Botulinum Neurotoxin: The Ugly Duckling

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Key Words

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Abstract

This review presents a brief account of the most significant biological effects and clinical applications of botulinum neurotoxins, in a way comprehensive even for casual readers who are not familiar with the subject. The most toxic known substances in botulinum neurotoxins are polypeptides naturally synthesized by bacteria of the genus *Clostridium*. These polypeptides inhibit acetylcholine release at neuromuscular junctions, thus causing muscle paralysis involving both somatic and autonomic innervation. There is substantial evidence that this muscle-paralyzing feature of botulinum neurotoxins is useful for their beneficial influence on more than 50 pathological conditions such as spastic paralysis, cerebral palsy, focal dystonia, essential tremor, headache, incontinence and a variety of cosmetic interventions. Injection of adequate quantities of botulinum toxins in spastic muscles is considered as a highly hopeful procedure for the treatment of people who suffer from dystonia, cerebral palsy or have experienced a stroke. So far, numerous and reliable studies have established the safety and efficacy of botulinum neurotoxins and advocate wider clinical therapeutic and cosmetic applications.

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Introduction

During the last decade, the word ‘botox’ became widely known – particularly among women, for many of whom ‘botox’ is something that diminishes face wrinkles and promises to ‘rejuvenate’ skin [1]. The name ‘botox’ was initially given to a medical preparation containing traces of a toxin called *botulinum toxin*, after the anaerobic, Gram-positive bacterium *Clostridium botulinum*, which produces this substance. Today, several pharmaceutical products based on botulinum neurotoxins are commercially available. Although botulinum toxin became initially known for its high lethality for people and its first application in warfare, botulinum toxin now plays – at least in financially and socially advanced countries – a considerable role in pathological cases related to extreme smooth and skeletal muscle spasticity, and is widely applied in a variety of cosmetic and aesthetic interventions, but in this case the target is the physiological muscle [2].

Botulinum toxin is the most poisonous substance known. Inhaled by a person, 1 µg would certainly kill him or her, i.e. 1 g can theoretically kill 1,000,000 persons, a property exploited negatively by various military regimes for the production of lethal weapons. Indeed, several decades ago, this toxin killed a lot of Europeans. Botulinum toxin exerts its effects by paralyzing muscles innervated by the somatic (e.g. striated muscles) or the autonomic (e.g. exocrine glands and smooth muscles) nervous system [2, 3]. Medical doctors have learned to

use this toxin beneficially to treat some pathological conditions caused by neurogenic overactivity and muscle spasticity, i.e. involuntary, continuous muscle contraction and hyperactivity. The unique nerve-paralyzing property of this toxin is studied intensively for the betterment of mankind through clinical applications to exploit its qualities and usefulness and to restrict dissemination [2, 4]. In many countries, health care professionals are allowed to use low doses of this toxin for the treatment of specific cases of somatic and autonomic muscle spasticity such as craniocervical dystonia, blepharospasm, strabismus, hyperhidrosis, pain syndromes, and they may inject the toxin between the brows to eliminate interbrow wrinkles [2]. Nevertheless, the exact relationship of this drug with traditional therapies such as neurotomy or chemical treatment remains to be elucidated.

Reviewing the literature on epidemiology, medical, cosmetic and military applications of botulinum toxin is not an easy task. There are more than 10,000 interesting articles and the relevant papers published every week all over the world contain much more information than the average human brain can assimilate. Even the most recent part of the literature dealing with the impact and safety of botulinum toxin in clinical applications is rather enormous, including short [4], extended [5], classical [6] and new [7–11] articles. An additional difficulty originates from the fact that the research field is spread over a wide range of various and frequently not very much related disciplines such as biochemistry, bioterrorism, chemical synthesis, cosmetics, ecology, medicine, microbiology, neurology. The aim of the present work is to provide an account of the most important aspects of the biological activity and applications of botulinum neurotoxins, from the pioneering to the most recent developments. The intention of this article is to give sufficient background information and help the reader who may not be familiar with the subject to feel informed. It is not the purpose of this review to consider all or even a significant proportion of the existing literature. Here, I only consider briefly some of the literature concerned with overall applications of this substance, which, once a dreadful poison, is now considered as a highly promising therapeutic and cosmetic instrument.

Botulinum Neurotoxins Cause Botulism

Botulism is a muscle-paralyzing disease caused by neurotoxins usually produced by anaerobic, Gram-positive, spore-forming bacterial species, such as *Clostridium*

botulinum, *C. baratii* and *C. butyricum* [12]. *C. botulinum* causes 99.5% of the cases of botulism. Today, botulism has a relatively minor impact on human health. Botulinum neurotoxins block voluntary and autonomic motor cholinergic neuromuscular junctions, thus preventing motor fiber stimulation [2]. So far, 7 (A–G) serologically different botulinum neurotoxins have been detected. Neurotoxins A, B, E, F and G are harmful for people; A is the most poisonous, followed by B and E [13]. Only neurotoxins A, B and F are coded by the *C. botulinum* genome, whereas the rest are coded by genes carried by bacteriophages and plasmids [14]. In their native states, these neurotoxins are bound to complexing nontoxic proteins (hemagglutinins and nonhemagglutinins) which enhance neurotoxin molecular stability, thus creating what is called the bacterial toxin. These neurotoxins are targeted against different proteins of the acetylcholine-exocytosing system.

Intravenous or intramuscular injection of 0.1 µg, inhalation of 1 µg and oral administration of 70 µg of toxin type A may be fatal [15, 16]. Spores of *C. botulinum* are ubiquitous in the environment, but bacterial growth and toxin elaboration occur only under particular conditions that include an anaerobic, low-salt, low-acid environment. Bacterial growth is inhibited by refrigeration below +4°C, heating above 121°C, high water activity, acidity (pH <4.5, e.g., vinegar) and high osmotic pressure (e.g., excess of salt and sugar). The neurotoxin is destroyed by heating to 85°C for at least 5 min, and spores are inactivated by heating to 121°C under a pressure of 15–20 lb/in² for at least 20 min. The canning and fermentation of foods are particularly conducive to creating anaerobic conditions that allow *C. botulinum* spores to germinate. There are three main kinds of botulism: (a) food-borne botulism occurs when a person ingests preformed toxin that leads to illness within a few hours to a few days. Food-born botulism is a public health emergency because the contaminated food may still be available to other persons besides the patient. Early in the 20th century, the proportion of botulism outbreaks caused by contaminated, commercially produced foods declined; however, improperly home-canned foods have long constituted a major source of botulism worldwide. (b) Infant botulism occurs in a small number of susceptible infants who harbor *C. botulinum* spores in their intestinal tract, usually as a result of eating honey. (c) Wound botulism occurs when wounds are infected with *C. botulinum* that secretes the toxin. As expected, wound botulism lacks the initial gastrointestinal manifestations (nausea, vomiting, abdominal discomfort, diarrhea) of food-borne botulism. Re-

cently, a fourth kind, i.e. iatrogenic botulism, has been proposed.

Clinical illness from botulism is characterized by cranial nerve palsy followed by descending flaccid muscle paralysis and devastating autonomic nervous system perturbations. The paralyzing effect is exerted at the level of the neuromuscular junction. Paralysis usually peaks 2 weeks after toxin injection and can involve the respiratory muscles. Symptoms of botulism include dilated pupils, double vision, blurred vision, drooping eyelids, slurred speech, difficulty in swallowing, dry mouth, muscle weakness that always descends through the body: first the shoulders are affected, then the upper arms, lower arms, thighs, calves [11]. Although ptosis (drooping of the upper eyelids) and dysarthria (impaired articulatory ability) may be mistaken for signs of encephalopathy, patients are fully alert and the results of a sensory examination are normal. Cognitive and sensory functions in botulism are almost always totally spared [17]. Paralysis of breathing muscles can cause a person to stop breathing and die unless assistance with breathing (mechanical ventilation) is provided and equine antitoxin is administered, optimally within 12 h of presentation [18]. Antitoxins must be administered early since they effectively neutralize only free neurotoxin molecules, and not those already bound by neural tissue. Surviving persons recover partially to completely after weeks or months [19] as a result of production of new neuromuscular junctions [12, 20].

Botulinum Neurotoxins Cause Muscle Paralysis by Inhibiting Synaptic Transmission

The botulinum neurotoxins are synthesized by the *Clostridium* bacteria and excreted in the form of inert, 150-kDa polypeptides which, produced inside the human body or gaining direct access into it, enter nerve endings. Within the human body, botulinum neurotoxins exert their detrimental effects (or beneficial effects, in cases of therapeutic treatment) on neuromuscular junctions by inhibiting the transmission of action potentials. Botulinum neurotoxins, in particular, exert their effects on the level of the motor unit. A motor unit is defined as a motor neuron plus all its innervating muscle fibers. The region where an axon makes functional contact with a muscle fiber constitutes the neuromuscular junction (fig. 1). The cell body of the motor neuron is usually located at the ventral horns of the gray matter of the spinal cord (lower neurons). The nerve cell stimu-

Fig. 1. Neuromuscular junction. **a** A motor neuron synapses via axonal endings with a striated muscle cell. **b** Every axonal ending includes thousands of vesicles containing the neurotransmitter acetylcholine. The action potential causes influx of Ca^{2+} into the axonal ending and acetylcholine exocytosis into the synaptic gap. **c** Binding of acetylcholine to its receptor causes influx of Na^{+} into the muscle cell and consequently its contraction. The presence of botulinum neurotoxins within the axonal ending of the motor neuron inhibits fusion of the presynaptic membrane with the membrane of the synaptic vesicles; acetylcholine is not released into the synaptic cleft, and the corresponding receptor remains inactivated so that the muscle remains flaccid.

lates the muscle cells of its own motor unit to contract by transmitting the action potential. The action potential generated at the axonal hillock is transmitted along the surface of the axon and, when the depolarization wave arrives at the synapse, the neurotransmitter acetylcholine is liberated and transmits the stimulation to the corresponding muscle cell, which contracts. Immediately, acetylcholinesterase destroys acetylcholine and the muscle stops receiving contractive messages. At the neuromuscular junction, each neurotoxin is cleaved by specific proteases into two fragments of 100 and 50 kDa which reunite differently by bisulfite bonds, acquires enzymatic activity and cleaves some presynaptic, endosomal proteins (e.g. SNAP-25, VAMP, Syntaxin) so that it prevents fusion of the presynaptic membrane with the membrane of the presynaptic vesicles and inhibits release of acetylcholine into the neuromuscular junction, thus inducing flaccid paralysis of the corresponding muscle [21]. In this way neurotoxins paralyze smooth and striated muscles, inhibit spinal reflexes and impede exocrine gland function. The carboxyl-terminal domain of the 100-kDa subunit recognizes specific binding molecules on the motorneuron endings. These binding molecules are the abundant polysialogangliosides and protein receptors. After binding, the cycling of the synaptic vesicle brings the toxin inside the nerve terminal [22, 23]. The tripartite mechanism of botulinum neurotoxin endocytosis and action, i.e. binding of the neurotoxins to cell surface receptors, translocation of the active light chain into the cytosol and cleavage of the presynaptic endosomal proteins, is currently the target of the relevant scientific community in order to develop products against intoxication [24].

Neurotoxin action on the central nervous system (reflex inhibition) is not immediate since toxins do not cross the blood-brain barrier and are neutralized during retrograde axonal transport [7]. Apart from inhibition of acetylcholine release, it has been shown that botulinum neurotoxin also inhibits secretion of other neurotransmitters such as substance P, glutamate and noradrenaline.

Bioterrorism

C. botulinum is a cosmopolitan species, easily isolated even from the soil, whereas culture, enrichment and drug storage do not pose any particular difficulties. Because of its high lethality, the toxin was misused about 80 years ago by military regimes against their opponents, both soldiers and civilians. Despite that, most authorities agree

that this toxin would be very difficult to deploy as a weapon of mass destruction because the toxin rapidly degrades in the environment and becomes nonlethal minutes after release. Due to its high weight (>900 kDa, complexing proteins included) the toxin does not penetrate into the skin. Antitoxins against those neurotoxins are already available, but antitoxin A does not neutralize antitoxin B and vice versa [25].

The Revelation of the Swan: Clinical Exploitation of the Muscle-Paralyzing Quality of Botulinum Neurotoxins

In mature organisms, the function of spinal (lower) motor neurons is under the control of upper motor neurons located in the brain cortex. When a lower motor neuron is destroyed, the classical notion states that the corresponding muscle fibers usually become permanently paralyzed. This might not be entirely true, since data are continuously accumulating that support possibilities for lower motor neuron restoration [26, 27]. However, when an upper motor neuron is destroyed, then the corresponding lower, intact motor neuron does not receive adequate messages, so that the corresponding muscle fibers do not receive the message of voluntary contraction and are regarded as paralyzed, which means that they cannot contract under voluntary control [28]. Despite the absence of an upper motor neuron, the corresponding lower neuron and the related muscle fibers may be stimulated by various causes, resulting in painful and dysfunctional muscle spasticity. This debilitating condition is a muscular disorder induced by various brain damages, such as dystonia, cerebral palsy, cerebral stroke, and accidental injury [8, 19, 29, 30].

Focal Dystonia

Focal dystonia is a neurological disorder which occurs usually in older individuals, women being more likely to develop it. The main symptoms, which can be mild or severe, include involuntary muscle contraction and uncontrollable twisting of the affected body part (eyelids, neck, face, hand, foot). The exact causes of dystonia are still elusive, but it has been linked to communication problems with neurons located in the basal ganglia [31]. The first most frequent indication of botulinum toxin treatment among focal dystonias is cranial dystonia, notably blepharospasm, sometimes bruxism, trismus, oromandibular dystonia and rarely lingual dystonia. The second most frequent use of this treatment is cervical

dystonia (spasmodic torticollis), and more rarely pharyngeal or laryngeal dystonia. The other indications of botulinum toxin therapy for focal dystonia are hand dystonia especially writer's cramp and foot and toe dystonia. Injections of pharmaceutical preparations of botulinum toxin are satisfactory, providing relief to many patients for about 3–6 months, depending on the location and the severity of dystonia [32].

Cerebral Palsy

Since the development of the human brain lasts up to about age 3, developmental disorders and/or damages are likely to occur. A group of disturbances characterized by disorders of movement and posture is known as cerebral palsy, although not all symptoms are attributed to the cerebrum. It is estimated that 2 out of 1,000 live births suffer from cerebral palsy. The brain damages can occur during pregnancy (75%), during childbirth (5%) and after birth (15%) [33–35]. The damaged area may involve the motor cortex, the corticospinal tract (e.g., spastic cerebral palsy), the cerebellum (ataxic cerebral palsy), the extrapyramidal/pyramidal system and the basal ganglia (athetoid cerebral palsy). The symptomatology of cerebral palsy is very diverse, ranging from mild facial gestures to severe impairments of movement and posture. The most characteristic symptoms include spasticity, unsteady gait, imbalance, decreased muscle mass; 'fortunately', the symptoms are not progressive [35]. The medical arsenal against cerebral palsy has recently been enriched by the recruitment of botulinum toxins. Presumably, the toxin relieves the symptoms of spasticity by disrupting the hyperactive spinal reflex at the level of the neuromuscular junction [36]. Although there are numerous contradictory results concerning the efficacy of botulinum toxins and classical treatments (e.g., serial casting) on the spastic muscle [37, 38], accumulating evidence suggests that higher than usual doses of botulinum toxin are both safe and effective in improving the patient's health state [36, 39].

Cerebral Stroke

More than 2 million people are affected by vascular cerebral stroke every year worldwide. About one-half of stroke survivors who live for more than 6 months lose voluntary control of half of their bodies (hemiparesis). Twenty-two percent cannot walk, approximately 50% are partially or completely dependent on others to care for them and one third suffers from depression [40, 41]. One threatening and progressive outcome of upper motor neuron cell death is spastic paralysis, whereby stretch-

sensitive (spastic) muscle overactivity emerges along with paresis and soft-tissue contracture [8, 28, 42]. The resulting loss of brain function is usually due to post-stroke neuron cell death [28, 43]. Death of primary motor cortex neurons initiates loss of voluntary control over the corresponding skeletal muscles because the lower neurons of the spinal cord do not receive adequate signals. Nevertheless, spinal cord neurons still receive excitatory signals from other sources, so that the innervated muscles may still twitch, though in an uncontrolled fashion which is frequently painful and prevents purposeful movements [44–46]. However, if a physiotherapeutic rehabilitation program is implemented immediately after stabilization, the patient may regain a substantial part of his/her previous abilities [7, 47, 48]. Therefore, a caring team should immediately intervene in the hope to prevent muscle rigidity and shortening [49]. If rigidity and spasticity are already established, rehabilitation can still be achieved by applying a physiotherapeutic training program preceded by relaxation of the spastic muscles with various drugs. Some of them, e.g., diazepam, dantrolene, zanaflex and baclofen, cause serious side effects, such as apathy, sedation and confusion. Recently, intrathecal infusion of baclofen displayed spectacular results, especially concerning the lower limbs [50, 51], although untoward effects are sometimes encountered [50]. Other chemical substances (e.g. phenol, alcohol) partially destroy peripheral nerves, thus blocking muscle contraction; however, for special cases or combination with other drugs the lack of specificity and the many side effects caused by such substances enable their application and acceptance [52, 53].

Two decades ago, scientists found that the botulinum neurotoxin, a deadly poison, as has already been referred to above, could be safely used in very low doses to treat various muscle disorders by exploiting knowledge and experience derived from botulism [8, 54–56]. Recently, the use of botulinum toxins has found a wide range of applications, including treatment of several muscular disorders such as achalasia (inability of esophageal muscles to relax), anal fissures (a tear in the lining of the lower rectum), blepharospasm (any abnormal tic or twitch of the eyelid), bruxism (grinding of teeth), cerebral palsy, cramps, dysphagia, dysphonia, dystonia, tics, pain, spasms, strabismus, tremors, urinary retention, hyperhidrosis and hypersalivation [57–59].

Pharmaceutical Preparations of Botulinum Neurotoxins

Although the clinical effects of botulinum neurotoxins have been long known [7, 60, 61], it was not until 1985 that scientists used this substance off label to treat muscular disorders [62–64]. In 1989, the Food and Drug Administration of the USA granted American doctors the license to use these toxins for the treatment of strabismus and blepharospasm. A carefully purified and defined quantity of the bacterial product injected by a trained surgeon within the spastic muscle considerably diminishes muscle hyperactivity/contraction, while leaving some strength for the physiological function. Although they are incredibly toxic, these toxins have one of the safest usage records. One reason is that upon injection the protein does not diffuse beyond 2 cm, exerting its paralyzing activity around the injection site with very limited spreading. Several pharmaceutical preparations of botulinum toxins for the treatment of human diseases in ophthalmology, neurology and dermatology are currently marketed under the trade names *Botox*[®], *Dysport*[®] and *Xeomin*[®] (based on botulinum neurotoxin A), and *Myoblock*[®]/*Neuroblock*[®] (based on botulinum neurotoxin B) [56, 65–67]. These different products exert their action by inhibiting acetylcholine release, but their effects are clinically comparable at different doses that might vary up to several orders of magnitude [2, 56, 65–67].

With the exception of Xeomin, which is practically devoid of complexing proteins [56, 68, 69], the other commercial formulations of botulinum toxins include, besides the neurotoxin, other bacterial complexing hemagglutinins and nonhemagglutinin proteins as well. Several additional substances (e.g., albumin, benzyl alcohol, sucrose, lactose) are included in most of such pharmaceutical preparations and aim at drug stabilization and facilitation of administration by intramuscular injection. In their lyophilized or frozen forms the toxins may be kept in long storage; however, if diluted with saline for injection, they must be used within a few hours. The biological potency of these preparations is expressed in mouse units. One mouse unit is defined as the intraperitoneally injected quantity of each pharmaceutical product required to kill 50% (LD₅₀) of an experimental group of female Swiss-Webster mice, each of 20 g body weight. Comparative studies performed to evaluate efficacy and safety indicated that 1 Botox unit equals 1 unit of Xeomin, 3–5 Dysport and 50–100 Myoblock/Neuroblock units [2, 13, 56, 66, 67]. Results concerning the therapeutic effects of Xeomin are promising; however, those ana-

lyzing its biological potency are contrasting [68–70]; therefore, further research is required. The lower amount of the included neurotoxin A and the lack of complexing proteins from this product result in its lower antigenicity [71].

By dividing the mouse units of each marketed preparation by the quantity of the active neurotoxin included, one obtains the specific biological potency. Comparative studies rank Xeomin first with 167 mouse units per nanogram, followed by Dysport with 100 mouse units per nanogram, Botox with 60 mouse units per nanogram and last Neuroblock[®] with a specific biological potency of 5 mouse units per nanogram [67]. The duration of the clinical results of botulinum toxin A treatments is higher than those of type B. Botulinum neurotoxins type B might have relatively stronger effects on the autonomic system. For clinical purposes, type A should be distinguished from type B and the two substances should not be used interchangeably [2, 13].

Usually, the commercial Botox products, currently the number one in terms of documented clinical trials and practical use around the world, contain 5 ng of botulinum neurotoxin (100 mouse units + complexing proteins), 0.5 mg human albumin and 0.9 mg NaCl. The lethal dose of the Botox A[®] preparation for a person of 70 kg is calculated to be 2,500–3,000 units. The recommended dose for large muscles (e.g. gastrocnemius) is 100–400 units, whereas for cosmetic purposes usually less than 30 units are injected directly into the targeted muscle. Large muscles are localized by touch, whereas smaller or deeper muscles are detected through electrostimulation. For smaller muscles (e.g. orbicularis oculi) 1–2 sites of injection and a quantity of 3–4 units are effective, whereas a large muscle (e.g. gastrocnemius) requires 4–5 injections and 300–400 units [72, 73]. Typically, a spastic muscle initially receives a small quantity (e.g., 10 units) of the toxin in order to evaluate several reactions (e.g., paralysis, allergy). If no paralytic reactions are observed then a higher dose (e.g., 25 units) is administered and, if the muscle fails to paralyze even in this case, the treatment is interrupted. If the expected muscle reaction (paralysis) is evident, then approximately 100 units are injected per site.

Botulinum neurotoxins, injected into the human body, as foreign proteins, may induce the formation of antibodies [71]. However, practically, only quantities >100 units may raise antibodies. Nevertheless, even with such large doses, antibodies are raised only in 3–5% of the neurological cases, although sometimes proportions of 15–20% are reported. These antibodies do not aggravate

the patient's current state, but they render the next toxin injection ineffective [74, 75]. In cases of antibody-induced failure of botulinum toxin treatment, another can be substituted for the inactive type after careful calculations. Besides neutralizing antibodies, the paralysis signs of the injected muscle finally disappear due to the regeneration of the physiological axonal ending, so that injections of the toxin must be repeated every 2–4 months; however, cases of 1 year of paralysis duration have been recorded. Despite its extremely high toxicity, the drug, as used today, has proved to be extremely safe. Recently, alert reports were issued by the Food and Drug Administration concerning 180 cases of clinically induced botulism after treatment of spastic muscles, including 16 reported deaths, mostly in children treated for cerebral palsy [76]. Although the alert by the Food and Drug Administration might be justified and well done, neurologists starkly protested, claiming that their patients would benefit from this treatment, whereas complications were scarce and trivial [77]. In a few cases, numbness, edema and allergic reactions have been reported at the site of injection, whereas nausea, headache, blepharospasm, dysphagia and dyspnea have been rarely reported following drug injection into the face. Many of those side effects may be avoided if a careful professional injection technique is applied, and the injection site is kept clear of touching or rubbing after application. Toxin appears to be more efficient in small muscles but less efficient in larger muscles, whereas approximately 4% of the patients remain unaffected by the drug. Needless to emphasize that botulinum neurotoxin is strongly contraindicated for neurological disturbances (e.g. myasthenia gravis, Lambert-Eaton syndrome) as well as during pregnancy and breast feeding. Patients scheduled for treatment with botulinum neurotoxins should not take antibiotics such as acetaminophen or aminoglycosides (they augment the toxic effects), chloroquines (they weaken toxin efficacy), alcohol, aspirin, warfarin, vitamin E, nonsteroidal anti-inflammatory drugs, green tea, red wine (they cause cutaneous hematomas) 1 week before and 1 week after injection. In addition, patients should be examined for their tolerance to stabilizing albumin [78].

Botulinum Neurotoxins and Rehabilitation Medicine

Muscle spasticity is a serious pathological condition of neurological origin that can be treated by botulinum neurotoxins. Muscle spasticity is defined as the involuntary, speed-dependent increase of muscle resistance to

muscle elongation. Spastic muscles exhibit exceedingly high muscle tone due to neuron hypersensitivity; nevertheless, the exact pathophysiological causes of the development of spasticity are still elusive [79]. Although the pathology is manifested in the muscles, the causes of spasticity must be attributed to central nervous system damage. This damage disrupts the equilibrium between inhibitory and excitatory signals, resulting in increased muscle stimulation [80, 81]. The clinical features of muscle spasticity are mainly characterized by pain, permanent muscle shortening and lack of voluntary movements. Muscle spasticity may be congenital (parts of the brain did not develop) or acquired (cerebral stroke, accident). The coordination and accuracy of voluntary movements are achieved by the cooperation of higher pyramidal and exopyramidal motor neurons, lower motor neurons and lower inhibitory and activating spinal internuncial neurons. The stretch reflex plays a prominent role in muscle spasticity. Under normal conditions, this reflex protects muscles from extensive stretching by activating their antagonists.

Several cerebral lesions were formerly considered as irreversible and permanent. The current concept, however, is that the brain is endowed with the ability to adjust to circumstances as those described above, and this ability has generated a plethora of rehabilitating procedures [82–85]. Today, we know that the brain contains several somatic (adult) stem cells which may differentiate to functional neurons [86]. Apart from stem cells, neural circuits are established anew if intact neurons, sensing the lack of their normal neighbors, manage to send new axons to other neurons, make synapses and acquire some of the lost functions [87]. Since axon regeneration is extremely slow, a physiotherapeutic program must start immediately after the patient's stabilization in order to inhibit muscle shortening as long as regeneration proceeds [49]. Apart from that, there is clear evidence that physiotherapy facilitates axon regeneration [88]. In order for a muscle to avoid shortening, the muscle must remain relaxed, and this can be achieved by various medications (e.g., baclofen, carisoprodol, chlorphenesin, chlorzoxazone, cyclobenzaprine, dantrolene, diazepam, metaxalone, methocarbamol, orphenadrine), with serious side effects, however [89]. Injectable phenol and alcohol are effective in causing long-duration chemical nerve dissection, and are cheap as well, but exposure of the targeted nerve is frequently required [90]. Botulinum neurotoxins are being extensively tested clinically and experimentally in human therapy and cosmetics as both approved and unlicensed drugs.

Fig. 2. Facial muscles usually treated with botulinum neurotoxins to cause their paralysis and disappearance of the relative wrinkles.

Cosmetic Applications of Botulinum Neurotoxins

The human face comprises a number of muscles which serve various functions (e.g., masseter – mastication, buccinators – keep food in the mouth, and orbicularis oris – shapes and controls the size of the mouth opening). Many of these muscles play a significant role in the display of facial expressions. The frequent contraction of these muscles, in various moods, results in the alteration of the form and structure of the overlying connective tissue. This alteration is manifested externally as several anaglyphs, known as wrinkles and lines. Wrinkles are due to skin attenuation, whereas lines are deeper and involve the whole dermis. The deepest lines extend to the subcutaneous connective tissue.

In recent years, millions of women and men all over the world have visited specialized medical centers with the desire to eliminate the unavoidable traces of aging which develop gradually on the human face as the years pass. The eliminating procedure is very simple and, in the hands of an experienced surgeon, it is considered fairly safe, although this might not be entirely true [91]. Knowledge and skill are the major tools for preventing adverse effects following botulinum toxin cosmetic injections. The use of correct injection techniques is mandatory, since most unwanted effects are caused by inappropriate technique. Knowledge of the target structures, e.g., the facial and extrafacial muscles, allows physicians to select the optimal dose, time and technique. Proper techniques

of dilution, storage and injection, as well as the careful exclusion of patients with any contraindications are most important for avoiding most adverse effects. However, the injection of botulinum neurotoxins for cosmetic reasons to adults aged between 18 and 65 years was licensed only in 2002. The majority of these interventions are carried out on the procerus muscle (also known as the depressor glabellae or pyramidalis nasi; it forms transverse wrinkles in the glabellar region), the frontalis (draws the eyebrows and skin of the forehead upwards and forms horizontal wrinkles running across the forehead) and the corrugator muscle (forms vertical wrinkles in the glabellar area and horizontal wrinkles at the bridge of the nose) (fig. 2). The beneficial effects (muscle paralysis and, therefore, disappearance of the wrinkles) become gradually visible within 3–10 days and last for approximately 4 months. After this period, the drug usually has to be reinjected. Pain, hematoma, ecchymosis and bruising can be prevented by cooling the skin before and after injection of the toxin. Rarely, and usually as a result of a human error (e.g. unsuccessful injection/dilution/storage), the toxin diffuses, which may induce blepharospasm. Injections to orbicularis oculi and orbicularis oris are not infrequent, but one tends to avoid them due to their proximity to the eyes (diplopia) and mouth (paresis, dysarthria), respectively. Since subtypes A and B differ in their diffusion characteristics, subtype B is recommended for injection into muscles in close proximity to the eye [92]. Frequently, after 3–4 sequential injections, the desired results tends to become permanent since the subject tends to not use these muscles anymore. Recently, Botox proved significantly more efficacious and led to greater patient satisfaction than local application of widely used topical creams [93].

Today, the cost of a single cosmetic intervention amounts to approximately EUR 500. The globally estimated expenses for 2008 amount to EUR 10 billion. To date, more than 25,000,000 individuals have improved their facial appearance with botulinum neurotoxins. Besides the tremendous financial costs, society must also be concerned for the millions of animals sacrificed in order for the manufacturers to estimate the exact number of biological units of each preparation (LD 50%).

Discussion

Numerous reliable, well-designed double-blind studies concerning the clinical application of botulinum toxins [75, 80, 93, 94] have confirmed beyond doubt that toxin administration under clinical supervision is fairly safe.

The de facto confirmation of the safety of such interventions advocating wider, clinically supervised use of botulinum neurotoxins is reinforced by the existence of a strong theoretical background. This undoubted reality forced the governmental institutions to grant permission to use those neurotoxins in various pathological cases and cosmetic conditions. Despite that, many scientists still remain skeptical as to whether these toxins should be freely used in clinical routine for the treatment of many other conditions caused by muscle spasticity, where the toxins have already been administered experimentally [8, 95, 96]. One serious argument is based on several reports and unconfirmed rumors about many (about 180) adverse effects including 16 deaths related to botulinum toxin types A and B in clinical treatment [97, 98]. Since no one may ignore the risks of every medical intervention, some serious complications might be expected after botulinum neurotoxin injection, but they are usually attributed to iatrogenic reasons and/or to underlying neuromuscular disorders [99].

One of the main reasons for this skepticism is the justified uncertainty about the long-term therapeutic potential of the drug [100] as well as immunobiological concerns [101, 102] and the cost of botulinum toxin treatment [103]. The experimental clinical studies carried out so far to test the efficacy of botulinum neurotoxins in various pathological circumstances are not considered fully reliable on universal standards. It must be stressed that even in well-designed and well-performed experimental studies (i.e., double-blind, fully randomized), the evaluation of the outcome, e.g., walking ability of a patient, joint range motion, is highly affected by two doubtful parameters. First, it is practically impossible to recruit a reliable control group of matched patients receiving the placebo [36]. Second, there is a lack of objectivity since both medical doctors and patients do not use absolute standard quantitative criteria for assessing treatment success; instead, this is based solely on a subjective evaluation which is highly affected by the patient's mood [104]. Despite the skepticism, the undoubted reality of the frequently reported nontoxicity of the treatment and the health improvement advocate the administration of the toxin even in open trials, without a control group.

Although the total number of patients subjected to botulinum neurotoxin therapeutic treatment to date worldwide cannot be characterized as insufficient, the description of their health improvement ranges from reserved to enthusiastic. An additional inherent difficulty in the assessment of the mentioned results is the fact that these studies are hardly comparable since they describe

cases of various seriousness, were performed on a plethora of different muscles – from extremely large to extremely small, the studied parameters vary (e.g. speed of gait, angle of bending, pain, muscle tone) and the methods of assessment are frequently quite different [96, 104, 105].

It seems that the number and the quality of both long-term and controlled experimental and clinical studies performed up to date in an attempt to elucidate the efficacy of botulinum neurotoxins and assess the progression of the patients – particularly those suffering from spastic hemiparesis displayed at the lower limbs immediately after a cerebral stroke – are not sufficient for scientific standards [83]. In addition, in many of these studies, the use of placebo seems to end up in improvement of the patient's condition, which augments ambiguity and unreliability. Another parameter complicating the drawing of useful conclusions is the rigorism of the therapeutic protocols, which may range from very stringent to quite flexible. For obvious reasons, flexible protocols are unavoidable in clinical practice, and the statistical assessment of the results is difficult [106–108].

Despite the existing numerous contestations and doubts, there are a lot of data and evidence in favor of continuing similar studies. There are still some open questions which should be answered in the near future, specifically (a) which muscles are better targets for maximalization of functional profit for each patient, (b) what are the optimal doses, (c) does an increase in the injected dose restrict the number of treated muscles, and (d) what is the most effective combination of various treatments for each case?

Concluding, the absence of serious side effects has allowed wide application and trials of the botulinum neurotoxin for numerous, different pathological conditions; the following reliable and promising results were obtained: botulinum neurotoxins are absolutely safe in experienced hands. They really achieve their goal and actually transiently improve the patient's physical state, particularly when the target is a small lower limb muscle. Although they do not remove the generating cause, beneficial effects of short duration are quite frequently reported. They relieve pain caused by muscle spasticity and allow spastic muscles to learn purposeful movements.

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