REVIEW ARTICLE

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The non-neuronal and nonmuscular effects of botulinum toxin: an opportunity for a deadly molecule to treat disease in the skin and beyond*

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Summary

There is growing evidence that botulinum neurotoxins (BoNTs) exhibit biological effects on various human cell types with a host of associated clinical implications. This review aims to provide an update on the non-neuronal and nonmuscular effects of botulinum toxin. We critically analysed recent reports on the structure and function of cellular signalling systems subserving biological effects of BoNTs. The BoNT receptors and intracellular targets are not unique for neurotransmission. They have been found in both neuronal and non-neuronal cells, but there are differences in how BoNT binds to, and acts on, neuronal vs. non-neuronal cells. The non-neuronal cells that express one or more BoNT/A-binding proteins, and/or cleavage target synaptosomal-associated protein 25, include: epidermal keratinocytes; mesenchymal stem cells from subcutaneous adipose; nasal mucosal cells; urothelial cells; intestinal, prostate and alveolar epithelial cells; breast cell lines; neutrophils; and macrophages. Serotype BoNT/A can also elicit specific biological effects in dermal fibroblasts, sebocytes and vascular endothelial cells. Nontraditional applications of BoNT have been reported for the treatment of the following dermatological conditions: hyperhidrosis, Hailey–Hailey disease, Darier disease, inversed psoriasis, aquagenic palmoplantar keratoderma, pachyonychia congenita, multiple eccrine hydrocystomas, eccrine angiomatous hamartoma, eccrine sweat gland naevi, congenital eccrine naevus, Raynaud phenomenon and cutaneous leiomyomas. Experimental studies have demonstrated the ability of BoNT/A to protect skin flaps, facilitate wound healing, decrease thickness of hypertrophic scars, produce an anti-ageing effect, improve a mouse model of psoriasiform dermatitis, and have also revealed extracutaneous effects of BoNT arising from its anti-inflammatory and anticancer properties. BoNTs have a much wider range of applications than originally understood, and the individual cellular responses to the cholinergic impacts of BoNTs could provide fertile ground for future studies.

What's already known about this topic?

- Botulinum neurotoxins (BoNTs) have been used therapeutically in different medical conditions, predominantly in relation to muscle relaxation.

What does this study add?

- Recent research indicates that serotype BoNT/A can also elicit specific biological effects in skin cells, leading to profound clinical changes in dermatological conditions.
- BoNTs appear to have a wider range of applications than originally understood.

The structure and function of botulinum neurotoxins

The structure of botulinum neurotoxins

In nature, botulinum neurotoxin (BoNT) is produced by *Clostridium botulinum* bacteria. The distinct toxin subtypes, designated A through G, have different structures and mechanisms of action. The A, B and E subtypes cause botulism in humans owing to their action inside the axon terminal, leading to paralysis of the respiratory muscles and death resulting from respiratory failure. Pure botulinum toxin A was first synthesized as an inactive 150-kDa protein complexed with varying amounts of nontoxic companion proteins. Botulinum neurotoxin is activated when the polypeptide chain is proteolytically cleaved into the 100 kDa heavy chain and the 50 kDa light chain. The nontoxic BoNT-associated proteins include three haemagglutinin (HA) proteins and one nontoxic non-HA protein. In nature, it is believed that these associated proteins protect the inherently fragile BoNTs from the hostile environment of the gastrointestinal tract and help BoNTs pass through the intestinal epithelial barrier before they are released into general circulation (reviewed by Peng Chen *et al.*).[1]

Mechanism of action of botulinum neurotoxin

With regard to muscle contraction under normal conditions, depolarization of the axon terminal results in acetylcholine release from the cytosol of cholinergic neurons into the synaptic cleft with subsequent muscular contraction. Various BoNT subtypes will inhibit this acetylcholine release, blocking the induction of muscular contraction. This blockade of acetylcholine release is referred to as 'chemical denervation'. For exocrine tissue, glandular secretion is blocked.

Acetylcholine release is performed by proteins from the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE), which mediates synaptic vesicle docking/fusion with the inner surface of axonal plasma membrane at the release sites. However, the inhibition of acetylcholine exocytosis is reversible by natural SNARE protein complex turnover.

The toxic mechanism of action of BoNT comprises several distinct steps. The internalization of BoNT is achieved by endocytosis after the toxin's heavy chain has attached to cell-surface structures specifically found on cholinergic nerve terminals, such as ganglioside moieties, a vesicular protein (SV2) for BoNT/A and synaptotagmin for BoNT/B (reviewed in Peng Chen *et al.* and Giordano *et al.*).[1,2] BoNT/A can also attach to some other cell-surface proteins, such as E-cadherin,[3,4] fibroblast growth factor receptor (FGFR)3[5] and vanilloid receptors.[6] The light chain, which has zinc metalloprotease activity at its N-terminal, is released from the endocytotic vesicles upon acidification, and then reaches the cytosol wherein it cleaves one or two SNARE proteins, such as synaptosomal-associated protein (SNAP)-25 (BoNT/A, C and E), syntaxin (BoNT/C) and vesicle-associated membrane protein also known as synaptobrevin II (BoNT/B, F and G) (Fig. 1). However, the BoNT receptors and intracellular targets are not unique for neurotransmission, as several of these receptors and targets have been found in both neuronal and non-neuronal cells.

The non-neuronal cell types targeted by botulinum neurotoxin

Based on published data, several types of non-neuronal cells may be directly affected by BoNT in human skin and other tissues that produce a biological effect. The cells expressing one or more of the BoNT/A-binding proteins SV2, FGFR3 or vanilloid receptors, and/or BoNT/A cleavage target SNAP-25, include epidermal keratinocytes,[7,8] mesenchymal stem cells from subcutaneous adipose,[9] nasal mucosal cells,[10] urothelial cells,[11] intestinal epithelial cells,[12,13] prostate epithelial cells,[14] alveolar epithelial cells,[15] T47D, MDA-MB-231 and MDA-MB-453 breast cell lines,[16] neutrophils[17] and macrophages.[18] Importantly, it has been reported that, in addition to SNAP-25, BoNT/A can also cleave SNAP-23,[11,19,20] which is ubiquitously expressed in human tissues.[21] BoNT/A can inhibit SV2 expression in breast cancer cell lines.[16] Moreover, as will be discussed in detail below, BoNT/A can elicit specific biological effects in dermal fibroblasts, mast cells, sebocytes and vascular endothelial cells.

Differences in botulinum neurotoxin binding to neuronal vs. non-neuronal cells

There is growing evidence regarding the differences in how BoNT binds to and acts on neuronal vs. non-neuronal cells.[22] For example, the BoNT/A heavy chain enters neuronal cells mainly via a clathrin-dependent pathway, in contrast to intestinal cells where it follows a Cdc42-dependent pathway.[12] HA, one of the nontoxic components of BoNT large protein complexes, disrupts the intercellular epithelial barrier by directly binding E-cadherin.[3] It has been demonstrated that binding of the HA complex sequesters E-cadherin in the monomeric state, compromising the E-cadherin-mediated intercellular barrier and facilitating paracellular absorption of BoNT/A and BoNT/B.[4,23] In contrast, BoNT/C HA disrupts the barrier function by affecting cell morphology and viability in a ganglioside GM3-dependent manner.[24]

Differences in botulinum neurotoxin action on neuronal vs. non-neuronal cells

BoNT/A exhibits differential effects on gene expression in neuronal and non-neuronal cells. Microarray analysis of gene-expression changes upon exposure to BoNT/A revealed that in human HT-29 colon carcinoma cells, 167 genes were upregulated while 60 genes were downregulated, whereas in SH-SY5Y neuroblastoma cells, about 223 genes were upregulated and 18 genes were downregulated.[25] Modulation of genes and pathways involved in neuroinflammatory ubiquitin-proteasome degradation, phosphatidylinositol, calcium

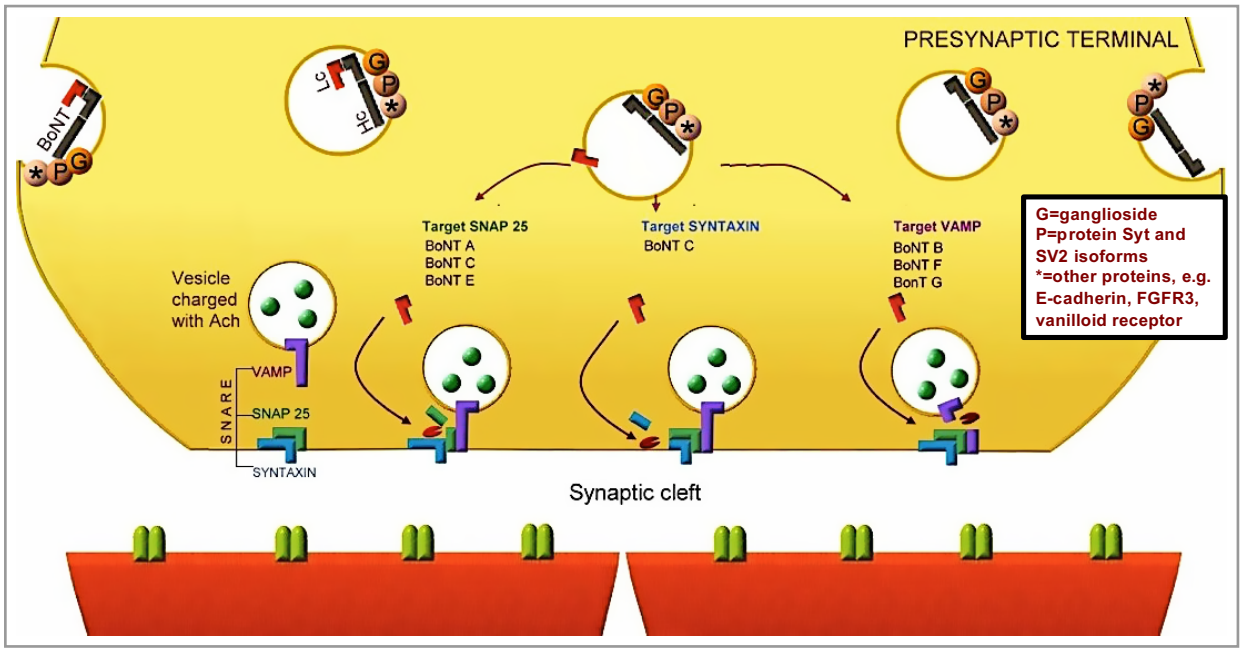

Fig 1. Molecular mechanisms of botulinum neurotoxins (BoNT) action (modified from Peng Chen *et al.*).[1] BoNT subtypes inhibit the release of acetylcholine (ACh) from the synaptic vesicle of the nerve ending into the synaptic cleft, resulting in 'chemical denervation'. Within the presynaptic terminal, ACh release is performed by the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE), which mediates docking/fusion of the synaptic vesicle with the inner surface of axonal plasma membrane at the release sites. BoNT is internalized by endocytosis after the toxin's heavy chain (Hc) has attached to ganglioside moieties, a vesicular protein (SV2) or synaptotagmin (Syt) present on the surface of cholinergic nerve terminals and some other cell-surface proteins, such as E-cadherin, fibroblast growth factor receptor (FGFR) 3 and vanilloid receptors. The toxin's light chain (Lc), which has zinc metalloprotease activity at its N-terminal, is then released from the endocytotic vesicle upon acidification, and reaches the cytosol wherein it cleaves one or two SNARE proteins, such as synaptosomal-associated protein (SNAP) 25 (BoNT/A, C and E), syntaxin (BoNT/C) and vesicle-associated membrane protein (VAMP) also known as synaptobrevin II (BoNT/B, F and G).

signalling in SH-SY5Y cells and genes relevant to focal adhesion, cell adhesion molecules, adherens- and gap-junction-related pathways in HT-29 cells suggested that affected genes play a distinct role in the biological effects of BoNT neuronal and non-neuronal cells.[25] The global transcriptional profiling of the murine alveolar macrophage cell line RAW264.7 revealed that altered genes were mainly involved in signal transduction, immunity and defence, protein metabolism and modification, neuronal activities, intracellular protein trafficking and muscle contraction.[18]

Clinical applications of botulinum neurotoxins

Botulinum neurotoxin products

The BoNT types A and B have been used therapeutically in various medical conditions for muscle relaxation, and more recently for their analgesic and cosmetic effects. The conditions approved for treatment with BoNT are cervical dystonia, blepharospasm, severe primary axillary hyperhidrosis, chronic migraine and strabismus. The global market for BoNT products, driven by their cosmetic applications, is rapidly growing with estimated annual sales in excess of 5–6 billion U.S. dollars.[26] In the global market, BoNT/A is sold as: Botox (onabotulinumtoxinA) by Allergan (Irvine, CA, U.S.A.); Dysport (abobotulinumtoxinA) by Ipsen Ltd (Slough, U.K.) and Galderma Laboratories (Fort Worth, TX, U.S.A.); Puretox by Mentor Corporation (Santa Barbara, CA, U.S.A.); Evosyal by Alphaeon (Irvine, CA, U.S.A.); Linurase by Prollenium (Aurora, Canada); and Xeomin (incobotulinumtoxinA) by Merz Pharmaceuticals (Frankfurt, Germany). BoNT/B is sold as Myobloc (rimabotulinumtoxinB) by Solstice Neurosciences (Malvern, PA, U.S.A.). In addition, there are several BoNT/A products marketed as: Siax (Neuronox) by Medy-Tox (Seoul, South Korea); Botulax by Hugel, and Nabota (DWP-450) by Daewoong (Seoul, South Korea); Lantox (Prosigne) by the Lanzhou Institute of Biological Products (Lanzhou, China); and Relatox by Mikrogen (Moscow, Russia). Several products, such as ANT-1207 (Anterios/Allergan), CosmeTox (Transdermal Corporation, Birmingham, MI, U.S.A.) and RT001 (Revance, Newark, CA, U.S.A.), have been developed for topical delivery of BoNT.[27,28] Each commercially available product has its toxin complexed with a varied quantity of unique proteins, and its activity has been determined using the assay specific to each manufacturer. These differences complicate an adequate comparison of clinical responses to similar products.[29]

Nontraditional applications of botulinum neurotoxins in various nondermatological conditions

BoNT/A has been used to treat certain neurological, musculoskeletal, ophthalmological, upper aerodigestive, gastrointestinal, urological and gynaecological disorders, including the following conditions: torticollis, dystonic tics/Tourette syndrome, spasticity related to stroke, essential tremor, Bell palsy, deformities related to cerebral palsy, cervical dystonia, upper and lower limb spasticity, chronic migraine, neuropathic pain (trigeminal and diabetic neuralgia), postherpetic neuralgia, head and neck cancer survivors with neck contractures following radiosurgical therapy, occupational cramping (i.e. writer's cramp), Parkinson disease, myofascial pain syndrome, ischaemic digits, movement disorders associated with injury, multiple sclerosis, focal dystonias affecting the limbs, face, jaw and vocal cords, and other vocal cord dysfunction, strabismus, hemifacial spasm, blepharospasm, corneal astigmatism, tear-film conditions, nystagmus, oscillopsia, benign eyelid fasciculation, chronic anal fissures, diffuse oesophageal spasm, oesophageal achalasia not amenable to surgery, anorectal outlet obstruction, rectal spasms, refractory gastroparesis, laryngeal dystonia, oromandibular dystonia, bruxism, cricopharyngeal spasm, stuttering, hot flashes, obesity (by increasing the gastric emptying time), overactive bladder, neurogenic detrusor overactivity with spinal cord injury and multiple sclerosis, benign prostatic hyperplasia, bladder pain syndrome, provoked vestibulodynia, pelvic floor spasm, vaginismus, urinary incontinence owing to detrusor sphincter dyssynergia, benign prostatic hyperplasia, gastric cancer and allergic rhinitis (reviewed in Giordano *et al.*,[2] Wollina[30] and https://en.wikipedia.org/wiki/Botulinum_toxin). Of particular interest is the use of BoNT to treat patients with major depressive disorder (MDD). Recent studies consistently showed significant reduction in depressive symptoms with BoNT/A injected into the glabellar muscles, indicating that this may be a safe and sustainable intervention in the treatment of MDD.[31–33]

Nontraditional applications of botulinum neurotoxins in dermatological conditions (Table 1)

In cosmetic dermatology, BoNTs have been traditionally used for facial muscle relaxation and to improve cutaneous elasticity, pliability and viscoelastic properties, in addition to the organization and orientation of collagen fibres of facial skin.[34] Aside from cosmetic indications, i.e. when treatment is intended to restore or improve a person's appearance, BoNTs have been used for various noncosmetic dermatological indications. Recent studies have demonstrated that BoNT/A affects not only skin texture but also sebum production leading to local skin dryness at the injection site,[35,36] which is in keeping with the experimental finding of reduced amounts of sebaceous cells and hair follicles in skin grafts of Wistar rats injected with BoNT/A.[37] Two female patients and one male patient with severe cystic acne were reported to have achieved a complete resolution of acne following intracutaneous injections of 2·5 U aliquots of botulinum toxin A spaced approximately 1·5 cm apart from one another.[38] Although the number of patients tested was small, all had recalcitrant acne that had not responded to conventional treatment. More recently, a double-blind, placebo-controlled, split-face study with 20 volunteers demonstrated that botulinum toxin effectively reduced sebum production and pore size in the oily skin group, but had no effect in the dry-to-normal skin group.[39] Interestingly, intramuscular injection of BoNT/A significantly reduced sebum production at the injection site but increased the sebum production of the surrounding skin at a radius of 2·5 cm.[40]

BoNT/A is used in different dermatological conditions associated with hyperhidrosis, including pompholyx, dyshidrosis, chromhidrosis and bromhidrosis, hidradenitis suppurativa and Frey syndrome, in addition to skin diseases worsened by hyperhidrosis, such as Hailey–Hailey disease, Darier disease, inverse psoriasis, aquagenic palmoplantar keratoderma, pachyonychia congenita (reviewed in Wollina, Messikh *et al.* and Campanati *et al.*).[30,41,42] BoNT/A showed efficacy in various eccrine gland abnormalities, such as multiple eccrine hydrocystomas,[43] eccrine angiomatous hamartoma,[44] eccrine sweat gland naevi and congenital eccrine naevus,[45,46] and as a vasodilator in treating Raynaud phenomenon.[47] BoNT/A improved the quality of life and alleviated pain in patients with painful cutaneous leiomyomas.[48] It was proposed that other painful cutaneous conditions with and without neurological involvements, such as anal fissures, leg ulcers, lichen simplex, postherpetic neuralgia and notalgia paraesthetica, might benefit from BoNT therapy.[41] BoNT/A has also shown therapeutic activity in patients with linear IgA bullous dermatosis,[49] lichen simplex,[50] alopecia areata,[51] androgenetic alopecia,[52] facial erythema and flushing (reviewed in Campanati *et al.*)[42] and various forms of itch in human skin (Table 1).[53–55]

A search of the ClinicalTrials.gov website, (a registry/results database of publicly and privately supported clinical trials conducted around the world), revealed the following studies concerning botulinum toxin in the following dermatological conditions: alopecia areata (NCT00999869, NCT00408798); recalcitrant alopecia totalis and alopecia universalis (NCT00997815); postexcisional scarring (NCT02623829); forehead scars following Mohs micrographic surgery and reconstruction for skin cancer (NCT01459666); itching from hypertrophic scars (NCT02168634); psoriasis (NCT00816517, NCT02577185); scleroderma-associated Raynaud syndrome (NCT02165111); localized vitiligo (NCT01051687); cutaneous leiomyomas (NCT00971620); herpes labialis (NCT01225341); acne (NCT00765375, NCT01293552); epidermolysis bullosa simplex and pachyonychia congenita (NCT00936533); Hailey–Hailey disease and Darier disease (NCT02782702); and for the itch-relieving effect of BoNT/A in healthy subjects (NCT02639052). On the other hand, several undesirable effects from BoNT/A injections have been reported, including vasculitis with panniculitis,[56] thrombosis of subcutaneous anterior chest veins (Mondor disease),[57] and

Table 1 Reports of noncosmetic use of botulinum neurotoxins in dermatology

Skin disease category	Name of skin disease
Blistering diseases	Linear IgA bullous dermatosis
Disorders of cutaneous pigmentation	Localized vitiligo
Disorders of cutaneous vasculature	Raynaud phenomenon (Raynaud syndrome), facial erythema and flushing
Eczematous diseases	Lichen simplex
Genodermatoses	Epidermolysis bullosa simple, pachyonychia congenita, Hailey–Hailey disease, Darier disease
Hair disorders	Alopecia totalis, alopecia universalis and androgenetic alopecia
Mucocutaneous viral infections	Herpes labialis and postherpetic neuralgia
Papulosquamous disorders	Psoriasis
Pruritus	Notalgia paraesthetica and various other forms of itch
Sebaceous gland disorders	Increased sebum production and acne
Skin wounds and wound healing disorders	Anal fissures, leg ulcers, postexcisional scarring, forehead scars following Mohs micrographic surgery and reconstruction for skin cancer
Smooth muscle tumours	Cutaneous leiomyomas
Sweating and sweat gland disorders	Hyperhidrosis, pompholyx, dyshidrosis, chromhidrosis, bromhidrosis, Frey syndrome, hidradenitis suppurativa, multiple eccrine hydrocystomas, eccrine angiomatous hamartoma, eccrine sweat gland naevi and congenital eccrine naevus
Miscellaneous dermatological conditions	Aquagenic palmoplantar keratoderma

cutaneous granulomatous reaction, possibly associated with cutaneous sarcoidosis.[58]

Experimental studies of cutaneous effects of botulinum neurotoxins

The results of *in vivo* and *in vitro* experiments have identified a number of direct effects of BoNT/A on non-neuronal cells in the skin, which help to explain the mechanisms and therapeutic benefits of BoNTs for nontraditional dermatological applications. Current literature on the experimental use of BoNT/A discussed below demonstrates its ability to protect skin flaps, facilitate wound healing, decrease the thickness of hypertrophic scars and produce an anti-ageing effect. Moreover, BoNT/A has been shown to decrease the infiltration of cutaneous lymphocytes and improve acanthosis in the KC-Tie2 mouse model of psoriasiform dermatitis,[59] protect from atopic dermatitis-like skin lesions of NC/Nga mice,[60] and produce an anti-itch effect in acute and mouse models of chronic dry skin itch.[61] The latter two functions may be related, in part, to the ability of BoNT/A to decrease mast-cell activity.[62]

Cutaneous effects of botulinum neurotoxin A action on cutaneous vasculature

In studies on skin-flap survival in rats, BoNT/A was injected into the entire flap and compared with a control group that received a saline injection.[63] The BoNT/A group showed nearly complete survival of the flaps owing to increased perfusion compared with the control group. The protective effects of BoNT/A on flap survival in rats have been independently reported by several research groups.[64–66] In one study, BoNT/A was used to prevent unfavourable effects of cigarette smoke in Wistar albino rats.[67] In another study, it increased skin-flap viability in diabetic rats, which was associated with an increase of lumen diameter, external arterial diameter and lumen/wall thickness ratio.[68] At the molecular level, BoNT/A affected the expression of vascular endothelial growth factor, platelet endothelial cell adhesion molecule 1, CD31, CD34, interleukin (IL)-1 and tumour necrosis factor-α in cutaneous flaps.[69–71] BoNT/A reduced the hypoxic area and the number of oxidative stress-associated DNA-damaged cells and apoptotic cells in the cutaneous ischaemia–reperfusion injury sites.[72] In an *in vitro* assay, BoNT/A significantly prevented the oxidant-induced intracellular accumulation of reactive oxygen species in vascular endothelial cells.[72]

Cutaneous effects of botulinum neurotoxin A action on remodelling of dermal connective tissue

The direct effects of BoNT/A on dermal fibroblasts are well characterized and may mediate its pharmacological effects on dermal tissue remodelling, which is characteristic of skin ageing, wound closure and scar formation. In cultured 3T3 fibroblasts, BoNT/A induced morphological changes, such as a loss of normal fibroblast morphology and the cytoplasmic retraction and spread phenomena.[73] The antiphotoageing potential of BoNT/A through a reduction in senescence-related proteins has been demonstrated in the studies of ultraviolet-B-induced premature senescence of human dermal fibroblasts *in vitro*. The irradiated fibroblasts that were additionally treated with BoNT/A demonstrated a decrease in the expression of senescence-associated β-galactosidase, levels of tumour suppressor and senescence-associated proteins, G1 phase cell proportion and secretion of matrix metalloproteinase (MMP)-1 and MMP-3, and also had an increase in the production of collagen types I and III.[74]

BoNT/A has been shown to alter fibroblast activities associated with wound healing. In cultures of human fibroblasts, BoNT/A inhibited expression of collagen types I and III, but enhanced the

expression of MMP-2 and MMP-9.[75] Gelatin zymography experiments confirmed enhanced MMP-2 activity in collagen degradation. BoNT/A also inhibited phosphorylation of Smad2 *in vitro*, and inhibited silicon-induced capsule formation through the transforming growth factor (TGF)-β/Smad signalling pathway *in vivo*.[75] According to one study, BoNT/A may interrupt the differentiation of fibroblasts to myofibroblasts by blocking TGF-β1 signalling.[76] However, in another study, BoNT/A upregulated the expression of type I collagen and decreased the production of some MMPs.[77] In Wistar rats, BoNT/A injections reduced wound and graft contraction,[78] and in another rat model of burn wound healing, BoNT/A improved both the healing process and the cosmetic appearance of the burn scar, and was associated with faster regeneration, less inflammation and an increase in the number of fibroblasts.[79]

BoNT/A decreases the thickness of hypertrophic scars, suggesting that its use may prevent hypertrophic scars after trauma, burns or surgery. Indeed, in the rabbit ear hypertrophic scar model, BoNT/A injection decreased scar thickness.[80] The mechanisms apparently involve the ability of BoNT/A to alter major biochemical events associated with fibroblast growth and differentiation. It has been documented that BoNT/A upregulates *Rac1*, *Cdc42* and *RhoA* gene expression in a dose-dependent manner in human dermal fibroblasts *in vitro*,[81] inhibits fibroblast proliferation and fibroblast-to-myofibroblast differentiation,[82] induces apoptosis and decreases expression of α-smooth muscle myosis and myosin-II, regulates S100A4 and collagen type I, and downregulates the *TGF-β1*, *VEGF*, *MMP-1* and *PDGFA* genes, and some other genes relevant to invasive growth in keloid fibroblasts.[83–85] Furthermore, treatment with BoNT/A affected cell cycle distribution, slowed proliferation and inhibited connective tissue growth factor expression in fibroblasts isolated from tissue specimens of hypertrophic scar.[86–88]

Experimental studies of extracutaneous effects of botulinum neurotoxins

Experimental studies have revealed extracutaneous effects of BoNT arising from its anti-inflammatory and anticancer properties.

Anti-inflammatory properties

In experimental cystitis induced by cyclophosphamide in rats, intravesical BoNT/A administration decreased the inflammatory reaction, expression of COX-2 and the prostaglandin E2 receptor EP4 subtype, and also suppressed bladder hyperactivity.[89] In a complete Freund's adjuvant-induced arthritic knee-joint model of the hind leg in rats, BoNT/A abolished joint inflammation and destruction, reduced infiltration of monocytes and macrophages, lowered IL-1β immunoreactivity and decreased the number of IL-1β-positive immune-reactive cells.[90] The *in vivo* anti-inflammatory effects of BoNT/A might be related to its ability to suppress lipopolysaccharide-induced nitric oxide and tumour necrosis factor-α production at the transcriptional level by blocking activation of JNK, ERK, and p38 MAPK, which was demonstrated in *in vitro* experiments with RAW264.7 macrophages.[90]

Anticancer properties

The anticancer effects of BoNT/A have been identified in three types of cancer cell line, i.e. prostate, breast and colon carcinoma. It was documented that BoNT/A inhibits the growth of LNCaP human prostate cancer cells *in vitro* and *in vivo*,[91] and also increases the phosphorylated form of phospholipase A2, which may represent one mechanism that explains how the toxin reduces cell growth and proliferation.[14] In rats, intraprostatic BoNT/A injection altered cellular dynamics by inducing apoptosis, inhibiting proliferation and downregulating α1-adrenergic receptors, which was associated with prostate apoptosis and atrophic change.[92,93] The toxin-treated rats showed reduced epithelial staining of Bcl-xL and consistently increased Bax and caspase-3 staining when compared with saline-treated animals.[94]

As breast and colon cell lines have been shown to respond to BoNT by changes in gene expression at the RNA and protein levels,[25,73] other types of cancer may become a potential target for the anticancer activity of BoNT.

Conclusions and perspectives

There is overwhelming evidence that BoNTs exhibit biological effects on many human cell types, which is of enormous clinical relevance. Apparently, these toxins have a much wider zone of influence than originally understood, and these ubiquitous events are based on individual cellular responses to the cholinergic impacts of BoNT/A. The BoNT receptors and intracellular targets are not unique for neurotransmission, as several of these receptors and targets have been found in non-neuronal cells. There are differences in the characteristics of how BoNT binds to and acts on neuronal vs. non-neuronal cells. Much is yet to be learned about these toxins and their mechanism of action. As such, the evidence indicates that there is fertile ground for future study, which is highly likely to result in impactful discoveries. If there was ever a drug that was likely to affect every cell of the body, this is it.

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