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Botolinum neurotoxin: its history and use in dermatology

Botulinski nevrotoksin: odkritje in uporaba v dermatologiji

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Abstract

Botulinum neurotoxin is a highly toxic protein produced by the anaerobic Gram-positive bacterium *Clostridium botulinum*. The discovery of botulinum neurotoxin began with unexplained sausage poisonings, hence its name. The Latin word for sausage is *botulus*. The knowledge of neurotoxin has grown through centuries of research, and clinical trials are still underway to support new indications with the increasingly widespread use of this toxin in medicine. Botulinum neurotoxin is a protein molecule that, in its active form, becomes a proteolytic enzyme exclusively specific for SNARE complex fusion proteins in presynaptic nerve endings. In the process of chemodenervation, it temporarily inhibits the action of the target tissue, causing, for example, temporary flaccid muscle paralysis on skeletal and smooth muscle. Botulinum neurotoxin is used in neurology to treat bladder overactivity and in dermatology, where botulinum neurotoxin is still widely associated with aesthetics alone. Recent findings reveal that botulinum neurotoxin reduces pain and itching and also affects some non-neuronal cells, such as epidermal keratinocytes, dermal fibroblasts, inflammatory cells, sebaceous cells, vascular endothelial cells, and mesenchymal stem cells in subcutaneous fat. The findings are promising for expanding the range of indications in dermatology too. The following article presents the history of botulinum neurotoxin development, its structure, mechanism of action, as along with current and promising indications for use in dermatology.

Izvleček

Botulinski nevrotoksin je izredno strupena beljakovina, ki jo tvori anaerobna po Gramu pozitivna bakterija *Clostridium botulinum*. Zgodba o odkritju botulinskega nevrotoksina se je pričela s pojavljanjem nepojasnjenih zastrupitev s klobasami, iz česar tudi izvira njegovo ime. Latinska beseda za klobaso je namreč *botulus*. Zgodba o tem se je nadaljevala s stoletji raziskav in še poteka z njegovo vedno širšo uporabo v medicini. Botulinski nevrotoksin v aktivni obliki postane proteolitični encim, izključno specifičen za fuzijske beljakovine kompleksa SNARE v presinaptičnih živčnih končičih. Prek kemodenervacije začasno zavre delovanje tarčnega tkiva, na skeletnih in gladkih mišicah povzroči npr. začasno ohlapno paralizo mišic. Botulinski nevrotoksin se uporablja v nevrologiji, za zdravljenje čezmerne aktivnosti sečnega mehurja in tudi v dermatologiji, kjer njegovo uporabo še vedno mnogi povezujejo zgolj z estetiko. Novejša dognanja pa razkrivajo, da botulinski nevrotoksin zmanjšuje bolečino in srbež ter vpliva tudi na nekatere nenevronske celice, kot npr. epidermalne keratinocite,

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dermalne fibroblaste, vnetne celice, lojnice, žilne endotelne celice in mezenhimske matične celice v podkožnem maščevju. Odkritja napovedujejo tudi možno razširitev nabora indikacij v dermatologiji. Članek predstavi zgodovino razvoja botulinskega nevrotoksina, njegovo sestavo, mehanizem delovanja in trenutne ter obetavne indikacije za uporabo v dermatologiji.

1 Introduction

The botulinum neurotoxin (BoNT) is one of the most toxic natural substances for humans. Assuming an average person's weight of 70 kg, just 39.2 g of this substance could eradicate almost the entire human race (5.6 billion people) (1). Despite being one of the deadliest known toxins, it is successfully used in small doses for the treatment of numerous medical conditions (2). As a proteolytic enzyme, it temporarily prevents the release of a neurotransmitter from presynaptic nerve terminals, thereby inhibiting the working of the target tissue (3).

In addition to its increasing use in neurology, for example for the treatment of strabismus, cervical dystonia, various types of spasms and chronic migraine headaches, the range of indications for its use is expanding to other areas. Its analgesic properties hold great promise (4).

In dermatology, many still associate it solely with use for aesthetic purposes, but recent findings prove just the opposite. Use of the most commonly used botulinum neurotoxin serotype A (BoNT-A) in dermatology has increased by an unprecedented 6,956.6% between 1997 and 2016 (5). Researchers and physicians in this area are also discovering ever-widening indications for the use of BoNT that go beyond purely aesthetic purposes. Encouraging results are available for the use of BoNT in the treatment of many dermatological diseases, but further research is still needed for wider use in clinical practice (6).

2 History

The story of the discovery of the protein we know today as BoNT is long and varied. Mankind has encountered this substance through unexplained poisonings since time immemorial. A review of Indian history reveals that the Maharajas used "sausage poison" to kill enemies. With civilizational development, various attempts at food preservation have emerged. Those less successful provided a favourable environment for the growth of microorganisms and often caused various poisonings (7). The development of BoNT to its currently used form required more than a hundred years of clinical observations and laboratory experiments (8).

2.1 Mass poisonings

The first surviving written records of unexplained poisonings date back to the Napoleonic Wars (1803-1815). Due to the economic crisis, the hygienic standard of food preparation and storage has fallen sharply (9). At that time (in 1793), an unexplained mass poisoning broke out in the village of Wildbad in southwestern present-day Germany. It was soon discovered it was not the first poisoning of its kind. Similar symptoms were observed in all patients, including mydriasis, so at first, deadly nightshade (*lat.* Atropa belladonna) was blamed for the poisoning. (10). Mass food poisonings, including the ones with fatalities (mortality rate 5-50%), were described in Stuttgart by Duke Frederick I, who ruled Württemberg at the time. Poisoning was soon associated with the consumption of improperly stored food, especially various sausages. Therefore, the unknown toxin was called "sausage poison". In 1802, the Stuttgart authorities issued a public warning about the dangers of eating smoked sausages (11). The main topic of discussion became speculation about the composition of the substance that caused the poisoning (8). Several chemicals were mentioned, and hydrocyanic acid was suspected as the most likely cause. Experts from the Tübingen Medical School were also consulted, as they were the first to mention that the most likely cause of the poisoning was organic. Professor Autenrieth then proposed the systematic collection of all reports of poisonings. Based on these, he was the first to collect and publish the findings about the disease we know today as botulism (11).

The next major shift in toxin detection was credited to Justinus Kerner, a German physician and poet who carefully studied the poisoning outbreaks. He published two monographs, in 1820 and 1822. In the first, he devoted himself mainly to the collection of cases and detailed clinical descriptions of the symptoms of sausage poisoning (vomiting, abdominal cramps, mydriasis, ptosis, dysphagia, respiratory failure). However, his interest in the cause of the poisonings fuelled a growing desire to isolate this unknown toxic substance. He began with post-mortem examinations of the victims and experiments on various animals, to which he gave an extract, obtained from spoiled sausages (11). He also performed

experiments on himself, noticing dryness of the oral mucosa after applying a few drops of this substance on the tongue (10). In his second monograph, he published the results of his research and a hypothesis about the poison's origin (11). He hypothesized that a substance found in the lipid-rich part of food and thriving in an anaerobic environment was responsible for the poisoning (9). He believed that this "fatty poison", as he called it, had a biological origin. He hypothesized that it travelled through the nervous system and interfered with the signal transduction in the neural pathways. He compared the damage it caused to rusty electrical wires (8). For the first time, his work suggested that the unknown poison could also be used for medical purposes, such as treating excessive irritability of the nervous system, involuntary muscle movements and reducing excessive secretion of sweat and mucus (9). It seems almost unbelievable that approximately 200 years after he made these predictions, BoNT is also used for these indications (8).

2.2 Discovery of the bacterium and neurotoxin

A Belgian professor of microbiology, Emile van Ermengem, was the first to discover the bacterium responsible for the food poisonings. He published a report on this in 1897 (12). He proved that the posthumous tissue samples of a group of poisoned people in a Belgian village and the spoiled ham that everyone ate contained a large number of the same Gram-positive bacteria, which he named Bacillus botulinus. The name comes from the Latin word for sausage, botulus (10,11). In the early 20th century, microbiologists began to discover that these bacteria are a part of a heterogenous group and not a single species. At that time, the bacterium was added to the genus of anaerobic bacteria *Clostridium*. It was renamed *Clostridium botulinum* (*C. botulinum*) (8).

In 1928, botulinum neurotoxin was isolated for the first time as a stable acid precipitate by Dr. Herman Sommer in California (9). It was the basis for further research. Interest in more detailed research and production of larger quantities of the toxin grew during World War II (11). The toxin was intensively studied at the U.S. Microbiological Laboratory at Fort Detrick Military Base, where a concentration and crystallization technique was developed (8). Despite efforts, attempts to use BoNT as a biological weapon were unsuccessful (7). In 1946, dr. Schantz et al in Fort Detrick developed the technique of preparing purified BoNT, which paved the way for its production in larger quantities, and later, its use in medicine. It was intended for government stockpiles and research institutes in the United States. In 1969, the United States announced the destruction of all its stockpiles of biological weapons. Three years later, the Biological and Toxin Weapons Convention was signed by more than 100 countries. The military microbiological laboratory at Fort Detrick was also closed at this time (11). Dr. Schantz continued his research at the University of Wisconsin. In 1979, he produced a large series of BoNT-A suitable for clinical use. This original series was in use in medicine until 1997 as the only BoNT series approved for use by the US Food and Drug Administration (FDA) (9).

2.3 Beginnings of the medical use of botulinum neurotoxin

The first major results in detecting the BoNT mechanism of action date back to 1949. At that time, Burgen et al reported that BoNT-A injected into hyperactive muscle prevents the release of the neurotransmitter acetylcholine (ACh) from nerve endings at the neuromuscular junction (NMJ), or motor end plate, and thus temporarily paralyzes the muscle (7). Experiments on chicken embryos into which BoNT was injected were also considered. These experiments have shown that BoNT injection causes dose-dependent muscle atrophy and weakness (8). The findings have caused much interest in the therapeutic use of BoNT in medicine. American ophthalmologist Scott, in collaboration with Dr. Schantzem, his BoNT supplier, was the first to inject these substances into monkeys in 1973 to treat strabismus (13). The results were promising and in 1980, he also published the first report on experiments on human volunteers, which were approved by the FDA (14). Scott published the results of his research, which demonstrated the effectiveness of BoNT in reducing or even preventing unwanted facial muscle movements, such as blepharospasm and hemifacial spasm (8). Following his efforts, the FDA approved the use of BoNT-A (then known as Oculinum, renamed Botox two years later) to treat strabismus, blepharospasm, and hemifacial spasm in 1989. This paved the way for the discovery of other indications for the use of BoNT (11).

Events over subsequent decades remain one of the most remarkable stories of biological substance development for medical use. Numerous major multicentre studies have led to new insights into BoNT and paved the way for its use for other medical indications. A powerful and frightening bacterial toxin, it has evolved into an effective medication for treating and relieving over a hundred medical symptoms. Over time, and with the discovery of different serological toxin types, other

BoNT products from different manufacturers have been developed under different brand names (8).

The discovery of the aesthetic effects of BoNT was accidental, as patients began to report a beneficial side effect - smoothing facial wrinkles - while treating spasms and unwanted facial muscle movements. The first formal report on the possible aesthetic effects of BoNT was published in California by Clark and Berris in 1989. A patient with paresis of the left facial nerve (*lat.* Nervus facialis, seventh cranial nerve) who was bothered by facial asymmetry was injected with BoNT on the unaffected side of the face. They reported beneficial effects of BoNT on smoothing wrinkles and reducing facial expressions, which also reduced facial asymmetry (15).

In 2000, the FDA approved the use of BoNT to treat cervical dystonia (11). It took a few more years for BoNT to be approved for use in dermatology. In 2002, the FDA approved the use of BoNT for smoothing glabellar wrinkles (vertical wrinkles between the eyebrows), and in 2004 for the treatment of axillary hyperhidrosis (excessive armpit sweating). This was followed by approvals for other medical indications, such as overactive bladder, migraine headaches and muscle spasticity in various neurological diseases (8). In 2013, the FDA approved its use for smoothing periocular wrinkles, and four years later for smoothing forehead wrinkles. However, off-label use is still common in dermatology (7). Ongoing studies are exploring new possibilities for BoNT use. There is also a lot of research on pain treatment options. Also, there are prospects in dermatology for expanding the indications for use, which we also describe in the continuation of the article (10).

3 Botulinum neurotoxin

3.1 Origin and structure

Botulinum neurotoxin is produced by the anaerobic Gram-positive bacterium *C. botulinum*. The bacterium is found on plants, in soil, water and also in the digestive tract of animals (16). It forms highly resistant endospores and the strongest natural toxin, which allows it to survive in a variety of environments. Years of research have led to the discovery of several different strains. Based on physiological, metabolic and genetic traits, they were classified into six groups. Each of them forms at least one of the seven known serological types of BoNT, and some even more (17,18).

Botulinum neurotoxin consists of a polypeptide chain with a molecular weight of 150 kDa and is less active in this form. The polypeptide chain consists of a heavy (100 kDa) and a light (50 kDa) chain linked by a disulphide bridge (18). The heavy chain has two functional units. The first is a domain that allows binding to a target receptor through an extremely selective mechanism. It has a high affinity for specific membrane ganglioside receptors and the SV2 protein on the presynaptic membrane of motor neurons. The second is the translocation domain (19,20,21). The main heavy chain purposes are the interaction of BoNT with the target cell, its entry into the cell and the transfer of the light chain to the target site. The light chain, on the other hand, is a zinc-dependent proteolytic enzyme and thus an active part of BoNT (22). The active form of BoNT therefore forms only after degradation of the polypeptide chain (18). The bacterium does not form BoNT in such a "pure" form but binds it to another protein. These are both non-haemagglutinin and haemagglutinin proteins (23). Together, they form a macromolecular protein complex with a molecular weight of up to 900 kDa. These auxiliary proteins are not toxic but stabilize the complex and protect BoNT from environmental influences (pH and temperature changes, enzyme action). Some also allow adhesion to target cells (e.g. gastrointestinal cells) (24).

Different types of BoNT are differentiated with serological methods. Currently, seven antigenically different BoNT serotypes are known, which are classified into more than 40 subtypes (25). Serotypes are named alphabetically from A to G (BoNT-A to BoNT-G). They are structurally similar to each other but differ serologically and antigenically (11). The strongest of these is the BoNT-A serotype, followed by the BoNT-B and BoNT-F serotypes. Serotypes A, B and E are most commonly associated with human botulism (16). Since 2013, an 8th serotype called BoNT-H has been mentioned (25). So far, serotypes A and B have been used clinically with only serotype A approved for use in dermatology (16). The three main products of the BoNT-A serotype derived from the Hall strain of *C. botulinum* are available on the market. They are known under the generic names abobotulinum toxin A (trade name Dysport, Azzalure), incobotulinum toxin A (trade name Xeomin, Bocouture) and onabotulinum toxin A (trade name Botox, Vistabel). After FDA approval, onabotulinum toxin A became the first made commercially available for use in dermatology. The original batch was produced by Schantz in 1979 and was in use until 1997. Abobotulinum toxin A and onabotulinum toxin A contain a toxin bound to a macromolecular protein complex. The peculiarity of incobotulinum toxin A is that it contains pure 150 kDa BoNT-A without a protein complex, which is supposed to reduce the likelihood of the formation of neutralizing

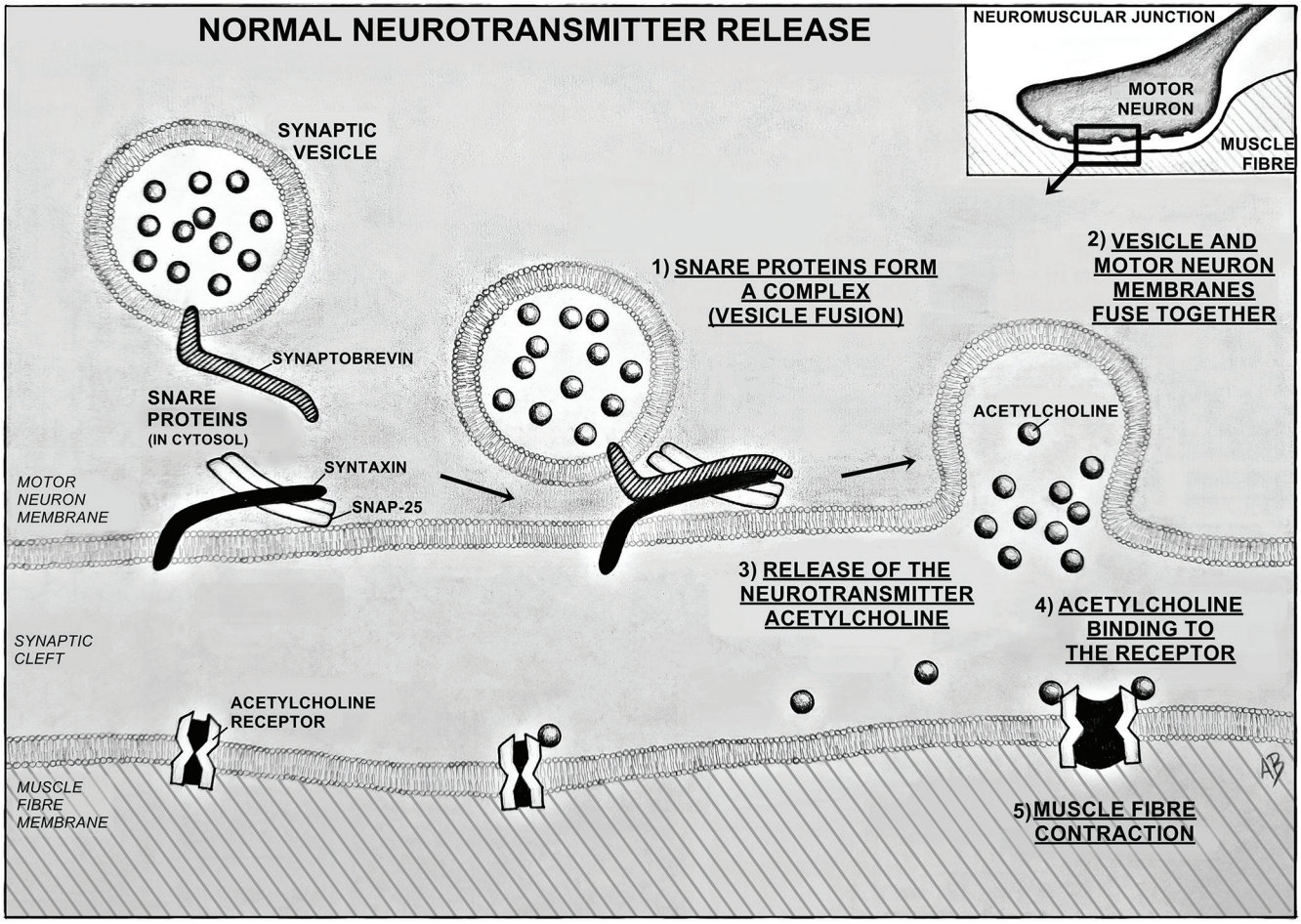

Figure 1: Normal neurotransmitter release in a neuromuscular junction (motor end plate).

antibodies (22). All three products are stored with an excipient that further stabilizes them. The excipient is human albumin with the addition of lactose (abobotulinum toxin A), sucrose (incobotulinum toxin A) or sodium chloride (onabotulinum toxin A). The preparations are stored and sold in lyophilised form and must therefore be dissolved in 0.9% aqueous sodium chloride solution before use. BoNT dosing units are product-specific and non-interchangeable. The BoNT-B serotype is also available on the market for medical use and is sold under the brand names Myobloc and NeuroBloc but is not currently used for aesthetic purposes (16).

3.2 Mechanism of action

Botulinum neurotoxin is a zinc metalloproteinase that temporarily prevents the release of the neurotransmitter ACh from peripheral cholinergic nerve endings into the synaptic cleft. This prevents the transmission of the nerve signal to the target tissue, e.g. skeletal and smooth muscles and sweat glands (24). When a nerve impulse is transmitted, the content of synaptic vesicles - a neurotransmitter - is released from the presynaptic nerve endings into the synaptic cleft. This occurs in the process of calcium-dependent exocytosis by fusion of vesicles with the presynaptic membrane of a nerve fibre (26). The fusion is facilitated by special fusion proteins that together form the soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex. These are synaptobrevin (vesicle associated membrane protein, VAMP) on the synaptic vesicle membrane and on the presynaptic nerve ending membrane syntaxin and SNAP-25 (synaptosomal associated protein, 25 kDa) (27). The SNARE complex enables the attachment of the synaptic vesicle to the presynaptic membrane and, in the case of an appropriate nerve impulse (depolarization), its fusion with the membrane and the release of the neurotransmitter into the synaptic cleft. There, ACh binds to its receptor on the postsynaptic membrane of the target tissue and elicits an appropriate response (e.g., skeletal and smooth muscle contraction, sweat secretion) (Figure 1).

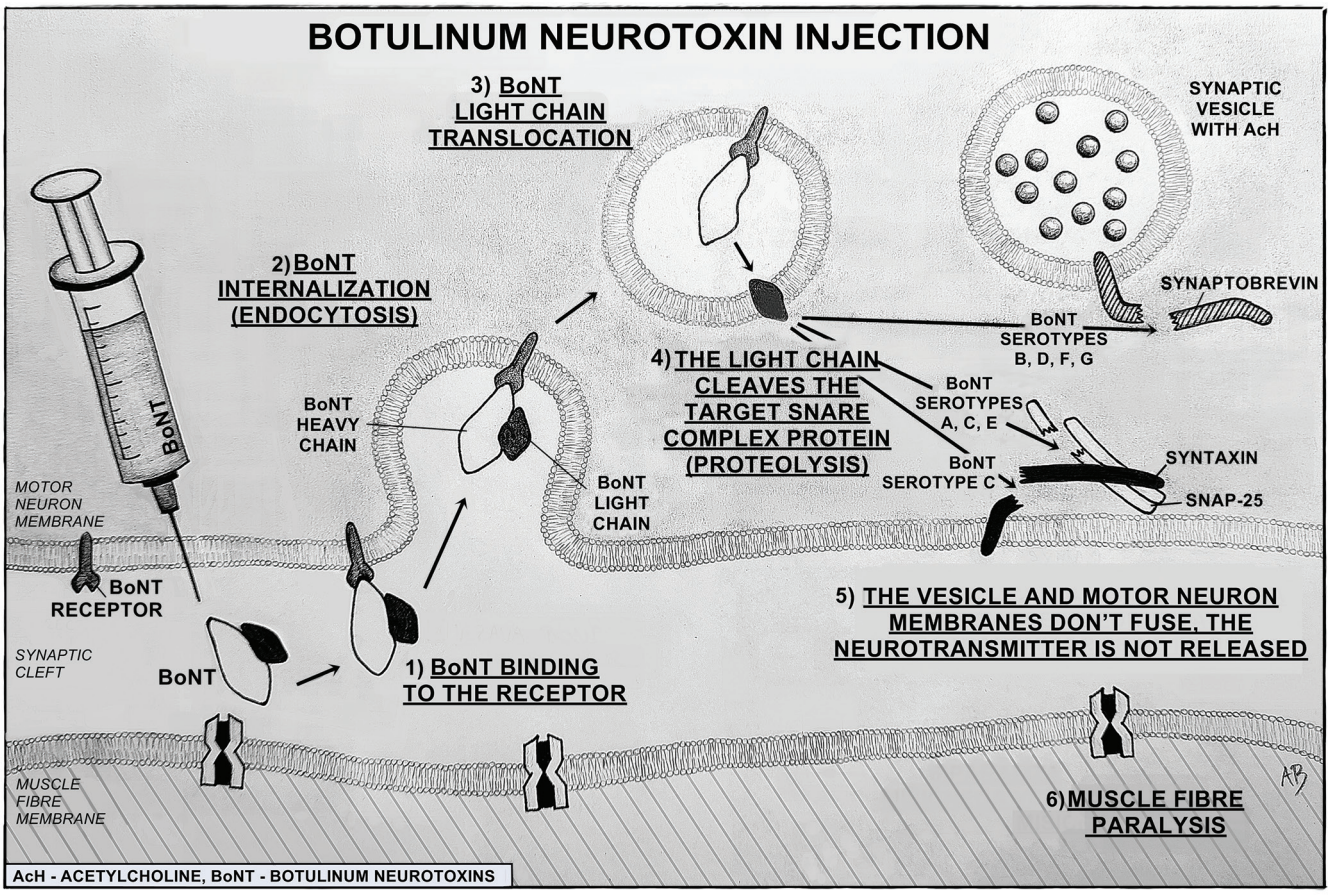

Figure 2: Botulinum neurotoxin mechanism of action in the neuromuscular junction (motor end plate).

The BoNT mechanism of action can be divided into four consecutive steps: binding, internalization, translocation and proteolysis of the SNARE complex target protein within the presynaptic neuron (Figure 2). Botulinum neurotoxin is initially present in the synaptic cleft after injection. From there, it must enter the presynaptic neuron to function. The BoNT heavy chain domain, responsible for binding to the target receptor, specifically binds to the ganglioside receptors of cholinergic presynaptic nerve endings. In the process of internalization, it enters into the neuron with endocytosis. Low pH in the endocytic vesicle causes certain BoNT structural changes, followed by disintegration of the disulphide bond between the heavy and light chains. The light chain crosses the membrane of the endocytic vesicle and enters the neuron's cytosol (24). The heavy chain translocation domain plays a key role in this light chain translocation (22). The light chain, finally released into the cytosol and now catalytically active, binds highly specifically to one of the three proteins of the SNARE complex in the presynaptic neuron and prevents its formation. To which protein of the SNARE complex the light chain of the toxin will bind depends on the serological BoNT serotype. Serotypes B, D, F and G bind to synaptobrevin, serotype C to syntaxin and SNAP-25, serotype E and the most commonly used serotype A only to SNAP-25 (24). The binding of the BoNT light chain to a specific protein of the SNARE complex is based on an extremely precise molecular structure that allows the toxin to match this specific target protein. Binding is very sensitive to the slightest structural changes. For example, the BoNT-A light chain highly specifically cleaves the peptide bond Gln197 – Arg198 on the SNAP-25 protein (28,29). The end effect - prevention of synaptic vesicle fusion with the presynaptic membrane in neuromuscular contact - is the same in all BoNT serotypes. The most researched targets of BoNT are skeletal and smooth muscle fibres and exocrine glands. (24). BoNT acts on muscle fibres by causing chemodenervation and thus flaccid muscle paralysis. It can impair up to 80% of the original muscle function (3). BoNT acts on the exocrine glands by inhibiting the secretion of their substances (saliva, sweat) (24).

Botulinum neurotoxin is a stable molecule with a half-life of 60 days. Binding of BoNT to SNARE complex

proteins and their degradation are irreversible processes. However, the final effect caused by BoNT is only temporary. The recovery of muscle strength and other functions of the target tissue is due to continuous cell renewal. The process begins a few weeks after the BoNT injection and gradually reaches a pre-injection state after an average of 3–6 months. Proliferation of new nerve endings and the formation of new synaptic contacts are key to regaining function. It also contributes to the replacement of degraded SNARE complex proteins with new ones and the promotion of genes for the synthesis of ACh receptors. Signalling via insulin-like growth factor 1 (IGF-1) plays a key role in these processes. There is evidence that repeated injection of BoNT leads to slower recovery of function. After a few months, the effects of BoNT cease with a complete restoration of target tissue function (30).

Recent research proves that not all BoNT effects can be explained by its action on peripheral nerve endings. BoNT (similar to its related tetanus neurotoxin) appears to be able to travel along nerve fibres. It also has a direct effect on the central nervous system. Experiments confirm that after peripheral injection of BoNT, it can affect both motor skills and pain perception through a direct effect on the anterior and posterior horns of the spinal cord (31). The analgesic effect of BoNT was initially associated solely with its effect on muscle relaxation. However, numerous studies have shown that the analgesic effect and muscle relaxation do not always coincide. There is evidence that BoNT affects the afferent translation of the pain signal. It inhibits the release of substance P, which is involved in pain perception, vasodilation and neurogenic inflammation. Neurogenic inflammation occurs due to the release of vasoactive neuropeptides from the peripheral sensory neurons nerve endings - a peptide associated with the calcitonin gene (calcitonin gene-related peptide, CGRP, substance P). It can be triggered by tissue injury or a painful stimulus. Botulinum neurotoxin also inhibits the release of certain neurotransmitters, such as glutamate, from which it can be concluded that the analgesic effect of BoNT is associated with inhibition of signal transduction in sensory neurons. These findings are used to further explore the potential use in the treatment of pain syndromes, postherpetic neuralgia, and new indications in neurology (4,32).

Research into additional BoNT uses in dermatology is ongoing. Recent studies suggest that BoNT also affects the function of other cells that express adhesion receptors for it and contain the SNAP-25 protein in the cell itself, which is the target of the most commonly used BoNT-A serotype. These are e.g. epidermal keratinocytes, dermal fibroblasts, inflammatory cells, sebaceous cells, vascular endothelial cells and mesenchymal stem cells in subcutaneous fat, which is promising for expanding the set of indications in dermatology as well. This topic is discussed in more detail in the next chapter (5,33).

3.3 Indications for use in dermatology

In 2002, the FDA approved the use of BoNT-A to smooth glabellar wrinkles. Since then, its use for aesthetic purposes has significantly increased, therefore many people still associate it with aesthetic dermatology only. Recent findings, however, suggest that BoNT-A has as much scope of use than originally anticipated. It affects different types of cells, including the skin. Therefore, research is now focused on discovering the possibilities of using BoNT-A that go beyond aesthetic indications. In the following text, we present both established and newly discovered possible indications for BoNT-A use in dermatology (16).

3.3.1 Smoothing facial wrinkles

The peculiarity of facial mimetic muscles is that they insert on the skin. With each contraction, the above-lying skin also folds, thus creating facial expressions. Facial wrinkles are formed perpendicular to the direction of muscle contraction. These are initially visible only when the muscles contract (dynamic wrinkles), but over the years, due to simultaneous structural changes in the skin, they become permanent (static wrinkles) (34). Botulinum neurotoxin prevents the release of ACh on the motor end plate and thus paralyzes the mimetic muscles at the injection site. It is used to smooth frontal and glabellar wrinkles, eliminate fine wrinkles around and under the eyes, and to raise the eyebrow arch. As part of its non-intended use, it is also used to inhibit drooping of the mouth corner (*lat.* m. depressor anguli oris), emphasizing the contour of the jaw (*lat.* m. masseter) or on the neck (*lat.* platysma) and chest (16).

Muscle paralysis usually occurs within 48 hours after injection. The time taken for the onset of paralysis depends on the anatomy, injection site and individual (genetic) muscle differences. The effect is also greater if BoNT-A is injected into the middle of the muscle fibre, where there is a higher concentration of neuromuscular junctions (neuromodulation). Accurate knowledge of anatomy is crucial as the muscle fibres of different mimetic muscles are organized differently. Some also use an electromyogram to confirm the most suitable injection site (30).

3.3.2 Hyperhydrosis

Hyperhidrosis is a condition characterized by excessive secretion of the eccrine sweat glands, which are located mainly on the palms, soles and armpits (5). The result is excessive sweating that exceeds the body's thermoregulatory needs (2). Primary hyperhidrosis, which does not occur because of another medical condition or taking certain medications, affects about 3% of the population. It is a significant psychological burden for affected individuals. BoNT-A has been shown to reduce or inhibit sweat secretion at the injection site and thus significantly improve quality of life. Therefore, treatment of primary hyperhidrosis with BoNT-A is one of the most common indications (5). We use it particularly after topical treatment is unsuccessful (2). BoNT-A is injected into the skin with the aim of inhibiting the transmission of nerve signals at cholinergic nerve endings that innervate the sweat glands. This temporarily inhibits the secretion of sweat. The use of BoNT-A has been approved by the FDA for the treatment of axillary hyperhidrosis with good treatment success. Data on the treatment of palm and soles hyperhidrosis are more modest. Caution should be exercised when injecting into the thenar eminence, as there is a risk of inadvertently paralyzing the muscles. Treatment of other body areas due to excessive sweating has also been described, as has the treatment of osmidrosis (5). In the treatment of hyperhidrosis, the occurrence of excessive sweating in other, previously unaffected areas of the body is also a problem (2).

3.3.3 Possible indications

In addition to the already established indications for BoNT-A use in dermatology, recent findings are promising. It appears that BoNT-A would also be suitable for the treatment of some other dermatological diseases. Botulinum neurotoxin serotype A has already been tried in the treatment of hypertrophic scars and keloids, as it reduces muscle contraction in the scar area. In this way, the pressure on wound borders, micro-injuries and inflammation are reduced. Botulinum neurotoxin serotype A is thought to inhibit the expression of transforming growth factor beta (TGF-beta), which is a major mediator in the formation of hypertrophic scars (5). Some studies have shown that local injection of BoNT-A is just as effective compared to corticosteroid injections. Additional benefits of BoNT-A include reduced pruritus and pain at the site of the scar (35). However, further research is needed on the actual role of BoNT-A in scarring, as the study results are still very contradictory.

BoNT-A has also been shown to be successful in the treatment of oily skin, a common dermatological disorder (36). Excessive sebum secretion blocks the opening of the pilosebaceous unit. Bacterial growth causes inflammation such as acne and seborrheic dermatitis. The BoNT-A mechanism of action after it is injected into the skin to reduce sebum production has not been fully explained. Local ACh receptors in sebaceous glands are thought to be the target. Further research is needed to determine the appropriate amount and technique of injection (6).

Many dermatological conditions are accompanied by intense pruritus. In small pilot studies, BoNT has been shown to have an antipruritic effect, which holds great promise. The mechanism of action is not yet fully understood, but the effect of BoNT-A on the inhibition of the release of neurotransmitters substance P, glutamate, ACh, CGRP and other mediators that may be involved in the otherwise complex pathogenesis of pruritus is particularly important (37). In many dermatological diseases, such as atopic dermatitis, acetylcholine is an important mediator in pruritus. Substance P also triggers the release of histamine through mast cell activation. Just like CGRP, it is a potent vasodilator. BoNT-A also has a direct effect on inhibiting mast cell degranulation. Pruritus is normally associated with skin inflammation. As BoNT-A reduces neurogenic inflammation, improvement of the primary skin condition is to be expected in addition to pruritus improvement (6). This is also confirmed by studies in patients with psoriasis and atopic dermatitis, such as findings on psoriasis improvement after accidental peripheral nervous system injury (38). Local BoNT-A injection in inverse psoriasis also has a beneficial effect due to the reduction of sweating, and thus less frequent intertriginous skin inflammation (6). The BoNT-A mechanism of action in patients with Hailey-Hailey disease (benign familial pemphigus) is also thought to be similar. It is a rare genetic skin disease with outbreaks of blisters and ulcers. The intertriginous skin is particularly affected; pruritus may be present (39). BoNT-A has a beneficial effect by reducing sweating, tissue maceration and pruritus (37).

Also interesting is the discovery that BoNT-A has a beneficial effect on the treatment of rosacea, which is a chronic inflammatory skin condition of complex aetiopathogenesis, also associated with mast cell function. BoNT-A reduces the neurogenic inflammatory component with inhibition of the neuropeptide substance P release, which otherwise activates mast cells in the process of neurogenic inflammation. Additionally, BoNT-A also cleaves SNARE complex proteins in mast cells, which are involved in their activation. With their degradation, BoNT-A directly inhibits mast cell degranulation. These

effects of BoNT-A are anti-inflammatory. Due to its multi-targeted action, BoNT-A could offer an advantage over currently established rosacea medications (40).

BoNT-A has been shown to be effective in relieving pain associated with other skin conditions, such as painful cutaneous leiomyomas and periorbital syringomas (5). Even in multiple eccrine hydrocystomas, local BoNT-A injection is a safe and effective treatment (41).

3.4. Side effects

Botulinum neurotoxin is one of the most well-known substances, toxic for humans. Toxicity is often expressed in toxic units (TU). One toxic unit (1 TU) of BoNT corresponds to the median lethal dose (LD50) for a 20 g Swiss mouse. However, susceptibility to BoNT varies from species to species. LD50 for monkeys is 39 U/kg. Based on these findings, it was estimated that for a 70 kg human, an intramuscular or intravenous injection of approximately 40 U/kg of crystalline BoNT-A corresponds to LD50. The single dose used in dermatology is significantly lower than this dose. In the treatment of large skin surfaces (e. g. in plantar hyperhidrosis), the amount of onabotulinum toxin A injected is up to 250 U per treated area. The doses used for aesthetic purposes are even lower (5,34,42).

Based on clinical experience, retrospective studies and meta-analyses, treatment with BoNT is safe for a wide range of therapeutic and aesthetic indications. Local side effects are caused by the excessive toxin effects and diffusion into the surrounding tissues. On the face, the temporary side effects due to mimetic muscle weakness include facial asymmetry, ptosis of the eyebrows, upper eyelids or lips and ectropion; additionally, strabismus and abnormal facial expressions. Dysphagia, dry mouth and a feeling of neck weakness are also possible with platysmal BoNT injection. The incidence of side effects can be reduced by following the BoNT guidelines and choosing the right patient, appropriate dose and injection technique for a particular procedure. Possible side effects include bleeding, redness and pain at the injection site. Headaches, lasting up to four weeks, may also appear (34,43).

Approved indications for BoNT usage in dermatology use such small toxin amounts that there are no reports of systemic BoNT effects in any approved BoNT product in healthy adults. Studies confirm that the recommended doses do not lead to systemic effects and BoNT is not measurable in peripheral blood after injection. A BoNT overdose could cause systemic signs such as muscle weakness, blurred vision, ptosis, and a rare life-threatening condition known as botulism. There are no reports of long-term side effects from the approved BoNT use in dermatology.

Like any foreign protein, BoNT can elicit an immune response. This is more likely with frequently repeated BoNT injections. In addition to the 150 kDa neurotoxin, some commercially available products contain BoNT bound in a complex with other proteins, which further increases the risk of neutralizing antibody formation. Therefore, subsequent BoNT injections may not be successful. Immunogenicity is also affected by the dose used. The results vary significantly between patients and between individual studies. The frequency and dose that determine the immune response are still unknown. It is also noted that in some patients, despite the formation of antibodies, the effect of BoNT does not necessarily decrease in subsequent treatments. In dermatology, particularly for aesthetic purposes, the amounts of BoNT used are small and the occasionally more frequent injections do not increase the risk of subsequent treatments failure (44). A major US study from 2009, which analyzed antibody formation in BoNT treatment for glabellar wrinkles in 1,554 individuals, found that none of them developed neutralizing antibodies. The success of subsequent treatments was not reduced (45). We can conclude that the absence of clear evidence of antibody formation in the dermatological use of BoNT suggests that their formation is very unlikely (44).

4 Conclusion

Whilst the research into BoNT use sometimes appears to be complete, recent findings challenge prejudices and radically change the view on BoNT use. It is one of the medications with a promising wide range of indications for clinical use, although at the moment, there is still significant off-label use. Botulinum neurotoxin holds great promise in many areas, such as neurology, pain relief, pruritus, and others. There continues to be more research that reveals new possible indications for treatment in the field of dermatology.

However, there are already enough reports to conclude that the association between BoNT and aesthetics alone is a thing of the past.

Conflict of interest

None declared.

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