Classic toxin review

A closer look to botulinum neurotoxin type A-induced analgesia

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ABSTRACT

Chronic pain indicates a type of pain that lasts over time and is accompanied by diagnostic and therapeutic difficulties. It follows that treatment failures are common and patients roam from doctor to doctor in search of an effective care program. So there is an urgent need for long-acting and effective therapeutics to alleviate symptoms of the varied forms of chronic pain. During the past few years, a good success has been achieved with a derivative of a neurotoxin. It has been shown that administration of this toxin can block the release of neurotransmitters and pain mediators. Botulinum neurotoxin type A (BoNT/A) is well known as a treatment for neuromuscular conditions such as dystonia and spasticity. However, the clinical application for BoNT/A has continued to expand. Its analgesic effect has been used in clinical practice with satisfactory results. This review provides an introduction of a hypothesis for the mechanism by which BoNT/A eases chronic pain. It also summarizes the clinical therapeutic effects of BoNT/A in different types of chronic pain and its potential prospects.

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1. Introduction

Pain is an unpleasant sensory experience produced by noxious stimuli, inflammation or damage to the nervous system. Patients suffer because of the long-lasting uncomfortable feeling. Therefore, there is a pressing need to find a long-acting and effective therapeutics to alleviate the symptoms of different forms of pain. Some groups came up

Abbreviations: BoNTs, Botulinum neurotoxins; BoNT/A, Botulinum neurotoxin type A; CCBs, Calcium channel blockers; CCI, Chronic constriction injury; CGRP, Calcitonin gene-related peptide; CNS, Central nervous system; CPRS, Complex regional pain syndrome; GABA, Gamma-aminobutyric acid; HC, Heavy chain; LC, Light chain; MFPS, Myofascial pain syndrome; RA, Rheumatoid arthritis; SNAP, Synaptosomal associated protein; SNARE, Soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor; SP, Substance P; TCAs, Tricyclic antidepressants; TRPV, Transient receptor potential vanilloid; VAS, Visual analogue scale; TN, Trigeminal neuralgia.

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with a new strategy to explore potent and specific inhibitors of the neuronal exocytosis of transmitters and pain mediators that exhibit unique antinociceptive activity. Based on the results of the progressively increasing studies, Botulinum neurotoxin type A (BoNT/A) met the requirements perfectly. In this review, we have provided a hypothesis for the mechanism of action of BoNT/A and explain how it eases chronic pain using the latest evidence from animal models. Furthermore, we have summarized the clinical therapeutics of BoNT/A in different types of chronic pain. Finally, we have presented the reason behind its potential in protein engineering.

2. Development of BoNTs and their analgesic effect

Botulinum neurotoxins (BoNTs), the most poisonous biological substances known, are produced by anaerobic bacteria of the genus Clostridium (Simpson, 1981; Gill, 1982). However, it was not until nearly 30 years later that the first batch of crystalline toxin was produced. Apart from the well-known therapeutic use in muscular hyperactivity and certain autonomic disorders (Mahant et al., 2000), BoNTs were also used in the treatment of pain. The beneficial effects of BoNTs include the remission of migraine, neuropathic pain, joint pain and back pain. In 2010, Qerama et al. reported the hypothesis that BoNTs inhibit the local neurotransmitter that is released from sensory nerve endings by peripheral SNAP-25 (Synaptosomal associated protein of 25 kDa) cleavage; which is similar to the activity in cholinergic neurons (Qerama et al., 2010; Cui et al., 2004). The recent studies of mirror pain and polyneuropathy models (paclitaxel-induced polyneuropathy, diabetic neuropathy) (Favre-Guilmand et al., 2009; Bach-Rojecky et al., 2010) cannot be explained only by local action on the sensory nerve endings adjacent to the site of injection because of the unilateral BoNTs and their bilateral effects. Furthermore, the destination of the transported BoNTs has remained unknown and the functional significance of BoNTs axonal transport from periphery to the CNS (Bach-Rojecky et al., 2010) needs to be explained. In the end, these are some of the issues that need to be urgently resolved.

3. The connections between the structure of BoNT/A and its unique function

BoNTs are a group of homologous di-chain proteins (serotypes A-G) with distinct characteristics (Fig. 1). It originates from Clostridium botulinum whose active form consists of a Zn$\text{2}^{+}$-dependent proteolytic light chain (LC, 50 kDa) linked to a heavy chain (HC, 100 kDa) via a disulphide and non-covalent bonds (Dolly and O’Connell, 2012). When BoNTs are injected into a target tissue, its heavy chain binds to glycoprotein structures specifically found on cholinergic nerve terminals; which can explain its high selectivity for cholinergic synapses. After internalization, the light chain binds to the SNARE protein complex with a catalytic domain expressing the protease activity (Montal, 2010). The other two domains (H-chain domain) are the central domain, whose function is related to the membrane translocation of L-chain into the neuronal cytosol and the COOH-terminal domain (HC); which consists of two equally size subdomains and is for the neuropathic region.

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3.1. The analgesic effect of BoNT in the animal model of inflammatory pain

3.1.1. Formalin model

The formalin model is a preclinical model used to investigate the analgesic effect of some drugs. It always elicits pain-related behavior, such as licking, biting and shaking. Injection of formalin into the plantar surface of the hind paw produced a biphasic response of neuronal excitation (Lee et al., 2011). Cui et al. (Aoki, 2005) showed that subcutaneous injection of BoNT/A into the rat paw significantly reduced formalin pain during phase two, inhibited the glutamate release in the hind paw, reduced the number of formalin-induced Fos-like immunoreactive cells in the dorsal horn of the spinal cord and significantly inhibited the excitation of wide dynamic range neurons of the dorsal horn in phase two. All of these findings demonstrated that the BoNT/A does not exert a local analgesic effect but reduces central sensitization (Aoki and Francis, 2011).

3.1.2. Capsaicin model (TRPV1, SP and CGRP)

The capsaicin model of inflammatory pain is to excite the sensory neurons with capsaicin; which is an irritant derivative from chilli peppers. It binds to the cation channel of the transient receptor potential vanilloid type 1 (TRPV1); which is located on C-fibers (Lomas et al., 2008). This model can cause intense pain due to the release of neuropeptides such as substance P and CGRP (Bach-Rojecky and Lackovic, 2005). Bach-Rojecky et al. reported that pre-treatment with BoNT/A for six days before induction significantly provided an outline of BoNT protein design and function. The HC, HN and LC regions are responsible for binding, translocation and protease activity; respectively (Montal, 2010). In this study, we have tried to combine the information provided to us through literature with the evidence we have found in the animal models in order to reasonably explain the molecular mechanism of BoNT action. Never the less, further details need to be gathered by more extensive studies.

Fig. 1. Botulinum neurotoxins consist of three domains. The NH2-terminal domain (L-chain domain) is a zinc endopeptidase that represents the catalytic domain expressing the protease activity (Montal, 2010). The other two domains (H-chain domain) are the central domain, whose function is related to the membrane translocation of L-chain into the neuronal cytosol and the COOH-terminal domain (HC); which consists of two equally size subdomains and is for the neuropathic region.

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attenuated mechanical and thermal stimuli (Bach-Rojecky and Lackovic, 2005).

3.2. The analgesic effect of BoNT in the animal model of neuropathic pain

3.2.1. The chronic constriction injury model

The chronic constriction injury (CCI) of sciatic nerve was used as a model of neuropathic pain. This model was originally proposed by Bennett and Xie (1988) and can be adapted for both rats and mice. The study conducted by Marinelli et al., in 2010 (Marinelli et al., 2010) mainly investigated the effects of BoNT/A on neuropathic pain. They demonstrated that the BoNT/A counteracted the neuropathic pain induced by chronic constriction injury (CCI) to the sciatic nerve both in mice and in rats. They suggest that this effect was already present after a single intraplantar (i.pl.) or intrathecal (i.t.) neurotoxin administration. This significantly reduced the sciatic nerve ligation-induced mechanical allodynia in mice and rats along with the thermal hyperalgesia in rats. This effect on the CCI model indicated the BoNT/A interfering function mediated by blocking neuroexocytosis through the cleavage of synaptosome-associated protein of SNAP-25. Meanwhile, according to the previous reports, the inhibitory effects on GABA (Verderio et al., 2004), glutamate (Cui et al., 2004), CGRP (Lucioni et al., 2008) and SP (Ishikawa et al., 2000) are also involved in CCI model. Therefore, the mechanism should be similar to that of the inflammation model. Furthermore, Marinelli et al. reported that a single injection of BoNT/A was sufficient not only to reduce the mechanical allodynia and cold hyperalgesia but also to improve the functional recovery of injured paw and to enhance the regeneration processes in the injured nerve (Marinelli et al., 2010). It is extremely important that BoNT/A exerts analgesic effects and simultaneously is able to accelerate the process of nerve regeneration (Marinelli et al., 2010), which opens promising prospects on the development of new pharmacotherapeutic approach against neuropathic pain.

3.2.2. Model of diabetic neuropathic pain

The model of diabetic neuropathic pain is another frequently-used neuropathic pain. Rats were induced to become diabetic by a single intraperitoneal injection of streptozotocin (80 mg/kg). In 2010, Bach-Rojecky et al. (Bach-Rojecky et al., 2010) reported that the diabetic animals with at least 25% lower pain thresholds compared to that of the non-diabetic group were considered neuropathic and were injected with BoNT/A either subcutaneously (3, 5 and 7 U/kg) or intrathecally (1 U/kg). The results presented as pain reduction after BoNT/A injection in the animals with diabetic neuropathy. They also shared their hypothesis on the mechanism of this effect based on their results. Basically, they believed that the bilateral pain reduction after unilateral toxin application and the effectiveness of lower dose with the faster onset after the intrathecal injection was suggestive of the involvement of the central nervous system in the antinociceptive action of BoNT/A in painful diabetic neuropathy. This is in agreement with the results in the inflammation model (Aoki and Francis, 2011).

3.3. A summarized proposed mechanism based on the animal model

Summing up all the results reviewed earlier and referring to the mechanism of action discussed in the literature (Dolly and O’Connell, 2012) (Ishikawa et al., 2000), we suggest a potential mechanism of action for BoNT/A analgesic effect on pain transmission (Fig. 2). The administration of BoNT/A in peripheral nociceptive neurons plays a direct role in its peripheral analgesic effect and an indirect role in its central analgesic effect because of retrograde transport. It can also have analgesic effects by inhibiting the release of the neurotransmitter with its administration in the central nociceptive neurons.

4. BoNT-based neurotherapeutics in the patients with chronic pain

The international association for the study of pain has defined the chronic pain as what persists after the injury when healing has ceased. Chronic pain is involved in some major health problems in a range of conditions including: diabetic polyneuropathy, chronic back and shoulder pains, myofacial pain, arthritis pain and multiple sclerosis pain. This kind of pain has disturbed the life balance of many people and imposed an enormous impact on both the economy and the quality of life of many sufferers. Unfortunately, the majority of the sufferers do not use the currently available non-addictive medicines. What is worse is that the commonly used analgesics are short-acting and cause unwanted adverse effects; which raises serious problems upon repeated use over long period (Dolly and O’Connell, 2012).

It has been proven that BoNT/A causes selective weakness in the painful muscles and disrupts the spasm–pain

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**Fig. 2.** When we inject botulinum neurotoxin type A into the peripheral nociceptive neurons, its analgesic effect can be direct, peripherally and indirectly, centrally because of the retrograde transport (Dolly and O’Connell, 2012). When intrathecally injected, botulinum neurotoxin type A may inhibit the release of neurotransmitters from central terminals (Dolly and O’Connell, 2012). The analgesic effect may occur in both peripheral and central level at the same time. Symbols: SP: Substance P; CGRP: Calcitonin gene-related peptide; TRPV1: Transient receptor potential vanilloid 1; Fos; Glutamate.
cycle that provides sustained pain relief. This allows the patients to perform physical exercises that are fundamental for long-term recovery (Ishikawa et al., 2000). Furthermore, an enormous number of studies showed that BoNT/A offered a new direction to ease the chronic pain.

4.1. Migraine

Migraine is a chronic neurovascular disorder that accounts for suffering of 2%–15% of the world’s population. Migraine is characterized by severe headaches and is often accompanied with nausea, vomiting and increased sensitivity to sound and light. Some sufferers cannot receive an effective therapy from the doctors and as a result, they don’t even consult a physician in future occasions. The commonly used prophylaxis agents for migraine include β-adrenergic blockers, calcium channel blockers (CCBs), tricyclic antidepressants (TCAs) and anticonvulsants. Due to the adverse effect profile and limited efficacy of the currently available therapies, the potent neurotoxin, BoNT/A has been introduced to intensive clinical investigation for the treatment of migraine and other types of headache (Colhado et al., 2009). A double-blind, randomized placebo-controlled study of 30 migraine sufferers revealed that BoNT/A treatment was well tolerated and the frequency of the attacks were significantly reduced at day 90. Likewise, the frequency of the severe bouts was significantly reduced at days 60 and 90 (Barrientos and Chana, 2003).

Another double-blind, placebo-controlled trial was performed to investigate if this bio therapeutic would be suitable to treat chronic tension-type headache. All suffers responded positively to local injections of BoNT/A that resulted in less headaches and precranical muscle tenderness (Dolly and O’Connell, 2012) (Relja and Telarovic, 2004). Furthermore, Elza compared BoNT/A with other currently available drugs for the treatment of migraines. Their results suggested that the BoNT/A was more effective for the group of patients with frequent episodic migraines. However, considering the clinical benefits and the lack of undesirable side effects such as weight gain and constipation, they argued that BoNT/A should be considered for use in the patients with chronic headaches as an alternative therapy or in patients with contraindications for the use of other classes of drugs. They also reminded that further investigation is needed to define patient subgroups that might benefit from BoNT/A (Magalhães et al., 2010).

4.2. Arthritis joint pain

Arthritis is an important and growing public health problem (Lawrence et al., 2008). There is a growing need for novel treatments of refractory arthritis joint pain as aging population is expanding with many sufferers who cannot receive the joint replacement surgery. In 2008, Jasvinder et al. reported the use of intra-articular BoNT/A in two rheumatoid arthritis (RA) patients with persistent painful monoarthritis in ankle/feet joints. Both patients had monarticular pain despite a good response of all other joints to a combination therapy that also included anti-tumor necrosis factor therapy. All intra-articular corticosteroid injections and declined surgical options were failed in both patients. They began with a single “off-label” intra-articular injection of BoNT/A into the right ankle (100 units) and left first metatarsophalangeal joint (25 units). As a result, their pain and function improved significantly (40%) in both patients and the function lasted 15–18 months. They concluded that the intra-articular BoNT/A may provide an additional therapeutic option in RA patients with persistent monoarthritis (Singh and Mahowald, 2009). In 2009, Maren et al. conducted several small open label studies in which they injected BoNT/A into the joints with arthritis. They found that two third of the patients had more than 50% reduction in the joint pain severity that was associated with a significant improvement in function. Importantly, no serious adverse effects of BoNT/A were reported. They continued their studies using the same method in shoulders and knees. The results showed that BoNT/A produced a significant decrease in shoulder pain severity in one month (6.8–4.4 on VAS, p = 0.22). Furthermore, BoNT/A produced a significant 48% decrease in McGill Total Pain Score in the knees in one month (p = 0.11). This was still significant three months after the injection (p = 0.02). This group pointed out that more studies need to be conducted for the dose range and the interval of the BoNT/A injections (Chou et al., 2010).

4.3. Myofascial pain syndrome

Myofascial pain syndrome (MFPS) is characterized by the presence of trigger points, palpable muscle abnormality and referred pain distal to the trigger point. Most of its treatments are aimed to reduce the pain in trigger points and to reduce the muscle spasm. The traditional treatments of MFPS consist of physical therapy, oral medications and trigger point injections (Annaswamy et al., 2011). In 2010, Delaram et al. reported two cases where proximal myofascial pain in complex regional pain syndrome (CPRS) was treated with an injection of 20 units of BoNT/A in each trigger point. The therapeutic effect was reported to be satisfactory. However, there are limited number of reports on myofascial pain syndrome in the literature. Therefore, this area needs more continued research and exploration (Safarpour and Jabbari, 2010).

4.3.1. Trigeminal neuralgia

Trigeminal neuralgia (TN) is a severe chronic pain syndrome characterized by an excruciating, brief electric shocklike paroxysmal pain in one or more divisions of the trigeminal nerve. It can occur either spontaneously or upon gentle tactile stimulation of a trigger zone on the face or in the oral cavity (Fields, 1996; Cheshire, 2007; Devor et al., 2002). There are two major methods of treatment for TN: pharmacotherapy and neurosurgical procedures. Pharmacotherapy is the routine way of treatment and includes the use of antiepileptic drugs like carbamazepine with the secondary drug choice to be baclofen, lamotrigine, oxcarbazepine, phenytoin, gabapentin or sodium valproate (Merrison and Fuller, 2003). This is generally safer and more suitable for medically compromised patients who cannot undergo surgery. For those patients who do not respond well to medical management, surgery is the only
option. In the past few years, several reports on the successful use of BoNT/A in patients with TN seem to give us a new way to subside this kind of refractory chronic pain. In 2005, Piovesan et al. reported their success in nearly complete pain relief in all of their 13 patients with subdermal injections of BoNT/A at a mean dose of 3.22 units/cm² directly into the affected facial regions for 10 days. The patients were followed up for 60 days (Piovesan et al., 2005). Allam et al. reported a longer duration of pain relief for 90 days in their single patient (Allam et al., 2005). In 2009, Wei et al. achieved a longer pain-free duration of five months. However, the doses used in the study were several times higher (100 units) than that of the former studies. The injection was performed subcutaneously into the right external nasal trigger zone (60 units) and to the right mental nerve region (40 units). The pain recurred five months later and the site was again injected with 100 units of BoNT/A. In their study, the repeated injections were useful in promoting a continuous pain-free state. However, the patient lost the nasolabial fold on the right side of the face (Ngeow and Nair, 2010). Therefore, the doses need further confirmation with more studies.

4.3.2. Other types of chronic pain

Beside the therapeutic effects reviewed above, there are some other kinds of chronic pain that can be treated. In 2010, Santamato et al. reported the treatment of the neck pain that was related to nocturnal bruxism with BoNT/A. In this study, each masseter muscle was injected with a dose of about 40 units and the temporal muscle was bilaterally injected with 25 units. After three days of treatment with BoNT/A, a decrease in bruxism symptoms was noted (Santamato et al., 2010). Furthermore, Jason Abbott also used BoNT/A in women with chronic pelvic pain in 2009. They indicated that BoNT/A (20–40 units) used in the vulva may have a continued benefit for 3–6 months after injection with limited side effects (Abbott, 2009).

5. The bright future for the BoNT-based neurotherapeutics

The LC in the type E BoNT gives rise to a more extensively truncated SNAP-25 product that is unable to form functional complexes with its SNARE partners. Therefore, it offers a more fast acting effect compared to that of BoNT/A. Besides, it can also pseudo irreversibly abolish release of neurotransmitters. Generally speaking, BoNT/E blocks the neurotransmission more quickly and more potently compared to BoNT/A. However, the clinical application of BoNT/A is restricted by its neuromuscular paralytic action being transient (less than 4 weeks) in contrast to BoNT/A (more than 4 months). In the past few years, Meng J reported the construction of a chimera of BoNT/A and E by introducing a nucleotide sequence encoding the acceptor binding Hc domain of type A into the BoNT/E gene (Fig. 3). The recombinant EA chimeric protein can then be expressed in Escherichia coli and be purified. They found that it cleaved SNAP-25 in the trigeminal neurons and blocked CGRP release triggered by all stimuli tested, including capsaicin (Wang et al., 2011). After that, some people proved that it was possible to show this dramatic increase in persistence of neuroparalysis (Dolly and O’Connell, 2012). In these days, a faster and more efficient BoNT-based neurotherapeutics becomes a possibility considering the advances in protein engineering.

BoNT/A has been under clinical trials for treatment of migraine and other chronic pain for many years. Therefore, the translation of the encouraging results from preclinical studies in animal pain models to clinical treatments of more various types of chronic pain in human sufferers can be a significant step. However, more in depth studies are necessary to reach to a point where it can be clinically applicable. None of the previous studies have established the exact mechanism responsible for analgesic effects of BoNT/A; which could provide the essential foundation of developing future therapeutic strategies. Besides, there is a lack of precise applicable doses and injecting sites to refer to. Therefore, more studies are required to determine the best and accurate method of using BoNT/A is the goal of many ongoing efforts. With all this said, there is still a great potential for BoNT/A in the treatment of chronic pain.

Competing interests

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript. The authors who have taken part in this study do not have a relationship with the manufacturers of the drugs used either in the past or present and do not receive funding from the manufacturers to carry out their research.

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Conflict of interest

None.

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