box.

Health Canada has approved various reconstitutions for all three products. ONA can be reconstituted as per the approved indication for wrinkles with 0.9% sterile non-preserved NaCl 1.3 - 4.0 milliliters (mL) per 100 U vial, resulting in dilutions of 7.5 - 2.5 U, per 0.1 mL (**Table 1**).³ The product monograph recommends dilutions between 4.0 – 7.0 U per 0.1 mL, and the package insert suggests using 2.5 mL to dilute a 100 U vial for a resulting dose of 4 U per 0.1 mL.²⁻³ Generally, a lower reconstitution volume with a higher concentration is desired for

cosmetic injections. This allows for more precision while injecting the product, thereby lowering the risk of diffusion and complications. In practice, many clinicians use a dilution of 10.0 U per 0.1 mL for ONA, which corresponds to 1 U per 0.01 mL marking on a syringe. This formula for dilution is not specified in Health Canada's approved labelling or in the package insert for ONA but is specified for ONA therapeutic which has many different indications.⁷ The on-label recommended reconstitution for ONA for axillary hyperhidrosis is 4.0 mL for a 100 U vial (**Table 1**).⁷ In practice, it is common to dilute ONA with 5.0 mL NaCl for hyperhidrosis, as more spread and diffusion is desirable. For INCO, the approved dilutions in the product monograph result in 10.0 – 2.5 U per 0.1 mL, with dilutions of 1.0 – 4.0 mL NaCl for a 100 U vial (**Table 1**). For ABO, the recognized dilutions on the product monograph result in 20 U – 10 U per 0.1 mL, with dilutions of 1.5 mL – 3.0 mL NaCl for a 300 U vial (**Table 1**).⁵

Although the units are not equivalent between different commercially available neuromodulators, equivalence ratios can be used to determine comparable dosing and clinical outcomes. Studies suggest that 1 U of ONA is equivalent to 2-2.5 U of ABO, based on efficacy, intensity, safety, and duration of the desired paralysis or anhidrosis.⁸⁻¹⁰ Another study demonstrated that a dose equivalence ratio of 2:1 U (ABO:ONA) results in similar field effects on muscle and sweat gland activity, but at a higher dose equivalence of 2.5:1 (ABO:ONA), ABO affects a greater area and horizontal diameter.¹¹ This should be taken into consideration when injecting this product to

Product	Units per vial	Volume of diluent	Dilution per 0.1 mL
ONA (BOTOX COSMETIC®) ⁽¹⁾	100 U	4.0 mL	2.5 U
		2.0 mL	5.0 U
		1.3 mL	7.5 U
ONA (BOTOX®) ⁽²⁾	100 U	4.0 mL	2.5 U
INCA (XEOMIN COSMETIC™)	100 U	4.0 mL	2.5 U
		2.5 mL	4.0 U
		2.0 mL	5.0 U
		1.25 mL	8.0 U
		1.0 mL	10.0 U
ABO (DYSPORT AESTHETIC™)	300 U	3.0 mL	10.0 U
		2.5 mL	12 U
		1.5 mL	20 U

Table 1. Health Canada approved reconstitution volumes with preservative free sterile saline.^{3-5,7}

Notes: (1) For facial lines (2) For axillary hyperhidrosis.

prevent unwanted spread and complications.

The same dose of ONA or INCO will typically achieve comparable results.¹²⁻¹⁴ In practice, many clinicians reconstitute a 300 U vial of ABO with 1.0 mL, 1.1 mL or 1.2 mL of bacteriostatic NaCl for an approximate ratio of 1:3 (ONA:ABO), which is considered comparable to 1 mL of diluent for a 100 U vial of ONA or a 100 U vial of INCO. These commonly used off-label dilutions allow for the same volumes of ABO and ONA to be injected, to achieve the same clinical results. The equivalence ratios for ABO of 1:2, 1:2.5 or 1:3 (ONA:ABO) can be achieved with the following volumes in **Table 2.1-2.3**, respectively.

If reconstituted with preservative free NaCl, the product should be refrigerated between 2°C and 8°C.³⁻⁵ The products should not be frozen once reconstituted. The product monographs recommend

using the products within 24 hours of reconstitution. Studies suggest that some of these products (ONA and ABO) are still effective and safe to use for 2 – 6 weeks, however.¹⁵⁻¹⁷

Various syringes can be used to administer the reconstituted product intramuscularly for lines, or intradermally for hyperhidrosis.⁷ For patient comfort and to minimize complications, such as bleeding, bruising, and inadvertent placement of the product, a short, small bore, sterile needle is recommended. The ONA monograph recommends a 1.0 mL tuberculin syringe, the ABO monograph a 30-gauge needle, and the INCO monograph a 13 mm long 30 – 33-gauge needle.³⁻⁵ In clinical practice, a 0.3 mL, or 0.5 mL, BD Ultra-Fine II syringe with a short 30-gauge needle works well. It is precise, and the small bore minimizes patient discomfort.

There are several other subtleties between ABO, INCO and ONA that make each product unique. Many factors are important to consider when making the proper selection for patients to meet their needs.

The onset of action of ONA and INCO starts within 1 week and can take up to 2 weeks for full onset, whereas the onset of action of ABO is shorter. Muscle relaxation for INCO can be observed between 2 – 5 days, with a median onset at 7 days, maximum effect at 2 weeks, and a duration of 9 – 16 weeks.⁴ ONA reduces the severity of facial lines for up to 120 days.³ The onset of ABO is the fastest, with observable results as early as 24 hours, and a median time to onset of three days.⁵ This is a good option for patients looking to achieve results quickly. The effects of ABO last up to 4 months.⁵ The clinical effect of ABO is known to locally migrate further than ONA or INCO, which also needs

Diluent for 100 U of ONA (BOTOX COSMETIC® or BOTOX®) (mL)	Diluent for 100 U of INCO (XEOMIN COSMETIC™) (mL)	Diluent for a 300 U vial of ABO (DYSPORT AESTHETIC™) (mL)
1	1	1.5
2	2	3
2.5	2.5	3.75

Table 2.1. Regular dilutions of 0.8 mL, 1 mL, 2 mL and 2.5 mL for ONA and INCO to achieve the 1:2 U equivalent dosing for ABO; courtesy of Dr Chloé E. Ward

Diluent for 100 U of ONA (BOTOX COSMETIC® or BOTOX®) (mL)	Diluent for 100 U of INCO (XEOMIN COSMETIC™) (mL)	Diluent for a 300 U vial of ABO (DYSPORT AESTHETIC™) (mL)
1	1	1.2
2	2	2.4
2.5	2.5	3

Table 2.2. Regular dilutions of 0.8 mL, 1 mL, 2 mL and 2.5 mL for ONA and INCO to achieve the 1:2.5 U equivalent dosing for ABO; courtesy of Dr Chloé E. Ward

Diluent for 100 U of ONA (BOTOX COSMETIC® or BOTOX®) (mL)	Diluent for 100 U of INCO (XEOMIN COSMETIC™) (mL)	Diluent for a 300 U vial of ABO (DYSPORT AESTHETIC™) (mL)
1	1	1 – 1.2
2	2	2
2.5	2.5	2.5

Table 2.3. Regular dilutions of 0.8 mL, 1 mL, 2 mL and 2.5 mL for ONA and INCO to achieve the 1:3 U equivalent dosing for ABO; courtesy of Dr Chloé E. Ward

to be considered in dosing. It is a good option in anatomical areas where several injections are needed, but this migration must be considered by clinicians in anatomical areas where spread needs to be minimized to prevent complications. Anecdotally, it has been described that effects of INCO can wear off more quickly in some patients, however, several clinical studies comparing INCO to ONA for axillary hyperhidrosis, blepharospasm, cervical dystonia,

and facial rhytides, including a split face study, did not reveal any significant differences in the duration of clinical efficacy. Results will vary from patient to patient and are dose dependent. Injection volume, injection angle and speed, needle size, muscle mass and skin thickness are also thought to have an effect on the field of treatment.

ONA and ABO contain an accessory protein to carry the botulinum toxin, and it is thought

that antibody formation can occur over time, diminishing the efficacy with continued use. INCO in comparison, is marketed as a product that does not contain additives or accessory proteins, as the therapeutic component of the toxin complex is isolated from accessory proteins, thereby reducing the risk of immunogenicity or allergic reaction to the accessory protein that other products contain. Although there are differences

Generic name	onabotulinumtoxinA	incobotulinumtoxinA	abobotulinumtoxinA
Brand name	BOTOX COSMETIC®/ BOTOX®	XEOMIN COSMETIC™	DYSPORT AESTHETIC™
Health Canada approved dermatologic Indications	• Hyperhidrosis of the axilla in patients ≥ 18 years of age • Upper facial rhytides, including forehead, lateral canthus, and glabellar lines in adults	• Moderate-to-severe horizontal forehead lines, lateral canthal lines, and glabellar lines in adults	• Moderate-to-severe glabellar lines and/or lateral canthal lines (crow's feet) in adults < 65 years of age
Contraindications	• Hypersensitivity • Infection at the proposed injection site(s)	• Hypersensitivity • Infection or inflammation at the proposed injection site(s) • Generalized disorders of muscle activity (e.g. myasthenia gravis, Lambert-Eaton syndrome)	• Hypersensitivity • Infection at the proposed injection sites • Cow's milk protein allergy • Generalized disorders of muscle activity (e.g. myasthenia gravis, Lambert-Eaton syndrome or amyotrophic lateral sclerosis)
Purified neurotoxin complex molecular weight	900 kD	150 kD	150 kD
Active substance	BoNT-A with complexing proteins	BoNT-A free from complexing proteins	BoNT-A with complexing proteins
Number of units per vial	100	100	300
Suggested equivalent dosing ratio for 1 U ONA	1 U	1:1	1:2 – 1:3
Toxin protein load in dose equivalence range	5 ng/100 U	0.6 ng/100 U	2.7 ng/300 U
Frequency of dosing	Every 3 months	Every 3 months	Every 3 months
Storage before reconstitution	-5°C	< 25°C	2–8°C
Storage once reconstituted with NaCl (preservative-free)	2–8°C	2–8°C	2–8°C
Shelf-life (unopened)	36 months	36 months	24 months
Shelf-life reconstituted	< 24 hours	< 24 hours	<24 hours
Excipients	• Human albumin 0.5 mg • NaCl 0.9 mg	• Human albumin 1 mg • Sucrose 4.7 mg	• Human albumin 0.125 mg • Lactose 2.5 mg

Table 3. Comparison of commonly used commercial preparations of BoNT-A in Canada^{1-5,7}
Notes: Kilodaltons (kD), unit (U), nanogram (ng), milligram (mg), degrees Celsius (°C)

in the molecular weights of the purified protein complexes, the variance in size among the products is now thought to be inconsequential with respect to clinical outcomes.¹ One study demonstrated that dilution of the concentrated ONA and ABO complexes with normal saline, results in dissociation or release of 85% or more of the free 150 kDa neurotoxin, prior to treatment injection.¹⁸ If a patient stops responding to ONA over time, the patient can still be treated with ABO or INCO, and vice versa.

In terms of cost, the dosing of INCO is most similar to ONA, but the cost is typically slightly lower. ABO requires higher units of the product to achieve the same outcomes, however, the cost per unit is lower than ONA.

In summary, ONA, ABO and INCO are all considered safe and effective cosmetic treatments for wrinkles and facial asymmetries, and, with good patient selection, injector experience and expertise, clinicians can be confident of achieving excellent outcomes for their patients. They all originate from the same bacterium source, clostridium botulinum and are synthesized as BoNT-A formulations. Their safety and efficacy have been demonstrated through clinical trials, regulatory oversight and approval, and extensive worldwide use. Although there are subtle differences between the commercially available products, similar results of temporarily paralyzing targeted muscles to improve the appearance of wrinkles and asymmetries can be similarly achieved with all three products.

References:

1. Hexsel D, Hexsel CL. Botulinum Toxins. In: Robinson JK, ed. *Surgery of the Skin: Procedural Dermatology*, 3e. London, New York, Oxford, Philadelphia, St Louis, Sydney, Toronto: Saunders Elsevier Inc; 2015: 427-440.
2. BOTOX Cosmetic (onabotulinumtoxinA). Package insert. Allergan, inc. 2020.
3. Health Canada; 2020. ONA Product Monograph. Available from: https://pdf.hres.ca/dpd_pm/00057811.PDF
4. Health Canada; 2019. XEOMIN COSMETICTM Product Monograph. Available from https://pdf.hres.ca/dpd_pm/00051826.PDF
5. Health Canada; 2020. ABO Product Monograph. Available from https://pdf.hres.ca/dpd_pm/00058388.PDF
6. Hexsel D, Mazzucco R, Dal'Forno T, et al.: Botulinum toxin for facial wrinkles: history and future. *Expert Rev Dermatol*. 2007; 2:417-427.
7. Health Canada; 2021. BOTOX® Product Monograph. Available from: https://pdf.hres.ca/dpd_pm/00060199.PDF
8. Carruthers JA, Lowe NJ, Menter MA, et al.: A multicenter, double-blind, randomized, placebo-controlled study of the efficacy and safety of botulinum toxin type A in the treatment of glabellar lines. *J Am Acad Dermatol*. 2002;46:840-849 2002.
9. Ascher B, Zakine B, Kestemont P, et al.: A multicenter, randomized, double-blind, placebo-controlled study of efficacy and safety of 3 doses of botulinum toxin A in the treatment of glabellar lines. *J Am Acad Dermatol*. 2004;51:223-233.
10. Monheit G, Carruthers A, Brandt F, et al.: A randomized, double-blind, placebo-controlled study of botulinum toxin type A for the treatment of glabellar lines: determination of optimal dose. *Dermatol Surg*. 2007;33(Suppl. 1):51-59.
11. Hexsel D, Brum C, do Prado DZ, et al.: Field effect of two commercial preparations of botulinum toxin type A: a prospective, double-blind, randomized clinical trial. *J Am Acad Dermatol*. 2012;67(2):226-232.
12. Kerscher M, Roll S, Becker A: Comparison of the spread of three botulinum toxin type A preparations. *Arch Dermatol Res*. 2012;304:155-161.
13. Dressler D, Mander G, Fink K: Measuring the potency labelling of onabotulinumtoxinA (Botox®) and incobotulinumtoxinA (Xeomin®) in an LD50 assay. *J Neural Transm*. 2012;119(1):13-15.
14. de Moraes OO, Reis-Filho EM, Pereira LV, et al.: Comparison of four botulinum neurotoxin type A preparations in the treatment of hyperdynamic forehead lines in men: a pilot study. *J Drugs Dermatol*. 2012;11(2):216-219.
15. Hexsel DM, Almeida AT, Rutowitsch M, et al.: Multicenter, double-blind study of the efficacy of injections with botulinum toxin type A reconstituted up to six consecutive weeks before application. *Dermatol Surg*. 2003;29:523-529.
16. Hexsel D, Castro IA, Zechmeister D, et al.: Multicenter, double-blind study of the efficacy of injections with botulinum toxin A reconstituted up to six consecutive weeks before application. *Dermatol Surg*. 2004;30:823.
17. Hexsel D, Rutowitsch M, Castro LC, et al.: Blind multicenter study of the efficacy and safety of injections of a commercial preparation of botulinum toxin type A reconstituted up to 15 days before injection. *Dermatol Surg*. 2009;35:933-939.
18. Eisele KH, Fink K, Vey M, et al.: Studies on the dissociation of botulinum neurotoxin type A complexes. *Toxicon*. 2011; 57(4):555-565.