

Gastrointestinal Smooth Muscles and Sphincters Spasms: Treatment with Botulinum Neurotoxin

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Abstract: More than fifty years following the discovery that botulinum neurotoxins inhibit neuromuscular transmission, these powerful poisons have become drugs with many indications. First used to treat strabismus, local injections of botulinum neurotoxin are now considered a safe and efficacious treatment for neurological and non-neurological conditions. One of the most recent achievements in the field is the observation that botulinum neurotoxin is a treatment for diseases of the gastrointestinal tract. Botulinum neurotoxin is not only potent in blocking skeletal neuromuscular transmission, but also block cholinergic nerve endings in the autonomic nervous system. The capability to inhibit contraction of smooth muscles of the gastrointestinal tract was first suggested based on *in vitro* observations and later demonstrated *in vivo*; it has also been shown that botulinum neurotoxin does not block non adrenergic non cholinergic responses mediated by nitric oxide. This has further promoted the interest to use botulinum neurotoxin as a treatment for overactive smooth muscles and sphincters, such as the lower esophageal sphincter to treat esophageal achalasia, or the internal anal sphincter to treat anal fissure.

Information on the anatomical and functional organization of innervation of the gastrointestinal tract is a prerequisite to understand many features of botulinum neurotoxin action on the gut and the effects of injections placed into specific sphincters. This review presents current data on the use of botulinum neurotoxin to treat diseases of the gastrointestinal tract and summarizes recent knowledge on the pathogenesis of disorders of the gut due to a dysfunction of the enteric nervous system.

Keywords: Anus; pathology; physiopathology; surgery; Autonomic Nervous System Diseases; Botulinum Toxin; pharmacology; therapeutic use; Enteric Nervous System; Esophageal Achalasia; drug therapy; Esophageal Diseases; Exocytosis; Fissure-in-ano; therapy; Gastric Emptying; Gastrointestinal Diseases; Gastrointestinal Motility; Membrane Fusion; physiology; Membrane Proteins; chemistry; Neuromuscular Agents; Spasm.

INTRODUCTION

Following the discovery that botulinum neurotoxin (BTX) inhibits neuromuscular transmission, this powerful poison has been under clinical investigation since the late 1970s and it has become drug with many indications [1,2]. Beginning with the pioneering work of Dr Alan Scott in the treatment of strabismus [3], the range of clinical applications has grown to encompass several neurological and non-neurological conditions. Over the years, the number of primary clinical publications has grown exponentially, and continues to increase every year. One of the most recent achievements in the field is the observation that type A BTX is a treatment for diseases of the gastrointestinal tract (GIT). BTX-A is not only potent in blocking skeletal neuromuscular transmission, but also block cholinergic

nerve endings in the autonomic nervous system [4-6]. The capability to inhibit contraction of GIT smooth muscles was first suggested based on *in vitro* observations and later demonstrated *in vivo*; it has also been shown that BTX-A does not block non adrenergic non cholinergic responses

Table 1. Target Site for the Injection of BTX-A in Different Conditions

Condition	Muscle
Achalasia	LES
Cricopharyngeal dysphagia	Cricopharyngeal muscle
Diffuse esophageal spasm	LES
Isolated hypertensive LES	LES
Delayed gastric emptying	Pylorus
Infantile hypertrophic pyloric stenosis	Pylorus
Sphincter of Oddi dysfunction	Sphincter of Oddi
Chronic anal fissure	IAS or EAS
Outlet-type constipation	Puborectalis muscle or EAS
Anterior rectocele	Puborectalis muscle and EAS

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mediated by nitric oxide (NO) [7]. This has further promoted the interest to use BTX-A as a treatment for overactive smooth muscles and sphincters (Table 1), such as the lower esophageal sphincter (LES), to treat achalasia, or the internal anal sphincter (IAS), to treat anal fissure.

Information on the anatomical and functional organization of GIT innervation is a prerequisite to understand many features of BTX-A action on the GIT and the effects of injections placed into specific sphincters. This review presents current data on the use of BTX-A to treat GIT diseases and summarizes recent knowledge on the pathogenesis of GIT disorders due to a dysfunction of the enteric nervous system (ENS).

NEUROMYOGENIC PROPERTIES OF THE GIT SMOOTH MUSCLES

Motility of a gut segment depends on its extrinsic and intrinsic innervation [8,9]. Preganglionic parasympathetic nerves and postganglionic sympathetic nerves, which constitute the autonomic nervous system, provide extrinsic innervation; the ENS provides the intrinsic innervation [9]. It is a highly complex system, responsible for the coordination of motility, secretion, and microcirculation in the GIT and for the regulation of the local response to immune inflammatory processes. The ENS can function independently of the central nervous system (CNS), which nevertheless maintains a coordinating role for diverse functions of GIT neurons via sympathetic and parasympathetic motor and sensory pathways [9,10]. A

deficiency of enteric neurons causes obstruction and lack of intestinal propulsion.

FUNCTIONAL ORGANIZATION OF THE ENS

The ENS is composed of nerve cell bodies and their processes embedded in the gut wall, which form two main ganglionated plexuses: Auerbach's myenteric plexus and Meissner's sub-mucous plexus. Other, non-ganglionated, plexuses, such as the longitudinal muscle plexus, the circular muscle plexus, the plexus of the muscularis mucosae, and the mucosal plexus, supply GIT layers "Fig. (1)". In addition, perivascular plexuses are found around arteries and arterioles in the gut wall [9].

In the ENS, intraparietal neurons encompass motor neurons (excitatory and inhibitory), interneurons and intrinsic sensory neurons. Sympathetic and parasympathetic neurons also innervate the GIT. Excitatory motor neurons innervate longitudinal and circular muscles and the muscularis mucosae. Their primary transmitter is acetylcholine (ACh), but they also release tachykinins, substance P, neurokinin A, neuropeptide K and neuropeptide Y. Inhibitory motor neurons relax smooth muscles and are involved in reflexes that facilitate the passage of food along the GIT. Inhibitory neurons release a combination of at least three transmitters: NO, adenosine triphosphate (ATP), and vasoactive intestinal polypeptide (VIP). In most neurons, NO is the primary transmitter, while the roles played by VIP and ATP may vary. A neuronal NO synthase is expressed in ENS neurons where it is usually co-localized with VIP.

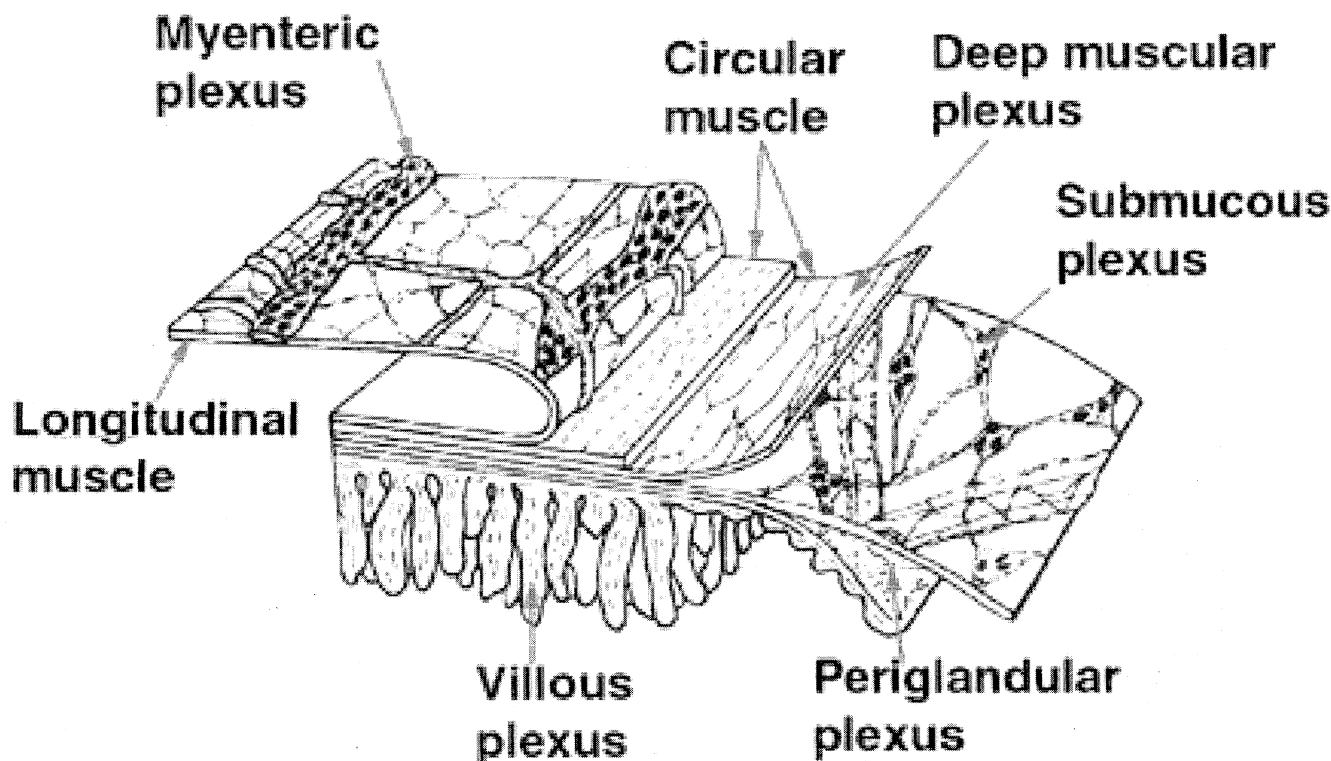


Fig. (1). Organization of neurons located in the enteric nervous system.

During nerve stimulation, NO regulates VIP release and diffuses to muscle cells to participate in relaxation. In turn, VIP acts on smooth muscle cells to regenerate NO, which constitutes the predominant component (up to 60%) of NO produced during nerve stimulation [10].

Sympathetic pathways to the GIT are noradrenergic; they inhibit motility, constrict the sphincters and in general inhibit contractile activity, by means of a presynaptic action on the myenteric plexus. The vagus nerve includes the axons of neurons located in the brain stem. A variety of central effects, including relaxation of the proximal stomach, enhancement of gastric peristalsis, secretion of acid, and promotion of gastrin secretion, are mediated by the vagus nerve [9,11]. Vagal neurons form synaptic connections with neurons, whose cell bodies are in the intrinsic ganglia of the GIT. They are involved in complex circuits integrating various enteric reflexes with signals that derive from the CNS and from other GIT regions; ACh principally mediates this transmission [9].

Smooth Muscle Contraction

This function is regulated by changes in cytosol calcium levels [12]. Calcium regulation is affected by a variety of regulatory proteins, including myosin light chains, calmodulin, and calponin. Contraction is dependent on an increase in cellular calcium. The activation of myosin may induce relaxation or allow cross bridges to enter a state of prolonged contraction, the so-called "latch state" [13]. The precise mechanisms responsible for the maintenance of smooth muscle tone are still not entirely known. Relaxation of smooth muscles occurs when there is a resulting decrease in cytosolic calcium. These functions depend on the intrinsic electrical and mechanical properties of GIT smooth muscles and are regulated by the ENS and by sympathetic and parasympathetic influences. An increase in cellular calcium can be produced by the influx of calcium through membrane channels or by stimulation of $\alpha 1$ adrenoceptors, resulting in a release of calcium from the sarcoplasmic reticulum mediated by inositol triphosphate [12,13]. Stimulation of $\beta 2$ adrenoceptors brings about cAMP-mediated return of calcium to the sarcoplasmic reticulum. Stimulation of NO induces cGMP-mediated decrease in cellular calcium.

Hormones and autoacids also regulate muscle activity and influence GIT motility [9,10]. Interstitial cells of Cajal act as local pacemakers to generate the rhythmic activity of the circular muscle layer throughout the GIT. Motor neurons control the musculature indirectly, through their action on Cajal's cells that are innervated by the myenteric plexus in the stomach and in the small intestine and by the submuscular plexus in the colon [9]. Cajal's cells are lost in Hirschsprung's disease and in infantile pyloric stenosis [14,15]; their deficiency contributes to the motor abnormalities associated with such diseases.

As a rule, hormonal influences on smooth muscle activity occur during meals and in between. Chemical substances, such as histamine, serotonin, adenosine, and eicosanoids, produced by non-neural cells in the muscle layers, can influence smooth muscle activity. Receptors for

most of these agents have been identified on GIT smooth muscle cells [9]. They can act directly on muscle cells and indirectly by stimulating or inhibiting the release of neurotransmitters.

Sphincteric Modulation in the GIT

Muscle tone of the LES results from the interaction of neurogenic and myogenic conditions [10,16]. Neurogenic tone in humans is partly due to cholinergic innervation; various other excitatory and inhibitory neurotransmitters are present in the sphincter, but their physiologic importance is unclear. Myogenic tone is mediated by shifts of intracellular stores of calcium in the sphincter muscle. The modulation of LES tone that occurs with activity of migrating motor complex (MMC) is largely mediated through the vagus nerve [10]. Relaxation of the sphincter induced by swallowing is mediated through the dorsal nucleus of the vagus. Efferent stimuli travel to the sphincter through the vagus nerve and the myenteric plexus. ACh is the presynaptic neurotransmitter; postsynaptic transmission is mediated by NO, but VIP is also thought to contribute [9,16,17].

At the anal level, the sphincter complex consists of two overlapping sphincters [1,2,12,13,18]. The external anal sphincter (EAS), that forms the outer layer, is composed of a voluntary, striated, skeletal muscle. The IAS is the inner, involuntary, smooth muscle component of the anal sphincter complex. Being in a state of continuous maximal contraction, the IAS is a natural barrier to the involuntary loss of stool and gas. This is due to a combination of intrinsic myogenic and extrinsic autonomic neurogenic properties [9]. The IAS is responsible for 50-85% of resting anal tone [17]. Being of visceral origin, it is supplied both by sympathetic and parasympathetic nerves; in addition, the ENS modulates its tonic activity [9,17]. Noradrenergic sympathetic nerves are considered excitatory and the parasympathetic inhibitory to the IAS. Sympathetic neurons that supply the IAS are noradrenergic. Vagal neurons do not act directly, but rather form synaptic connections with neurons whose cell bodies are in the intrinsic GIT ganglia. This transmission is principally mediated by ACh acting on nicotinic receptors [9].

Recently, it has been shown that the longitudinal layer and the circular smooth muscle in the human rectum receive an intrinsic NO-mediated inhibitory innervation [19-21]. The exact site of involvement remains to be determined, but recent data suggest that carbon monoxide and heme oxygenase pathway may have a role in neurally mediated relaxation of the IAS [22,23].

In vitro studies on strips of human IAS have revealed excitatory α -adrenergic receptors, inhibitory β -adrenergic receptors, excitatory or biphasic cholinergic receptors, and inhibitory non-adrenergic non-cholinergic receptors [9,12,13,17,24]. Experimental evidence suggests that norepinephrine induces contraction of isolated IAS strips, while adrenaline has a variable response and isoprenaline induces relaxation [25]. Furthermore, blockade of $\alpha 1$ -adrenoceptor by a single oral dose of indoramin causes a

lasting reduction in resting pressure along the length of the anal canal either in patients with fissure or in healthy subjects [26].

BOTULINUM NEUROTOXINS

Chemistry and Pharmacology

The clostridial neurotoxin family is composed of tetanus neurotoxin and 7 serotypes of BTX, designed as serotypes A-G [27,28]. BTX produced under anaerobic conditions by *Clostridium botulinum* exert their paralytic effects at the neuromuscular junction by inhibiting the ACh release [29,30]. Although the 7 BTX are antigenically distinct, they are released by autolysis as a prototoxin, have a similar structure and molecular weight, consisting of a heavy (H) chain and a light (L) chain joined by a disulfide bond [30-35]. Regions of sequence homology among 7 serotypes, and between BTX and tetanus toxin, suggest that all employ similar mechanisms of action [32-34].

When BTX-A is isolated from bacterial cultures, it is normally associated with nontoxic macromolecules, such as proteins or nucleic acids; for instance, BTX-A is associated noncovalently with hemagglutinin. When toxin is administered orally, these nontoxic proteins may enhance toxicity, compared to toxin without the proteins, possibly by protecting BTX-A from proteolytic enzymes in GIT. However, when administered parenterally, as in therapeutic uses, these proteins do not enhance BTX-A toxicity and may even interfere slightly. Nevertheless, they may enhance stability and retard diffusion, leading greater therapeutic control.

BTX-A is synthesized as single chain polypeptides with a molecular mass of approximately 150 kDa (1296 aminoacids) [30,36,37]. In this form, the toxin molecules have relatively little potency as neuromuscular blocking agents. The BTX-A toxicity is a result of a multi-step mechanism [36,38,39]. The toxin is post-translationally proteolyzed to form a 1285 aminoacids di-chain molecule, in which the two chains remain linked by a disulfide bond [30]. The result is a H-chain (approximately 100 kDa) tethered by a disulfide bond to a L-chain (approximately 50 kDa), that is associated with one atom of zinc. The protein is composed of three functional domains where 1) the catalytic function is confined to the L-chain (residues 1-437), that acts as a zinc endopeptidase, with proteolytic activity concentrated at the N-terminal end, 2) the translocation activity with the N-terminal half of the H-chain (residues 448-872), and 3) receptor binding with its C-terminal half (residues 873-1295) [30].

For most forms of BTX, including type A, this cleavage is accomplished by the bacterium itself, before the toxin is released. It is in this form that BTX-A enters the axon terminal. The second activating step, disulfide reduction, occurs only after internalization by the target cell.

Mechanism of Action

BTX-A binds to the presynaptic nerve endings of cholinergic neurons and enters by receptor-mediated

endocytosis. Acidity in the endosome is believed to induce pore formation, which allows translocation of the catalytic domain into the cytosol. The zinc-dependent catalytic domain of the 7 serotypes specifically cleaves one of different proteins essential for synaptic vesicle fusion [27,40]. This cleavage results in inhibition of ACh secretion, ultimately leading to muscle relaxation. There are three steps involved in BTX-A mediated paralysis: A. internalization; B. disulfide reduction and translocation; C. inhibition of neurotransmitter release.

BTX-A must enter the nerve ending to exert its effect. Binding of toxin to both peripheral and central nerves is selective and saturable. Susceptibility of cholinergic and non-cholinergic neurons to specific BTX-A may be related to their ability to bind to receptors on presynaptic terminals [31-35]. Pharmacologic and morphologic data suggest that internalization is via a receptor-mediated endocytotic/lysosomal vesicle pathway. The process is independent of Ca^{2+} concentration, is partially dependent on nerve stimulation, and is energy dependent [41]. In experimental models, internalization is hastened in an acid medium, and slowed by cooling. Binding to receptors on the unmyelinated presynaptic membrane is mediated by the C-terminal of H-chain [42]. Recently, it has documented that the binding domain appears as two distinct sub-domains roughly equal in size. The entire binding domain tilts away from the long helical axis of the translocation domain such that there is no contact between the C-terminal sub-domain and the translocation domain and all of the surface loops are accessible for binding [30].

The interaction between BTX-A and the presynaptic nerve endings is proposed to occur through both a polysialoganglioside, so-called GD_{1b} or GT_{1b} , and a yet unidentified protein receptor [43]. These could represent a general binding site for all the clostridial neurotoxins.

Following cell surface binding and receptor-mediated endocytosis of BTX-A, an acid-induced conformational change in the toxin's translocation domain is believed to allow the translocation domain to penetrate the endosome and form a pore. The membrane interaction and pore formation is thought to facilitate the passage of the catalytic domain across the membrane into the cytosol. The pore-forming segment of type A BTX-A has been identified: the candidate segment (residues 659-681) was shown to increase permeability of lipid bilayers [30,43-46]. After internalization, either within the acidic endosome or upon exposure to the cytosol following translocation, the disulfide bond connecting the catalytic and translocation domains (Cys 429 – Cys 453) is reduced, and the catalytic domain is released into the cytoplasm [30]. If the disulfide bond that links the two chains is broken before BTX-A is internalized by the target cell, the L-chain cannot enter, and there is a virtually complete loss of toxicity.

Like tetanus neurotoxin, BTX-A is a zinc (Zn^{2+}) endopeptidase. The final step in toxicity is the cleavage of a presynaptic protein by the zinc protease catalytic domain, which is located in the N-terminal of the L-chain. The binding of the zinc to the L-chain is reversible; aminoacids with side chains closest to the zinc include His 222, Glu

223, His 226, Glu 261 and Tyr 365 [38,40]. The observed orientation of these residues support a model in which the His 222, His 226 and Glu 261 directly coordinate the zinc, and Glu 223 coordinates a water molecule as the fourth ligand. The Tyr 365 is more likely to be involved in secondary interaction with substrate [30].

At motor nerve endings, action potentials cause depolarization, that activates voltage-gated Ca^{2+} channels. Ca^{2+} entry initiates interactions between secretion-related proteins: these protein interactions result in the ACh release from 10 to 200 vesicles (each vesicle contains 5,000 to 20,000 neurotransmitter molecules). ACh release is inhibited when the endopeptidase cleaves one or more neuronal proteins of the vesicle transport pathway. The ATP-dependent translocation of an intact vesicle from the cytosol to the plasma membrane requires a suite of specific proteins. Cleavage of any of these proteins interferes with the proper binding and fusion of a vesicle to the plasma membrane, and therefore impedes exocytosis-mediated neurotransmitter release.

In every cell, membranes move and fuse [47]. Trafficking pathways shuttle vesicles to and from the plasma membrane, within the endoplasmic reticulum, Golgi apparatus, endosomal compartments, and between these and several other organelles. All of these trafficking events involve membrane fusion, and a central role in these events is attributed to a protein complex known as the core complex. Vesicle docking on the inner membrane surface involves the formation of a complex including cytoplasmatic proteins [γ -synaptosomal-associated protein (SNAP), α -SNAP, SNAP-25, N-ethylmaleimide-sensitive factor (NSF)], vesicle proteins [vesicle-associated membrane protein (VAMP), also called synaptobrevin] and proteins of the target membrane (syntaxin) [48-52]. Synaptobrevin, SNAP-25 and syntaxin forms a 1:1:1 stoichiometric complex. Interaction between vesicle-associated SNAREs [(v-SNARE), soluble NSF (N-ethylmaleimide-sensitive factor) attachment receptor] and target-associated SNAREs (t-SNARE) forms a high affinity complex.

Activated by α -SNAP/NSF, SNARE complex formation draws vesicles toward target membrane and leads to vesicle fusion with cell membrane. Interactions occur in fast-releasing synapses, at the active zone, near voltage-dependant Ca^{2+} channels [53]. Furthermore, it has been noted that syntaxin and synaptobrevin are aligned in parallel, with their transmembrane domains (C-terminal) at one end of the particle and the N-terminal at the other end of the particle, and that v-SNARE participates in synaptic vesicle formation mediated by the AP3 adaptor complex.

A vesicle first contacts the target membrane in a reaction called docking. This represents a recognition event that probably determines the specificity of fusion, but little is known about its mechanism. After docking, a multi-step reaction leads to complete integration of the vesicle membrane into the target membrane. Priming and fusion depend on proteins of the vesicle and target membranes that interact with each other to form the core complex and that are referred to as SNAREs. In presynaptic terminals, the vesicular SNARE is synaptobrevin/VAMP while the target

membrane SNAREs are syntaxin and a synaptosomal associated protein of 25 kDa (SNAP-25). SNAREs and the core complex are linked to exocytosis, and synaptobrevin; syntaxin and SNAP-25 are substrates for tetanus neurotoxin or BTX-A [50]. These toxins completely block exocytosis without interfering with vesicle docking. A vesicle is thought to dock without participation of SNAREs. After docking, synaptobrevin, syntaxin and SNAP-25 form the core complex during priming and fusion. Before core complex formation, syntaxin is largely complexed to munc18-1, a synaptic *sec1*-homolog that performs an essential but unknown role in fusion [54].

Two alternative models of the function of the core complex formation in priming and fusion have been proposed [50,51]. The first hypothesis is based on the high stability of the core complex and predicts that complex formation itself drives membrane fusion. The second hypothesis for the function of the core complex postulates that formation of the core complex primes the membranes for fusion but is not directly responsible for the actual fusion reaction. In either case, core complex formation and fusion are inhibited by tetanus or BTX-A action.

Recently, based on physical properties, as well as genetic and biochemical evidence, Misura and coworkers [55-58] propose that *sec1*/syntaxin1 complex communicates with Rab, a G protein on the vesicle membrane, and Rab-E to recognize and guide the neurotransmitter-filled sac to its appointed fusion site on the target membrane. They further propose that syntaxin1a and *Sec1* collaborate with VAMP and SNAP-25 to bring about fusion itself. In this model, n*Sec1* performs a chaperone-like role for syntaxin1a, first shielding it from other proteins, and later facilitating its interactions with them [59].

BTX are now known to target some of these proteins of the core complex "Fig. (2)". Intracellular substrate for both type B BTX and tetanus neurotoxin is the vesicle protein VAMP/synaptobrevin 2. Type D and type F BTX also cleave VAMP/synaptobrevin 2, but in a different site than B serotype. Type A and type E cleave another translocation protein, SNAP-25, and C serotype acts by cleaving syntaxin [60-63].

After synaptic transmission is blocked by BTX-A, the muscles become clinically weak and atrophic. The affected nerve terminals do not degenerate, but the blockage of neurotransmitter release reverses when the toxin effect wanes and the vesicle cycling process starts again. Function can be recovered by the sprouting of nerve terminals and formation of new synaptic contacts; this usually takes two to three months.

Preparations of BTX-A

Serotype A is widely commercially available. Two preparations are available for clinical use: the European preparation (trade name Dysport®, manufactured by Speywood Biopharm Ltd, UK, and distributed by Ipsen Spa, Italy) and the US preparation (trade name Botox®, manufactured and distributed by Allergan, USA).

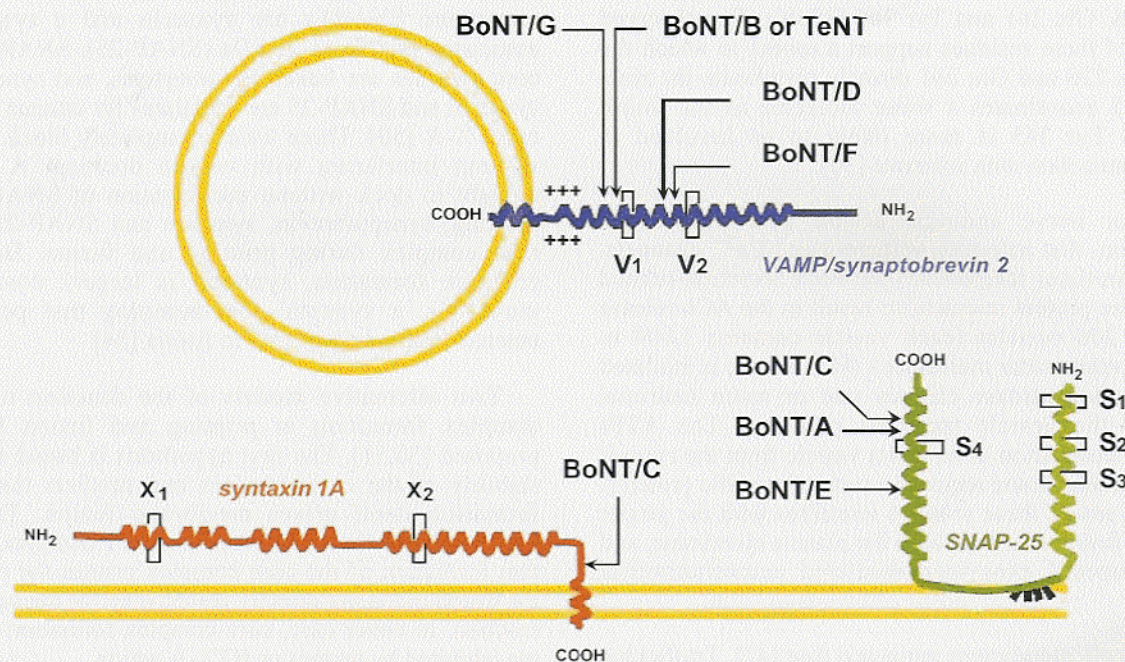


Fig. (2). Sites of action of botulinum neurotoxins.

Unfortunately, there has been much confusion over the doses and units of potency of the two preparations. Although doses are quoted in mouse units (which is the amount of toxin that kills 50% of a group of 18-20 g female Swiss-Webster mice), implying some standardisation, Botox® seems to be more potent. It is suspected that one Botox® unit is approximately equivalent to 3-5 Dysport® units. Furthermore, it has suggested a Dysport®/Botox® equivalency ratio of approximately 3:1 or 5:1 at standard vial dilutions. A vial of Botox® contains 100 units, while a vial of Dysport® contains 500 units.

The use of BTX serotypes B was studied recently, and is available commercially (MyoBloc® in the USA, NeuroBloc® in Europe, manufactured by Elan Pharma, Ltd). Use of BTX type F also is under investigation in patients who have become immunologically resistant to serotypes A and B.

Practical Aspects of BTX-A Treatment

BTX-A has to be injected into affected muscles or glands. Doses have to be tailored according to the mode of use and individual patients. Generally, the effective dose depends on the mass of muscle being injected: the larger the muscle the higher the dose required. However, susceptibility to BTX-A varies. Overactive muscles are identified by muscular hypertrophy, stiffness, tenderness, and visible abnormal muscular activity. In addition, clinical observation of abnormal movements or postures may help identify an overactive muscle. Electromyography can also be useful.

The weakness induced by injection with BTX-A usually lasts about three months [60]. Patients will then need further injections at regular intervals, although the interval varies widely depending on the dose and individual susceptibility.

Response after the injections should be assessed both by using patient reported benefit in terms of pain relief and improvement of disability, and by objective measures on clinical examination.

Most patients treated with BTX-A require repeated injections over many years. Some patients who respond well initially develop tolerance to the injections. As indicated by Borodic and coworkers [64], sensitization is a major cause of decreased or absent clinical response after repeated injections. This can be caused by the development of neutralising antibodies to BTX-A. To minimize immunoresistance, it has been recommended to use the smallest possible effective dose, to extend the interval between treatment as long as possible, with at least 3 months between treatments, and to avoid using booster injections.

Cautions and Contraindications

BTX-A injections are generally well tolerated. After injection, BTX-A diffuses into the muscles and other tissues. Its effect diminishes with increasing distance from the injection site, but spread to nearby muscles is possible, particularly when high volumes are injected. Distant effects shown by electromyographic tests can also occur, but weakness of distant muscles or generalised weakness, possibly due to BTX-A spreading in the blood, is very rare.

However, BTX-A should be used with caution in patients with disturbed neuromuscular transmission, such as myasthenia gravis or Lambert-Eaton myasthenic syndrome, or during treatment with aminoglycosides. BTX-A is contraindicated in pregnancy and while breast feeding. Other systemic side effects include an influenza-like illness and, rarely, brachial plexopathy, which may be immune mediated. No severe allergic reactions have been reported.

Other reports have documented detectable abnormalities in some cardiovascular reflexes [65,66]. These abnormalities do not appear to have any clinical significance, and it is not known how long they persist. Controversy exist regarding safety of BTX-A treatment. Effects are seen at autonomic ganglia where it block autonomic transmission acutely, producing autonomic symptoms. A study of four patients with botulism found an impairment of heart rate and blood-pressure responsivity as well as neuromuscular transmission. The sympathetic tests normalized earlier than the parasympathetic tests on follow-up. Systemic side effects such as skin and allergic reactions, increased residual urine volume, muscular weakness, abnormalities on electromyography of distant muscles, and postural hypotension have been reported, and BTX-A diffusion is thought to be responsible for the occurrence of a heart block following treatment for esophageal achalasia in a patient with severe cardiac disease.

To evaluate safety of this treatment, we have been studied six patients, from 22 to 62 years old, without detectable cardiovascular or autonomic diseases, who underwent treatment with 150 units (0.75 ml) of BTX-A (Dysport, Ipsen SpA, Italy) for chronic anal fissure. We conducted the Ewing protocol in basal condition (before BTX-A treatment) and repeated the tests within 96 hours and within 30 days after treatment. The Ewing protocol, including measurement of heart rate changes during deep breathing, Valsalva maneuver, and during standing up; blood-pressure measurement during handgrip and during standing up, has been developed to assess the autonomic control of cardiovascular system and to diagnose autonomic neuropathy. Three of these tests (responses to the Valsalva maneuver, to deep breathing and to standing up, based on measurement of heart rate changes) are an index of parasympathetic activity; the other two are index of sympathetic activity. To classified the severity of the effect on the ANS, a score (0 = normal response; 1 = borderline; 2 = abnormal) is given to each test; an ending score can change from 0/10-1/10 (normal pattern), to 2/10-4/10 (borderline pattern), to 5/10-10/10 (abnormal pattern). Anorectal manometry was performed before and one month after Dysport treatment.

No patient had worsening of test scores after BTX-A injections. In particular, before treatment a borderline pattern (2/10 score) was found in 4 patients. At 96-hour evaluation, a borderline pattern (2/10 score) was found in 1 patient; at 30 days evaluation, all patients who had previously an abnormal score no longer had such score, and a normal pattern (0/10) was found in all treated patients. At 30 days evaluation, the fissure in all six patients had healed, and resting anal tone was reduced from 126 ± 23 mm Hg to 82 ± 7 mm Hg ($P = 0.001$), while maximum voluntary contraction was not significantly changed (from 86 ± 23 mm Hg at baseline to 85 ± 8 mm Hg at one month).

CRICOPHARYNGEAL DYSPHAGIA

The cricopharyngeal muscle, or upper esophageal sphincter (UES), constitutes a sphincter separating the hypopharynx from the esophagus. It is activated during

swallowing to prevent the inlet of air into the esophagus and esophageal reflux to the pharynx during inspiration. Cricopharyngeal dysphagia derives from a dysfunction of the cricopharyngeal muscle [9], which can be primary or secondary to several conditions, such as cerebrovascular accidents, amyotrophic lateral sclerosis, oculopharyngeal muscular dystrophy, lesions of the skull base, and postoperative (laryngectomy). Proximal dysphagia is the typical clinical presentation and pharyngeal (Zenker's) diverticula are possible aftermaths.

Increased function of the cricopharyngeal muscle has been traditionally treated with myotomy, mechanical dilatation, or neurectomy of the oropharyngeal plexus. Localized BTX-A injections have also been successfully used [67,68]. The outcome of swallowing disorders due to UES spasticity, hypertonus, or delayed relaxation has been documented with cinematic radiograms and manometry in 7 patients. The dose ranged between 80 and 120 Botox units per patient and was decided based on the severity of symptoms. After having identified the UES by direct esophagoscopy and under EMG guidance, the injections were performed into the dorsomedial part and on either side in the ventrolateral part of the muscle. The procedure required general anesthesia and admittance to hospital for 2 days on average. All patients, but two, experienced a marked improvement or a complete relief of symptoms that lasted up to 5 months. BTX-A injection (16 Botox units) provided marked functional recovery in an 86-year-old man with multifocal dystonia and severe dysphagia, who had persistent tonic UES activity. BTX-A injections were used to reduce pharyngoesophageal spasms in 5 patients after laryngectomy. The injection site was identified using EMG under fluoroscopic control; swallowing or voice function improved in all patients, but one.

The treatment of cricopharyngeal dysphagia has been performed in non-anaesthetized outpatients using CT-guided injections [69]. Appropriate intramuscular placement could not be achieved in one case, but in the remaining cases the dose of BTX-A was progressively increased until a good control of symptoms occurred. A dose as low as 5 Botox units produced onset of benefit after about 10 days, lasting for approximately 2 weeks; it was proposed to identify patients who can benefit from BTX-A treatment by starting with a low test dose.

A simplified out-patient percutaneous technique with electromyography guidance has also been developed to inject the UES [70-72]. The muscle is reached after manual rotation of the larynx by inserting an electromyography recording needle through the skin and advancing it behind the thyroid lamina until muscle motor unit potentials are recorded. Two injections of 2.5 Botox units were administered in each side to 6 patients; in one case with unsatisfactory response endoscopic injection under general anesthesia had to be performed. The best results were obtained in the patients with pure cricopharyngeal spasm and Zenker's diverticula.

Local injections of BTX-A into the UES are a relatively simple, safe and effective means of improving dysphagia. They can be used during diagnostic evaluation to ascertain the role played by cricopharyngeal spasm in determining the

patient's clinical picture; in addition, they help to sift out those patients who are more likely to benefit from myotomy. If BTX-A fails to improve the symptoms despite evidence of cricopharyngeal muscle weakening, myotomy will be unlikely to provide benefit. If BTX-A improves symptoms, the patient could either be treated with repeated injections or undergoes myotomy.

It is worth recalling that secondary oropharyngeal dysphagia can occur in 14-50% of patients, who receive BTX-A injections for cervical or oromandibular dystonia. Women, patients with a thin neck, or those who receive large doses into the sternocleidomastoid muscles are more likely to suffer from dysphagia. The symptoms are transient, but sometimes they are severe enough to require a liquid diet. It has not been tested whether BTX-A injections into the cricopharyngeal muscle can accelerate recovery in such cases.

ACHALASIA

Achalasia is a failure to relax the LES that remains tonically contracted, resulting in a functional obstruction of the esophagus [9,16]. The lower two thirds of the esophagus containing smooth musculature is affected, and no peristaltic contractions take place "Fig. (3)".

The cause may be a generalized loss of enteric neurons or a selective loss, or dysfunction, of the inhibitory VIP- and NO-containing neurons located in esophageal myenteric plexus. NO synthase is undetectable in the myenteric plexus and in the nerve fibers reaching the esophagus, the cardias and the gastric fundus [16,73]. Esophageal ganglion cells and neurons of the dorsal motor nucleus of the vagus are decreased in number, and the vagus nerves degenerate. The etiology is unknown. NO-containing neurons are primarily involved, while cholinergic neurons may be spared [74,75].

The preserved cholinergic innervation to LES induces increased sphincter pressure that is a characteristic feature of this disorder. It has been postulated that an autoimmune reaction may cause achalasia: two thirds of patients have autoantibodies against DARPP-32, a dopamine carrier protein located on the cell surface in the myenteric plexus [76]. The degenerative neural lesions of achalasia cannot be corrected by the treatment, which is therefore symptomatic. Peristalsis rarely recovers and treatment is aimed at reducing LES pressure by drug therapy, by forceful dilatation, or by surgical myotomy.

Some diseases may cause ENS dysfunction resulting in secondary achalasia; they include paraneoplastic syndromes, Chagas' disease, and Parkinson's disease (PD). Herpes infections have been reported with increased frequency in the myenteric plexus of patients with achalasia; this virus has a preferred tropism for the squamous epithelium lining the esophagus.

Familial achalasia occurs in a number of genetic disorders, and clinical severity may vary among family members. A positive association with the HLA allele DQw1 has been reported in several populations; the allele DRw53 is also reduced among Caucasians [77,78].

Treatment

Classic therapies were aimed to mechanically reduce LES tone by means of pneumatic dilation or surgical myotomy. These procedures are efficacious in the majority of patients (65-90%), but carry a significant risk of complications [16,79]. Perforation occurs in 2-5% of patients and gastroesophageal reflux is reported by 10-50% of patients after myotomy. In addition, repeated procedures are sometimes required after dilation. In addition, the therapeutic benefit of these procedures declines from 10 to

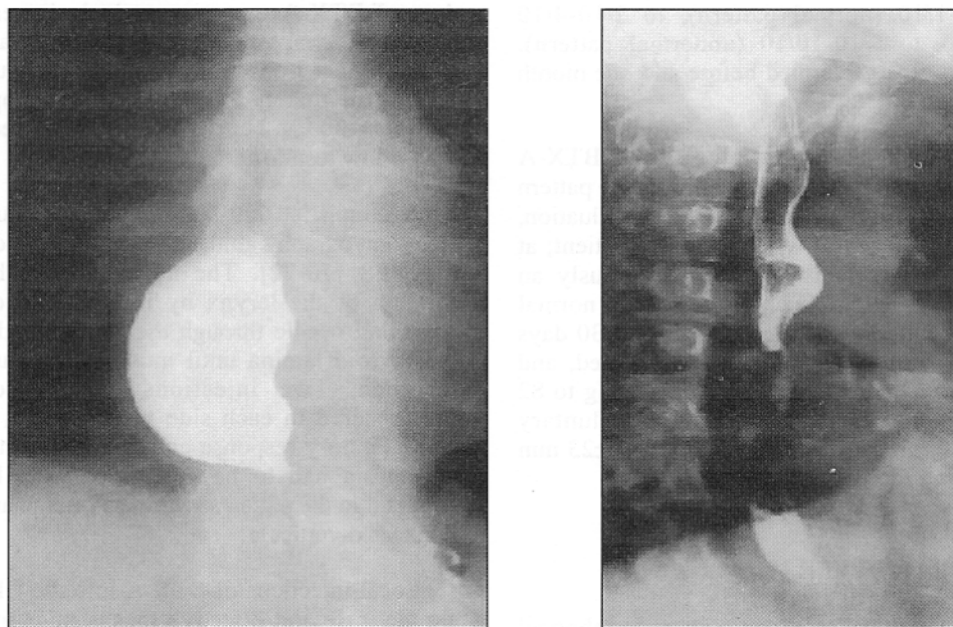


Fig. (3). Esophagogram of a patient with achalasia before treatment (a) and 12 weeks after treatment with BTX-A (b).

20 years after surgery. The limitations of classical surgical procedures prompted the use of injections of BTX-A into the LES, based on the rationale that BTX-A injections would inhibit an unbalanced cholinergic influence on the sphincter and improve esophageal emptying [80-105].

Results of BTX-A Treatment

The LES of patients is easily injected during an upper endoscopy using a 5-mm sclerotherapy needle. Endoscopic ultrasonography has been employed to guide injections, in an effort to maximize the amount of BTX-A delivered to LES. The infiltration appears as an enlarging anechoic zone around the needle, while performed.

Short-term symptomatic relief occurs in 70-100% of patients (Table 2) [80-82,87,88,106-109]. The occurrence of vigorous achalasia has been described as the principal determinant of the response to BTX-A [86,110,111]. LES pressure decreases and esophageal emptying improves in parallel to symptomatic relief, but to a lesser degree than after dilation. Other drawbacks of the treatment with BTX-A have been recently observed. Classic surgical procedures and BTX-A treatment have equal success rates, but in the long-term the efficacy of BTX-A is significantly shorter than that of balloon dilation. BTX-A is more expensive than pneumatic dilation, particularly due to the need of repeated treatments. Another limitation of BTX-A treatment is the lack of an initial symptomatic response and a high residual LES pressure (≥ 18 mm Hg). Nevertheless, there are obvious advantages of BTX-A treatment: the procedure is endoscopic, long-term efficacy is reasonable and the treatment is safe in older patients with tortuous megaesophagus or epiphrenic diverticulum [105], in whom pneumatic dilation is highly risky. It has been recently noted that BTX-A is also efficacious on pediatric patients suffering from achalasia [112,113]. The response is sustained beyond 6 months in 43% of the cases, but in one half of these young patients an additional injection is required approximately 7 months after the first treatment. For this reason, it has been recommended that BTX-A be used only for children with achalasia, who are poor candidates for pneumatic dilation or surgical myotomy.

The effect of BTX-A inevitably wanes over time some months after treatment. Only three studies report a follow-up longer than one year [81,84,91]. A decline of effects, from 65 to 30%, has been observed despite repeat injections in 31 patients followed for 2.4 years. One year after treatment, 36% of 33 patients had a good to excellent response after a single injection and 39% required subsequent treatment. Another study with an observational period of 18 months reported that 72% of 57 patients had a good to excellent response provided that therapy is repeated at relapse of symptom. Both commercial preparation of type A BTX-A have been used and the results obtained were comparable.

The injection of BTX-A in achalasia is remarkably safe and well tolerated. Mild chest pain, which has been observed in some patients (less than 10%) shortly after the injection, does not require a specific treatment. A single case of

esophageal ulceration and hematemesis has been observed two weeks after the injection. Moreover, a failure of BTX-A treatment does not influence the efficacy of subsequent surgical myotomy or dilation. BTX-A diffuses from the injection site into the surrounding tissues; the effects diminish as the distance from injection site increases. Spread to nearby muscles is possible, particularly when high volumes are injected, but weakness of distant muscles or generalized weakness is very rare. Diffusion of BTX-A is thought to be responsible for the detection of abnormalities of cardiovascular reflexes and for the occurrence of a heart block following treatment for achalasia, in a case report of a patient with pre-existing cardiac conduction disease [114].

The available data on the endoscopic injection of BTX-A into the LES are promising, but they pose a number of issues that still remain to be addressed. Namely: the identification of predictors of outcome, the definition of the optimal dosage and treatment schedule, ways to prolong BTX-A efficacy, a long-term evaluation of cost effectiveness and quality of life, and a proper definition of BTX-A action on smooth muscles.

OTHER INDICATIONS IN THE UPPER GIT

Recently, interest has focused on the use of BTX-A to treat motility disorders of the esophagus other than achalasia or cricopharyngeal dysphagia. BTX-A may have a potential for treating these motility disorders, which are not as debilitating as achalasia, considering that surgery may be judged too invasive and pneumatic dilation too risky.

Diffuse Esophageal Spasm

This is a poorly understood hypermotility disorder, where patients experience chest pain or dysphagia as a result of repetitive and simultaneous esophageal contractions of high-amplitude. The etiology is unknown. A history of some other functional GIT complaint is common, suggesting an involvement of the ENS. Antispasmodic drugs, such as anticholinergics or benzodiazepines, are occasionally helpful. The response to sublingual nitroglycerin is variable and sometimes dramatic; calcium channel blockers may also be of benefit. Dilation may relieve dysphagia and chest pain for several weeks to some months and needs to be repeated if required. Thoracic esophagomyotomy has been performed in cases of unresponsive spasm, but the results are less reliable and favorable than those observed in achalasia. A small series of ten patients with manometrically proven diffuse esophageal spasm has been treated with BTX-A injections into the LES [115]. Two months after treatment 80% of patients had scored a 50% or greater decrease in a scale for dysphagia, chest pain and regurgitation.

Isolated Hypertensive LES

This is a rare esophageal motor disorder characterized by high LES pressure, LES relaxation and normal esophageal

Table 2. Review of Experience Using Botulinum Toxin for the Treatment of Achalasia

Author / Year	Description	Patients	Results / Conclusions
Pasricha <i>et al.</i> , 1995	BTX-A vs Placebo	21	67% were improved at 6 weeks.
Annese <i>et al.</i> , 1996	BTX-A vs Placebo vs PBD	16	100% were improved at 1 month. 88% required repeated injections. BTX-A is as effective as pneumatic dilation.
Fiorini <i>et al.</i> , 1996	BTX-A vs placebo	13	72% were improved at 3 months.
Pasricha <i>et al.</i> , 1996	BTX-A	31	60% (82% of those aged >50) were improved at 3 months.
Fishman <i>et al.</i> , 1996	BTX-A	65	60 idiopathic cases: BTX-A treatment improved symptoms of dysphagia, chest pain and regurgitation in the majority of patients. 5 secondary cases: there was no response to BTX-A in 4 patients. Patients, who respond to a first BTX-A injection, but relapse, may respond to a second treatment.
Cuilliere <i>et al.</i> , 1997	BTX-A	55	60% were improved at 6 months.
Brant <i>et al.</i> , 1999	BTX-A in Chagas' disease	3	Clinical improvement occurred in all patients. Mean LES pressure dropped by 29%.
Kolbasnik <i>et al.</i> , 1999	BTX-A	30	Symptomatic improvement for >3 months was seen in 77% of patient. 7 patients had a sustained response after a single injection; 16 relapsed and required re-treatment.
Annese <i>et al.</i> , 1999	Botox vs Dysport	78	Type A BTX-A have comparable efficacy in esophageal achalasia after up to 6 months after treatment.
Muehldorfer <i>et al.</i> , 1999	BTX-A vs PBD	24	The two treatments had equal initial success rate (dilation 83%, BTX-A 75%). In the long-term the efficacy of BTX-A injection was statistically significantly and shorter than that of balloon dilation.
Panaccione <i>et al.</i> , 1999	BTX-A vs PBD	NR	Intrasphincteric BTX-A injection was more costly pneumatic dilation (USD 5,033 compared to USD 3,608). BTX-A treatment may be less costly if life expectancy is less than 2 years.
Greaves <i>et al.</i> , 1999	BTX-A	11	The relapse rate was 73% within 2 years from treatment. There were a beneficial effect on dysphagia, no improvement in chest pain or regurgitation scores, and no reduction of mean LES pressure.
Wehrmann <i>et al.</i> , 1999	BTX-A in high risk patients	20	80% were improved at 6 weeks. Mean cardia diameter was increased from 2.1 mm to 3.2 mm. The patients who initially had a symptomatic relapse after an average of 5 months. BTX-A re-injections were efficacious.
Hurwitz <i>et al.</i> , 2000	BTX-A in children	23	The mean duration of effect in 19 responders was 4.2 months. 50% of the patients required an additional procedure (pneumatic dilation, surgery) on average 7 months after the first treatment.
Annese <i>et al.</i> , 2000	BTX-A dose rasing study	118	82% of the patients were responders at 1 month. No dose related effect was observed. Vigorous achalasia was the main determinant of the response to BTX-A.
Ip <i>et al.</i> , 2000	BTX-A in children	7	100% were improved at 4 months. Sustained response beyond 6 months occurred in 43% of patients.
Hep <i>et al.</i> , 2000	BTX-A plus PBD	3	Propulsive peristalsis of the esophagus was restored in all patients.
Mikaeli <i>et al.</i> , 2001	BTX-A vs PBD	40	Cumulative 12-month remission rate was significantly higher after a single pneumatic dilation (53%) compared to a single BTX-A injection (15%, $P < 0.01$). The 12-month estimated adjusted hazard for relapse and need for retreatment for BTX-A group was 2.69 times that of the pneumatic dilation group.
Allescher <i>et al.</i> , 2001	BTX-A vs PBD	37	After 24 months a single pneumatic dilation was superior to a single BTX-A injection, and after 48 months all patients treated by BTX-A injection had experienced a symptomatic relapse.
Ghoshal <i>et al.</i> , 2001	BTX-A vs PBD	17	Both therapies resulted in a significant reduction in LES pressure.
Zarate <i>et al.</i> , 2002	BTX-A	17	The effect of BTX-A injection wanes with time in elderly patients, necessitating repeated injections to keep the patients symptom-free.
D'Onofrio <i>et al.</i> , 2002	BTX-A	37	Of the 35 patients followed, 12 had a relapse and were retreated; 4 out of 12 did not respond after retreatment. One or two injections of BTX-A result in a clinical and objective improvement in about 84% of achalasia patients and are not associated with serious side-effects; patients over 50 years showed better benefit than younger patients.

Abbreviations: BTX-A, botulinum toxin; LES, lower esophageal sphincter; PBD, pneumatic balloon dilation.

peristalsis. The mechanism of dysphagia in these patients remains unclear, but incomplete LES relaxation may play a

role in a number of patients. Pneumatic dilation has been employed with good results in the patients with significant

dysphagia who failed conservative therapy. Still, since most patients with dysphagia due to hypertensive LES do not have progression of symptoms or weight loss, and dilation carries an appreciable risk for esophageal perforation, this technique is indicated only in the most extreme cases. Recent attention has focused on endoscopic injection of BTX-A directly into the LES as a means to reduce LES pressure and improve dysphagia. A 45-year-old woman with a 3-year history of intermittent solid food dysphagia was treated with 80 Botox units into the LES. The patient had a 75% subjective improvement in dysphagia for 8 months. Short-term responses have been favorable without any significant complication, but long-term studies are needed to fully evaluate the efficacy of this treatment.

Delayed Gastric Emptying and Gastroparesis

BTX-A has been used to facilitate gastric emptying in patients who underwent pylorus-preserving duodenopancreatectomy [116]. Six patients were treated with 80 Botox units injected into the pylorus at the end of the surgical procedure. In the postoperative period, only 1 patient reported symptoms related to delayed gastric emptying.

Furthermore, BTX-A has been recently used to treat idiopathic gastroparesis [117-119]. Antral hypomotility is thought to be a major factor in this condition, and increased gastric outlet resistance due to pyloric dysfunction or pylorospasm has been described in gastroparesis, primarily the diabetic kind. An initial pilot study suggests that BTX-A injection into the pylorus in patients with idiopathic gastroparesis improves both gastric emptying and symptoms. In a preliminary report, all 3 patients treated with BTX-A intrapyloric injection had significant symptomatic improvement afterwards [117]. Similar results have been obtained in the treatment of 5 patients with diabetic gastroparesis [118]. Despite BTX-A injection of the pylorus seems to be a safe and well-tolerated procedure in patients with idiopathic or diabetic gastroparesis, further studies are required to determine whether BTX-A leads to long-term symptomatic improvement.

Infantile Hypertrophic Pyloric Stenosis

This is a congenital hereditary disorder characterized by a functional gastric outlet obstruction that occurs in 1 per 750 births. Pyloric obstruction is the result of a gradual hypertrophy of the circular smooth muscle of the pylorus. Although myenteric neurons have a normal appearance, neurons that innervate the circular-muscle layer of the pyloric sphincter lack NO synthase. Recently it has been observed lack of response to BTX-A injection in two patients with pyloric stenosis [120].

Sphincter of Oddi Dysfunction

Recurrent upper abdominal pain is a common clinical problem affecting 10% or more patients undergoing cholecystectomy. Sphincter of Oddi dysfunction has been

implicated in the etiology of 10-20% of these cases. Unfortunately, it is difficult to diagnose this disorder without performing a manometry of the sphincter, a procedure with a potential risk of pancreatitis. Sphincterotomy is considered the most effective treatment because response to medical therapy is poor; however, it is not easy to establish a direct relationship between Oddi dysfunction and pain before sphincterotomy.

Experimental studies have demonstrated that local injections of BTX-A significantly reduce wave amplitude and phasic contractile activity in the sphincter of Oddi, by means of a selective inhibition of cholinergic influences [121,122]. At least two potential uses of BTX-A can be hypothesized: first, an intrasphincteric injection may serve as a handy test to select patients whose pain is directly related to Oddi dysfunction; second, repeated BTX-A treatments may provide a treatment modality alternative to sphincterotomy [123-125]. Further prospective studies are needed to investigate the potential use of BTX-A in these patients.

Recently, 15 consecutive patients with frequent attacks of acute pancreatitis during 6 months and manometrically proven pancreatic sphincter of Oddi dysfunction have been treated by endoscopy with 100 Botox units into the major papilla. No side effects have occurred. Twelve patients (80%) have remained asymptomatic for 3 months after BTX-A treatment. One of the three patients who had no symptomatic benefit suffered from continued elevated pancreatic sphincter pressure and benefited from pancreatic sphincterotomy later on. Eleven of the 12 patients, who initially responded to BTX-A, had a symptomatic relapse on average 6 months after BTX-A treatment and then achieved long-term clinical remission when treated with pancreatic or combined sphincterotomy [124].

CHRONIC ANAL FISSURE

A chronic fissure is a cut or crack in the anal canal or anal verge that may extend from the muco-cutaneous junction to the dentate line [1,61,126]. It is a common complaint in young adults with a roughly equal incidence in both sexes, and shows great reluctance to heal without intervention. Classic symptoms are pain on or after defecation that is often severe and may last from minutes to several hours. Often there is bright blood on the toilet paper. The patient may report that constipation is the antecedent event, but once pain develops the fear of act of defecation and refusal of the call to stool can exacerbate this problem.

The majority of fissures occur in the posterior midline of the anal canal; fissures are located in the anterior midline in 10 per cent of women and 1 per cent of men. Multiple fissures or fissures occurring in the lateral position arise suspicion of other diseases including Crohn's disease, ulcerative colitis, tuberculosis, human immunodeficiency virus or syphilis [25].

The fissure can be seen as the buttocks are parted "Fig. (4)". It is often suspected because there is marked spasm of the anus making examination difficult. Digital or

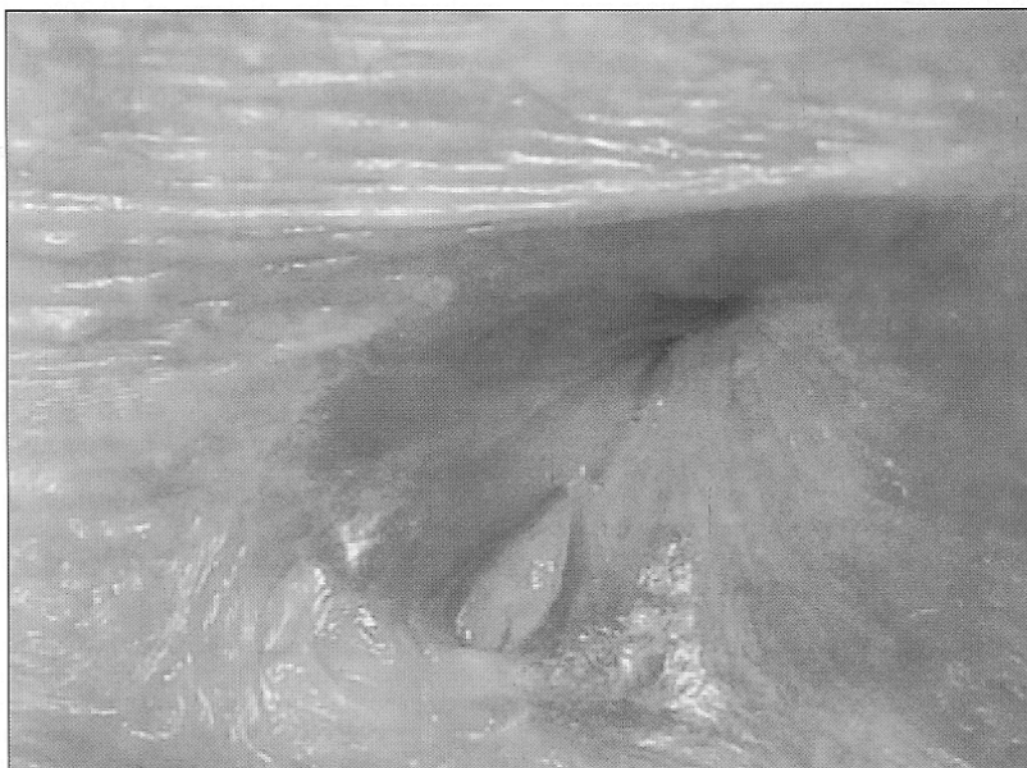


Fig. (4). Direct observation of a chronic anal fissure, with sharp borders and exposure of fibers of the internal anal sphincter.

proctoscopic examination is often impossible because of pain experienced by the patient. Under general anesthesia, the fissure is seen as a linear or pear-shaped breach in the lining of the anal canal below the dentate line. Spasm of the IAS has been noted in association with chronic fissure and for many years treatment has focused on alleviating IAS hypertonia.

Pathophysiology of Chronic Fissure

The cause of chronic fissures and the reasons for their failure to heal remain unclear. Also unexplained are the main characteristics of this painful condition, including the predilection for posterior midline and the lack of granulation tissue at the fissure site. Several theories have been advanced to unravel the underlying cause of anal fissure. Most of them are conflicting and none gives a satisfactory explanation for the characteristic features of chronic fissure.

The passage of a hard stool bolus has traditionally been thought to cause anal fissure. Fibers of the superficial EAS decussate to form a Y shape posteriorly; the overlying skin has been said to be poorly supported and to be prone to tear on passage of a large stool. However, a history of constipation preceding the onset of anal fissure is obtained only in one of four cases and diarrhea is seen to be a predisposing factor in about 6% of patients [25,61].

Fissure is associated with childbirth in 3 to 11% of patients and commonly occurs in the anterior midline. Shearing forces from the fetal head on the anal mucosa or

mucosal tethering after childbirth rendering it more susceptible to trauma have both been incriminated; postpartum anal fissure is associated with reduced anal canal pressures [127].

IAS spasm has been noted for many years in association with anal fissure; thus surgical treatment has been generally aimed at overcoming the spasm. It has been found that the IAS of patients with anal fissure is fibrotic, compared with that of controls [128]. It was then postulated that a myositis might occur early in the course of a fissure and that this is the underlying cause of both spasm and fibrosis. Although the cause of the spasm remains obscure, it has been consistently found that resting anal pressure is higher in the patients with anal fissure than in normal controls, suggesting that the high resting pressure be related to IAS hypertonicity. It is interesting to note that resting pressure of patients healed by conservative treatment tends to increase to pretreatment level while the patient remains symptom free. This probably means that sphincter hypertonus does not directly cause pain.

It has been postulated that the increased incidence of fissure in the anterior and posterior midline positions is related to the distribution of vessels supplying blood to the anal canal [129,130]. Relief of symptoms and healing induced by treatment could be attributed to a decrease in anal pressure, that would increase the mucosal blood flow and relieve ischemia. The inferior rectal arteries branching from the internal pudendal artery provide blood supply to the distal anal canal. These vessels cross the ischioanal fossa; their divisions pass through the anal sphincters to reach the

mucosa. Post-mortem angiography of the inferior rectal artery has revealed a paucity of inferior rectal artery branches at the posterior commissure in 85% of 41 subjects [131]. A morphologic study of the capillaries revealed a reduced density in the subanodermal space and within the IAS in the posterior midline in the majority of subjects. The predilection of anal fissures for the posterior midline and the lack of granulation tissue seen in the base of a chronic fissure may be explained by ischemia.

Decreased anodermal blood flow may be promoted by endothelial cells dysfunction associated with reduced NO synthesis, which is known to be involved in the regulation of local blood flow. Interruption of the endothelial continuity does not only remove the anticoagulant and vasodilator functions of the endothelium, but also exposes the subendothelium that has several pro-coagulant functions. In addition, even in the absence of detectable microscopic changes, endothelial function can change from vasodilator to vasoconstrictor and from anticoagulant to procoagulant. These changes may be induced by inflammatory or immune cytokines. Activation of the endothelium may express antigens as the endothelial cells can act as antigen-presenting cells. Anti-endothelial cell antibodies have been found in many patients with anal fissure, but not in healthy controls [132]. In antibody-positive patients, higher resting anal tone, with no change of maximum voluntary contraction, has been observed. This supports a role of the endothelium in the pathogenesis of anal ischemia. Circulating antibodies may activate the endothelium to produce vasoactive autacoids, which could contribute to the increased basal tone and aggravate the ischemia at the level of the posterior anal commissure. The observation that the topical application of nitroglycerin ointment may induce healing of anal fissure in up to 60% of cases [133] supports a pathogenic role of endothelial NO synthesis.

A primary IAS disturbance may be a contributing etiologic factor. IAS supersensitivity to β_2 -agonists has been observed in the patients with chronic fissure [24]. This may be induced by a prolonged absence of the neurotransmitter, by abnormalities at neurotransmitter or metabolic level, or by a modification of cholinergic and adrenergic receptors. Increased IAS adrenergic or cholinergic activity is likely to occur in patients with chronic fissure, as demonstrated by the efficacy of BTX-A in inducing fissure healing and reduction of resting tone following injection into the IAS [134,135].

Conventional Therapies

Since the passage of a hard stool is thought to contribute to the development of anal fissure, the control of constipation has been considered the main treatment for years [25]. Patients with a history suggestive of anal fissure of recent onset are often treated successfully by conservative measures, such as stool softeners, bulking agents, a high-fiber diet and sitz bath. To prevent recurrence, a patient should be encouraged to continue with the diet and to use bulked laxative agent, if required, even after symptoms have resolved.

Anal dilators have been employed in the treatment of anal fissure for many years. Dilators have not been helpful in treating anal fissure. Furthermore, it has been shown that the addition of an anal dilator is not more useful than stool softeners or topical anesthesia alone. It has been reported that more than half of the patients were initially cured using this technique, but that half of those cured relapsed.

If symptoms fail to resolve or have been present for a long period of time, resolution without surgery becomes increasingly less likely. Lateral internal sphincterotomy (LIS) has been the most commonly used treatment for chronic fissure since the 1950s [1,25]. It may be performed under local or general anesthesia, through a radial or circumferential incision, or using a subcutaneous approach. Open or subcutaneous methods produce adequate and equivalent falls in anal pressure. The IAS may be divided from medial to lateral or vice versa. The results of LIS have been reported from many centers. Surgery is associated with several complications, most of which can be prevented by the use of a judicious technique and, of course, by familiarity with anorectal anatomy. Although lateral internal sphincterotomy heals and relieves symptoms of chronic fissure in nearly all patients (96%), the incidence of incontinence varies [136]. The largest studies report impairment of continence in up to 30% of patients. Although most episodes of incontinence are minor and transient, in a subset of patients incontinence is permanent.

An incision of the IAS throughout its whole length is inadvisable, but it is uncertain how much of the sphincter should be divided. A common practice is to divide the sphincter for the length of the fissure. It has been suggested that the LIS length does not affect the incidence of recurrence and the alterations of continence, but some disagreement has occurred on this point.

Results of BTX-A Treatment

BTX-A can be used to treat anal fissure, particularly when a patient is at high risk of incontinence (Table 3) [137-142]. With the patient lying on a side (usually the left side when the operator is right-handed), the IAS can be easily palpated and injected. The lower rounded edge of the IAS can be felt on physical examination about 1-1.5 cm to the dentate line. The groove between the internal and the external sphincters can be visualized or easily palpated.

In an open-label study, we have observed that 60% of ten patients healed following a single infiltration of 15 Botox units into the IAS [143]. In a subsequent double blind study, a success rate of 76% was achieved following a single treatment with 20 units. A prospective comparison between two dose regimens (15 and 20 units) showed negligible side effects and no complications; symptomatic improvement was achieved in both groups of patients, but the healing rate was higher in the 20 U group. We have also been able to demonstrate that fissure healing is induced more effectively by BTX-A treatment than glyceryl trinitrate and that IAS hypertonia is also alleviated more effectively. Compared to baseline values, one month after treatment resting pressure

Table 3. Comparison of Published Results on the Treatment of Patients with Anal Fissure

Authors/Year	Nr of patients	Dose of toxin (Units)	Healing rate at 6-8 weeks %	Temporary incontinence %	Recurrence %	Complications %
Gui <i>et al.</i> , 1994	10	15 Botox	70	10	20	10
Jost and Schimrigk, 1994	12	5 Botox	83	0	8	0
Jost <i>et al.</i> , 1995	54	5 Botox	78	6	6	11
Mason <i>et al.</i> , 1996	5	Nr Dysport	60	0	0	0
Jost, 1997	100	5 Botox	82	7	6	0
Espi <i>et al.</i> , 1997	36	10/15 Botox	65/81	0	0	0
Maria <i>et al.</i> , 1998	25	20 Botox	88	4	0	0
Maria <i>et al.</i> , 1998	57	15/20 Botox	44/68	0	0	0
Minguez <i>et al.</i> , 1999	69	10-21 Botox	48-70	0	37-52	0
Jost and Schrank, 1999	50	20/40 Dysport	76/80	4/12	4/8	0
Brisinda <i>et al.</i> , 1999	25	20 Botox	96	0	0	0
Fernandez Lopez <i>et al.</i> , 1999	76	40 Botox	67	3	0	1
Khademi and Feldman*, 2000	11	25 Botox	82	0	0	0
Maria <i>et al.</i> , 2000	50	20 Botox	74	0	0	0
Lysy <i>et al.</i> *, 2001	30	20 Botox	73	0	0	-
Madalinski <i>et al.</i> *, 2001	14	25-50 Botox	54	0	8	-
Tilney <i>et al.</i> , 2001	10	Nr Dysport	Nr	Nr	Nr	20
Brisinda <i>et al.</i> , 2002	150	20/30 Botox	89/96	3	4	0

*Plus topical nitrates. Nr: not reported.

was reduced by 26.2% in BTX-A-treated patients and by 16.6% in the patients treated with nitrate ointment. Two months after treatment, resting anal pressure was reduced by 28.4% in the BTX-A group and by 13.7% in the nitrate group.

The therapeutic efficacy of different BTX-A doses in chronic fissure have been reported recently [135,138,139]. It has been found that the healing rate does not differ significantly when the total dose and the number of injection sites are varied. The healing rate was 83% in the patients treated with 10 Botox units, 78% in those treated with 15 units and 90% in the group treated with 21 units. Between-group comparison did not reveal significant differences. The injections were administered through the intersphincteric groove in the direction of the IAS; however, one month after treatment the mean squeeze pressure was reduced more than resting pressure, suggesting diffusion of the toxin to the external sphincter.

Based on the theory that low anodermal perfusion at the base of the fissure contributes to the pathophysiology, additional infiltration of BTX-A has been performed at this point. In our experience, patients with a posterior chronic fissure have better results, represented by a lowering of

resting anal tone and early development of an healing scar, when BTX-A is injected anteriorly into the IAS. The practice of anteriorly placed injections induces a higher fall in resting pressure and improves the clinical outcome. Fibrosis of the IAS, that is more prominent in the site of the fissure than elsewhere in the smooth muscle, may reduce IAS compliance and limit BTX-A diffusion. It is known that the myenteric plexus with myenteric ganglia is located between the circular and longitudinal smooth muscle layers along the entire extent of the IAS. A chronic reduction of perfusion in the posterior part of the anus may affect the myenteric nervous fibers at this location and make them less sensitive to the action of BTX-A.

In a recent study, the influence of different dosage regimens injected anteriorly in the internal anal sphincter on the clinical outcome of patients with a posterior chronic anal fissure has been investigated [135]. Fissure healing and symptomatic improvement were achieved in both groups of patients. At one month after injection, complete healing was present in 73% of patients treated with 20 units (Group I) and 87% of patients treated with 30 units (Group II). A symptomatic fissure persisted in only 5 patients in Group I. At two months after injection, 89% of patients in Group I had a healing scar, while 96% in Group II had a healing scar;

3 patients in Group II had a persistent fissure in the absence of symptoms. These results confirm that higher doses lead to higher success rate [135]. Resting anal pressures were significantly lower than pre-treatment values in both groups; although maximum voluntary pressure was unchanged in patients treated with 20 units, it was significantly lower than pre-treatment value in patients treated with 30 units, probably related to a diffusion of BTX-A to EAS. Five of these patients reported mild incontinence of flatus that lasted two weeks after treatment and disappeared spontaneously. BTX-A diffusion in the tissues is a dose-dependent phenomenon: histochemical staining of acetylcholinesterase suggested that higher doses produced a biological effect throughout the entire muscle, whereas smaller doses produced a gradient down the length of the muscle studied.

It has been observed that BTX-A injections into the striated EAS are also effective for treating fissure. The mechanism is probably mediated by diffusion to the IAS, as shown by the observation that maximum squeeze pressure and resting pressure are both reduced with this procedure. Since the fundamental pathogenic event in chronic fissure is the IAS spasm, the injection into the EAS is not the first choice for treatment. In addition, the IAS is readily visible and easier to inject than the EAS.

Complications of the treatment have been reported by several authors. Reported side effects, other than mild and transitory incontinence for flatus or feces, encompass perianal thrombosis and hematoma [144]. However, the development of the potential complications does not seem to influence the overall efficacy of the treatment [135,140,143-157]. Recently, Minguez and co-workers [139] analyzed the long-term outcome (42 months) of 57 patients in whom an anal fissure had healed after botulinum toxin injections. The authors state that the late recurrence rate of chronic anal fissure is high when the effect of botulinum toxin disappears. A fissure recurrence has been noted in 22 patients (41.5%). Furthermore, they showed that the highest risk of recurrence is associated with anterior location of the fissure, prolonged illness, the need for reinjection and for high doses to achieve healing.

In conclusion, BTX-A appears as a safe treatment for patients with chronic anal fissure. It is easier to perform than surgical treatment and does not require anesthesia. It is also more efficacious than nitrate therapy. In the patients with a posterior chronic fissure better results are achieved when BTX-A is injected anteriorly into the IAS. No adverse effects or permanent IAS damage have resulted from BTX-A injection.

OTHER APPLICATIONS IN THE LOWER GIT

Chronic Constipation Due to Pelvic Floor Dysfunction

A diagnosis of constipation is made when less than three bowel movements per week and at least one of the following occur on a minimum of 25% of occasions: hard bowel movements, difficulty passing the bowel movement, sense of inadequate defecation. Patients with chronic idiopathic constipation can be classified in two pathophysiological

groups: slow transit constipation and pelvic floor dysfunction.

Pelvic floor dysfunction is characterized by a failure of the puborectalis muscle to relax during efforts to defecate, or by its paradoxical contraction. With an effort to evacuate the rectum, the puborectalis and the EAS normally relax to straighten the anorectal angle and open the anal canal. The diagnosis is suggested by the demonstration of a persistent impression of the puborectalis on the posterior surface of the anal canal during attempted evacuation of barium paste and, more reliably, by EMG evidence of increased electrical activity in the puborectalis muscle during straining.

The etiology of pelvic floor dysfunction is unclear. As in other forms of constipation, the patients are commonly females, who developed constipation as adolescents or young adults. Prolonged efforts to empty the rectum may aggravate the condition. It has been suggested that paradoxical puborectalis contraction during straining represents a focal dystonia, characterized by excessive recruitment of synergistic and antagonistic muscle groups during voluntary activity and lack of reciprocal inhibition. Signs of anismus have been noted in normal subjects, in patients with anorectal pain, and even in patients with fecal incontinence [1,2].

BTX-A has been used to selectively weaken the EAS and puborectalis muscle in constipated patients. It has been observed that BTX-A relaxes the puborectalis muscle; as a consequence, the anorectal angle increases during straining and evacuation becomes possible. However, despite good results, the effect of BTX-A injections is fairly short term. From 6 to 15 Botox units were injected under electromyography guidance into the EAS or the puborectalis muscle; this brought about short-term improvement of all four treated patients, who had failed to respond to conventional biofeedback treatment, but in the long-term only half of the patients remained successful. In a recent study, a symptomatic improvement was noted in 13 out of 15 patients after injections of 25 Botox units into the EAS. Improvement was maintained for a mean of 5 months, and re-injection was thereafter necessary. In a group of 50 patients with chronic outlet obstruction constipation, 4 patients with a puborectalis syndrome were studied using anorectal manometry, defecography and electromyography [141]. They were then treated with a total of 30 Botox units, injected into two sites on either side of the puborectalis muscle, under ultrasonographic guidance. One patient was lost to follow up; in the remaining three, the frequency of natural bowel movements increased from 0 to 6 per week and only one patient needed laxatives. Anorectal manometry demonstrated decreased tone during straining from 96 ± 12 mm Hg to 42 ± 13 mm Hg at four weeks ($P = 0.003$) and 63 ± 22 mm Hg at eight weeks ($P = 0.009$). Defecography performed 8 weeks after treatment showed improvement in the anorectal angle (increased from $94^\circ \pm 11$ to $114^\circ \pm 13$, $P = 0.01$) and evacuation of barium paste [141,142]. EMG demonstrated that the puborectalis muscle was still capable of producing a mild paradoxical contraction. Still, one of the patients suffered from symptomatic recurrence 16 weeks after treatment. He was re-treated with 50 units; eight months later he received a further 60 units. Seven months after the

last injection he had normal daily bowel movements with no use of laxatives.

Outlet-type constipation may also occur in Parkinson's disease (PD) [1]. Following the observation of the dramatic improvement of a PD patient with outlet-type constipation treated with BTX-A injections into the puborectalis muscle "Fig. (5)", we performed a prospective study to identify the prevalence of this condition among PD out-patients. Patients with a diagnosis of PD filled an inventory of gastrointestinal function (evaluating the number of bowel movements and defecatory function) and received a proctological evaluation. A total of 138 patients met the inclusion criteria for chronic constipation; 18 of them (13%) had isolated or prominent outlet-type constipation. Ten patients (1 woman and 9 men, mean age $\pm$ SD 69.1 $\pm$ 9.2 years, mean duration of PD 70 $\pm$ 53 months, mean duration of constipation 35.3 $\pm$ 11 months) were studied. All the patients were treated with levodopa, dopamine agonists, and domperidone. They were evaluated by means of manometry, defecography and electromyography before treatment and twice at monthly intervals after the injection. BTX-A (Botox, Allergan Inc, California, USA) was injected in the puborectalis muscle (two sites on either side of the muscle) under transrectal ultrasonography guidance; the total dose per session was 100 units in each patient. Following treatment, anal tone during straining was reduced from 97.4 $\pm$ 19.6 mm Hg at baseline to 40.7 $\pm$ 11.5 mm Hg one month after treatment ($P=0.00001$); no further change was observed at the two-month evaluation (38.2 $\pm$ 10.4 mm Hg; $P=0.00001$ vs. baseline values). Resting anal tone and maximum voluntary contraction were unchanged compared to baseline values.

The anorectal angle during straining (as measured with defecography) increased from a mean of $99^\circ \pm 7.9$ before treatment to $122.2^\circ \pm 15$ ($P=0.0004$); 9 patients evacuated the barium paste without the need for laxatives or enemas.

This observation indicates that outlet obstruction is the main cause for constipation in a minority of PD patients and provides evidence that BTX-A may be a remedy for them. The duration of efficacy of the injections remains to be measured, and repeated treatments are probably necessary [158]. The BTX-A optimal dose also remains to be determined; a placebo controlled study with long-term follow up is warranted.

Anterior Rectocele

Rectocele is a hernia of the anterior rectal wall into the lumen of the vagina. It is a frequent finding in women and its clinical relevance is questionable: from 20 to 81% of non-symptomatic women and of constipated people may present a rectocele. If less than 2 cm in diameter, rectocele is considered a normal finding in constipated or healthy subjects. When the diameter increases beyond 2 cm, the rectocele can cause outlet obstruction and rectal emptying difficulties [159,160].

It has been suggested that in some instances the rectocele is caused by failure of relaxation or paradoxical contraction of the puborectalis muscle occurring during attempted evacuation, but the reason for its establishment is not clear. It is important to identify a rectocele when it is the primary

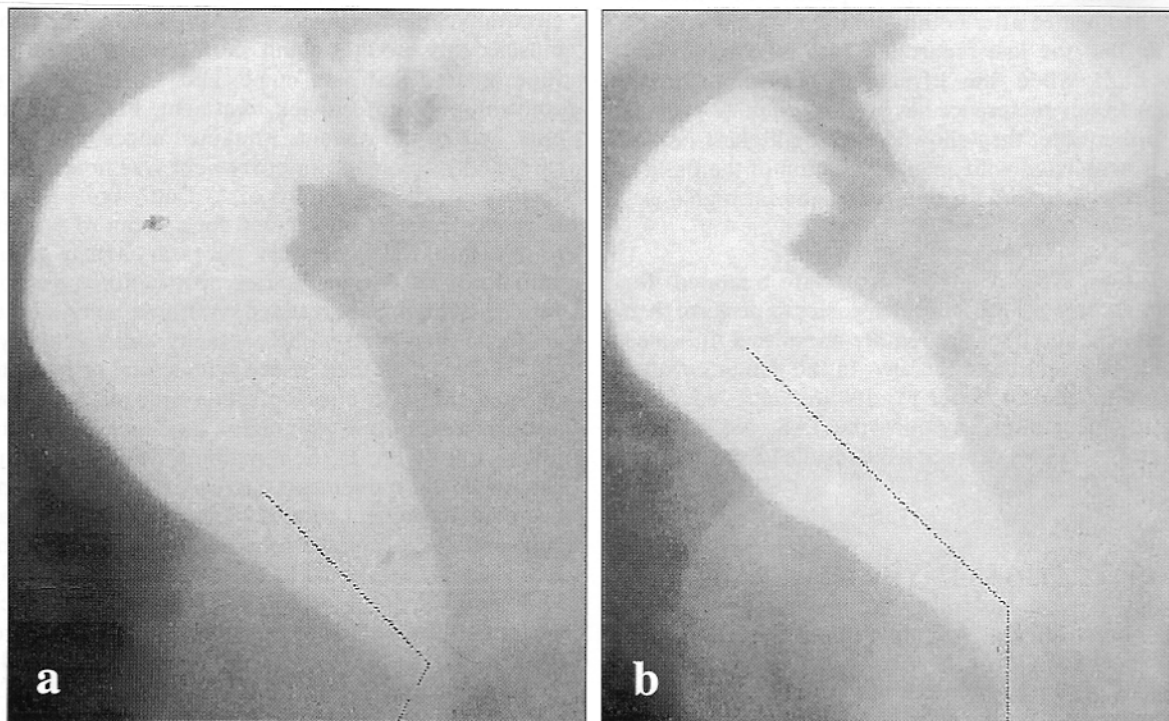


Fig. (5). Treatment of outlet-type constipation in PD by injection of BTX-A in the puborectalis muscle, before (a) and after (b) BTX-A treatment. Defecographies a and b have been taken during straining. Following treatment, during straining the pelvic floor descends about 3 cm and the anorectal angle becomes obtuse.

cause of intractable obstructed evacuation. A rectocele may cause mild to severe anorectal symptoms, which are usually associated with chronic constipation. It is conceivable that straining aggravates the rectocele, enlarges it, and makes evacuation even more difficult. When there is a rectocele or a paradoxical sphincter reaction, defecation has to occur through the non-relaxed pelvic floor.

Although simple to diagnose, the rectocele has proven difficult to treat. The initial phase of management usually involves a high-residue diet to try to elicit rectal voiding. The next step is to use increasing doses of laxatives and enemas. Neither procedure is effective in solving the problem. A wide variety of surgical approaches have been proposed with the aim of assuring rectal emptying by reducing the dimension of the rectocele. However, the results of surgery are often disappointing with regard to emptying difficulties. Surgical repair, either vaginal, transperineal, or transanal, does not always alleviate symptoms, and in some patients causes impaired fecal continence. Furthermore, transanal repair may compromise anal sphincter pressures and an alternative approach should be considered when the anal sphincter is lax. From 30 to 72% of patients still had difficulties with evacuation in the postoperative period. 54 per cent of patients still complained of constipation, often severe, after the surgical procedure, 17% of them noted persistent anorectal pain and 34% had impaired fecal continence. Six months after successful transanal repair of rectoceles, a significant impairment in resting and squeezing anal pressures, probably correlated to stretch of the anal sphincters, were still found. Moreover, increased incidences of rectovaginal fistulas and vaginal narrowing and significant postoperative pain have been reported when the transvaginal approach is used [61].

Recently, 14 women (mean age of 55 ± 11 years) were treated with a total of 30 Botox units evenly divided into three sites, two on either side of the puborectalis muscle and the third anteriorly in the EAS [159]. At one month, inspection revealed a symptomatic improvement in 7 patients. Incomplete evacuation had disappeared in 7 patients and was reduced in 2 patients. The pressure during straining was reduced by 41% as compared with the baseline value, and was significantly lower than the resting pressure value at the same time. At two-month evaluation, a symptomatic improvement was found in 9 patients. At defecography, the rectocele depth was reduced from 4.3 ± 0.6 cm to 1.8 ± 0.5 cm ($P < 0.001$) and the rectocele area was reduced from 9.2 ± 1.3 cm² to 2.8 ± 1.6 cm² ($P < 0.001$). The anorectal angle measured during straining increased from a mean of $98 \pm 15^\circ$ before treatment to a mean of $121 \pm 19^\circ$ ($P = 0.001$) [159]. At one-year evaluation, there was no report of digitally assisted rectal voiding and rectocele was no found at physical examination.

Chronic Idiopathic Anal Pain

Chronic idiopathic anal pain is part of a rather ill-defined group of disorders termed chronic idiopathic perineal pain, which also includes proctalgia fugax and coccygodynia [161,162]. The main feature of these syndromes is that no objective abnormalities are found on clinical examination,

and the distinction between the different groups of perineal pain is based solely on the patient's description of the pain and location of tenderness by palpation.

Patients classified as having chronic anal pain usually describe a pain located at the anus, often with radiation to the upper anal canal or lowest part of the rectum. In the majority of patients, the pain is present constantly, usually intense, sometimes burning, often with some irradiation; it was usually aggravated by sitting whereas defecation had no constant effect, and is relieved by lying down. A feeling of obstructed defecation has also been described. The cause and pathogenesis of the syndrome are unknown, but in a few patients, more advanced, instrumental investigations of the anal canal, such as intra-anal ultrasound and magnetic resonance scan, may reveal treatable organic disorders, such as chronic intrasphincteric abscesses and fistulas. There is no satisfactory treatment for chronic anal pain [163]; nonetheless, anal stretch and LIS are still used in several patients on the assumption that the pain might be caused by a hypertonic IAS, because no objective changes can be demonstrated. Recently, injections of BTX-A (20 units placed intersphincterically in four quadrants and/or at the lower rim of the puborectalis muscle under ultrasonographic guidance) resulted in improvement in 4 patients with chronic anal pain that persisted during a follow up of 12 to 24 months [164].

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