Botulinum Neurotoxin and Its Potential Role in the Treatment of Erectile Dysfunction

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ABSTRACT

Introduction: Botulinum toxin type A (BoNT-A) has been used to treat several striated and smooth muscle disorders. During the past year, human and animal studies conducted in Egypt and Canada by two different groups of investigators have suggested a possible role for the intracavernosal injection of BoNT-A in the treatment of erectile dysfunction (ED).

Aim: To discuss BoNT-A and its current medical uses, the rationale for its new potential use in the treatment of ED, and the available evidence and concerns.

Methods: A literature search was conducted. This review was based on the available studies presented at the European Society for Sexual Medicine, Sexual Medicine Society of North America, and International Society for Sexual Medicine meetings in 2016 by the two groups.

Main Outcome Measures: Sinusoidal diameter; penile color Doppler study; Erection Hardness Score; Sexual Health Inventory for Men questionnaire; and Sexual Encounter Profile questions 2 and 3.

Results: Two human studies conducted by the authors and two animal studies (one from the authors' group and one from Canada) were reviewed. These seemed to suggest generally favorable outcomes with the use of BoNT-A in the treatment of ED.

Conclusion: BoNT-A could be a potential therapy for ED. In addition to the findings of the three pilot studies, larger multicenter trials need to be conducted to further explore the true therapeutic efficacy and clinical safety of BoNT-A in the treatment of ED. **Ghanem H, Raheem AA, AbdelRahman IFS, et al. Botulinum Neurotoxin and Its Potential Role in the Treatment of Erectile Dysfunction. Sex Med Rev 2018;6:135–142.**

Key Words: Botulinum Neurotoxin Type A; Botox; Erectile Dysfunction; Intralesional Treatment

INTRODUCTION

Botulinum neurotoxin (BoNT) is one of the most potent toxins known to humans. It is produced by *Clostridium botulinum,* an anaerobic spore-forming, gram-positive bacterium. Poisoning with BoNT can cause botulism, resulting in generalized paralysis including respiratory arrest and death.[1,2]

The capacity of BoNT to relax muscles has been used during the past four decades to treat several striated and smooth muscle disorders in addition to its wide use in esthetic medicine. More recently, researchers have investigated whether the muscle-relaxing capacity of BoNT could be used within the corpora cavernosa to enhance penile erections, thus introducing a possible new line of treatment for erectile dysfunction (ED). During the past year, human and animal studies have been conducted in Egypt and an animal study has been conducted in Canada by two different groups of investigators.[3–8]

MECHANISM OF ACTION OF BONT

There are seven distinct biochemical and serologic forms of BoNT (A, B, C1, D, E, F, and G). BoNT-A, BoNT-B, and BoNT-E can cause botulism in humans, whereas the remaining BoNT forms can cause disease only in animals. BoNT-A is the most commonly used form in medicine.[9,10]

The seven BoNT forms cause flaccid paralysis by preventing the release of acetylcholine at the presynaptic membrane. They act on different aspects of the soluble N-ethylmaleimide sensitive

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factor attachment protein reception (SNARE) complex. The SNARE complexes are important groups of polypeptides that mediate the fusion of the synaptic vesicle to the presynaptic membrane at the neuromuscular junction, thus allowing acetylcholine release into the synaptic cleft. The SNARE complex influenced by BoNT-A is synaptosome-associated protein-25 (SNAP-25; Figure 1).[11,12]

BoNT-A is formed by a heavy polypeptide chain and a light polypeptide chain joined by a disulfide bond. The heavy chain has high affinity to cholinergic neurons and forms an irreversible bond at the presynaptic surface with synaptic vesicle protein-2. Once this bond is formed, the toxin-receptor complex can enter the cell through the process of endocytosis. When the complex enters the cytoplasm, the disulfide bond breaks, separating the heavy and light chains. It is the light chain that interacts and cleaves SNAP-25. Once BoNT-A has cleaved the polypeptide of SNAP-25, the presynaptic nerve terminals are affected irreversibly. It takes up to 3 months for the production of new nerve terminals, at which point function returns.[11,12] The polypeptides of SNAP-25 and synaptic protein-2 are central to the effect of BoNT-A. They have been identified in the urothelium of human bladders and are found widely throughout various smooth muscles.[13]

The inhibitory effects of BoNT on the release of acetylcholine from parasympathetic and cholinergic neurons are well understood. There also is evidence suggesting that BoNT can inhibit noradrenaline, dopamine, glycine, and γ-aminobutyrate. The effect on these additional neurotransmitters is often less profound than on acetylcholine and is dose and site specific. BoNT has been used for prostatic smooth muscle relaxation in the management of lower urinary tract symptoms.[14] It also has been found to inhibit noradrenaline release in the urethra and anococcygeus of rats[15,16] and prostatic tissue of dogs.[17] The duration of effect of BoNT-A in striated muscle is roughly 2 to 3 months; however, in smooth muscle, its effects are believed to last longer.[11]

CURRENT MEDICAL USES OF BONT

BoNT-A is the most commonly used serotype for medical application and was the first to be licensed for medical use. There are several commercially available forms; Botox (Allergan Pharmaceuticals, Parsippany, NJ, USA) is the most widely used and has the most medical applications. Different formulations of BoNT-A are available, each produced by a different company. Each formulation varies slightly in structure, efficacy, duration, and safety profile.[13] The effect of BoNT is site specific; it is administered by local injection (subcutaneous or intramuscular) into the targeted area. It can be administered using endoscopic procedure and by injection directly through the skin. Given the high affinity of BoNT to cholinergic neurons, its effects are consistent and, given at a low dose, have limited systemic adverse effects.[9]

BoNT-A was first used in medicine in 1977 for the treatment of strabismus in children. Since then, it has been widely used for different conditions and by different specialties. It is best known for its use in the cosmetic industries; however, it also is established practice in the treatment of overactive striated muscles

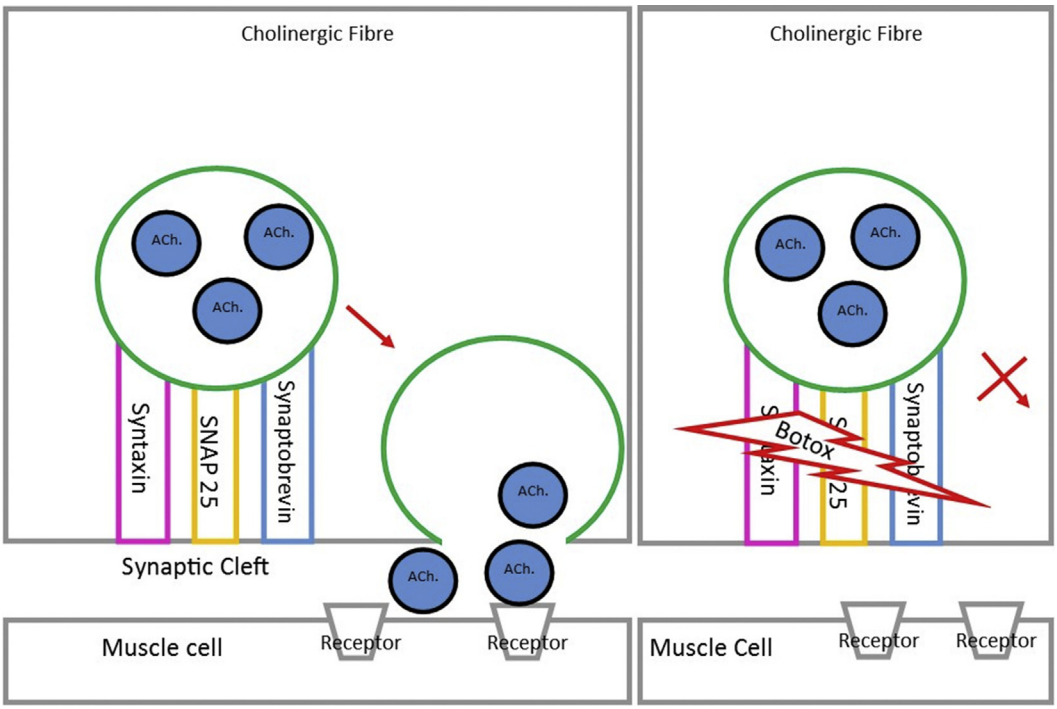

Figure 1. Mechanism of action of botulinum neurotoxin type A on muscle. SNAP-25 = synaptosome-associated protein-25. Figure 1 is available in color at www.smr.jsexmed.org.

disorders, such as strabismus, esotropia, exotropia, focal dystonias, spasticity, and movement disorders.[18]

There is growing evidence for the use of BoNT in smooth muscle disorders. It has been used in the management of different conditions with varying success, such as achalasia, esophageal spasm, ptyalism, hyperhidrosis, and intrinsic rhinitis. The safety and efficacy of BoNT in smooth muscle relaxation is well established in the treatment of overactive bladder syndrome and neurogenic detrusor overactivity.[19]

The EMBARK clinical research program conducted two large, phase III, randomized, placebo-controlled trials that found a significant improvement in overactive bladder symptoms and quality of life in patients who received BoNT-A injection into to the detrusor muscle by cystoscope compared with controls. No significant adverse effects were noted.[20–22]

Furthermore, there have been two large international, multicenter, randomized, placebo-controlled trials conducted by the DIGNITY research group. They compared BoNT-A with placebo for neurogenic detrusor overactivity in patients with multiple sclerosis and patients with spinal cord injury; 691 patients were randomized to the intervention and control groups. Maximum cystometric capacity, maximum detrusor pressure during first involuntary contraction, and incontinence quality of life also were significantly improved in the intervention vs control group.[20,23–25]

Intravesical BoNT-A is very well tolerated. Urinary tract infections, hematuria, and urinary retention are the most commonly reported adverse events.[26] Systemic adverse effects are very uncommon.

A NOVEL ROLE FOR BONT-A IN ED

Development of the Concept

In the initial clinical trial in Egypt, the authors ran an ED clinic and a related antiaging clinic in which antiaging procedures were performed, including BoNT-A injections. Having developed experience in these two areas, the authors contemplated that because cavernosal smooth muscle relaxation is an integral part of the development of an erection and BoNT-A is a strong inducer of smooth muscle relaxation, then BoNT-A might be effective in the treatment of ED not responding to other forms of non-surgical treatment.[3–6]

Physiology of Penile Erection

The penile erection is a neurovascular event that is strongly influenced by psychological and hormonal factors. Cavernosal smooth muscle relaxation plays a central role in penile erection. In the flaccid state, the cavernosal smooth muscle of the penis exhibits tonic contraction, which allows only a small volume of blood into the corpus cavernosa. Neuronal activation after sexual stimulation results in cavernosal smooth muscle relaxation and penile tumescence. Cavernosal smooth muscle relaxation is mediated by nitric oxide production resulting from stimulation of parasympathetic cholinergic and non-adrenergic non-cholinergic neurons. Sympathetic neurons that release noradrenaline cause detumescence. Stimulation of parasympathetic neurons causes the release of acetylcholine, which binds with muscarinic receptors, and the production of endothelial nitric oxide synthase and the subsequent production of nitric oxide. Stimulation of non-adrenergic non-cholinergic neurons results in the production of neuronal nitric oxide synthase and subsequent nitric oxide release.[27]

Dilatation of arteries and arterioles increases inflow into the penis. This causes the sinusoids to expand and the blood becomes trapped in this space. This expansion compresses the subtunical venous plexus against the tough tunica albuginea, limiting venous outflow. As the pressure increases, the tunica albuginea begins to stretch, compressing the emissary veins between the inner circular and outer longitudinal tunica layers. At this stage, the intracavernosal pressure increases and venous outflow is at a minimum (full erection stage). With contraction of the ischiocavernosus muscle, the pressure increases further (rigid erection phase).[27]

As phosphodiesterase type 5 (PDE5) converts cyclic guanosine monophosphate to guanosine monophosphate, the penile vasculature begins to develop vasoconstriction, resulting in detumescence.[28]

PATHOPHYSIOLOGY OF VASCULOGENIC ED

ED of vasculogenic origin is very common. It is difficult to accurately predict how common, because of the often mixed and multifactorial etiologies in ED. ED of vascular etiology can be further divided into arteriogenic and venogenic.

Arteriogenic ED shares the same risk factors as cardiovascular disease and is likely an alternative manifestation of the same disease process. A recent systematic review suggested that ED might precede future cardiovascular disease, citing the arterial size hypothesis as a possible reason.[27] The pathophysiologic process of atherosclerosis results in occlusive arterial disease that causes a decrease in perfusion pressures in the penile arteries. This decrease in pressure causes a longer duration for penile tumescence and a decrease in penile rigidity.[29]

In addition, there is evidence suggesting that cardiovascular risk factors (eg, essential hypertension) can increase sympathetic basal tone in humans and rats, resulting in vasoconstriction and cavernosal smooth muscle contraction.[30] There also is evidence of impaired endothelial-dependent vasodilatation in response to acetylcholine in patients with essential hypertension.[31]

Furthermore, a decrease in oxygen partial pressure (secondary to arterial insufficiency) has been found to cause structural changes in collagen synthesis and decrease smooth muscle content in the trabeculae of the corpus cavernosa. These structural abnormalities have been found to increase the incidence of veno-

occlusive dysfunction.[32–34] In a rabbit model, Azadzoi et al[35] found that cavernosal ischemia from arterio-occlusive disease could predict impaired venous compression.

Venogenic (veno-occlusive) ED occurs when there is inadequate compression of the subtunical and emissary veins. Different pathophysiologic processes have been identified as possible causes.

The integrity of the tough elastic tunica albuginea is key in providing a surface against which the subtunical and emissary veins can be compressed. Any condition (Peyronie disease, penile fracture, or diabetes mellitus) that interrupts or alters the microstructure of the tunica can result in veno-occlusive ED.[36–38] Furthermore, ischemic priapism refractory to injection of α-adrenergic agonist is managed by creating a venous shunt. This channel provides an alternate route of venous drainage, thus weakening the effectiveness of venous compression.

Changes in the structure or function of the cavernosal smooth muscle affect erectile function. The content of smooth muscle is correlated with cavernosal expandability.[33] Sattar et al[39] found a significantly smaller mean percentage of cavernous smooth muscle fibers in men with ED compared with normal controls. Furthermore, cardiovascular disease risk factors have been associated with a decrease in the elasticity of the smooth muscle fibers and an increase in collagen deposition.[40,41] Junemann et al[42] found that a high cholesterol diet in rabbits resulted in degeneration of cavernous smooth muscle.

Insufficient cavernosal smooth relaxation prevents effective compression of the subtunical and emissary veins. Without effective compression, it is not possible to achieve a full penile erection. Any endogenous or exogenous α-adrenergic source can increase cavernosal smooth muscle tone. This is demonstrated in the use of α-adrenergic agonists as prophylaxis in recurrent ischemic priapism.[43] Increased basal sympathetic tone from organic or psychosexual factors also can affect erections.[29,30] Pickard et al[44] found smooth muscle functional impairment in men with venogenic ED; the smooth muscle of these men was less responsive to nerve-evoked relaxation. Furthermore, aging is associated with structural and physiologic changes that lead to ED in the absence of any other risk factors.[45]

HUMAN AND ANIMAL STUDIES

The available studies were presented in 2016 at the European Society for Sexual Medicine, Sexual Medicine Society of North America, and International Society for Sexual Medicine meetings, two of which also were registered on clinicaltrials.gov since 2015 (Table 1). A further Medline search did not yield more literature.

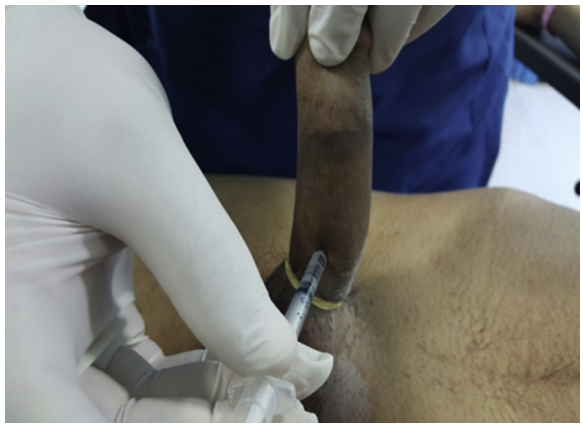

Figure 2. Intracavernosal injection technique of botulinum neurotoxin type A. Figure 2 is available in color at www.smr.jsexmed.org.

Human Studies

Two human studies are available from Egypt. The first is a phase I pilot randomized controlled trial (RCT) of 24 patients that was completed and presented at meetings of the International Society for Sexual Medicine and affiliated societies.[3–6] The second is a phase II RCT of 160 patients that is in progress.[7] Two animal studies of 30 and 10 rats also were conducted.[3–6,8]

The human RCT involved 24 men with severe vasculogenic ED diagnosed by penile duplex and refractory to PDE5 inhibitors (PDE5Is) and intracavernosal injection (ICI) therapy with tri-mix, with penile prosthesis insertion being their only option. Only patients with a "no" response to Sexual Encounter Profile questions 2 and 3 were included in the study. The patients were randomized to the intervention and control groups (1:1). Assessment for the two groups was done by penile color Doppler study and the Erection Hardness Score at baseline and 2 weeks after treatment, respectively, in addition to the Sexual Health Inventory for Men (SHIM) questionnaire and Sexual Encounter Profile questions 2 and 3 at baseline and 4 weeks after treatment, respectively.[3–6]

The intervention group received a single ICI of Botox 50 U and the control group received a single ICI of 0.9% normal saline 1 mL. To decrease the risk of systemic absorption, compression of the penile base was applied using a rubber band placed over the base of the penis before the injection and removed after 20 minutes (Figure 2). After injection with BoNT-A, there was a statistically significant improvement in

Table 1. Registered BoNT-A in erectile dysfunction clinical trials

Investigators	Start	Status	Type	BoNT-A dose (U)	N
Ghanem et al[3]	September 2015	Completed	RCT	50	24
Ghanem et al[7]	April 2017	Ongoing	RCT	100	160

BoNT-A = botulinum neurotoxin type A; RCT = randomized controlled trial.

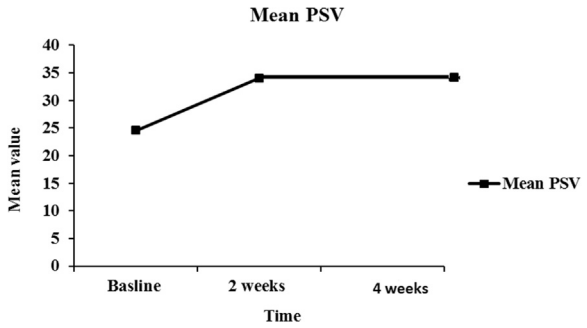

Figure 3. Penile duplex PSV changes after intracavernosal injection of botulinum neurotoxin type A. PSV = peak systolic velocity.

the mean peak systolic velocity in the treatment group from 24.6 cm/s at baseline to 34.9 cm/s ($P = 0.005$) but not in the control group (Figure 3). There also was a statistically significant improvement in the mean SHIM score (from 5.58 to 10.25; $P = 0.0075$) and the mean Erection Hardness Score (from 2 to 2.75; $P = 0.01$; Figure 4). Of the 12 patients in the treatment group, 7 could engage in penetrative sex with their partners with the help of sildenafil 100 mg compared with two patients from the control group, with the erection lasting long enough to complete intercourse in 3 of the 7 patients from the treatment group vs none from the control group (Figure 5). One patient in the treatment group experienced a 2.5-hour prolonged erection during the post-treatment penile color Doppler study with the tri-mix injection that required an ephedrine ICI. However, there were no episodes of priapism or systemic toxicity.[3–6]

The second study by the same group is an ongoing phase II randomized controlled trial of 160 patients who will be randomized to intervention and control groups (1:1); the intervention group will receive BoNT-A 100 U by ICI and the control group will receive saline 1 mL by ICI.[7]

Animal Studies

The Egyptian group also conducted an animal study. Of 30 male albino rats, 10 received an ICI of saline 0.1 mL (control), 10 received an ICI of BoNT-A 1 U, and 10 received an ICI of BoNT-A 2 U.[3–6]

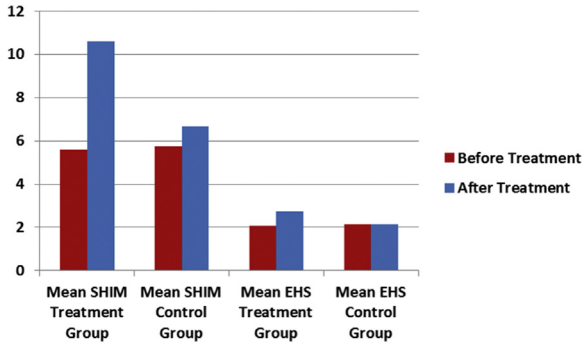

Figure 4. SHIM and EHS changes after intracavernosal injection of botulinum neurotoxin type A. EHS = Erection Hardness Score; SHIM = Sexual Health Inventory for Men. Figure 4 is available in color at www.smr.jsexmed.org.

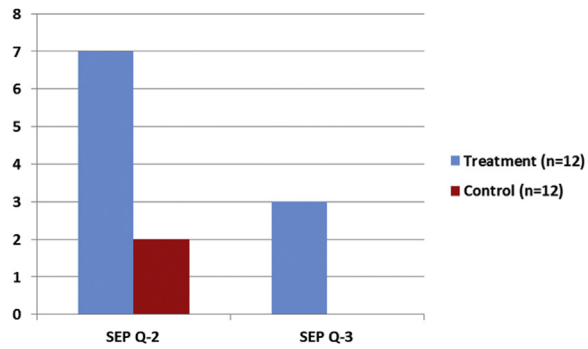

Figure 5. SEP questionnaire in treatment and control groups. Q-2 = question 2; Q-3 = question 3; SEP = Sexual Encounter Profile. Figure 5 is available in color at www.smr.jsexmed.org.

Four weeks after the ICIs, all rats were sacrificed and the penile tissues were harvested for histologic and immunohistochemical analyses with the pathologist blinded to which group the specimens belong to.

The results showed a statistically significant larger mean resting sinusoidal diameter in the two treatment groups (26.2 ± 6.5 μm, $P = .000027$; 22.57 ± 5.97 μm, $P = .0003$) compared with the control group (13.32 ± 2.8 μm). There were no local or systemic side effects (Figures 6 and 7).

The findings of this study suggested that BoNT-A induced relaxation of the surrounding smooth muscles as evidenced by the difference in resting sinusoidal diameter between the treatment and control groups. It also showed that it is safe to inject in the corpora cavernosa. Before this study; there were two concerns: (i) priapism from loss of muscle tone and (ii) occurrence of systemic toxicity after ICI of BoNT-A.

In another animal study, De Young et al[8] investigated the use of BoNT-A in smooth muscle relaxation as potential treatment for ED. They investigated the use of BoNT-A ICI in 10 rats. The treatment group (n = 5) received ICI of BoNT-A 10 U and the control group (n = 5) received ICI of saline. Then, the two

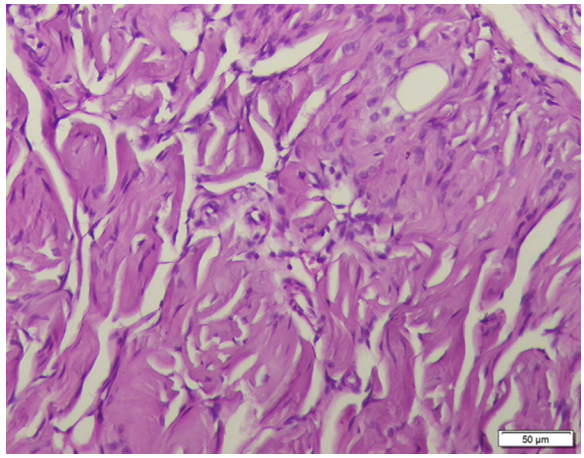

Figure 6. Histopathologic rat cavernosal tissue biopsy specimen from the control group. Figure 6 is available in color at www.smr.jsexmed.org.

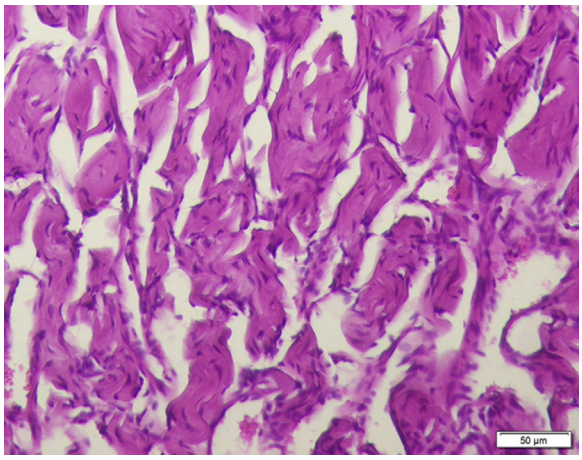

Figure 7. Histopathologic rat cavernosal tissue biopsy specimen from the treatment group. Figure 7 is available in color at www.smr.jsexmed.org.

groups underwent cavernosal nerve stimulation and the intracavernosal pressure was measured. They found a significant increase in intracavernosal pressure in the treatment vs control group. Similar to the findings of the first animal study, when they looked at the histologic specimens, they also found a larger resting sinusoidal diameter in the intervention vs control group.[8]

SUGGESTED MECHANISM OF BONT-A IN THE TREATMENT OF ED

Failure of adequate smooth muscle relaxation causing veno-occlusive dysfunction is the proposed pathophysiology in PDE5i and ICI non-responders (Figure 8).[46] Any therapy that can increase cavernosal smooth muscle relaxation might be able to turn PDE5I and ICI non-responders into responders.

The two animal studies found an increased resting unstimulated sinusoidal diameter in the treatment groups, which suggests that BoNT-A caused cavernosal smooth muscle relaxation by inhibition of the release of noradrenaline from the adrenergic neurons acting on the cavernosal smooth muscle. Thus, the dominant sympathetic basal tone of the cavernosal smooth muscle is essentially removed, facilitating the occurrence of an erection at stimulation, which then would be dependent on the nitric oxide produced from the non-adrenergic non-cholinergic neurons because the cholinergic neurons also would be inhibited by BoNT-A. Furthermore, decreasing smooth muscle tone would lead to an increase in penile blood flow, which was demonstrated by the human study. The human study also demonstrated that BoNT-A therapy can convert PDE5I and ICI non-responders to PDE5I responders, thus decreasing the number of patients requiring penile implant surgery.[3–6]

Safety Concerns

Several safety concerns need to be addressed: Is ICI of BoNT safe? Would injection of BoNT-A induce prolonged smooth muscle relaxation resulting in priapism? What about systemic side effects of the toxin?

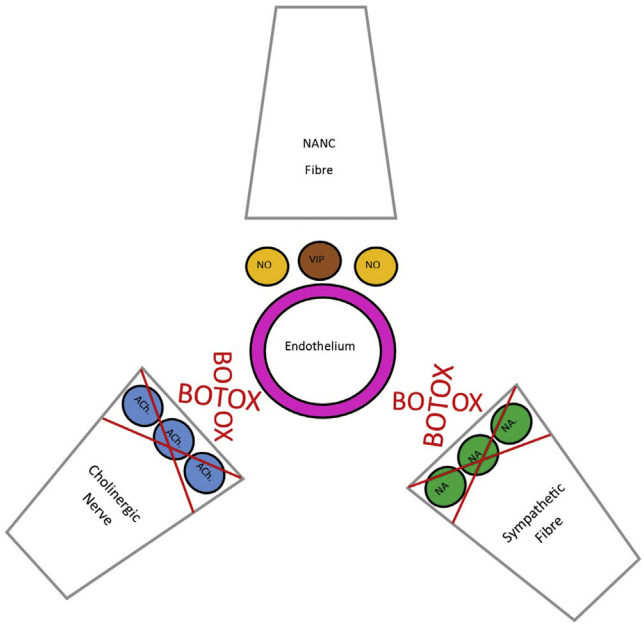

Figure 8. Proposed mechanism of action of botulinum neurotoxin type A on the cavernosal smooth muscle. NANC = non-adrenergic non-cholinergic; NO = nitric oxide; VIP = vasoactive intestinal peptide. Figure 8 is available in color at www.smr.jsexmed.org.

To address safety concerns, the initial pilot study included only men who were candidates for penile prosthesis surgery, because all other lines of therapy were not effective. This study aimed at decreasing the risk of prolonged erection or priapism and avoiding the possibility of prosthesis placement in a patient who could have been treated through other non-surgical measures. Because of the safety concerns, BoNT-A 50 U was chosen in the human study, which is low compared with that used for other indications, such as detrusor muscle hyperactivity. In the animal histologic studies, 1 and 2 U were used, which is equivalent to 100 and 200 U in humans. No local or systemic adverse events were noted during the human and animal studies apart from one case of prolonged erection after ICI.[3–8] Priapism did not occur in any of the study subjects from the BoNT-A injection or the use of sildenafil. Based on these findings, a higher dose of 100 U is being used in the phase II human study, which is currently being conducted by the authors, because it could lead to a better response.

Efficacy Concerns

Concerns regarding efficacy were that BoNT-A is not a vasodilator drug, so would cavernous smooth muscle relaxation occur and become effective clinically and histologically, thus enhancing erections?

Efficacy was shown by the improved vascular parameters and SHIM scores in the human study and the cavernosal sinusoidal dilatation in rats in the two animal studies.[3–8]

LIMITATIONS AND RECOMMENDATION FOR FUTURE RESEARCH

The findings of the studies presented in this article suggest a possible role for BoNT-A in the treatment of ED; however, they are limited by the small samples. Furthermore, the long-term efficacy was not assessed because of the limited duration of the follow-up period. Further highly powered, double-blinded RCTs with longer follow-up periods are needed to assess the true therapeutic efficacy, optimal dose, and duration of action in various ED patient populations. In addition, the precise physiologic mechanism of action and histopathologic changes can be further explored by conducting more animal and laboratory studies.

CONCLUSION

BoNT-A as a potential therapy in vasculogenic ED appears promising. In addition to the findings of the three pilot studies discussed earlier, double-blinded RCTs need to be conducted to further explore the true therapeutic efficacy and clinical safety of BoNT-A in the treatment of ED. Ideally, these studies should be highly powered using larger samples from diverse patient populations across multiple clinical centers.

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