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SPECIAL TOPIC
Cosmetic

Sensory Symptoms Associated with Aesthetic Botulinum Toxin A Treatments

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Background: A retrospective review of patients who switched from onabotulinumtoxinA (onaA) and/or abobotulinumtoxinA (aboA) to incobotulinumtoxinA (incoA) found anecdotal reports of differences in "feel," including a "lighter" feel or treatment-associated tightness. We surveyed the frequency of these sensations as an initial proof of concept of toxin proprioception among our patients who switched toxins.

Methods: Seventy-nine patients who had past facial aesthetic treatments with more than one botulinum toxin A (BoNT/A) formulation completed a questionnaire on their experience of treatment-associated sensations, including stiffness or a frozen feeling.

Results: Treatment-associated sensations of tightness (47.3%), headache (41.8%), heaviness (38.2%), feeling frozen (29.1%), stiffness (20.0%), and weakness (20.0%) were reported by 55 of 79 patients. Furthermore, 78.2% of 55 patients noted an interformulation sensory difference. Of 79 patients surveyed, 68.4% of onaA-treated patients associated sensations with onaA, 39.1% of aboA-treated patients associated sensations with aboA, and 12.2% of incoA-treated patients associated sensations with incoA.

Conclusions: Some patients reported a different feel between toxins, and the difference in frequency of treatment-associated sensation varies between the different formulations used. Given the fine coordination of facial expressive muscles, we suspect that associated proprioceptive afferents are involved. Our findings confirm that post-toxin treatment-associated sensations can be detected by some patients, and this is likely due to the variations between the formulations. Failing to advise patients of this before switching formulations may cause a misperception that the treatment is not working well or that its effect has worn off prematurely, and some patients may consider switching formulations to reduce these conscious proprioceptive sensations. *(Plast Reconstr Surg Glob Open 2022;10:e4631; doi: 10.1097/GOX.0000000000004631; Published online 16 November 2022.)*

INTRODUCTION

Clostridium botulinum toxin A (BoNT/A) was first used in 1978 to treat strabismus[1] and then in 1992 for cosmetic purposes to treat facial wrinkles.[2] Since then, the therapeutic and aesthetic applications of BoNT/A have expanded,[1] including dystonias,[2] blepharospasms,[3] migraines,[4] sialorrhea,[5] glabellar frown lines,[6] and marionette lines.[7] Three commercial formulations are currently approved in Australia: onabotulinumtoxinA (onaA),[8] abobotulinumtoxinA (aboA),[9] and incobotulinumtoxinA (incoA).[10]

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Structurally, the commercial or pharmaceutical BoNT/A is a culture-extracted, single-chain polypeptide refined by proprietary processes.[8,9,11–13] The native BoNT/A is a complex of noncovalently bound proteins known as progenitor toxin complexes, which vary between 300 and 900 KDa.[14] These are comprised of the 150-KDa central core neurotoxin, which is common to all preparations,[15] a 140-KDa nonhemagglutinin protein (NTNHA),[16] and a variable combination of hemagglutinin proteins (Fig. 1). Manufacturing of onaA produces 900-KDa complexes, while aboA is produced as 500–600- and 900-KDa complexes,[17] though the exact composition, particularly

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its nontoxin components, is not publicized. The incoA preparation undergoes an extrachromatography step to extract only the pure 150-KDa neurotoxin.[18]

The mechanism of action and effects on cholinergic receptors by BoNT/A are well understood. All botulinum toxins inhibit acetylcholine release at the neuromuscular junction in a stepwise manner. Initial cleavage of the single-chain polypeptides by proteases results in the formation of a double chain with heavy and light chain moieties (Fig. 1). The heavy chain binds to a ganglioside receptor and a synaptic vesicle 2 (SV2) receptor[19] at the presynaptic nerve terminal, which enables the internalization of the toxin into endosomes. Disruption of the disulphide bond allows the light chain to translocate into the cytosol of nerve cells via the *N*-terminal translocation domain (H_N) part of the heavy chain. The light chain internally cleaves the membrane proteins (SNAP-25 within the SNARE or "SNAP receptor" complex) that facilitate acetylcholine vesicle docking, thus inhibiting the fusion of the vesicles with the nerve membrane and preventing acetylcholine release into the neuromuscular junction.

Although these formulations all derive from the same Hall strain of BoNT/A,[12,20] different manufacturing processes have led to observed clinical variations, only some of which have been explained.[21] In aesthetic medicine, the clinical efficacy of aboA, onaA, and incoA has been published for the treatment of upper facial lines.[22,23] IncoA demonstrated an equivalent efficacy to onaA and aboA,[24,25] and dose equivalence to onaA.[26,27] Other observed clinical differences among BoNT/A include spread, precision, speed of onset, and longevity, with variable evidence to support these claims. Injecting aboA into the forehead produced wider anyhdrotic halos than injecting incoA or onaA.[28,29] Thus, as onaA and incoA may have smaller fields of clinical effect than aboA, they are considered to facilitate more precise treatments.[30] IncoA onset of action has been shown at 3 days and aboA and onaA at 5 days.[31] Differences in immunogenicity and stability between the preparations are well documented.[32] In pain science, there is ongoing research into the actions of BoNT/A, which may have a greater biological influence than just through the acetylcholine neurotransmitter.[19]

A retrospective review of our patients who have switched from aboA and/or onaA to incoA found anecdotal reports of a difference in "feel." Some patients noticed a treatment-associated headache or tightness, while others reported a "lighter" feel with incoA. As this afferent aspect of BoNT/A has not been described in the literature, we aimed to document and survey the frequency of treatment-associated sensations identified by our patients as an initial proof of concept of toxin proprioception.

Takeaways

Question: Do patients who switch to a highly purified botulinum toxin A (BoNT/A) actually experience a different "feel," or toxin proprioception?

Findings: Patients previously treated with more than one BoNT/A formulation completed a survey on their experience of symptoms. A majority (69.6%) had symptoms such as tightness, headache, and heaviness, and most also felt a difference in sensation between formulations.

Meaning: Different botulinum toxin formulations can feel different, but patients should understand that their treatment is still effective.

METHODS

Patients who were identified as having had past treatment with more than one BoNT/A formulation for facial aesthetic indications completed a paper-based questionnaire (**See figure, Supplemental Digital Content 1**, which shows the proof-of-concept proprioception questionnaire, **http://links.lww.com/PRSGO/C235**.) upon presentation to our clinic. Patients were asked if they had ever experienced any of the following six symptoms associated with BoNT/A treatment and to identify the formulation with which this occurred: (1) a feeling of muscle weakness in the targeted area, (2) tightness, (3) stiffness, (4) headache, (5) heaviness, or (6) a frozen sensation. The patient-reported outcomes were collated in a spreadsheet, and chi-squared statistical analysis was conducted on the frequency of treatment-associated sensations reported after administration of at least one BoNT/A formulation.

RESULTS

Of the 79 patients who completed the questionnaire, 55 (69.6%) reported treatment-associated sensations (data not shown), and 43 of those 55 patients (78.2% of patients with sensations and 54.4% of all patients surveyed) noted a sensory difference between the formulations (Fig. 2). Of the 55 patients with treatment-associated sensations, 26 (47.3%) reported tightness, 23 (41.8%) reported headache, and 21 (38.2%) reported heaviness (Fig. 3). Additionally, 16 (29.1%) felt frozen, 11 (20.0%) felt stiffness, and 11 (20.0%) had the sensation of weakening in the targeted area. Furthermore, we analyzed the results

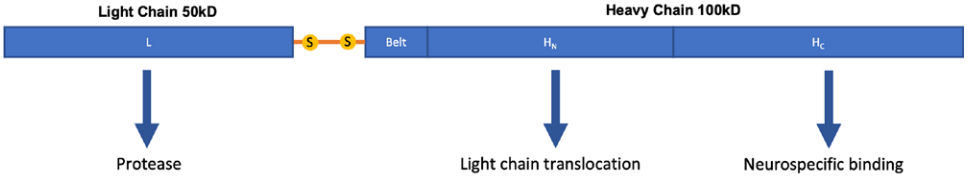

Fig. 1. Schematic of BoNT/A. The 150-KDa central core neurotoxin comprises a double chain with heavy and light chain moieties connected by a disulphide bond (not to scale). Adapted from the work by Lalli et al,[56] Pellizzari et al,[57] and Verderio et al.[58]

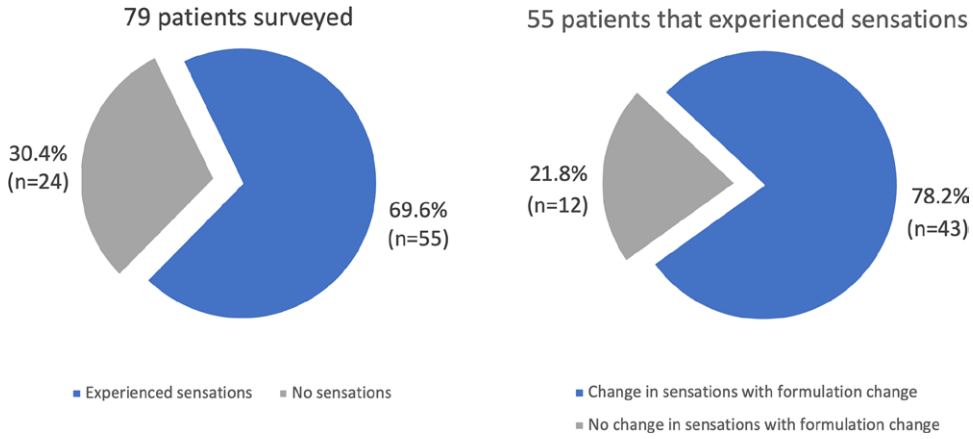

Fig. 2. Experience of treatment-associated sensations among cohort.

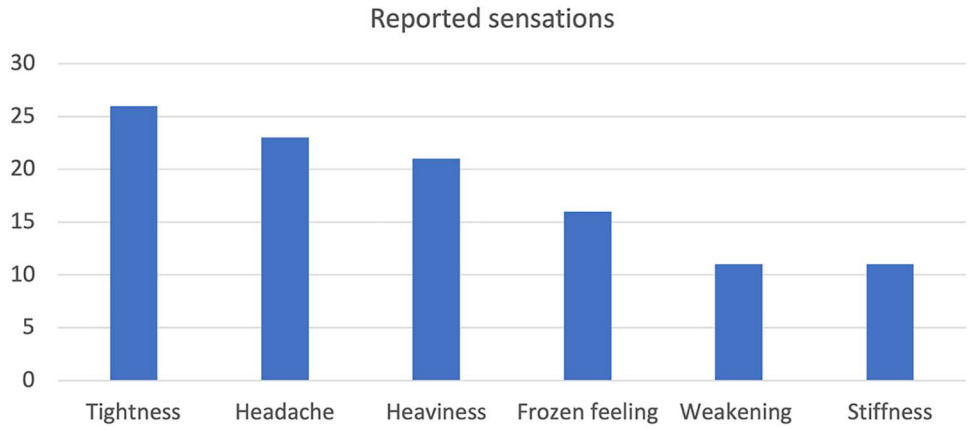

Fig. 3. Treatment-associated sensations reported by patients. Sensations were reported after receiving more than one BoNT/A formulation.

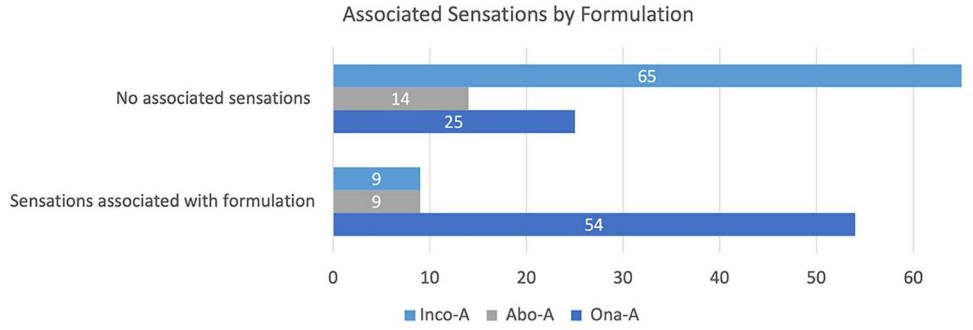

Fig. 4. Frequency of sensations associated with BoNT/A formulation. Frequency was assessed after receiving more than one BoNT/A product.

to see if treatment-associated sensations were associated differently with the formulations (Fig. 4). Fifty-four of 79 (68.4%) onaA-treated patients associated sensations with onaA, nine of 23 (39.1%) aboA-treated patients associated sensations with aboA, and nine of 74 (12.2%) incoA-treated patients associated sensations with incoA. We found that the difference in frequency of treatment-associated sensations versus no-sensations between formulations was statistically significant ($\chi^2 = 68.348$; $P < 0.00001$).

DISCUSSION

Among our patients switching between preparations, some reported a different feel between the toxins, which was not previously observed in the literature. We selected six symptoms for retrospective survey of these treatment-associated sensations among our patient cohort. The most common treatment-associated sensations reported by patients were tightness, headache, and heaviness. Given the minimal injection volumes used (0.01–0.05 mL per

injection point), treatment-associated sensations that are reported weeks after treatment are unlikely to be attributed to volumetric and local pressure effects.

Different manufacturing processes can produce minor modifications that may substantially alter the clinical profile of each BoNT/A formulation and explain the different clinical effects seen between the different BoNT/A products. Although we have not identified a cause for the treatment-associated sensations experienced by our patients, we propose several potentially contributory physiological and biochemical factors.

In 1906, the English neurophysiologist Sherrington[33] described proprioception as the perception of joint and body movement as well as position of the body or body segments in space. Proprioception is involved in regulating postural equilibrium and joint stability as well as initiating several conscious peripheral sensations. This conscious proprioception encompasses joint position sense, and the senses of resistance and heaviness. Sensory input is received from mechanoreceptors, such as muscle spindles, joint mechanoreceptors, Golgi bodies in tendons, and also cutaneous mechanoreceptors.[34] These varying sensory inputs are integrated at the spinal level, brain stem, brain cortex, and cerebellum. Higher levels of the central nervous system elicit the conscious awareness of proprioception.[35]

Many aspects of facial muscle proprioception remain unknown, such as the exact biochemical mechanism by which proprioceptive stimuli are conveyed to the central nervous system, and the exact function of the multiple trigemino-facial neural anastomoses found in the face.[36] Muscle spindles have not been detected in facial muscles, and it is assumed that skin mechanoreceptors provide the sole proprioceptive input.[37] The observed treatment-associated sensations in our cohort appear similar to those reported after facial nerve palsy, which is often accompanied by sensations of heaviness or numbness without associated sensory loss. The conscious proprioceptive sensation of heaviness in paralyzed limb muscles has been attributed to the unopposed firing of the intrafusal fibers of the muscle spindle, but in the absence of muscle spindles in the facial muscles, this remains unexplained.[37–39] However, given the fine coordination of facial expressive muscles, facial muscles may have associated proprioceptive afferents.

The CNV and CNVII cranial nerves comprise fibers that mediate both motor and sensory innervation of the face.[40] There are extensive interconnections between CNV and CNVII. The deep and superficial CNV nerve connections facilitate the proprioceptive and motor innervation of muscles of mastication.[41] It is currently thought that facial muscles may transmit proprioceptive impulses through the CNV branches[42] innervating the skin. However, it is likely that proprioceptive signals from the facial musculature transmit through connections between the facial nerve (the seventh cranial nerve; CNVII) and CNV to the mesenchymal trigeminal nucleus responsible for proprioception.[43]

In 2017, Cobo et al identified corpuscle-like structures within the zygomaticus major and buccal muscles. These structures were found in both muscles, were of variable size and shape, and contained numerous axon profiles arranged as a complex and resembling elongated or round Ruffini-like corpuscle. The acid-sensing ion channel 2 and transient receptor potential vanilloid 4 mechanoproteins present in mechanoreceptors, including muscle spindles, were detected in these corpuscle-like structures, confirming their mechanosensory nature.[36]

The mode of action of BoNT/A is still being evaluated beyond the well-understood, stepwise reduction of acetylcholine exocytosis across neuromuscular junctions. Receptors other than SV2 were found to be involved with the intracellular uptake of BoNT/A. Fibroblast growth factor receptor 3 was shown to be a high-affinity receptor for BoNT/A and pivotal to neuronal uptake.[44] The analgesic effect after BoNT/A treatment of dystonias and spasticity was assumed to be secondary to the muscle relaxation; however, other mechanisms of action were proposed for the afferent, antinociceptive effect after various pain conditions unrelated to muscular contraction were found to be relieved by BoNT/A treatment.[19] However, while preclinical studies showed that BoNT/A prevents neurotransmitter release and inflammatory changes, clinical studies failed to confirm this as a prime mode of action.[45]

BoNT/A can also inhibit exocytosis of other neurotransmitters such as calcitonin gene-related peptide (a pain mediator)[46] in afferent sensory pathways. In vitro, BoNT/A blocked peripheral sensory nerves[19] from releasing nociceptive neurotransmitters such as calcitonin gene-related peptide, substance P, and inflammatory mediators including serotonin and bradykinin. It also reduced transient receptor potential vanilloid subfamily member 1 (TRPV1) expression in trigeminal nerves, possibly preventing intracellular mobilization to the plasma membrane.[47] The effect of overactive bladder treatment has been postulated on efferent nerves to affect exocytosis of adenosine triphosphate as well as acetylcholine. In bladder sensory nerves, BoNT/A treatment may normalize key signaling receptors such as P2X and TRPV1 receptor.[48–50] In studies of BoNT/A on nerve cells, Li and Coffield suggested that BoNT/A might actually interact directly or indirectly with the transmembrane receptor TRPV1.[50]

We postulate that BoNT/A activity is mediated through the mechanoreceptor protein, TRVP4, in proprioceptive corpuscles observed by Cobo et al,[36] and accounts for the conscious proprioceptive sensations described by our patients. However, further in vitro and clinical investigations are needed to assess the variability of effects and the molecular mechanisms by which different BoNT/A formulations produce these outcomes.

We observed a difference in treatment-associated sensations between the formulations that we cannot readily explain. Complexing proteins, specifically the haemagluttanins,[51–53] present in onaA and aboA but not in incoA may have a role in nature through their ability to bind to E-cadherin and disrupt cell-to-cell adhesion, enabling the neurotoxin to pass through the small intestine into the circulation.[54] Johnson and Bradshaw[55] suggested that the hemagglutinin proteins may bind to other receptors hitherto unidentified that contain sialic acid and/or D-galactose on their surface.

When Wang et al[56] evaluated the binding of BoNT/A and/or the respective complexing proteins to neuronal and nonneuronal cells, they found that the pure BoNT/A (without complexing proteins) did not bind to nonneuronal cell lines, including skeletal muscle cells. However, both the BoNT/A complex (including complexing proteins) and complexing proteins alone did bind to these cells. Moreover, both the pure BoNT/A, BoNT/A complexes and complexing proteins could bind to neuronal cells, but only the BoNT/A complex and complexing proteins resulted in an increased release of inflammatory cytokines. Despite the limitations of this in vitro study, including the use of noncommercial botulinum toxins, their results point to the complexing proteins individually and as part of the BoNT/A complex binding to and exerting a cellular response independent to that of the pure BoNT/A in both neuronal and nonneuronal cells. The different formulations used clinically vary in their components. These studies indicate that they may be more than inactive components and can potentially influence clinical response despite having no role in BoNT/A's main mechanism of action to inhibit acetylcholine-mediated muscle relaxation.

Our study was limited by injector, dose, and technique variation, different BoNT/A formulations across the patient group, its retrospective nature, and most importantly, the inability to reliably identify BoNT/A products used in previous treatments at other clinics. As a retrospective study, it was also not possible to identify a precise postinjection timing after which treatment-associated sensations occurred, or for how long these sensations persisted, which also prevented us from linking a sensation's emergence to a particular formulation. Nevertheless, as an initial proof of concept, we collected sufficient data at the point of patient presentation at our clinic to warrant further investigations (eg, blind, prospective, and/or crossover investigations) of larger cohorts showing treatment-associated sensations due to specific formulations. More comprehensive analysis on the exact time of treatment-associated sensation onset and duration would also be beneficial to patients and clinicians, and allow better treatment planning.

Finally, we emphasize the importance of always performing BoNT/A therapy in an individualized manner, taking into full account a patient's needs or requests and the clinician's experience and expertise. The proof-of-concept insights presented here should be considered during treatment planning with regard to formulation choice. Injectors should also note that although many BoNT/A products are available worldwide, manufacturing processes are variable and may not be subject to equivalent regulatory standards. Consequently, BoNT/A product purity, stability, and potency can vary and manifest as a different or compromised predictability of clinical responses and safety to what would be expected.

CONCLUSIONS

Although this study has several limitations, a difference in patient-reported frequency of treatment-associated sensations was noted between BoNT/A formulations. Despite the limitations of this retrospective, proof-of-concept study, and the further research warranted, our findings are clinically meaningful and relevant as they confirm that some patients can indeed detect treatment-associated sensations after toxin treatment, and there is likely a difference between the formulations. Tightness, headache, and heaviness were the most commonly experienced. Some patients may regard their symptoms as a "normal" part of BoNT/A effect. Failing to advise patients of this before switching formulations may cause a misperception that the treatment is not working well or that its effect has worn off prematurely, purely because of the difference in sensation even when the motor effect is still evident. Furthermore, some patients may consider switching formulations to reduce the conscious proprioceptive sensations they associate with BoNT/A treatments.

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