Gupta *et al. Plast Aesthet Res* 2024;11:16
DOI: 10.20517/2347-9264.2023.90

Plastic and Aesthetic Research

Review

Open Access

Updated review on neuromodulators

Shreya Gupta[1], Rasika Sudharshan[1], Alena Shen[1], Sandy Zhang-Nunes[1], Robyn Siperstein[2]

[1]USC Roski Eye Institute, University of Southern California, Los Angeles, CA 90033, USA.
[2]Department of Medicine, Florida Atlantic University, Boca Raton, FL 33472, USA.

Correspondence to: Dr Robyn Siperstein, Department of Medicine, Florida Atlantic University, 9897 Hagen Ranch Road, Boynton Beach, Boca Raton, FL 33472, USA. E-mail: DoctorSip@SipDerm.com

How to cite this article: Gupta S, Sudharshan R, Shen A, Zhang-Nunes S, Siperstein R. Updated review on neuromodulators. *Plast Aesthet Res* 2024;11:16. https://dx.doi.org/10.20517/2347-9264.2023.90

Received: 26 Sep 2023 **First Decision:** 22 Feb 2024 **Revised:** 26 Mar 2024 **Accepted:** 17 Apr 2024 **Published:** 29 Apr 2024

Academic Editor: Nan-Ze Yu **Copy Editor:** Yanbing Bai **Production Editor:** Yanbing Bai

Abstract

As botulinum toxin is increasingly used cosmetically and medically, it is important to understand the differences between each formulation of this product. While the active molecule in each is a 150 kDa protein that prevents neurotransmitter release, there are currently five products FDA-approved for clinical use, with a sixth currently in its Phase 3 trial and a seventh applied under FDA review, each with different nontoxic accessory proteins and varying amounts of human serum albumin. These properties affect diffusion rates and storage methods, which are outlined in this review. Common complications and recommendations to avoid them are discussed.

Keywords: Botulinum toxin, botox, cosmetic botox, medical botox

INTRODUCTION

The history of botox

Botox injection has become the most common nonsurgical cosmetic procedure performed in the United States, with an annual estimate of 4.4 million injections of botulinum toxin type A administered by plastic surgeons in 2020, solely for nonsurgical cosmetic use[1]. The earliest reporting of botulinum toxin, however, dates back to the Napoleonic War in the late 1700s. Due to unsanitary conditions during the war, there were many deaths associated with eating smoked sausage, which was later discovered to be caused by botulinum toxin-related food poisoning. Several decades later, Justinus Kerner, a German physician and poet, began conducting experiments to better understand the effects of this toxin. He also conducted experiments on

himself where he would ingest small amounts of toxin and record his own symptoms including intestinal spasms, ptosis, strabismus, and even respiratory failure. He later published the first case of botulism, and importantly, he hypothesized that this toxin could be used in treating medical conditions such as movement disorders[2].

Botulinum toxin was first used in humans to treat strabismus medically in 1980 by Dr. Alan Scott, an ophthalmologist in California, USA, who sought to find an alternative treatment to the existing surgical treatment for strabismus. He was the first to report the positive therapeutic effects of this toxin. Dr. Scott named his original product *Oculinum*, and it was first approved as a treatment for strabismus and blepharospasm associated with dystonia by the FDA in 1989. Once an ophthalmologist, Jean Carruthers, used this medication to treat a blepharospasm patient and received feedback from the patient that it made her wrinkles disappear, she and her husband, a dermatologist Alastair Carruthers, started to experiment for the first time with the cosmetic use of botox. They published the first paper on this topic in 1992 in *The Journal of Dermatologic Surgery and Oncology*, which led to the widespread use of botulinum toxin in aesthetic medicine[3]. The familiar name, *Botox* (OnabotulinumtoxinA), was given to the product in 1991 by Allergan, the first manufacturer of botulinum toxin for cosmetic use. From this point on, various companies created and received FDA approval for several types of botulinum toxin type A, and one formulation of type B (Myobloc) for different aesthetic and medical uses [Table 1] and additional approvals are continually being sought[4]. The constant addition of products and indications requires a continual amendment to the current literature to maintain an updated and accurate summary[5-8].

PHARMACOLOGY

Botulinum toxin is produced by the bacteria Clostridium. There are seven known serotypes of botulinum neurotoxin (A-G), five of which the human nervous system is susceptible to (A, B, E, F, G), and two of which (A and B) have been approved by the FDA and are commercially produced for clinical use. The active subunit of botulinum toxin type A (BoNT-A) neurotoxin is a 150 kDa protein. This 150 kDa protein binds to cholinergic nerve terminals to enter the cell through receptor-mediated endocytosis enabled by the C-terminal of the 100-kD heavy chain. Once internalized, a disulfide bond is cleaved, creating a 100 kDa heavy chain and a 50 kDa light chain. The light chain cleaves and inactivates SNAP-25, a SNARE protein that is required for the fusion of the synaptic vesicle with the presynaptic membrane and subsequent neurotransmitter release (inhibits acetylcholine exocytosis)[4,5]. Without the release of acetylcholine into the synapse, muscle fibers do not depolarize or contract.

Serotype A botulinum toxins have a longer onset of action but also a longer duration of effect. Their duration of action, measured by how long they inhibit neurotransmitter release, varies based on the half-life of the light chain and how long it takes the neuron to restore SNARE proteins[9].

INDICATIONS

The FDA-approved uses of botulinum toxin are described in Table 1, with approved age ranges of use specified in parentheses.

OFF-LABEL USE OF NEUROMODULATORS

Off-label use of neuromodulators spans both medical and cosmetic indications[6]. BoNT-A has been used for many dermatological conditions such as scar prevention, burns, keloids, wound healing[10], of psoriasis, rosacea, bullous skin disease, Darier disease, eccrine nevus hyperhidrosis, chromhidrosis, bromhidrosis, pompholyx, hidradenitis suppurativa[7], lichen simplex chronicus, lichen planus, pachyonychia congenita, aquagenic keratoderma, and alopecia[8]. Neurological conditions include facial palsy, spasm, post-herpetic

Table 1. Formulations of botulinum toxin A with FDA-approved use, preparation and storage specifications[4,10,11]

Generic name	OnabotulinumtoxinA	AbobotulinumtoxinA	IncobotulinumtoxinA	PrabotulinumtoxinA	DaxibotulinumtoxinA-lanm
Brand Name	Botox	Dysport	Xeomin	Jeuveau	Daxxify
Manufacturer	Allergan	Galderma	Merz	Evolus	Revance
Packaging (U/Vial)	50, 100	300	500	100	100
Molecular weight (kDa)	900	500	150	900	150
Preparation	Vacuum-drying	Lyophilized	Lyophilized	Vacuum-drying	Lyophilized
Storage prior to reconstitution	2-8 °C	2-8 °C	Room temperature	2-8 °C	Room temperature
Shelf life post-reconstitution	36 h	24 h	36 h	24 h	72 h
Contains accessory proteins	Yes	Yes	No	Yes	No
Contains HSA	Yes; 500 μg	Yes; 125 μg	Yes; 1 mg	Yes	No
Excipients	Sodium chloride, HSA	Lactose, HSA	Sucrose, HSA	Sodium chloride, HSA	PS20, sugar, buffer, excipient peptide (RTP004)
FDA-approved medical use	Overactive bladder, urinary incontinence from detrusor overactivity (adult), neurogenic detrusor overactivity (5+), prophylaxis of headaches from chronic migraine (adult), cervical dystonia (adult), severe axillary hyperhidrosis, dystonia-associated blepharospasm (12+), strabismus (12+) (Botox)	Cervical dystonia (adult), spasticity (2+)	Chronic sialorrhea (2+), upper limb spasticity (2+), cervical dystonia (adult), blepharospasm (adult)		Cervical dystonia (adult)
FDA-Approved cosmetic use	moderate to severe glabellar lines, moderate to severe lateral canthal lines, moderate to severe forehead lines (Botox Cosmetic)	moderate to severe glabellar lines	moderate to severe glabellar lines	moderate to severe glabellar lines	moderate to severe glabellar lines

HAS: human serum albumin; PS20: polysorbate-20.

neuralgia[8], trigeminal neuralgia, diabetic neuropathy, tremor, tics, post-stroke pain, spinal cord injury, myofascial pain, and bladder pain[6,12,13]. Muscular and fascial conditions include temporomandibular joint dysfunction, plantar fasciitis[10], and anal fissures, while skeletal and vascular conditions include epicondylitis, thoracic outlet syndrome, and Raynaud phenomenon.

Cosmetic off-label uses include treatment for decreased sebum production in oily skin[14], facial flushing[8], nasal tip ptosis[15], gummy smile[16], drooping oral commissures[12,17], dimpled chin[18], mental crease[18], platysma[19], neck elongation[19], bunny lines on the nose[20], pretarsal orbicularis hypertrophy[21], perioral lines[22], lip ptosis[23], and masseter[24], shoulder[25] and calf contouring[26,27]. In addition, once one of the Botulinum toxin A brands is FDA-approved for an

indication, all other brands are often used off label despite not having that specific indication[13,14].

FORMULATIONS

There are five variations of botulinum toxin A that are FDA-approved for clinical use, with a sixth currently in its Phase 3 trial and a seventh applied under FDA review. Table 1 provides the preparation and storage specifications of each formulation. Botox, Dysport, and Xeomin have been in circulation for much longer compared to Jeuveau which was initially approved in 2019 and Daxxify in 2022. A new product, RelabotulinumtoxinA, developed by Galderma, is in Phase 3 trials for the use of improving glabellar and lateral canthal lines[28]. In addition, Hugel is currently seeking FDA approval for LetibotulinumtoxinA (Letybo).

Clostridium botulinum is a bacteria that produces botulinum neurotoxin. The neurotoxin is produced from a culture and purified by a series of steps and then excipients are added to minimize product inactivation before the product is either freeze-dried (lyophilized) or vacuum-dried. All formulations except Daxxify contain human serum albumin (HSA), a protein component of human blood. BoNT-A is a protein complex comprised of nontoxic accessory proteins (NAPs) bonded to the 150 kDa neurotoxin. The molecular weight of the formulation depends on the composition of the NAPs and the manufacturing process. Xeomin has the complexing proteins removed during the manufacturing process, yielding a solution of just the 150 kDa toxin[29]. The purpose and effects of complexing proteins in botulinum toxin A may include stabilization of the neurotoxin and delay of diffusion into adjacent tissues[30], but can also cause the formation of neutralizing antibodies against the toxin[31]. This lack of complexing proteins may decrease the immune response, as was shown in a meta-analysis that revealed the lowest rate of neutralizing antibody formation[32].

Daxxify is the first to be formulated with the positively charged, proprietary stabilizing excipient peptide, RTP004. This peptide has demonstrated enhanced binding of the neurotoxin to negatively charged extracellular structures such as neuronal surfaces and extracellular matrix proteins. It is hypothesized that the enhanced binding may increase the likelihood of internalization of the neurotoxin, correlating with the clinically observed longer duration of effect[33].

While the active unit of all formulations of BoNT-A is the 150 kDA protein, there are still significant differences in the quantity of neurotoxin in each potency unit, and therefore, units cannot be compared and the units for different BoNTA preparations are not equal. In cosmetic practice, the dose ratio of Dysport:Botox:Xeomin has been shown to be approximately 2.5:1:1[9]. When comparing Dysport, Botox, and Xeomin, they were all shown to have equivalent light chain (LC) activities per quantity of BoNT-A, which disputed findings from a previous paper published by Frevert[34] that stated the difference in specific activity could be attributed to varying light chain activities. However, there are significant differences in the quantity of neurotoxin in each potency unit of these three BoNT-A products. Dysport was found to have greater amounts of active neurotoxin per vial compared to Botox and Xeomin[34]. The higher quantity of neurotoxin in Dysport could explain not only its possible longer duration of action, but also the possibility of increased adverse effects outside the target region compared to Botox[35].

COMMON PREPARATION AND DILUTION

Botox is the oldest commercially available product and therefore the most studied botulinum neurotoxin. Most manufacturers recommend that botulinum toxins be reconstituted with unpreserved saline, but preserved saline (which contains 9 mg of sodium chloride and 9 mg of benzyl alcohol) has been shown to not affect the potency of the neurotoxin[36] while making the injections less painful[37]. In addition,

reconstitution using lidocaine with epinephrine reconstitution also reduced patient discomfort while not decreasing the efficacy or safety of botulinum toxin A[38].

Yen *et al.* demonstrated a synergistic effect among Botox and bupivacaine, where patients experienced less pain with this combination compared to Botox with unpreserved saline as well as greater muscle weakness in the experimental side compared to the control at one week. This could be due to the greater myotoxicity of the combined formulation, but no difference was noted between the control and experimental sides at one and three months[39].

DIFFUSION

Several studies have examined the diffusion potential of botulinum toxin in both animals and humans. Neither molecular weight nor the presence of complexing proteins seems to affect diffusion, with one possible exception being Daxibotulinum toxin, in which the complexing proteins impart a positive charge. Instead, modifiable factors such as the type of reconstitution solution, the volume, concentration, and dose all influence this property[39]. In animals, Borodic *et al.* demonstrated that with high enough doses, diffusion of botulinum toxin could reach up to 45 mm from the site of injection and only 15-30 mm with the use of lower doses. Patients who were treated with botulinum A for idiopathic spasmodic torticollis for a mean of 1.1 years with 150 *vs.* 100 units had a significantly greater incidence of dysphagia ($P = 0.026$). This was due to the spread of botulinum toxin to pharyngeal muscles from the site of injection in the sternocleidomastoid when high doses are administered[39,40]. Arezzo and Yaraskavitch *et al.* also showed that it spread to other muscles that were not injected in a dose-dependent manner[41,42]. In addition, a human study showed an effect of botulinum toxin on untreated muscles, which the investigators hypothesized to be due to local diffusion[43].

In a study by Hsu *et al.*, diffusion was shown to be affected by fluid volume. Patients had one side of their forehead treated with 5U of toxin in 0.25 mL of preserved saline and the other side with 5U in 0.05 mL of preserved saline. The side injected with a fivefold increase in volume had a 50% increase in the affected area[44]. In addition, in a review paper, Ramirez-Castaneda writes that "the spread of [botulinum toxin] is dependent on a number of factors, one of the most important is the volume"[45].

However, there are other studies that do not show any effect on diffusion to nearby muscles. This difference in outcomes is likely related to many possible variables such as the concentration, volume, and dose of the botulinum toxin, the concentration of receptors and distance to the target area, muscle activity, and the technique used to inject (speed of injection, size of needle, placement, and number of injections).

COMPLICATIONS AND HOW TO AVOID THEM

Overall, the use of botulinum toxin A is very safe; however, there are certain common side effects including bleeding, edema, erythema, and pain at injection site[46]. All side effects will resolve and there have been no reported serious permanent side effects from the on-label use of botox for cosmetic indications[12]. While there is a potential risk of spreading viral diseases such as Creutzfeldt-Jakob Disease (CJD) in those products containing human albumin, it is extremely rare, and there have been no reported cases of any transmission to date[47].

Bruising, especially around the lateral canthus region, can be limited by using a small gauge needle and being attentive to the superficial vessels[48]. While more rare, nausea, malaise, influenza-like symptoms, brow or lid ptosis, ectropion, or strabismus may occur[46].

Contraindications

The prescribing information advises caution when administering to patients with pre-existing cardiovascular disease, neuromuscular disorders (myasthenia gravis, multiple sclerosis, and Eaton Lambert Syndrome), and those with compromised respiratory function or dysphagia. In addition, caution is recommended for those on aminoglycosides, anticholinergic agents, cyclosporin, D-penicillamine, and muscle relaxants. There are no studies or adequate data on the risk associated with the use of BoNTA in those who are pregnant or breastfeeding, and therefore, it should not be used in such cases. The safety and effectiveness of BoNTA in patients under 18 have not been studied. BoNTA is contraindicated in those with known hypersensitivity or allergy to either BoNTA or any components such as human albumin and those with an infection at the injection site.

Injection site reactions

Side effects can occur at local injection sites, including pain, skin dryness, and infection. Localized pain can occur from the needle puncturing skin. This pain can be attenuated using topical anesthetic, adjusting the needle to puncture with bevel side up, injecting slowly, using smaller gauge needles, or diluting the botulinum toxin with saline containing preservative. Local skin can become dry from decreased sweat gland activity. Additionally, the injection site can develop an infection. This rare side effect can be prevented with proper antiseptic solution application prior to the procedure, aseptic technique during the procedure, and post-operative care to not touch the area for 6 h until the injection site has closed[49].

Ecchymosis and purpura could possibly be decreased with cold application to injection sites before and after treatment[50]. However, there are also publications showing the uptake of neuromodulator is temperature dependent[51] with decreased uptake at lower temperatures. In addition pressure on the injection site after treatment for at least 10 seconds may decrease post-injection bruising[52]. Finally, there are many conflicting studies regarding the use of arnica, bromelain, and vitamin K in reducing bruising and increasing the rate of reabsorption. A systematic review of oral bromelain and arnica treatments for bruising reported insufficient data to determine their efficacy and additional research is needed[53].

Two short-term side effects are headache and allergic reactions. Headaches occur commonly after treating rosacea, and the severity depends on which nerve branch is affected. These usually resolve by themselves within a few days of the treatment. Otherwise, they can be treated with nonsteroidal anti-inflammatory drugs or opioids. Allergic reactions can also vary in severity and symptoms, including edema, erythema, urticaria, and anaphylactic shock. Antihistamines can be used for erythema lasting over 24 h, and anaphylactic shock is treated as a medical emergency[49].

Ptosis, diplopia & assymetry

One of the most dreaded aesthetic complications that arises is brow or lid ptosis. Brow ptosis occurs when horizontal forehead lines are treated by injecting the frontalis muscle. This complication can be avoided by injecting at least 2-3 cm above the supraorbital margin or 1.5-2 cm above the eyebrow. This allows the inferior frontalis muscle fibers to be spared and therefore can prevent ptosis[54,55]. To assist in preventing lid ptosis, which occurs from affecting the levator palpebrae muscle, placing fingers on the orbital ridge to prevent the toxin from diffusing into the orbit can be done and avoiding deep injections above the midpupillary line where the toxin could enter the supraorbital notch or foramen is important[56,57].

Injection of botulinum toxin close to the orbital ridge near the lateral canthus can cause diffusion through the orbital septum, leading to diplopia and strabismus by weakening the lateral rectus and extraocular muscles[29,34,35]. In addition, injecting toxin too deeply into the upper lateral aspect of the periocular area can affect lacrimal gland secretions, leading to xerophthalmia, while diffusion of toxin into the palpebral portion

of the orbicularis oculi can lead to lagophthalmos[54,55]. Staying 1-2 cm away from the lateral orbital rim and injecting superficially can aid in decreasing the likelihood of these side effects[55].

Rare complications seen when injecting toxin below the superior zygomatic arch or too low along the nasal side walls are asymmetry and lip ptosis. Diffusion into the upper lip elevators, levator labii superioris alaeque nasi, the zygomaticus muscles, and levator labii superioris can even result in difficulty with speaking, smiling, and eating[58]. An asymmetric smile can also occur when treating the masseter too medially or too superiorly by affecting the risorius, or by hitting the depressor labii inferioris when treating either the depressor anguli oris too medially or the mentalis too laterally, and finally, when not treating the orbicularis oris equally[59,60].

While it can be safe to treat the platysma, diffusion to underlying muscles due to improper technique can precipitate complications. These cholinergic muscles are responsible for deglutition, phonation, and neck flexion, and higher doses of botulinum toxin or deeper injection can result in xerostomia, dysphagia, and neck weakness[61,62].

CONCLUSION

In summary, the active subunit of botulinum neurotoxin is composed of a 150 kDa protein that binds to cholinergic nerve terminals, enters, and inactivates SNAP-25, preventing neurotransmitter release, disabling muscle fiber contraction. On-label cosmetic use of BoNTA has been found to be a very safe and effective medication with no long-term side effects when used correctly. It has been studied for a wide variety of indications, from wrinkle treatment to dystonia, as well as for a myriad of off-label indications. While each indication carries its own risk of side effects, a thorough understanding of the anatomy of each area will decrease the risk of potential side effects.

Increasing formulations with different onset of action and duration are continually emerging in the market. As more companies seek approval for newer products, additional research will be required to compare those with existing ones. There are also newer indications being studied with current products that may lead to a better understanding of botulinum toxin and all its capabilities.

With botulinum toxin's various cosmetic and medical applications, it is important to understand the differences in properties and clinical implications of each formulation. This article provides a comprehensive review of established and more recent products and both their on-label and off-label indications and can serve as a guide to understanding the uses and complications associated with each. In addition, while we understand the basic mechanism of action of botulinum neurotoxin, there is still a lot yet to be discovered.

DECLARATIONS

Authors' contributions

Background research, writing, and editing: Gupta S, Sudharshan R, Shen A, Zhang-Nunes S, Siperstein R

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Dr. Robyn Siperstein is a primary investigator, consultant, speaker, and trainer for Allergan and Galderma. All other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. 2020 plastic surgery statistics report. Available from: https://www.plasticsurgery.org/documents/News/Statistics/2020/plastic-surgery-statistics-full-report-2020.pdf [Last accessed on 19 Apr 2024].
2. Erbguth FJ. From poison to remedy: the chequered history of botulinum toxin. *J Neural Transm* 2008;115:559-65. DOI PubMed
3. Carruthers JD, Carruthers JA. Treatment of glabellar frown lines with C. botulinum-A exotoxin. *J Dermatol Surg Oncol* 1992;18:17-21. DOI
4. Whitcup SM, Hallett M. Botulinum toxin therapy. Springer; 2021.p.1-289.
5. Park MY, Ahn KY. Scientific review of the aesthetic uses of botulinum toxin type A. *Arch Craniofac Surg* 2021;22p:1-10. DOI PubMed PMC
6. Bach K, Simman R. The multispecialty toxin: a literature review of botulinum toxin. *Plast Reconstr Surg Glob Open* 2022;10:e4228. DOI PubMed PMC
7. Coetzee S, Nunez N, Belaunzaran M, Mark J, Stickler MA. Beyond wrinkles: a comprehensive review of the uses of botulinum toxin. *J Drugs Dermatol* 2023;22:7243e. PubMed
8. Martina E, Diotallevi F, Radi G, Campanati A, Offidani A. Therapeutic use of botulinum neurotoxins in dermatology: systematic review. *Toxins* 2021;13:120. DOI PubMed PMC
9. Walker TJ, Dayan SH. Comparison and overview of currently available neurotoxins. *J Clin Aesthetic Dermatol* 2014;7:31-9. PubMed PMC
10. Gassner HG, Brissett AE, Otley CC, et al. Botulinum toxin to improve facial wound healing: a prospective, blinded, placebo-controlled study. *Mayo Clin Proc* 2006;81:1023-8. DOI
11. Lewandowski M, Świerczewska Z, Barańska-Rybak W. Off-label use of botulinum toxin in dermatology-current state of the art. *Molecules* 2022;27:3143. DOI PubMed PMC
12. Naik PP. Utilities of botulinum toxins in dermatology and cosmetology. *Clin Cosmet Investig Dermatol* 2021;14:1319-30. DOI PubMed PMC
13. Padda IS, Tadi P. Botulinum toxin. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557387/ [Last accessed on 19 Apr 2024].
14. Shuo L, Ting Y, KeLun W, Rui Z, Rui Z, Hang W. Efficacy and possible mechanisms of botulinum toxin treatment of oily skin. *J Cosmet Dermatol* 2019;18:451-7. DOI PubMed
15. Yi KH, Lee JH, Kim SO, et al. Botulinum neurotoxin injection for treating plunged nose and post-rhinoplasty: anatomical perspectives of depressor septi nasi, nasalis, leveator labii superioris alaeque nasi muscle. *Anat Cell Biol* 2023;56:409-14. DOI PubMed PMC
16. Fatani B. An approach for gummy smile treatment using botulinum toxin a: a narrative review of the literature. *Cureus* 2023;15:e34032. DOI PubMed PMC
17. Yi KH, Lee JH, Hu HW, et al. Novel anatomical proposal for botulinum neurotoxin injection targeting depressor anguli oris for treating drooping mouth corner. *Anat Cell Biol* 2023;56:161-5. DOI PubMed PMC
18. Yi KH, Kim SB, Kim HJ. Ultrasonographic observations of the paradoxical mentalis bulging in regard to botulinum neurotoxin injection for mentalis muscle. *Skin Res Technol* 2023;29:e13517. DOI PubMed PMC
19. Yi KH, Lee JH, Lee K, Hu HW, Lee HJ, Kim HJ. Anatomical proposal for botulinum neurotoxin injection targeting the platysma muscle for treating platysmal band and jawline lifting: a review. *Toxins* 2022;14:868. DOI PubMed PMC
20. Yi KH, Lee JH, Hu HW, Kim HJ. Novel anatomical guidelines on botulinum neurotoxin injection for wrinkles in the nose region. *Toxins* 2022;14:342. DOI PubMed PMC
21. Kontis TC, Lacombe VG. Cosmetic injection techniques: a text and video guide to neurotoxins and fillers. Thieme; 2019.p.1-226.
22. Semchyshyn N, Sengelmann RD. Botulinum toxin A treatment of perioral rhytides. *Dermatol Surg* 2003;29:490-5; discussion 495. DOI PubMed
23. Loyo M, Kontis TC. Cosmetic botulinum toxin: has it replaced more invasive facial procedures? *Facial Plast Surg Clin North Am*

2013;21:285-98. DOI PubMed
24. Yi KH, Lee HJ, Hur HW, Seo KK, Kim HJ. Guidelines for botulinum neurotoxin injection for facial contouring. *Plast Reconstr Surg* 2022;150:562e-71e. DOI PubMed
25. Yi KH, Lee HJ, Choi YJ, Lee K, Lee JH, Kim HJ. Anatomical guide for botulinum neurotoxin injection: application to cosmetic shoulder contouring, pain syndromes, and cervical dystonia. *Clin Anat* 2021;34:822-8. DOI PubMed
26. Phan K, Younessi S, Dubin D, Lin MJ, Khorasani H. Emerging off-label esthetic uses of botulinum toxin in dermatology. *Dermatol Ther* 2022;35:e15205. DOI PubMed
27. Yi KH, Park HJ, Kim JH, et al. Intramuscular neural distribution of the gastrocnemius for botulinum neurotoxin injection: application to cosmetic calf shaping. *Yonsei Med J* 2023;64:511-7. DOI PubMed PMC
28. Galderma R&D. Long-term Treatment of Moderate to Severe Glabellar Lines and Lateral Canthal Lines (READY-4). Available from: https://clinicaltrials.gov/study/NCT04225260 [Last accessed on 19 Apr 2024].
29. Frevert J, Dressler D. Complexing proteins in botulinum toxin type A drugs: a help or a hindrance? *Biologics* 2010;4:325-32. DOI PubMed PMC
30. Aoki KR, Ranoux D, Wissel J. Using translational medicine to understand clinical differences between botulinum toxin formulations. *Eur J Neurol* 2006;13 Suppl 4:10-9. DOI PubMed
31. Sharma SK, Singh BR. Immunological properties of Hn-33 purified from type A clostridium botulinum. *J Nat Toxins* 2000;9:357-62. PubMed
32. Fabbri M, Leodori G, Fernandes RM, et al. Neutralizing antibody and botulinum toxin therapy: a systematic review and meta-analysis. *Neurotox Res* 2016;29:105-17. DOI
33. Solish N, Carruthers J, Kaufman J, Rubio RG, Gross TM, Gallagher CJ. Overview of DaxibotulinumtoxinA for injection: a novel formulation of botulinum toxin type a. *Drugs* 2021;81:2091-101. DOI PubMed PMC
34. Frevert J. Content of botulinum neurotoxin in Botox®/Vistabel®, Dysport®/Azzalure®, and Xeomin®/Bocouture®. *Drugs RD* 2010;10:67-73. DOI
35. Field M, Splevins A, Picaut P, et al. AbobotulinumtoxinA (Dysport(®)), OnabotulinumtoxinA (Botox(®)), and IncobotulinumtoxinA (Xeomin(®)) Neurotoxin content and potential implications for duration of response in patients. *Toxins* 2018;10:535. DOI PubMed PMC
36. Kwiat DM, Bersani TA, Bersani A. Increased patient comfort utilizing botulinum toxin type a reconstituted with preserved versus nonpreserved saline. *Ophthalmic Plast Reconstr Surg* 2004;20:186-9. DOI PubMed
37. Alam M, Dover JS, Klein AW, Arndt KA. Botulinum a exotoxin for hyperfunctional facial lines: where not to inject. *Arch Dermatol* 2002;138:1180-5. DOI
38. Gassner HG, Sherris DA. Addition of an anesthetic agent to enhance the predictability of the effects of botulinum toxin type A injections: a randomized controlled study. *Mayo Clin Proc* 2000;75:701-4. DOI PubMed
39. Yen MT, Wall VK. Bupivacaine-induced myotoxicity and its effect on botulinum toxin paresis. *Ann Plast Surg* 2008;60:6-9. DOI PubMed
40. Borodic GE, Joseph M, Fay L, Cozzolino D, Ferrante RJ. Botulinum A toxin for the treatment of spasmodic torticollis: dysphagia and regional toxin spread. *Head Neck* 1990;12:392-9. DOI PubMed
41. Arezzo JC. NeuroBloc/Myobloc: unique features and findings. *Toxicon* 2009;54:690-6. DOI PubMed
42. Yaraskavitch M, Leonard T, Herzog W. Botox produces functional weakness in non-injected muscles adjacent to the target muscle. *J Biomech* 2008;41:897-902. DOI
43. Eleopra R, Tugnoli V, Caniatti L, De Grandis D. Botulinum toxin treatment in the facial muscles of humans: evidence of an action in untreated near muscles by peripheral local diffusion. *Neurology* 1996;46:1158-60. DOI PubMed
44. Hsu TS, Dover JS, Arndt KA. Effect of volume and concentration on the diffusion of botulinum exotoxin A. *Arch Dermatol* 2004;140:1351-4. DOI PubMed
45. Ramirez-Castaneda J, Jankovic J, Comella C, Dashtipour K, Fernandez HH, Mari Z. Diffusion, spread, and migration of botulinum toxin. *Mov Disord* 2013;28:1775-83. DOI PubMed
46. Jensen JD, Freeman SR, Cohen JL. Botulinum toxins. In: Draelos ZD, editor. Cosmetic Dermatology. Wiley; 2015. pp. 364-74.
47. DailyMed. BOTOX COSMETIC - onabotulinumtoxina injection, powder, lyophilized, for solution. Available from: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=485d9b71-6881-42c5-a620-a4360c7192ab [Last accessed on 19 Apr 2024].
48. Kassir M, Gupta M, Galadari H, et al. Complications of botulinum toxin and fillers: a narrative review. *J Cosmet Dermatol* 2020;19:570-3. DOI PubMed
49. Witmanowski H, Błochowiak K. The whole truth about botulinum toxin - a review. *Postepy Dermatol Alergol* 2020;37:853-61. DOI PubMed PMC
50. Ascher B, Talarico S, Cassuto D, et al. International consensus recommendations on the aesthetic usage of botulinum toxin type A (Speywood Unit)--Part II: Wrinkles on the middle and lower face, neck and chest. *J Eur Acad Dermatol Venereol* 2010;24:1285-95. DOI PubMed
51. Rzany B, Zielke H. Safety of botulinum toxin in aesthetic medicine. In: de Maio M, Rzany B, editors. Botulinum Toxin in Aesthetic Medicine. Berlin: Springer Berlin Heidelberg; 2007. pp. 119-25.
52. Yılmaz D, Düzgün F, Durmaz H, Çinar HG, Dikmen Y, Kara H. The effect of duration of pressure on bruising and pain in the subcutaneous heparin injection site. *Jpn J Nurs Sci* 2020;17:e12325. DOI

53. Ho B, Jagdeo J, Waldorf HA. Is there a role for arnica and bromelain in prevention of post-procedure ecchymosis or edema? a systematic review of the literature. *Dermatol Surg* 2016;42:445-63. DOI
54. Carruthers A, Carruthers J. Clinical indications and injection technique for the cosmetic use of botulinum A exotoxin. *Dermatol Surg* 1998;24:1189-94. DOI PubMed
55. Klein AW. Complications and adverse reactions with the use of botulinum toxin. *Dis Mon* 2002;48:336-56. DOI
56. Klein AW. Dilution and storage of botulinum toxin. *Dermatol Surg* 1998;24:1179-80. DOI PubMed
57. Carruthers A, Carruthers J. Botulinum toxin type A: history and current cosmetic use in the upper face. *Semin Cutan Med Surg* 2001;20:71-84. DOI PubMed
58. Matarasso SL, Matarasso A. Treatment guidelines for botulinum toxin type A for the periocular region and a report on partial upper lip ptosis following injections to the lateral canthal rhytids. *Plast Reconstr Surg* 2001;108:208-14; discussion 215. DOI
59. Klein AW. Contraindications and complications with the use of botulinum toxin. *Clin Dermatol* 2004;22:66-75. DOI PubMed
60. Bae JH, Choi DY, Lee JG, Seo KK, Tansatit T, Kim HJ. The risorius muscle: anatomic considerations with reference to botulinum neurotoxin injection for masseteric hypertrophy. *Dermatol Surg* 2014;40:1334-9. DOI PubMed
61. Klein AW. Complications and adverse reactions with the use of botulinum toxin. *Semin Cutan Med Surg* 2001;20:109-20. DOI PubMed
62. Blitzer A, Binder WJ, Aviv JE, Keen MS, Brin MF. The management of hyperfunctional facial lines with botulinum toxin. A collaborative study of 210 injection sites in 162 patients. *Arch Otolaryngol Head Neck Surg* 1997;123:389-92. DOI PubMed