Chapter 2

Botulinum Toxin Adverse Events

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Abstract

Botulinum toxin acts at the neuromuscular junction (motor plaque) blocking the release and effects of acetylcholine (ACh), a neurotransmitter of both the central nervous system (CNS) and the peripheral nervous system (SNP). By inhibiting the release of acetylcholine, botulinum toxin interferes with the nervous impulse and causes a characteristic flaccid paralysis of the muscles. This effect is used to decrease wrinkles of the facial skin and chin providing a smooth appearance and for the treatment of a variety of human syndromes characterized by hyperfunction of selected nerve terminals. Side effects of this treatment are rare, but are essentially related to the active ingredient of the drug or to medical malpractice. These adverse events and their possible therapy are discussed in this chapter.

Keywords: botulinum toxin, adverse events, therapy, esthetic, motor endplate

1. Introduction

Botulinum toxin is a neurotoxic protein produced by the anaerobic bacterium *Clostridium botulinum*. There are seven types of distinct botulinum toxin and are indicated with the alphabet letters: A, B, C, D, E, F, and G [1].

Recently, a novel botulinum neurotoxin (BoNT/X) has been identified [2] and the first botulinum-like toxin outside the *Clostridia* family has been described [3].

The currently used in esthetic medicine is botulinum toxin type A (BoNT-A). It is used for wrinkles of expression and for those dynamic wrinkles linked to the hypertonia of mimic muscles [4]. Botulinum toxin acts at the level of the neuromuscular junction (motor endplate) blocking the release and effects of acetylcholine, an ester of acetic acid and choline, responsible for neurotransmission both at the central nervous system (CNS) level and at the

peripheral nervous system (SNP) level. The enzyme acetylcholineesterase, present in the presynaptic nerve endings, continuously hydrolyses the acetylcholine which is then immediately resynthesized and stored through an active transport mechanism by means of a specific carrier protein, within synaptic cholinergic vesicles of storage. Within these cytosolic vesicles, acetylcholine is transported to the presynaptic region of the neuron (synaptic button) where it waits for the ionic signal (calcium ions) to release its role as a neurotransmitter [1].

Acetylcholine is normally released into the synaptic space trough a potential action that, by following the axon of the neuron at the last termination level of the final arborization of the axon, determines the opening of voltage-dependent ion channels: the channels of calcium. The calcium ions, present in the synaptic space, penetrate inside the synaptic button and start the realizing process of ACh into the synaptic space where it acts on specific receptors (ACh receptors). ACh receptors are located on the postsynaptic cell membrane of the muscle fibrocell, which are of two types: nicotinic and muscarinic. Interacting with ACh receptors, the neurotransmitter achieves its effects by determining, at the postsynaptic level, the opening of sodium-potassium ion channels through which the sodium ions penetrate into the muscle fibrocell which, thus, initiate muscle contraction. Immediately afterward, ACh is hydrolyzed by acetylcholinesterase. By inhibiting the release of acetylcholine, botulinum toxin interferes with the nervous impulse and causes a flaccid paralysis of the muscles. Botulinum toxin is in fact a real muscle relaxant [4].

Botulin toxin is a double-chain polypeptide consisting of a heavy chain and a light chain. The former has a molecular weight of 100 KDa while the latter has a molecular weight of 50 KDa. The heavy chain is linked to the light chain via sulfide bridges. The two chains perform different functions. The heavy chain binds to a receptor on the cell membrane of the synaptic button, the SV2 receptor, and begins the endocytosis phenomenon through which the botulinum toxin enters into the synaptic button. The heavy chain works like a sort of light chain conveyor [5].

Once penetrated into the synaptic button, the botulinum toxin releases the light chain that can perform its protease function capable of hydrolyzing the proteins of the SNARE complex (SNAP-25, syntaxin, synaptobrevin) of the neuromuscular junction preventing the release of ACh from synaptic vesicles [6].

The proteins of the SNARE complex play a crucial role in the release of ACh, because they favor the fusion between the membrane of the synaptic vesicles in which the acetylcholine and the membrane of the synaptic button are stored. The protein that is hydrolyzed is SNAP-25, and in this way, the fusion between the synaptic vesicle membrane in which the acetylcholine and the synaptic membrane are crammed is made impossible, and it is for this reason that the acetylcholine cannot be released into the synaptic space of the motor plate and the characteristic flaccid paralysis of the treated muscles is determined [7].

In 1980, botulin toxin was first described and used by ophthalmologists in humans for the treatment of strabismus [8], while its esthetic use was first reported in 1992 by Carruthers and Carruthers [9].

1.1. Commercial products of botulinum toxin

The most widespread toxin in the world has the trade name of Botox. Botox for esthetic use are called:

- Vistabel® 50 U (corresponding to Botox® used in pathology); the storage of the solution requires a temperature between 2 and 8°C, because the toxin is thermolabile. According to the technical sheet, it is maintained for up to 4 hours. According to some scientific works, in 12 hours, the effect is reduced to 50%. According to other works, the effect remains intact for 6 weeks. When inserting the needle into the bottle, the syringe must be sucked: this is a sort of test to verify that the product is actually under vacuum and has therefore been stored correctly;
- Azzalure® 125 U (corresponding to the Dysport® used in pathology); and
- Bocouture® 50 U (corresponding to Xeomin® used in pathology) is a bare toxin (not a complex protein like the previous ones). Units are not equivalent. The conversion rate is 2.5 (1 U Vistabel or Bocouture = 2.5 U Azzalure). Bocouture not requires the cold chain; it is stored at room temperature (0–25°) for 3 years and presents less risk of allergies as albumin is absent in the commercial preparation [10].

1.2. Therapeutic uses of BTX

In the last 20 years, the therapeutic spectrum of botulinum toxin has greatly increased. BoNT-A has been used for a wide range of established and emerging applications grouped into the following categories:

- neurological,
- otolaryngological,
- ophthalmological,
- urological disorders,
- esthetic,
- gastrointestinal/proctological disorders,
- pain, and
- symptomatic treatment of Parkinson's disease (PD) [11–13].

1.3. Esthetic uses of BoNT-A

In 2002, AIFA authorized the esthetic use of BoNT-A with the following indication: "Temporary improvement of vertical wrinkles, moderate to severe, between eyebrows to wrinkling, in adults aged <65 years, when the severity of such wrinkles has an important psychological impact on the patient." Although this is the only indication for esthetic use approved by the regulatory authority, many physicians use the toxin in off-label mode at injection sites other than those approved, in particular for periocular and frontal wrinkles [14]. Actually, botulinum toxin is approved by the US Food and Drug Administration (FDA) for esthetic use in the treatment of:

- axillary hyperhidrosis,
- glabellar lines, and
- lateral canthal lines.

Figure 1. Fronto-orbital balance of the eyebrows: levator muscles and depressor muscles. The fronto-orbital balance clarifies botulinum toxin action: relaxing of the frontalis muscle determines a strength increase of depressor muscles, with possible ptosis. Instead, relaxing of the depressor muscles causes a strength increase of the frontalis.

The dynamic rhytides of the upper third of the face are the best indication of botulinum toxin [15, 16].

These dynamic wrinkles depend on both the muscle factor and the photoaging. If the muscle factor (young subject) predominates and if the skin is fine, you can hope for a good result; if photoaging is predominant (older subject) and if the skin is thick, the result is less good. Despite the apparent ease of injections, the correction of these glabellar wrinkles in particular requires a good understanding of the anatomy and function of the fur muscles of the region. It is necessary to respect the depression/elevator balance, which is not the same for each face, and the type of frowning to choose the appropriate doses and to respect the recommended injection points (**Figure 1**) [17].

2. Adverse events

Side effects are essentially related to active ingredient of the drug and are referred to both therapeutic and esthetic use.

2.1. Effects related to the drug

Regarding the side effects related to the drug, those most frequently reported are:

- injection of high doses of this drug (more than 200 units in every injection); and
- booster within less than 1 month is dangerous [18].

Side effects of this treatment are rare, but can include bruising, headache, allergic reactions due to allergy to human albumin or sodium chloride present as an excipient in the drug, facial and palpebral edema, injection site pain, eye pain, erythema, psoriasis, skin infections, vertigo, nausea, fever, blepharitis, xerostomia, respiratory virosis, itching, asthenia, muscle weakness, psychiatric disorders, and pneumonia ab ingestis ineffectiveness of the drug (the formation of antibodies against botulinum toxin neutralizes the effect of the toxin itself).

In many cases, side effects can be minimized by lower injection doses [19].

2.2. Side effects of esthetic use

In esthetic, the dose of use is between 6 and 400 units; the maximum dose is between 400 and 600 U; the LD50 or toxic dose is between 2500 and 3000 U.

The most common reported side effects are mild and transient, and include injection site discomfort, erythema, bruising, temporary headaches, and rarely, prolonged migraine headaches [20].

A recent study on the safety of botulinum toxin described that the treatment-related adverse events were:

- eylid ptosis,
- brow ptosis,
- eye sensory disorders in the upper face,
- lip asymmetries, and
- imbalances in the lower face [18].

Eyelid ptosis is due to the interference with the function of the upper eyelid levator muscle. It can mainly occur when there is an excessive diffusion of the toxin to the frontalis muscle. It is therefore necessary to avoid high dosage, to inject slowly and firmly to press the eyeball with a free finger to prevent any possible diffusion of the drug into the orbital area. Eyelid ptosis appears after the 2nd day and can last from 1 to 2 months. Therapy is based on the administration of an eyedrop (Iopidine®) based on apraclonidine (α-adrenergic) which causes, in addition to mydriasis, the contraction stimulation of the Muller muscle of the upper eyelid, resulting in elevation of the lash margin (**Figure 2**) [21].

Eyelid ptosis is connected with the unwanted diffusion of the product toward the eyelid lift if the corrugator has been injected too low and too far outside. This complication is always feared, even if exceptional for an experienced operator, and hardly dissipates before 4–6 weeks. A possible asymmetry of the eyebrows can be corrected secondarily if it is an excessive and/or asymmetrical lift, while the lowering is more difficult to modify. The frontal muscle should not be injected too low, especially in men who already have eyebrows and a fairly low forehead [22, 23].

Lateral brow ptosis is due to chemodenervation of the frontal leaflet and therefore the orbicularis muscle of the eyes (pars superior) pulls down the lateral third of the eyebrow.

Medial brow ptosis can occur after excess of dosage or injections too low in the frontal muscle. Prevention consists of the injections at least 2 cm above the orbital rim.

Lateral brow elevation (mephisto sign) is caused by a compensatory contraction of the lateral portion of the frontal muscle. The remedy consists in the botulinum toxin treatment of the external portion of the frontalis muscle [24, 25].

A full blockage of the frontal mimic muscles can be avoided with intradermal injections to obtain a better distribution of botulinum toxin and with lower concentration in the underlying muscular tissue [26].

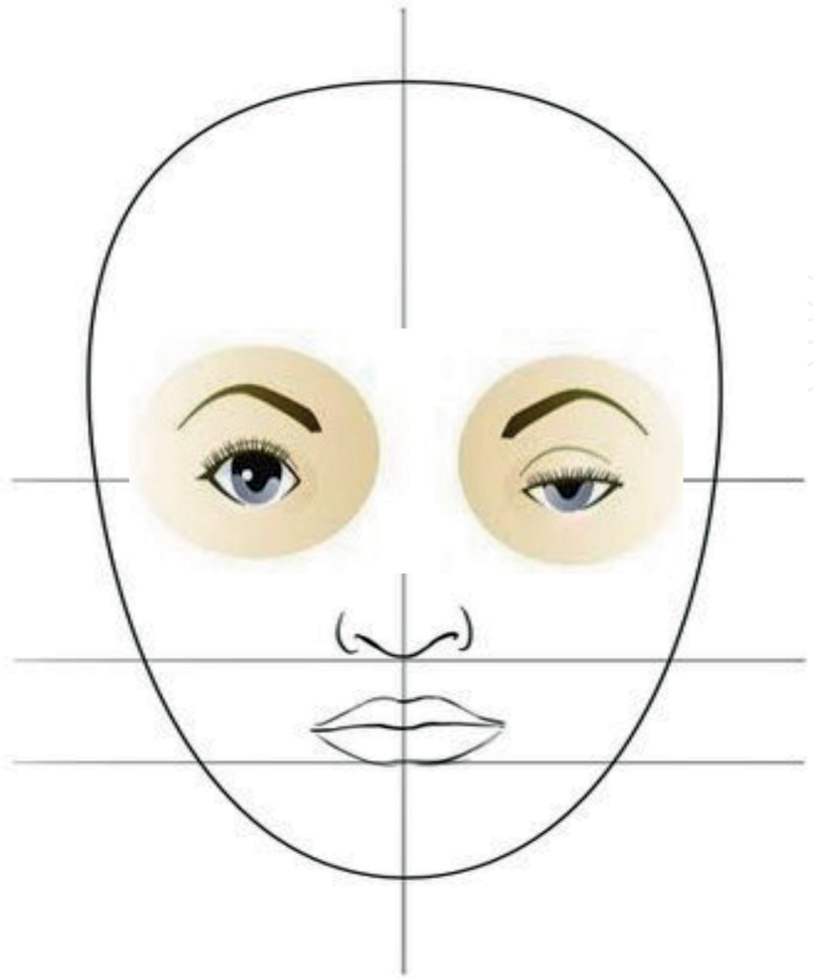

Figure 2. Schematic representation of eyelid ptosis complication of BoNT-A administration: uilateral eyelid ptosis.

A scleral show, greater evidence of sclera, can be verified after a functional deficit of the eye's orbicularis (pars inferior) following interference with the function of this muscle.

Ectropion, anomalous reversal toward the outside of the lower eyelid, is due to functional deficit of the orbicularis muscle of the eye (pars inferior) for chemodenervation of the orbicularis muscle.

A strabismus, deviation of the visual axes, is caused by the malfunction of the extrinsic oculomotor muscles (lateral rectus) with consequent inability of binocular representation at the retinal level.

Diplopia is caused by the involvement of the lateral rectus muscle through the diffusion of the toxin inside of the secondary orbitary cavity with inoculation too deep and close to the margin orbital. Temporary monolateral ocular bandage may be useful (**Figure 3**) [27–29].

Smile asymmetry is due to the toxin diffusion into the nearby zygomaticus major muscle and asymmetry of mouth mobility is caused by the blockage of the zygomatic muscle with ptosis of the lip (**Figure 4**).

Difficulty in whistling occurs after a functional deficit of the orbicular muscle of the mouth. Incidence may be reduced using diluted doses of botulinum toxin [30, 31].

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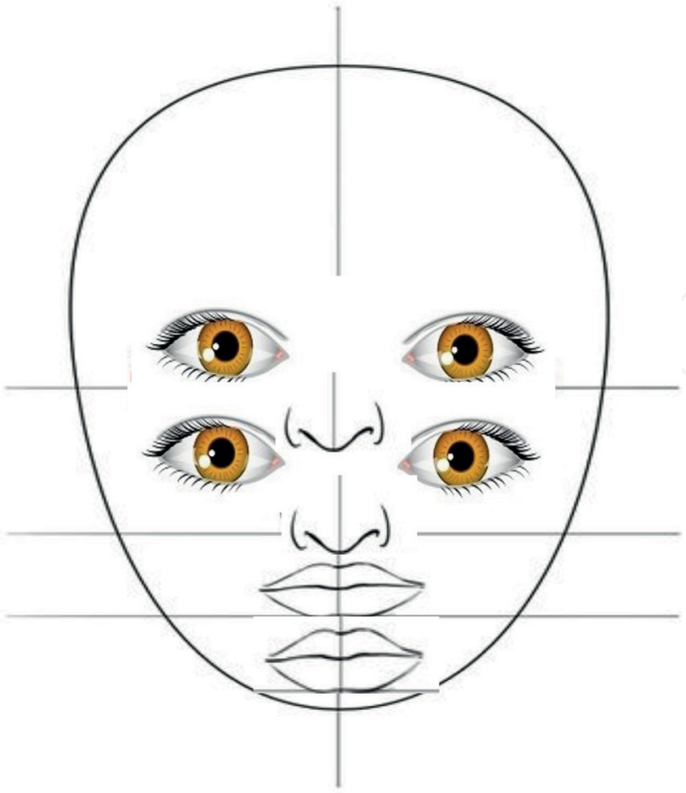

Figure 3. Schematic representation of diplopia complication of BoNT-A.

Botulinum toxin is often interesting to mitigate the fold of the marionette, which gives the face a sad and aged appearance, injecting the depressor of the corner of the mouth, which lowers the labial commissures. The injection must be low to prevent the lips from spreading to the orbicularis [32].

At the neck, the attraction through the posterior platysmal cords of the area in which the falling cheeks are delineated can be attenuated by the Nefertiti lift, injecting two or three small doses along the posterior platysmal chord and the mandibular edge. The anterior and posterior platysmal chords can be mitigated by small doses of botulinum toxin, injected every 2 cm, pinching and attracting the rope forward [33, 34].

All of these events resolved spontaneously maybe dose-dependent and were attributed to local diffusion of BoNT into adjacent areas [35].

Serious adverse events related to the cosmetic use of botulinum toxin include thyroid eye disease in a patient with Graves hyperthyroidism, sarcoidal granuloma, pseudoaneurysm of the frontal branch of the superior temporal artery, and respiratory damage [36–39].

2.3. Side effects of therapeutic use

Recent studies demonstrate that BoNT trafficking is not restricted to the neuromuscular junction, but also involves internalization of the toxin by spinal cord motor neurons and fast axonal retrograde transportation. Toxin's effect is sometimes observed beyond the site of local injection. Major adverse events can include:

- death,
- anaphylaxis,
- dysphagia,
- respiratory insufficiency, and
- muscle weakness.

These systemic events are rare and observed only at high dosages or in patients with underlying medical conditions predisposing to the complications [40–44].

Bahtia et al. reported on three patients in whom treatment of their dystonia with therapeutic doses of botulinum toxin resulted in clinical muscle weakness distant from the site of injections. It may be speculated that repeated injections at intervals of 10–12 weeks as in their patients may have an impact on toxin binding and diffusion. In fact, according to authors, the cause is most likely presynaptic inhibition due to systemic spread of the toxin [45]. Even in the case of repeated blepharospasm treatments with BoNT-A, an induction of acute myasthenic crisis has been demonstrated [46].

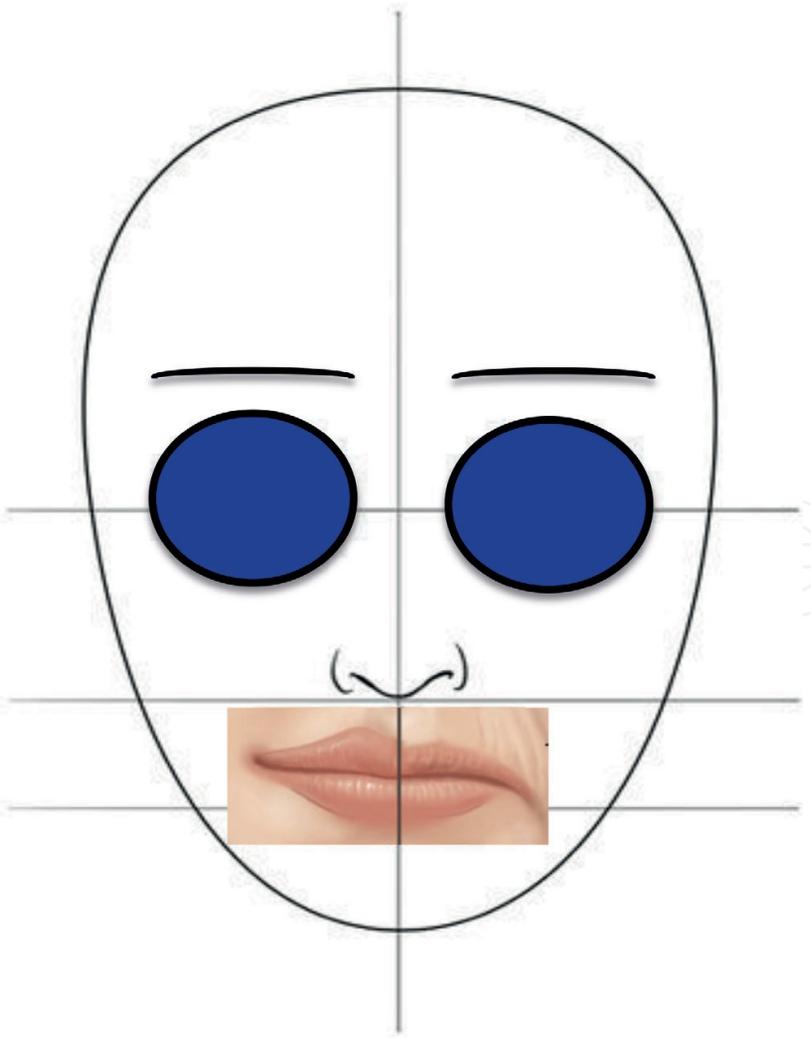

Figure 4. Schematic representation of asymmetry of mouth mobility of BoNT-A administration.

Systemic adverse events have been reported at the time of botulinum toxin A injection (6% injection episodes) and at follow-up (22% injection episodes) in children with cerebral palsy (CP), and children in Gross Motor Function Classification System (GMFCS) levels IV and V have increased rates of systemic adverse events [47].

Tugnoli et al. describes a first case of generalized muscular weakness associated with signs of systemic cholinergic autonomic impairment who was treated with 1400 U of BoNT-A for axillary and palmar hyperhidrosis. The authors assert that this case is consistent with a mild but diffuse Botulism-like syndrome, probably related to the high BoNT-A doses uses and to numerous intradermal injections and the slight build of their patient [48].

All these data demonstrate the possible risk of unwanted adverse effects due to spreading of the toxin [42].

2.4. Diffusion and migration of BoNT

In the diffusion phenomena, the concentration gradient and the BoNT molecular size determine the movement of the toxin beyond the immediate injection site through Brownian motion even if these muscles are separated by fasciae. In migration instead, a haematic and neuroaxonal transport of BoNT occurs, which is distant from the muscle and is related to systemic side effects that may be fatal if left untreated [49, 50].

Experimental studies in rodents have shown that botulinum toxin receptors exist in the central nervous system and a small amount of botulinum toxin crosses the blood-brain barrier. This raises the possibility that botulinum toxin is transported retrogradely, similar to tetanus toxin, and may cause centrally mediated side effects [51].

Botulinum toxin type-A can induce autonomic effects such as biliary colic, impairment of gastrointestinal and cardiovascular autonomic pathways, and inhibition of autonomic cholinergic pathways in the bladder. Cholinergic receptors in the pharyngeal and laryngeal sphincters are likely to be inhibited by systemic spread of BoNT and may be the main reason for dysphagia/dysphonia [52–54].

One of the suggested mechanisms for transport of the toxin from one part of the body (neck) to a remote location (toes) is the vascular spread via absorption through the capillary system and the retrograde axonal spread of the toxin. The injection of proximal upper extremity muscles with BoNT-A can determine diffusion of the toxin into the surrounding muscles resulting in dysphagia. These data suggest a systemic spread even when toxin is injected in sites anatomically adjacent to the locus of the side effect. Retrograde axoplasmic spread of the toxin is the second possible mechanism for the observed distant adverse events.

Recent studies show retrograde transport of enzymatically active toxin molecules via microtubules in the axon to both sensory and motor regions in the spinal cord after intramuscular and intraneural injections of BoNT-A. In fact, antinociceptive effect of BoNT-A may occur through retrograde spread of BoNT-A from the sensory nerves in the periphery to the central nervous system. Moreover, distant effects also may be caused by intrafusal uptake of the toxin in the muscles spindles as well as neuroplastic changes post-BoNT-A injections. Diffusion of BoNT is affected by a variety of factors; however, dose, concentration, and volume probably are the greatest contributors that increase the risk of diffusion. In general, the BoNT reduction in amplitude increased with increasing doses and with increasing concentration [55–57].

To limit diffusion is target muscle localization using EMG and endoscopic or imaging guidance [58].

2.5. Nonresponsiveness to treatment with BoNT

Nonresponsiveness to BoNT could be as a result of possible factors that include misdiagnosis, insufficient dose, problems with toxin storage and preparation, and administration. Another possible reason for lack of clinical effect is immunoresistance to BoNT, which refers to ineffectiveness of the toxin as a result of development of neutralizing antibodies against the toxin [59].

The formation of neutralizing antibodies to BoNT is increased by a short time period between injections, the administration of booster injections, and the use of high BoNT doses. To prevent antibody formation against BoNT, the practitioner can use a newer BoNT formulation with the lowest protein content [60].

3. Contraindications and interactions with some medications

BoNT is contraindicated in patients with known peripheral motor neuropathies or neuromuscular disorders, such as Eaton-Lambert syndrome, multiple sclerosis, and myasthenia gravis, because further chemodenervation may exacerbate muscle weakness. The cause is to be found in a reduced release of acetylcholine in the neuromuscular endplate, due to the effect of autoantibodies against the presynaptic channels of calcium [61].

The treatment can be performed in the 18–65 age range. Other contraindications are represented by:

- allergy to human albumin and/or sodium chloride,
- skin infections,
- presence of scleral show,
- senile ectropion,
- pregnancy,
- lactation,
- dysphagia, and
- psychiatric disorders.

Aminoglycoside antibiotics that can enhance the effect of botulinum toxin are netilmicin, tobramycin, gentamicin, neomycin, amikacin, kanamycin, and streptomycin. Other drugs that also interfere with neuromuscular transmission are muscle relaxants such as D-tubocurarine, baclofen, thiocolchicoside, tizanidine, diazepam, dantrolene, and pridinol [62, 63].

4. Rehabilitation of the motor endplate

The rehabilitation of the motor endplate can be very useful in case of side effects following treatment with botulinum toxin.

Radioiodinated botulinum toxin A (125I-BoNT/A-complex, 67 or 344 U free-125I-BoNT/A) was injected into the gastrocnemius muscle of rats and measured in various tissues at different time points. These "in vivo" studies allowed to establish that after 24 hours, the toxin is no longer present in the infiltrated muscle.

Thus, the side effects reported seem to be related to the damage caused by toxin caused and not to the presence of it in the muscles. These effects can be visible after 10–12 days [64].

For this reason, it is useless to administer the antitoxin which exerts its action by binding to the toxin still in circulation, complexing it and making it inactive. Furthermore, the healing capacity depends on the regeneration of the affected synaptic terminations.

Because the light chain of botulinum toxin causes proteolysis of the SNAP 25 protein, reducing its endocellular pool, one must reestablish its own physiological endocellular pool.

In practice, it is necessary to stimulate the biosynthesis of the SNAP25 protein to favor the structural and functional recovery of the motor endplate.

The aim of the therapy is to stimulate the biosynthesis of the SNAP 25 protein, consisting of about 200 amino acids. So, we can correct side effects such as ectropion, diplopia, palpebral ptosis, strabismus, scleral show, and asymmetries of smile and mouth mobility.

To improve the biosynthesis of the SNAP 25 protein, it is necessary to take:

a. A proteic diet (meat, fish);

b. Amino acids such as arginine and cysteine as they belong to the molecular composition of the SNAP-25 protein. Then, we supplement other amino acids: arginine, bioargin, and cysteine;

c. L-acetylcarnitine which is an agonist of the mitochondrial growth function and reparative agents (NGF), expounds an antioxidant activity in the neurons of the central and peripheral nervous system. L-acetylcarnitine is structurally similar to acetylcholine and plays an indispensable role for proper cellular energy, metabolism, and neurotransmission;

d. Alpha-lipoic acid (also called thioctic acid), a fat-soluble vitamin that participates in various antioxidant mechanisms such as the regeneration of reduced glutathione (GSH) and ascorbic acid; and

e. L-carnosine, a dipeptide composed of β-alanine and L-histidine; it has the ability to promote protein regeneration even in difficult situations such as in the late stage of the life cycle. It has antioxidant properties.

This therapy is able to guarantee fast responses (7–10 days) and in 80% of cases [65–68].

5. Conclusions

The use of BoNts continues to steadily expand and multiply. New indications of clinical use of BoNTs are continuously emerging in medical therapy and further applications will be developed in the future. Adverse events occur more frequently after the clinical use of the toxin, but may also disclose after its esthetic use. The safe utilization of BoNTs requires knowledge of its indications and pharmacology, anatomy of the treated muscles to avoid serious complications.

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