ORIGINAL ARTICLE

Chemical blockage for cerebral palsy spasticity treatment

Bloqueios químicos para o tratamento da espasticidade na paralisia cerebral

Maria Matilde de Mello Sposito[1]

ABSTRACT

The chemical blockades are considered important weapons in the modern treatment of the espasticidade even in adults as in children. They can be carried through with phenol, botulinum toxin of the type A or both (called mixing blockades). In this article we will review in details the different types and uses of chemical blockades for the treatment of the spasticity.

Keywords: Muscle Spasticity, Cerebral Palsy, Botulinum Toxin Type A

RESUMO

Os bloqueios químicos são considerados armas importantes no moderno tratamento da espasticidade tanto em adultos como em crianças. Eles podem ser realizados com fenol, com toxina botulínica do tipo A ou ainda com ambos, os chamados bloqueios mistos. Neste artigo discutiremos em detalhes os diferentes tipos de bloqueios químicos utilizados para o tratamento da espasticidade.

Palavras-chave: *Espasticidade Muscular, Paralisia Cerebral, Toxina Botulínica Tipo A*

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Conflict of Interest Statement

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HISTORY

Phenol has been used in medicine for over 50 years, however its indication for spasticity treatment is much more recent and it has been primary used intrathecally to block spinal nerves anterior roots.[1] But intrathecal phenol have inconsistent results and a high number of complications such as nerve roots damage, arachnoiditis, meningitis, spinal cord injury, motor paralysis, sensory loss, paresthesia, pain and eventually death.[1]

In 1966 an alternative administration route was described with the motor point blockage, with improvement for long term spasticity.1 At the same time, other agents such as lidocaine where described as suited for blockage. Nonetheless lidocaine presented a short effect duration and its use was then restricted to "Therapeutic Testing."

Phenolic blocages were widely used in spasticity treatment between the '70s and '80s, but were forgotten because of adverse reactions such as dysesthesia. With the advent of botulinum toxin in the '90s, this procedure almost fell into oblivion. But the toxin dose restriction, the toxin procedural costs and the knowledge that dysesthesia barely existed when blocking nerves with predominantly motor fibers, the procedure with phenol re-emerged in the form of a "mixed procedure" associated with botulinum toxin in cases of multifocal spasticity.

Phenolic Blocking

It is a focal and temporary neurolytic treatment, usually used for blocking the anterior obturator nerve branch in lower limbs and the musculocutaneous nerve in the upper limbs,[2-4] because they have small sensory function and thus present less risk of dysesthesia or anesthesia after phenol blocking.[4]

Phenolic block has also been used to reduce muscle tone by blocking motor points.[4] Motor point is defined as the area where the motor nerve branch enters the muscle or area where there was a greater concentration of motor endplates.[1] Its use is more appropriate in cases of severe focal and multifocal spasticity, where mixed blocks (botulinum toxin and phenol) may be useful for treating a larger number of muscles in a single procedure.[5]

Mechanism of Action

Phenols mechanism of action undergoes a first phase where the drug acts as an anesthetic on the gamma fibers[1] then occurs a protein denaturing (proteolysis), with the interruption of the efferent signals from the hyper excitable cells of the anterior horn of the spinal cord, through an induced axonal necrosis (Walerian degeneration), but which preserves the endoneurial tubes.[1,4,6]

The effects of pheenol use are not permanent and a functional re-innervation occurs over months to years.[1,7] Controversy exists as to which type of fibers are more affected by phenol. Some electromyography studies show that the alpha I nerve fibers are the most affected.[1]

Phenol is acidic and tends to spread badly, which increases its local inflammatory potential.[6] It spreads very little into the tissues, so the injection should be performed as close as possible to the target nerve in order to obtain results.

Drug characteristics and doses

Normally aqueous phenol solutions are used, they range between 3-5% to 7% (25% phenol in 60% glycerin solution diluted in sterile water at a concentration of 5%),[6] both in selected muscles motor points as in the perineural nerves region. We can also find oily or glycerin preparations, but only used to opened blocks.[1]

The concentrations for the alcoholic blocking vary from 30-50%, but these seem to last less then the phenolic blocks and therefore are less used.[1]

The doses are not fully established for children, but the 30mg/kg dose apears to be safe.[8] It is recommended to start with a dose of 0.5 g or 30mg/kg (10 ml of solution at 5%, total dose / procedure), 1-5ml/point (usually 2ml/point).[1,6] The estimated lethal dose is at 8.5 g-15g and it is recommended not to administer more than 1g in 24 hours, or 20 ml of phenol at 5%.[5,9] Phenol is excreted by the kidneys and can lead to darker-colored urine.[1]

Effects duration

When phenol is administered an almost instantaneous relaxation is noted.[9] For having low cost and long period of action, 6 - 12 months and even 18 months,[9] phenolic blocks are an attractive treatment option for selected patients with focal or multifocal spasticity. This procedure action time varies with concentration, injected volume, duration of exposure, injection technique and history of injections.[10,11]

Adverse Effects

If the phenol is injected around nerves which have predominantly sensory fibres it may cause dysesthesia or anesthesia that can last up to 4 months.[12] In cases of dysesthesia beneficial effects of the procedure can not be appropriated, for instance, a child with feet dysesthesia can continue to walk in an equino pathon, in an antalgic posture, despite having reduced the gastrocnemius spasticity. Thus sensory nerves with large sensory components should be avoided in this procedure.

The most frequent adverse effects in phenol treatment are: dysesthesia and pain due to local inflammatory process, varying from 0.4% to 5% in children[6,13] and 2-32% in adults.[14]

We can also observe: edema, rashes, deep vein thrombosis, infection and excessive relaxation. Soon after the procedure, patients can present: headache, feeling drunk, alcoholic breath, lethargy, nausea and vomiting, all can be reversed within 1 hour.[1,16] Histologically we can observe: injury or loss of skin tissue, muscle necrosis and interstitial fibrosis.[17] If the phenol is administered intravenously can observe: tremors, blackouts, respiratory failure and heart conditions.[1]

Indications and contraindications

In children with cerebral palsy the nerves most used for phenol neurolysis are: anterior obturator nerve branch, musculocutaneous nerve, popliteal medial branch of posterior tibial nerve and sciatic nerve.

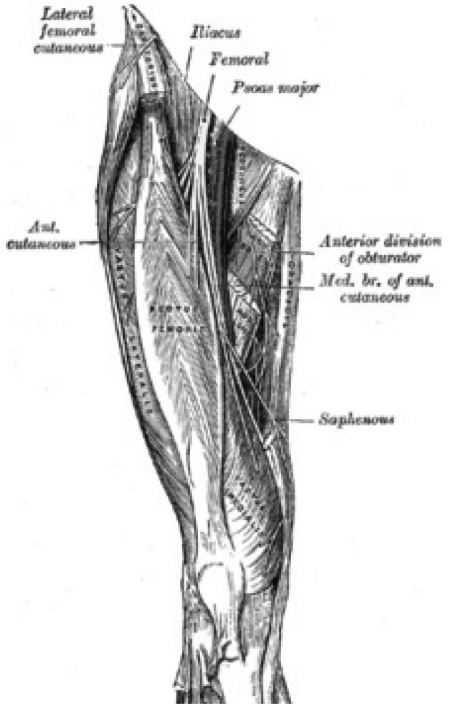

Figure 1 - Anatomic location of the anterior branch of the obturator nerve

The anterior obturator nerve branch is used to treat spasticity in the adductor thigh muscles, leading to a scissor inferior member's posture that difficults balance, hygiene and overall posture.

The musculocutaneous nerve innervates the biceps brachilis, brachialis and coracobrachialis, thus it is accessed to treat elbom flexion. This technique is often associated with botulinum toxin blockade (eg phenol for elbow flexors and toxin to wrist and fingers flexors).

The injection into the medial popliteal branch of the posterior tibial nerve may be limited by the increased risk of dysesthesia[10] and the injection in the sciatic nerve's motor branches is used to relax the hamstrings. An anesthetic block can serve as a therapeutic test for a more definitive phenolic block.

Phenolic block is indicated in cases of severe spasticity unresponsive to usual conservative treatments. Its advantage is to cause a specific long duration tone ablation.[1] It is also preferred during neurological recovery from acute injury, once the injection takes spasticity without interrupting the function.[1] It is also used in functional members or when extensive neurolysis is needed, usually associated with botulinum toxin.[1]

The contraindications for the use of phenol include: general discomfort, extensive and severe contracture.

Injection technique

Anatomical location[6]

Anterior branch of obturator nerve: the best approach is the anterior between the tendons of the adductor muscles of the thigh (Figure1).

Motor branch of the posterior tibial nerve: upper-lateral portion of the lateral gastrocnemius (Figure 2A and 2B).

Musculocutaneous nerve: the musculocutaneous nerve can be located at two points, one more proximal, above the armpit artery, and another distal near the anterior branch of the brachial artery (Figure 3).

Electrical stimulation technique

To localize the point for injection, nerves or motor points, the most used techinique is electrical stimulation.[7,10] However this technique is poorly tolerated by children and ultimately requires sedation or anesthesia.

The electrical stimulation used is 1.0 milli seconds and square wave pulse. Phenol should be slowly injected at the point of best response, i.e. maximum muscle contraction with minimal electrical stimulation (<1 mA), until the con-

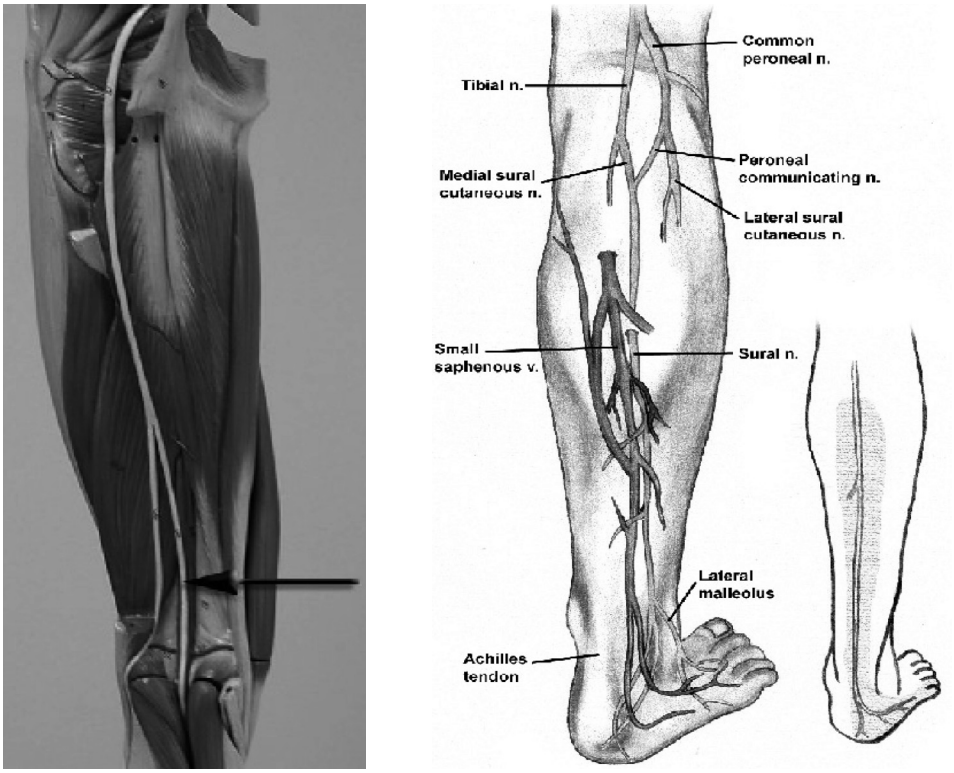

Figure 2A and 2B - Anatomical location of the posterior tibial nerve and its motor branch

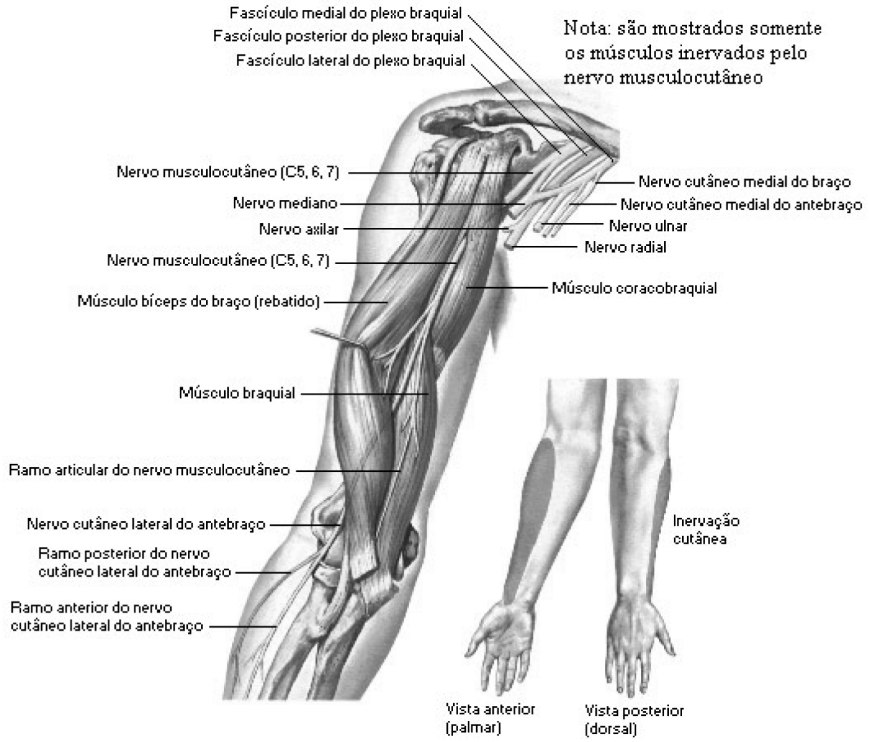

Figure 3 - Anatomic location of the musculocutaneous nerve

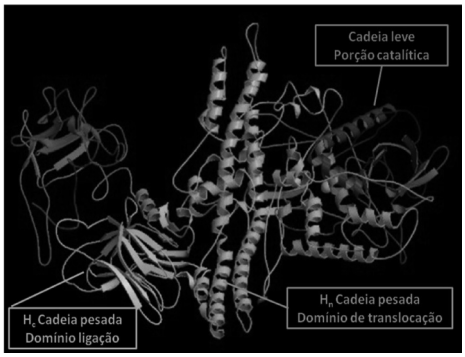

Figure 4 - Tridimentional Representation of the BoNT/A
(Image used with consent ©2003 Allergan, Inc.)

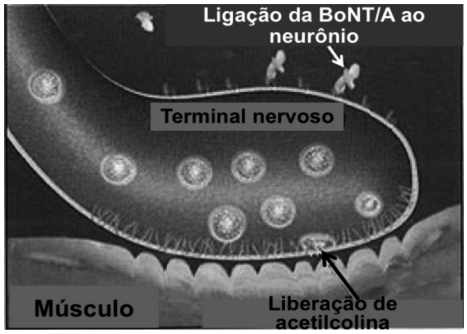

Figure 5 - BoNT/A binding to the neuromuscular junction receptors of peripheral motor nerves cholinergic neurons
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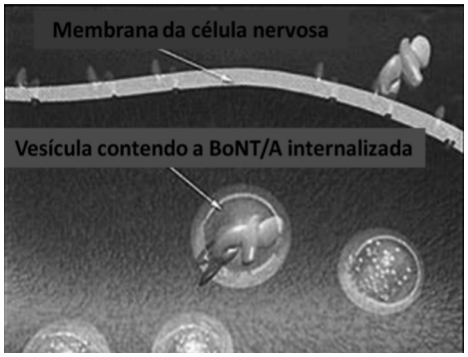

Figure 6 - BoNT/A molecule internalization
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traction is eliminated.[6] To locate the right injection point by electrical stimulation requires specific medical training and is best performed by physiatrists rather than neurologists.[6,18]

In children this procedure is usually performed under anesthesia, which increases costs and risks.

However, Kolaski K et al[6] demonstrated that the combined procedure of botulinum toxin and phenol under general anesthesia is safe and that related anesthesia complications are very low, even when dealing with children with cerebral palsy cases, whose associated complications rate is greater than general infant population.

The advantages of phenol compared to botulinum toxin are: low cost and not tot induce antigenicity.[7]

Botulinum toxin blockage

The botulinum neurotoxin (NTB) is produced by the anaerobic bacterium Clostridium botulinum and is considered one of the most potent toxins known. Its high toxicity combined with very specific mechanisms of action gives it unique high danger properties that are, nonetheless, associated with large medical utility.[19]

The active part of the botulinum toxin type-A (BoNT / A) molecule weighs 150 kDa and consists of two parts: the light chain with catalytic activity (50 kDa) and the heavy chain (100kDa) (Figura 4). The light chain weighs 50 kDa and is responsible for zinc dependent metalloprotease activity that prevents the release of neurotransmitters by blocking presynaptic vesicle fusion.[20] The heavy chain contains two domains: the connection represented by Hc (C-terminal half of heavy chain) and translocation represented by Hn (N-terminal half of heavy chain).[21,22] The heavy chain is responsible for binding itself to extracellular receptors and internalization in nerve cell, besides helping the translocation of the light chain into the neuron cytoplasm.[20]

Clostridium botulinum is the bacteria responsible for botulinum toxin synthesis into seven toxin serotypes named from A to G. Type A is more potent and more used in therapy being sold as a drug in different formulations.

Therapeutic botulinum toxin preparations contain an active complex plus non-toxic proteins, forming the so-called "protein complex" and excipients. The accessory proteins have a role in protecting the neurotoxin from degradation.[23,24]

Action Mechanism

Botulinum toxin basically inhibits exocytotic release of acetylcholine at motor nerve terminals leading to a decrease of muscle contraction.[25]

This property makes it useful, clinically and therapeutically, in a number of conditions where there is excess muscle contraction.[25]

Observation of botulinum toxin effects in different clinical conditions showed that the benefits extended to other aspects besides muscle relaxation, which led to studying the mechanism of action involving other neurotransmitters. Thus, today we must not only think in the classically described mechanism of action, on the inhibition of acetylcholine release in motor nerve terminals, but also in action on other neurotransmitters.19 So from a didactic standpoint the mechanism of action can be divided into the following topics:

A. Muscle Relaxation
   i. Action on striated muscles
   ii. Action on the stretch reflex
B. Antinociceptive Action
   i. Blocking the release of peptides related to pain
C. Autonomic Nervous System
   i. Glandular action: salivary, sweat and lachrymal
   ii. Action on the bladder and prostate
D. Direct and indirect effects on the Central Nervous System

In this chapter we discus only the muscle relaxation mechanism, but we recommend reading,[19] were they all are addresses extensively.

Muscle relaxation

I. Action on striated muscles

The classic Botulinum Toxin type A (BoNT/A) action mechanism is the acetylcholine release inhibition at the paripheral nerve terminal.[21,26]

Once injected into the muscle BoNT/A reaches the cholinergic nerve terminal by combining the dispersion and diffusion properties and starts its mechanism of action upon arrival. This mechanism is done in three steps: (a) binding to cholinergic nerve terminal, (b) internalization / translocation, (c) calcium dependent inhibition of neurotransmitter release (exocytosis).[21] To do so is required one molecule of BoNT / A with intact two chains (light and heavy), established as a zinc dependent endopeptidase that breaks specifically the essential proteins to mediate neurotransmitter exocytosis, in this case acetylcholine.[24]

(a) Blinding to cholinergic nerve terminal: BoNT/A binds itself to a high affinity receptor found predominantly in cholinergic motor nerves neurons through the heavy chain binding domain (Figure 4 and 5).[21,26,27]

(b) Internalization / translocation: Once BoNT/A binds itself to the neuron, begins the presumably immediate internalization process by an endocitosis receptor. These receptors are located at the amyelinated portion of the mammalian neuromuscular junction. There appears to be two phases of internalization: (a) rapid

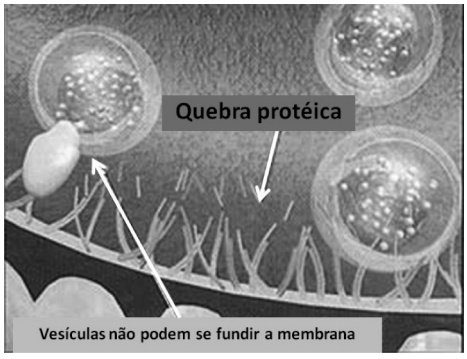


Figure 7 - Dependent inhibition of neurotransmitters calcium release (Image used with consent ©2003 Allergan, Inc.)

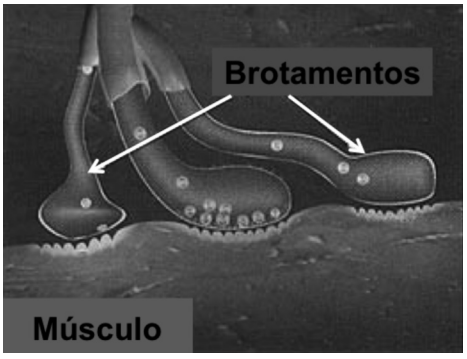


Figure 8 - Axonal sprouts and restoration of the synapse with the neuromuscular junction (Image used with consent ©2003 Allergan, Inc.)

entry: that uses vesicular system and (b) a slow entry: that requires hours and is less specific.[21]

Under acid conditions, low pH, changes occur in the structural protein molecule conformation (domain translocation – see figure 4), so that the heavy chain facilitates the light chain entry to the cytoplasmic compartment of nerve terminals.[21,26,27]

(c) Dependent inhibition of neurotransmitters calcium release (exocytosis): the neurotransmitters exocytosis inhibition, acetylcholine, occurs through a zinc-dependent proteolytic activity from the light chain, wich selectively breaks the peptide bonds of the SNARE protein (Soluble N- ethylmaleimide-sensitive factor attachment protein-receptor) essential for the neurotransmitter release that is calcium dependent (Figure 7).[21,26,27] Thus, the light chain exerts its effect by breaking down the proteins that are responsible for the fusion of acetylcholine vesicles with the cell membrane from the nerve terminals.

It is demonstrated that the loss of SNARE proteins by themselves do not prevent the SNARE fusion complex formation, but results in the formation of a non-functional complex in which the docking of the influx of calcium (Ca^2) is discontinued at the fusion time. The increase of calcium concentration in the synaptic terminal partially reverses the botulinum toxin effect.[28]

Neuromuscular junction response to blockage: After about two months, the nerve terminal initiates its expansion trough sprouts that extend across the muscle surface. When the sprouts/buds form a synaptic connection with the physical neuromuscular junction, motor nerve unit is restored (Figure 8).[21]

In vivo studies established that these sprouts produce a temporary re-innervation in the early stages of pos-blockage recovery. During the late stages the original neuromuscular junction regains exocytotic activity and these sprouts regress making the termination return to its original form fully functional.[29,30]

II. Action over the stretch reflex

Besides the direct action on the striated muscle, botulinum toxin also acts on the muscle spindle by reducing the centripetal information traffic. The mechanism by which this occurs is not yet fully elucidated.[31]

The striated muscle in humans contains cholinergic neuromotor junctions between the α-motoneurons and the extraspindel muscle fibers, and between γ-motoneurons and the intraspindle muscle fibers, forming the muscle spindles. When a muscle strain occurs, afferent signals originated in muscle spindle run trough the Ia and II fibers, stimulating α-motoneurons of the stretched muscle, as well as interneurons that inhibit the α-motoneurons of antagonistic muscles.

The γ-motoneurons of the stretched muscles are activated by colateral α-motoneurons (α and γ co-activation). This circuit is shown in figure 9. Afferent signals from muscle spindle are also related to supraspinal structures involving long latency responses to the stretch reflex and the generation of body image in space.[32]

Recently the involvement of afferent signals was studied in the pathophysiology of dystonia. The facilitation for Ia fibers can lead to increased involuntary movements in various disorders that cause dystonia, on the other hand, the lidocaine injection on the muscle spindles promotes a "muscle afferent block.[33]

Botulinum toxin produces different effects on muscle spindle. Intra and extra spindle atrophy has been demonstrated in animals, as well as blocking of γ-motoneurons reducing the Ia and II afferent signals from muscle spindles and therefore reducing also tone by reflex inhibition. The antispasmodic effect of botulinum toxin, howerver, affects not only the targeted muscle but also inhibits the spinal reflex.[32]

Moreover, botulinum toxin injection can cause a profound spasticity reduction in areas larger than expected and not related to the medicine's dispersal area.[34] This observation may be related to the botulinum toxin effects on the γ-motoneurons reducing the Ia afferent signals from muscle spindles. This Ia signals attenuation reduce feedback to α-motoneurons and other routes, reducing the activity of non-injected muscles.[34]

Indication for the botulinum toxin treatment

When thinking about a botulinum toxin treatment some factors should be considered: [35]

1 - Patient's personal characteristics: age, tolerance threshold to bear electrical stimulation to locate muscles motor points, treatment preferences, etc.

2 - Focus on the treated condition.

3 - Locate functional goals of treatment: comfort, hygine, improvement in the use of orthoses and the ability to walk.

4 - Spasticity degree and muscle hyperactivity.

5 - Preservation degree of of motor control and motor recruitment level that needs to be preserved for the muscle in question (eg, anterior tibial influencing the ankle inversion or plantar flexion influencing walk).

6 - Degree of motor control preservation from muscles adjacent to the treated.

7 - Selection of target muscles in order to result in objective improvements.

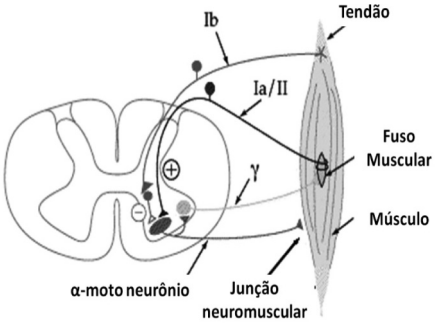
8 - Anatomical region and the need for resources use to locate exactly the target muscle (deep muscles X superficial muscles, obesity, etc.).

9 - Dose required to reach treatment goals (if the total dose available is insufficient for treatment purposes consider the use of mixed treatment).

10 - Access and adherence to complementary treatments such as physiotherapy and occupational therapy.

11 - Responses to previous treatments including former chemical blockage with botulinum toxin.

Indication for general botulinum toxin treatment in cerebral palsy reffers to the presence of dinamic contractures, interferring with function, in the absence of fixed contracture.[36] The best results are found in children that have the adequate selective motor control.[37] The selective motor control may be measured through the scale in Chart 1.



Afferent stimuli from muscle spindles and Golgi tendon organs monitor the activity of the alpha motoneurons in the innervation of striated muscles. When a striated muscle is stretched, the muscle spindles send a signal to the alpha motoneuron which in turn stimulates contraction of the fibers within and outside the spindle.

Figure 9 - Strecht reflex

Chart 1 - Selective motor control scale[37]

Definition	Degree
Without the abitity for active ankle dorsiflexion	0
Ankle dorsiflexion done mainly with hallucis or finger extensor	1
Ankle dorsiflexion done by the fingers extensor with some activity on the tibialis anterior	2
Ankle dorsiflexion done by the tibialis anterior, but with knee or hip associated flexion.	3
Ankle dorsiflexion done selectively by the tibialis anterior with knee extension	4

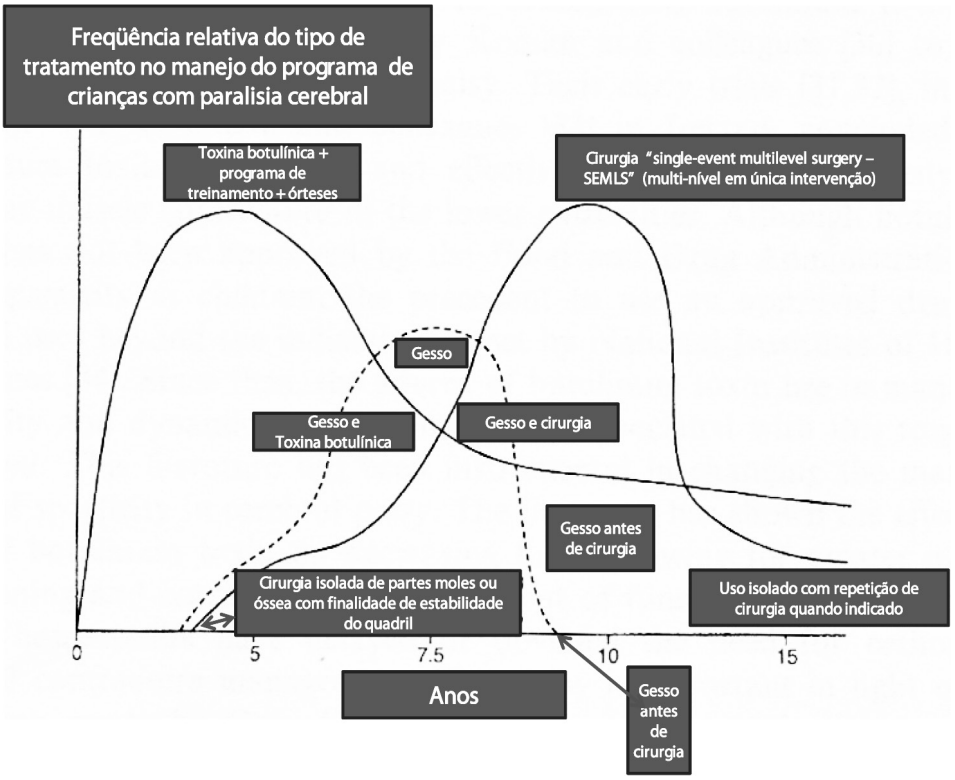


Figure 10 - Relative frequency of the type of treatment in managing the program for children with cerebral palsy

The conditions to achieve effective results with the botulinum toxin spasticity treatment are:[38]

1. Presence of reducible dynamic contracture that alters motor function.
2. That the objective be the improvement of a limited number of muscle groups. We must remember that there is a limitation of total dose to be used per procedure, but there is a worldwide trend to use multifocal treatment that seems to change the course of the disease.[36,39]
3. That the movement disorder depends primarily on the spasticity of a muscle group and not the weakness of the antagonists. This is not easy to determine especially if there is already some degree of tendons shortening.
4. Spasticity interfering with limb or body function. Remember that spasticity is not always negative. We expect a functional improvement by reducing spasticity.
5. Ensurement of muscle stretching several times a day through physical therapy and physical activity (eg walking or orthoses) in order to achieve maximum muscle growth.

Patients with better indication for the botulinum toxin injection have muscle imbalance with strong agonist muscles spasticity.[40] In children with less than 7 years, if the dynamic spasticity is eliminated there are great chances of improving the function.[41]

Early treatment with botulinum toxin favors the maximum response and induces longer therapeutic responses, thereby reducing the potential risk for the onset of contractures and the need for surgery.[37] The optimal age for this kind of procedure, in children with spastic cerebral palsy, is between 1-5 years old during the period of dynamic motor development, where there are more chances to alter the natural course of disease.[37]

The possibility of delaying possible orthopedic surgeries in later ages, between 6 and 12 years, is another advantage. At this age the surgeries are more likely to be definitive.[37]

Relative frequency of the type of treatment in managing the program for children with cerebral palsy and the influence of treatment with botulinum toxin in the disease course can be seen in Figure 10.[37,42]

Negative factors for botulinum toxin procedure include:[37]

1- Severe fixed contractures (moderate contractures may respond with the association of toxin and plaster).
2- Bone twists and joint instabilities.
3- Hemostasis disorders.
4- Many muscles to be treated (consider other treatment options).

Much has been argued about the safety factors related to the minimum age for the botulinum toxin procedure. In a recent study,[43] demonstrated the safety and advantages of the procedure performed in children younger than 2 years. Adverse events found are similar to those found in older children, both in type and frequency. Intervention within the first year of life, according to the authors, has the advantage of preventing spastic hip dislocation in cases of severe adductor thigh spasticity and treatment with botulinum toxin has proven useful in this regard. Another early indication would be for the correction of muscle imbalance in cases

Chart 2 - Contraindications for botulinum toxin type A blockage

Absolute	Relative
Known allergy to the drug or its components.	Neuromuscular disease associated (post-polio syndrome, miastemia gravis, amytrophic lateral sclerosis, etc).[35]
Infection at the blockage site.	Associated coagulopathy and / or decompensated.
Pregnancy and breast-feeding (categ. C).[35]	Autoimmune disease in activity.
	Lack of patient cooperation for the global procedure and unrealistic expectations.
	Use of enhancers such as aminoglycosides up to four weeks before the procedure.[35]
	Use of aspirin or nonsteroidal anti-inflammatory within four weeks prior to the procedure.

Chart 3 - Advantages and disadvantages of the botulinum toxin treatment[44]

Advantages	Disadvantages
Efectiveness regardless of the spasticity cause	Cannot treat widespread spasticity
Effective treatment for focal problems	potential economic implications
specific treatment	Reversible, require repetition.
Very safe drug	Not a long term solution
Reversible effects	Difficult to estimate the actual costs
Easy use	may require combination treatments
May reduce the need for systemic medications	Access to the muscles to be injected can be difficult
Few drug interactions	
Prevents complications	

of obstetric brachial palsy, where the toxin prevents the joint limitation and corrective orthopedic surgery indication.

Contraindication

The botulinum toxin type A blockage is, generally contraindicated under the conditions listed in Chart 2.35 Yet we must remember that as various pharmaceutical preparations of botulinum toxin type A have completely different characteristics and individualities, including differences in the components and formulations, each have their specific contraindications. Furthermore, for being biologic products they never may be considered generic.

General contraindications are divided into absolute and relative. The relative contraindications should be examined by medical criteria against the patient's condition.

In patients with cerebral palsy, botulinum toxin is contraindicated in the presence of severe and fixed joint contractures. When there is muscle fibrosis, tendon and articular capsule the therapeutic effects are very small.

Advantages and disadvantages of botulinum toxin tretament

The botulinum toxin treatment has show several advantages in relatin to others treatments. The advantages and disadvantages of this procedure are listed on Chart 3.

Regarding the botulinuim toxin's procedural cost, a study from Balkrishnan R et al,45 shows that the cost of this procedure, in the treatment of children with cerebral palsy, is not associated with an increase in spending by the health care system - Medicaid USA – with these patients.

Differences between the formulations

There are several commercial preparations of botulinum toxin type A in the international market. Botox® (Allergan, Inc.), Dysport® (Ipsen Limited), Prosigne® (Lanzou Institute of Biological Protucts) and Xeomin® (Mertz Pharmaceuticals). They are all biological products that have different formulations and potential requiring different dosages.[46] The dose equivalence between them does not exist. The differences between the formulations commercially available in Brazil can be seen in Chart 4.

Doses

The botulinum toxin dosage for children is based on the patient's weight, size and number os muscles to be injected, spasticity degree and functional objectives.[41] The initial studies have used dosagens ranging from 2 to 5 U/kg body weight; following studies have used doses from 10 to 12 U/kg without increase in the number of collateral effects. The spasticity study group[47,48] recommends pedriatric dosage guides for inferior and superior limbs, besides directives modifying doses and key points according to charts 5,6,7,8 and 9.

We should notice that the reccomendend dosages on the charts below are exclusive for BOTOX® formulation.

High dosage usage was reported by some authors. Hart DA,[41] recommends 20 U/kg, distributed over 50 U per injection point and 10 U/kg per muscle, with maximum dose varying between 300 - 400U. Molenaers G et al,[36] use 20 - 25U/kg on his patients; the same was reported by and that addressed up to 30U/kg. refers to botulinum toxin type A treatment in children with cerebral palsy, with weight lower then 45kg, in doses of 15-22U/kg of body weight or in young adults weighing 45kg or over, receiving doses from 800 to 1200U, without showing significant adverse effects.

We must remember that the estimated lethal dose for 70 kg adults is 3000U, based on the lethal dose for monkeys that is 40U/kg[41] and estimated lethal dose for mice is 81.4 U / kg for Botox® and 160.8 U / kg for Dysport®.[38,50-53]

The maximum dose of Dysport® per procedure is 1000U; higher doses increase the risk of adverse effects.[16,41]

Dose-response intensity corelation

There is a correlation between dose and extent of paresis provoked. On the other hand, a relatively low dose of BoNT/A can cause a substantial paresis. The observation of dose-response curves can be useful to optimize the use of the toxin.[32]

Dose-response duration correlation

There is also a correlation between dose and duration of therapeutic response. But this correlation is stronger when low doses of toxin are used, with high doses the duration of effects seems to be saturated in 3 months (for skeletal muscle).[32]

Dose Equivalency

The toxin is usually quantified by testing the *lethality endpoint* and the toxin lethality endpoint unit more commonly used is the median of the lethal dose (lethal dosis) DL_{50}.[50,54]

The DL_{50} for the standart crystalline toxin type A, for a 20g mouse was determined in 0.043ng.[50,54] The DL_{50} for toxin type A purified by chromatography, was estimated at about 6 picograms. Sometimes the lethality is reported as "Minimum Lethal Dose (MLD), which is the lowest dose able to cause death in over 50% of injected animals.[50,54]

The DL_{50} can not be interpolated and its accurate determination requires that the number of dilutions be increased little by little, slowly and carefully, and that 6 to 10 rats be tested each dilution.[50]

Chart 4 - Comparison between the characteristics of different botulinum toxin type A commercial presentations available in Brazil until July 2010

	BOTOX®	DYSPORT® RELOXIN®	PROSIGNE® BTXA	XEOMIN® NT-201	NEURONOX® SIAX® BOTULIFTING
Differential Name	OnabotulinumtoxinA	AbobotulinumtoxinA		IncobotulinumtoxinA	
Country of origin	United States	United Kingdom	China	Germany	South Korea
Release year	1989	1991	2001	2005	2006
Sorotype	A	A	A	A	A
Units per vial	50- 100-200	300 e 500	50 e 100	100	100
Presentation	Dried in Vacuum	Lyophilized	Lyophilized	Lyophilized	Lyophilized
Vial size	10ml	3ml	3ml	3ml	
Expíration Date	3 years	2 years	2 years	3 years	2 years
Composition	Human Albumin 0,5mg NaCl 0.9mg	Human Albumin Solution 20% 0.125 mg Lactose 2.5 mg	Bovine Gelatine 5 mg Dextran 25mg Sucrose 25mg	Human Albumin 20% = 1000mcg Sucrose 5mg	Human Albumin 0,5mg NaCl 0.9mg
Neurotoxin amount per vial (ng)	4.8	4.3	4.8	0.6	4.8
Molecular Complex (kD)	900	500-700 + something 900	500 ~ 900	150	900
Propagation	1,5 – 3cm Low	Higher ? High			
1U = DL50	0,04ng	0,025ng	0,04ng		0,04ng
Targeted Protein	SNAP-25	SNAP-25	SNAP-25	SNAP-25	SNAP-25
Strain	Hall	Ipsen strain NCTC 2916	Hall	Hall	Hall
Control Strain	Constant Selection	Renewal each 3 years			
Culture Medium	N-Z casein Yeast Extract Glucose		Tripsina Yeast Extract Casein		
Lyophilization Diluent	Human Albumin	Human Albumin	Bovine Gelatine	Human Albumin	Human Albumin
pH	7,4	7,4	6,0	7,4	6.8
Production	Multiple acid precipitation / dialysis	Purification Column	Multiple purification methodes/ dialysis		
Estabilization	Dried in Vacuum	Dried by freezing (Lyophilization)	Dried by freezing (Lyophilization)	Dried in Vacuum	Dried in Vacuum
Purification	Precipitation / chromatography	Precipitation / chromatography		Precipitation / chromatography	
Storage	2-8°C	2-8°C	-5 a -20°C	room temperature	2-8°C
After dilution	2-8°C for 3 days	2-8°C for 8 hours	2-8°C for 4 hours	2-8°C for 24 hours	2-8°C for 4 hours
Specific biological activity	20 MU-A/ng NC-B	100 MU-I/ng NC-D		167 MU-M/ng toxine	
Corrected specific biological activity	60 MU-EV/ng toxine	100 MU-EV/ng toxine		167 UM-EV/ng toxine	
ED50	4.4 ± 0.3 U/Kg	16.2 ± 1.1U/kg	5.7 ± 0.3U/kg		
Equivalency	None	None 1:3 / 1:5	None ≥30%	None ≥ 1 (10%)	None

Botulinum toxin type A, BOTOX®, is measured in biological units (U) defined by the DL_{50}, i.e. the dose that kills 50% of female Swiss-Webster mouse weighing 18-20g when injected intraperitoneally.[50,52] In nanograms the BOTOX® unit is approximately 0.48 ng.[53]

Being a Biological Product, mesurated in biological units (U), there is no equivalence between the different pharmacological presentations of botulinum toxin type A.[55]

In the analysis of both business presentations of toxin type A, BOTOX® and Dysport®, the tests of lethality Na (DL_{50}) and muscular paralysis (effective dose DE) measured by the DAS test (*digital abduction score*), discrepancies were found in the two preparations activity. In this study the efficient intramuscular dose for a DAS of 2 foi was 6,2 ± 0,6U/kg for BOTOX®

Chart 4 (continuation)

	BOTOX®	DYSPORT® RELOXIN®	PROSIGNE® BTXA	XEOMIN® NT-201	NEURONOX® SIAX® BOTULIFTING
Brazil Use	Adult and pediatric - 1992	Adult and pediatric – 2000	Adult and pediatric 2005	Adult 2009	Adult 2010
Uso at the country of origin	Adult (over 12 years)	Adult (over 2 years – april 2009)	Adult	Adult	Adult
Absolute Contraindications	Allergy to components of the formula Infectious process at the injection site Concomitant treatment with aminoglycosides or streptomycin Widespread disturbances of muscle activity Bleeding disorders and anticoagulant use Contraindications for intramuscular injection Pregnancy and lactation	The same + Lactose Allergy	History of anaphylactic reaction Allergy to components of the formula Infectious process at the injection site Bleeding Disorders Special precautions: Heart, liver and lung disease, active tuberculosis, pregnant women, children younger than 12 years the procedure should be performed with caution. It may lead to anaphylaxis reaction so to combat it medication should be available (epinephrine solution 1:1000)	The same	The same
Indications approved by ANVISA - Brazil (label indications)	Squint Blepharospasm Cervical dystonia Hemifacial spasm Muscle spasticity Hyperkinetic Facial Lines Palmar and axillary Hiperhidrosis Overactive bladder	Blepharospasm Hemifacial spasm Spasticity Spasmodic torticollis Hyperkinetic Facial Lines Palmar and axillary hyperhidrosis in adults	Squint Blepharospasm Hemifacial spasm Spasticity Spasmodic torticollis Cervical dystonia Cerebral Palsy Muscle Rehabilitation Hyperkinetic Facial Lines Palmar and axillary hyperhidrosis in adults	Blepharospasm Dystonia	?
Approval at the country of origin	Squint Blepharospasm Cervical dystonia Hemifacial spasm Muscle spasticity Hyperkinetic Facial Lines Palmar and axillary Hiperhidrosis Spasticity	Blefaroespasmo Hemifacial spasm Spasticity Spasmodic torticollis Hyperkinetic Facial Lines at the superior 1/3 of the face Hyperhidrosis	Squint Blepharospasm Hemifacial spasm	Blepharospasm Cervical dystonia	Squint Blepharospasm
Agências regulatórias internacionais	FDA/EMEA Latin American Agencies	FDA/EMEA Latin American Agencies	GMP China Latin American Agencies	EMEA Latin American Agencies	KFDA (Korea) Latin American Agencies
Health Ministry Brazil - Liberation	YES	YES	YES	YES	YES
Fabrication laboratory	Allergan Inc.	Ipsen Biopharm Medicis Inc.	Lanzhou Institute of Biological Products	Mertz Pharmaceuticals	Medy-Tox Inc.
Imporatiton and Commercialization Laboratory - Brazil	Allergan Produtos Farmacêuticos Ltda.	Ipsen Biopharm	Cristália Prod. Químicos e Farmacêuticos Ltda.	Biolab	Bergamo
Worldwide Presence	85 countries 5 continents Including USA e Canada	79 countries 5 continents excluindo Canadá Incluindo USA	China and 10 Latin America Countries	Germany, othe 12 european countries, Mexico , Brazil Argentina, Colombia	Korea , Brazil Colombia
Maximum dose per procedure	400U 600U – Brazil	1200U 1500U - Brazil	360U 600U – Brazil	300U	300U

and 22,9 ± 3,2U/kg for DYSPORT®. For the intramuscular DL_{50} the values obtained were 81,4 ± 3,5U/kg for BOTOX® and 106 ± 7,2U/kg for DYSPORT®. Therefore, the therapeutic indices were different being 13,9 ± 1,7 for BOTOX® and 7,6 ± 0,9 for DYSPORT®. These data show that BOTOX® is four times more effective and 2 times safer than DYSPORT®.[56]

These differences between the products become especially important when working with large doses. Thus, one should avoid simple units conversion from one preparation to another.[55]

Hyperdosage

There were no reports of systemic toxicity due to accidental oral ingestion of botulinum toxin type A. Based on reports of individual human poisoning cases, it is estimated that the lethal dose for humans could be 3.000 U or more.

Chart 5 - Pediatric doses for superior limbs[47,48]

Clinical Pattern	Potential muscles envolved	BOTOX® dose Untis/Kg	Number of injection sites
	pectoralis complex	2	2 – 3
	latissimus dorsi	2	2
	teres major	2	1 – 2
	subscapularis	1 – 2	1 – 2
Flexed Elbow	brachioradialis	1 – 2	1
	bíceps	2	2 – 4
	brachialis	2	1 – 2
Pronated forearm	pronator quadratus	0,5 – 1	1
	pronator teres	1 – 2	1
Flexed Wrist	flexor carpi radialis	1 – 2	1
	flexor carpi ulnaris	1 – 2	1
Thumb-in-Palm	flexor pollicis longus	0,5 – 1	1
	adductor pollicis	0,5 – 1	1
	flexor pollicis brevis/ opponens	0,5 – 1	1
Clenched Fist	flexor digitorum profundus	1 – 2	1 – 2
	flexor digitorum superficialis	1 – 2	1 – 2
Intrinsic Plus Hand	lumbricales/interossei	0,5 – 1	1

In the event of product ingestion, the patient should be monitored during several days, observing for signs of muscle weakness or paralysis. It is estimated that the entire content of a 100U viel is lower than the systemic toxic dose for humans weighing 6 kg or more.[57]

Storage and Conservation

The vacuum vial containing the neurotoxin, should be put under refrigeration at 2-8°C or in a freezer at -5 °C. After dilution in saline solution without preservatives, the solution must be used in the shortest time frame possible, and it may be stored at 2-8ºC for up to 3 days.[58-60]

Botulinum toxin type A is heat labile and can be inactivated by pH changes and by boiling.[50] Storage of the diluted product seems to make it lose potency over time. Refreezing the solution for two weeks, leads to a potency loss of 70%.[59] Other studies however, refer to several storage time frames after dilution without loss of product's potency.[41,61,62]

Prepare and Dilutions

Botulinum toxin type A is presented as a sterile lyophilized powder. So, to use, it is necessary to dilute the product. It is recommended that this dilution be performed among a saline solution without preservatives, saline 0.9%.[38,58] The use of distilled water or saline solution with higher concentrations makes the injection very painful. The use of saline solution with preservatives can alter the potency of botulinum toxin by changing the pH of the solution.

During the dilution, one must avoid bubbling or agitation of the vial content. The same care should be taken during the recovery of the drug into the injection syringe. Due to the large toxin molecule size and labile of its connecting bridges, the bubbling or agitation of the liquid will eventually break it and deactivate it, since the heavy segment separates from light one.[38]

Toxin saturation of the injected area will be responsible for the clinical block.[57] Therefore, dilution should be such that enables the dose control during injection, but also one that does not have excessive volume favoring the drug spread.[38,59,63] It is estimated that the major toxin action occurs at a 4-5 cm radius from the injection point.[40]

Toxin can be diluted in any volume, provided that during the application the dose relation be respected. Dilution is thus subject to convenience of the doctor, while the dose is subject to the need of the patient. The most common dilution on the other hand is 1-2ml/100U.[38,41]

Chart 10 shows some examples of commonly used dilutions.

Dilution effect

It is established that increasing the dilution favors the spread of toxin in the target muscle and this fact will impact on the therapeutic effects and adverse reactions. However it is not yet established what would be the optimal dilution ratio for each application of BoNT/A.[32] The area of botulinum toxin diffusion is estimated between 3-4cm with the 10U / 0.1 ml dilution.[64] The muscular fascia, a natural anatomical barrier, does not prevent the spread of the toxin.[41] Of course this fact depends on the characteristics of the formulation and other factors.

Beginnig and duration of effects

The effects of the injection can be felt between the 2nd and 10th day after application, peaked between 2-4 weeks, and last about six weeks to six months.[35,40] At this time the patient should be evaluated for the possibility of a new application. In patients using botulinum toxin type-A for a longer time it is reported a longer effect duration and increased break between applications.[35,65]

Electromyographic studies show that the amplitude of action potential of the injected muscles declines after 48 hours of injection and reaches its lowest point in 21 days. The same studies showed that 100 days after injection, the amplitude of action potentials was still reduced by 80%.[66]

The duration of clinical effects on the other hand, depend on several factors, including: total used dose, severity of clinical symptoms, presence of other therapies and individual factors such as neurological regeneration capacity. In patients undergoing rehabilitative programs, the spacing between two injections can reach up to a year or even 14 months.[39,42]

Controls are recommended at least at the time of each procedure, 1-2 months later, in order to observe the effects and compare with the following injections.[38]

When botulinum antitoxin antibodies are formed, the duration of action and extent of the maximum therapeutic effect tends to diminish (partial failure) before there is a total failure in treatment. The duration of action may vary among patients suffering from the same disease and among patients suffering from different pathological conditions. When the same patient is treated with the same parameters and did not develop antibodies, the effects tend to be stable.[32]

Complications

Possible complications to treatment with botulinum toxin can be divided between: comparative, rare and described, as shown by Chart 11.[67,68]

Comparative complications are preventable or easily resolvable; the rare actually have very low incidence, but the formation of antibodies is a highly undesirable effect and requires special care by the physician.

Described complications are usually due to technical error, error in clinical and functional assessment of patient for the procedure, error in dosage or dilution.

Adverse effects occur in less than 15% of cases and usually last a few days.[38] Their primary impact is estimated at 5-6% and related to discomfort at the injection site, pain and skin irritation, lasting 1-2 days.[1,37,39,40,41] Some patients experience transient muscle weakness with deterioration of gait, tendency to fall and early fatigue in walking for 1-2 weeks.[1,37,41]

This usually occurs in a dose-dependent relation where there is saturation of the muscle injected withtoxin and spread to adjacent muscles.[37]

Quadriplegic children, particularly those with pseudo-bulbar palsy should be carefully monitored after the administration of botulinum toxin. The period of risk in these cases is 1-3 weeks after injection.[37] Symptoms of botulism include: fatigue, ptosis, diplopia, and dysarthria associated to dysphagia with respiratory impairment. These symptoms can be treated with pyridostigmine administration and are usually resolved within 6 weeks.[49]

Chart 6 - Pediatric doses for lower limbs[47,48]

Clinical Pattern	Possibly envolved muscles	BOTOX® doses Unities/Kg	Number of application points
Flexed Hip	Iliacus	1 – 2	1
	psoas		
	rectus femoris	3 – 5	2 – 3
Flexed Knee	medial hamstrings	3 – 8	3 – 4
	gastrocnemius (as knee flexor)	3 – 6	2 – 4
	lateral hamstrings	2 – 6	1 – 2
Adducted Thighs	adductor longus/brevis/ Magnus	3 – 6	1 – 3
Stiff (Extended) Knee	quadriceps mechanism	3 – 6	2 – 4
Equinovarus foot	gastrocnemius medial/ lateral	3 – 6	1 – 4
	soleus	2 – 3	1 – 2
	tibialis posterior	1 – 2	1
	tibialis anterior	1 – 3	1
	flexor digitorum longus/ brevis	1 – 2	1
	flexor hallucis longus	1 – 2	1
Striatal Toe	extensor hallucis longus	1 – 2	1

Muscle Atrophy

When injected into hyperactive muscles, the paralysis induced by BoNT/A causes a reduction in diameter of muscle fibers in the targeted. When there is a muscular hypertrophy the BoNT/A normalizes muscle size. If administered for a long period of time, the BoNT/A can induce focal atrophy, but this is not a binding effect.[32]

Antigenicity

The botulinum toxins are proteins foreign to the human body and being so antibodies can be formed against the toxic portion or against its non-toxic proteins. Exposure to botulinum toxin antigens stimulate an immune response by activating B and T lymphocytes, immune memory cells, formation of cytokines and finally the formation of antibodies.[24]

The antibodies block the biological activity and induce therapeutic failure. These are called neutralizing or blocking antibodies. The antibodies formed against the non-toxic pieces of protein are called non-neutralizing.[31] Neutralizing antibodies bind to antigens of botulinum toxin reducing its effectiveness and memory cells will be triggered causing immune responses in sequential applications. This fact has high clinical relevance since repeated applications are usually necessary for the treatment of chronic conditions.[24]

Risk factors for treatment failure associated with antibodies include: the treatment dose and the interval between successive applications. The importance of dose is the correlation with the protein charge injected, and this fact is related to the formulation used. The risk is not associated with biological activity per se but with the amount and frequency with which the antigen presentes itself to the immune system.[31]

When a toxin is produced and stored changes in its conformation can lead to partial inactivation of molecules. The inactivation leads to biological activity loss, but the immune potential is maintained and therefore there may be the induction of antibody formation. Thus, the amount of inactive toxin contained in a preparation determines its immunogenic potential. The relation between biological potency and neurotoxin amount is called "specific biological activity" and is used as a parameter for determining the immune quality of a therapeutical preparation.[31]

Another risk factor for treatment failure is the immune system reaction, and this is an individual characteristic. Risk factors also include the injected tissue immune response and female gender. Cumulative dose, treatment time and age were not proven as risk factors.[31]

Chart 7 - Dosing guidelines for children[47,48]

Dosing guidelines for children
Total maximum body dose per visit = lesser of 16 Units per kg or 400 Units
Maximum dose per large muscle per visit = 6 Units per kg
Maximum dose per small muscle per visit = 1 – 2 Units per kg
Maximum dose per injection site = 50 Units
Maximum volume per site = 1.0 mL, except in select situations
Dilution: 1 – 5 mL per vial. More dilute solutions may be more effective in larger muscles.
Re-application ^{3} 3 months

Chart 8 - Children dose modifiers[47,48]

DOSE MODIFIERS		
CLINICAL SITUATION	**DOSE PER MUSCLE**	
	A dosage diminuition may be indicated if:	A dosage increase may be indicated if:
Patient weight	Low	High
Probable therapy duration	Cronic	Acute
Muscle mass	Very low	Very high
Numbers of muscles being injected simultaneosly	Many	Few
Ashworth Score	Low	Very High
Underlying voluntary control	Good	Precarious
Concern that treatment may result in excess weakness	High	Low
Previous therapy results	Too much weakness	Inadequate Response

Chart 9 - Key points to orient pediatric dosage[47,48]

KEY POINTS
Botulinum toxin type A rarely causes complications or significant adverse effects on pediatric patients
Children may benefit from ansiolitic and/or anestetic use before application
Treatment result evaluation depends on the early definition of objectives
Botulinum toxin type A effects are observed within a few days and last, on average, 3 to 4 months.
Children over 60Kg should follow adult dosage

Chart 10 – Dilutions

Units	Dilution/ml	1U	10U	Units /0.1ml
100	0,5	0,005ml	0,05ml	20U
100	1	0,01ml	0,1ml	10U
100	1,5	0,015ml	0,15ml	6,5U
100	2	0,02ml	0,2ml	5U
100	4	0,04ml	0,4ml	2,5U
100	5	0,05ml	0,5ml	2U
100	8	0,08ml	0,8ml	1,25U

An additional concept, in terms of antibody formation, is that the phenomenon of cross-reaction can occur between the serotypes of botulinum toxin and tetanus neurotoxin. Also the formation of antibodies against a second serotype is faster when there are already pre antibodies against another serotype.[24,34] Antibodies formation is a potential problem for botulinum toxin therapy, its formation should be prevented by using the lowest dose possible, together with the longest interval between two applications.[24,34]

Recommendations for preventing the formation of antibodies include control of doses and intervals between treatments, avoiding the practice of fillers or injections "boosters":[32,41]

1. Use the lowest dose possible
2. Applications interval with at least three months
3. Do not make "boosters" or reinforcement injections

The overall incidence of non-responsiveness to botulinum toxin is low, less than 10%, and incidence of neutralizing antibodies with the new formulation of Botox® is less than 1%.[16,38]

The non-responsiveness may also be due to errors in dosage, selection of patients or the injection technique with access to inappropriate muscles.[1]

Warnings and Precautions

The recommended dosages and administration frequency should not be exceeded. There were no reports of systemic toxicity resulting from accidental injection or oral ingestion. If any of these events occur, the patient should be accompanied by a doctor for several days for observation of systemic weakness or muscle paralysis signs or symptoms. The effect of botulinum toxin type A may be potentiated by aminoglycoside antibiotics or other drugs that interfere with neuromuscular transmission.

Histologically, by the action of botulinum toxin type A, occurs initially a change in the pattern of acetylcholinesterase activity, finding it more dispersed in the muscle fibers.[69] There is also atrophy of the muscle fiber, with a variation in the size of each individual fiber, the first 2 weeks.[70] In the following weeks this process can continue, but tends to stabilize and after 2 months appears an increased number of fibers of different sizes compared with controls. After four months of injection date, the pattern of cholinesterase activity and the size of the fibers return to normal.[71] Other authors, however, report that there are no significant histological changes with the use of botulinum toxin type A in long term.[57]

In an electromyographic study of muscle fibers treated with botulinum toxin A an abnormal neuromuscular transmission appears in muscles distant from the injection site, with no signs of muscle weakness, showing that the scattering power of the product may be greater than we imagine, when using high doses.[72]

Precautions

The efficacy and safety of botulinum toxin depends on the proper storage of the product, selecting the correct dose and appropriate techniques for reconstitution and application as a result of the condition being treated. Physicians who make use of this product must deeply understand the topography and functional anatomy, as well as be aware of any anatomical changes that have occurred with the patient due to prior surgical procedures. They must also meet the standard techniques of electromyography and electrical stimulation.

Chart 11 – Complications

Comparative	Rare	Described
Pain	Allergy - diffuse skin eruption (not described anaphylaxis)	Dysphagia can be followed by aspiration and pneumonia[37]
Hematoma	Focal atrophy	Functional alteration
Strength loss sensation	Diplopia, difficulty in visual accommodation	
Mild edema	Intense or generalized muscle weakness	Urinary and fecal incontinence (injection in the adductor region)[37,42]
Gastrointestinal and flu-like symptoms	Antibody formation (3-5%)	
Local infection	Altered sweating	

Botulinum toxin type A - BOTOX®, is presented in vials filled in vacuum. This presentation is a fabrication laboratory choice, not a condition for the stability of the product, as other pharmaceutical forms of botulinum toxin type A are not presented in vacuum vials. The use of vacuum is a safety factor. Beeing so, it is recommended that vials should not be used in case the vacuum was somehow affected. Nonetheless, in the

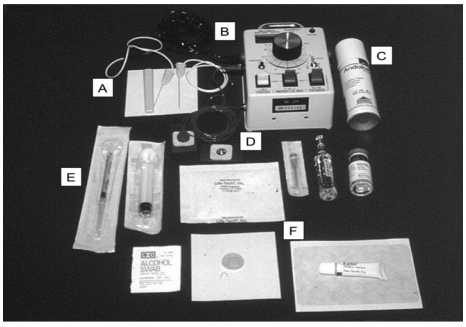


Figure 11 - Equipment required for the electrical stimulation technique (A - needles coated with insulating material, B - electroastimulator, C - refreshing spray for analgesia, D - Self-adhesive surface electrodes, E - syringes, F - sterile material and bandage)

presence of vacuum, one should be careful during dilution to avoid that the entry of saline solution occurs at speed, helped by the presence of vacuum, inducing drug turbulence and risk of possible molecules breakdown. The pseudo absence of vacuum may occur when the rubber sealing of the vial is frozen, the needle entry may cause micro cracks allowing air entrance.

Drug Interactions

The effect of botulinum toxin may be potentiated by drugs that interfere with neuro-muscular junction.[67] These are: aminoglycosides (kanamycin, gentamicin, streptomycin), calcium channel blockers, cyclosporine, aminoquineinins (chloroquine and hidrocloroquina), D-penicillamine, tubocurarine, pancuronium, gallamine and succinylcholine. Patients who use these drugs should be closely observed when treated with botulinum toxin. Caution should be exercised with patients treated with polymyxins, tetracyclines and lincomycin; the same for those using muscle relaxants. In this last case a muscle relaxant dose reduction is recommended.

Location and Injection Technique

Shaari & Sanders,[73] demonstrated in rats that injection of botulinum toxin near muscles motor points resulted in better responses to the drug. The injection 0.5 cm distant from motor point shows a 50% lower responce. One way to improve the botulinum toxin injection efficiency is to know the areas where motor points density is higher (usually aligned at the midpoints of muscle fibers) and turn them into target.[44,74] Another way to reach a larger number of motor points is increasing dilution of the toxin to be injected - 20U/ml.[74]

The choice of injection technique depends on the location of the muscles to be injected. Large superficial muscles can be injected through the palpation technique with muscle belly prick. For smaller or deeper muscles a guided technique of electromyography or electrical stimulation is recommended.[41,44]

In the electrostimulation technique, the device must be calibrated to repeated stimuli of 0.5 Hz. The intensity should be increased to cause visible muscle contractions, usually between 10 and 20 amps. This indicates that the placement of the needle is in the target muscle and should then be positioned to achieve maximum contraction. From this point on the current should be decreased, while it repositioning the needle, until getting the maximum response with the lowest current intensity that causes muscle contraction. Theoretically, this would be the point closest to the muscle motor point and the ideal injection site.[41] The equipment needed to perform this technique can be seen in Figure 11.

Especially in children the ultrasound guide technique has the advantage of locating deep and superficial muscles and being at the same time a non-invasive, painless, safe technique tha does not add stress to the patient. It is accurate but requires specific technical knowledge and it is a costly procedure.[44] The advantages and disadvantages of different injection techniques can be seen in Chart 12.

The use of combined location techniques is recommended, always including the technique of anatomical location and palpation. Especially when the required treatment is multifocal for children with cerebral palsy or if it is necessary the use of electrical stimulation, the procedure should be performed under anesthesia.[36,37]

RESULTS

Levels of recommendation by evidence-based medicine

Botulinum toxin, applied under the above criteria, for a period of 1 year, showed positive short and medium term results on measures of motion range, tone, and shows an average gain of 6% in the objectives related to the GMFM and their overall score. Children under 5 are the ones with more good results.[42] The GMFM is the scale most widely accepted today for the verification of functional benefits in children with cerebral palsy, although it is best applied to children with moderate compromising.[37]

Chart 12 - Location techniques for intramuscular injection of botulinum toxin[35]

Technique	Main Advantage	Disadvantage	Comments
Surface anatomy and palpation	Quick and easy to apply Painless	Inconsistent producitibility Does not confirm that the injection needle is at the target muscle; Is not usefull for deep muscles	May be acceptable as a single technique in case of treatment of very large or isolated muscles. Eg deltoid, quadriceps, hamstrings and braquial biceps braquillis.
Electromyography	Confirms the presence of the injection needle into hypertonic muscle Provides information related to muscular activity	Is not specific Makes no discrimination between hyperactivity of muscle and of adjacent synergistic muscle	It is an essential technique when treating spastic muscles; useful for deep muscles and can be combined with other techniques
Electrical stimulation	Confirms the presence of the injection needle into a specifc muscle or in its muscle fascicle	Cannot confirm the presence of the injection needle into hypertonic muscle Can be misleading if the injection needle is adjacent to the motor nerve branch	Essential technique when treating the muscles of the wrist, fingers and their issues.
Fluoroscopy	Useful for very deep muscles such as psoas	Fluoroscopic equipment access needed	It is not necessary for most commonly treated muscles.
Ultrasonography	Confirms the presence of the injection needle into a specific muscle	Ultrasound equipment access as well as knowledge of technique	Used in botulinum toxin injections in cases of achalasia, detrusor sphincter dyssynergia, spasticity in obese patients, in children and when there was prior tendon transfer surgery.

In a recent article of evidence-based medicine, Simpson et al[46] studied the papers published regarding the treatment of cerebral palsy with botulinum toxin. Six studies class I were selected (American Academy of Neurology - Appendix I), 3 made with Botox® formulation and 3 with Dysport®, which together totaled 376 patients treated. The evaluation criteria were based on the Ashworth scale for muscle tone, range of motion for passive function, gait, video documentation in kinematic gait analysis and the Global Disability Scale for activities.

Adverse events were pain, muscle weakness, tendency to fall, incontinence and dysphagia (a work with Botox® and 3 with Dysport®). The authors[46] found the best evidence for the treatment of equinus foot and conclude for the moment:

1. It is established that injection of botulinum toxin for gastrocnemius is effective for the treatment of equinus foot in cerebral palsy patients.
2. There is insufficient evidence to support or refuse the benefit gained by the use of plaster in the treatment after botulinum toxin injection for the gastrocnemius and soleus muscles, as well as injections in the hamstrings.
3. In patients with adductor spasticity, botulinum toxin injection is probably effective in improving arch of movement as well as decrease pain in post-operative lengthening surgery.
4. In the upper limbs the injection is probably effective in improving spasticity range of motion.

Based of the conclusions above, the authors Simpson et al[46] recommend for the moment:

1. The botulinum toxin injection in the calf can be offered as treatment for equinus foot in children with cerebral palsy. *(Level of Evidence A)*
2. The botulinum toxin injection should be considered as a treatment option for spasticity of the adductors and to control pain after lengthening surgery of the adductors in spastic cerebral palsy patients. *(Level of Evidence B)*
3. The botulinum toxin injection should be considered as a treatment option for upper limb spasticity in patients with spastic cerebral palsy. *(Level of Evidence B)*

So,

Conclusion	Recommendation
Stablished as safe and effective	**A** BOTOX ® e DYSPORT®

In sum according to the European Consensus 2009,[75] for the use of botulinum toxin in children with cerebral palsy, we have:

1. Treatment Indication - must be stablished to each severity degree.
2. Objective - correction of spastic dynamic misalignment of one or more joints (multilevel).
3. Principle - Local inhibition of acetylcholine release in the nerve endings management and muscles motor branches to reduce tone in the injected muscle (dose dependent). Reduction of muscle shortening by approximately 20%. Effect lasts for about 3-6 months (or more). Adhesion of ½ to 1/3 of patients treated 1-3 times a year. Inhibition local acetylcholine release in the management of nerve endings, and plates.
4. Examples - GMFCS I-III: *Functional Indication*: reduction of muscular hypertonia and prevention of the imbalance between flexors and extensors, responsible for deformities in upper and lower limbs. *Structural indication*: delaying the development of contractures and improving orthosis tolerance. *GNFCS IV-V: Functional Indicationt:* rare but potential to improve the operation of auxiliary equipment. *Structural Indication*: to reduce pain, simplify care, improve orthosis tolerance and reduce salivation.
5. Limitations and controversies - is a focal treatment for a non-focal disease, potential for action at distance and drug systemic action only work in dynamically active muscles. The action on the muscle and on its control circuits is partially understood. It is not an approved treatment in all countries.

CONCLUSIONS

Chemical blocks are now part of the spasticity treatment. They may be performed most commonly with Botulinum Toxin Type A and / or phenol. There is a tendency to consider mixed blocking (botulinum toxin type A associated with the phenol) to treat a greater number of muscles with lower or same Botulinum Toxin Type A dose.

The results indicate that the combined treatment of chemical blocks with plaster casts, orthoses and physical therapy improve the chances of reaching the treatment goals, increasing duration of treatment effects and improving the quality of patient's life. The reversal of the pathological process induced by spasticity can result in a modification of the underlying disease effects. In children this is extremely important because of the possibility to postpone or avoid surgical treatments.

The clinical experience tells us that the treatment with Botulinum Toxin Type A can reduce spasticity, increase voluntary movement and improve function in selected patients, although clinical trials have difficulty in showing functional improvements related to the decrease in spasticity. Botulinum Toxin Type A treatment reduces spasticity measured by Ashworth scale, reduces pain, spasms and other symptoms associated with upper motor neuron syndrome. This leads to an improvement in some functional objectives such as hygine, dressing, positioning and etc.

Physicians who wish to perform chemical blocks need to be trained specifically for this purpose.

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ATTACHMENT 1

AAN classification of evidence for therapeutic intervention

Class I: Randomized, controlled clinical trial with masked or objective outcome assessment in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences. The following are required: a) concealed allocation, b) primary outcome(s) clearly defined, c) exclusion/inclusion criteria clearly defined, and d) adequate accounting for drop-outs (with at least 80% of enrolled subjects completing the study) and cross-overs with numbers sufficiently low to have minimal potential for bias.

Class II: Prospective matched group cohort study in a representative population with masked outcome assessment that meets b-d

above OR a RCT in a representative population that lacks one criteria a-d.

Class III: All other controlled trials (including well-defined natural history controls or patients serving as own controls) in a representative population, where outcome is independently assessed, or independently derived by objective outcome measurement.*

Class IV: Studies not meeting Class I, II or III criteria including consensus, expert opinion or a case report.

*Objective outcome measurement: an outcome measure that is unlikely to be affected by an observer's (patient, treating physician, investigator) expectation or bias (e.g., blood tests, administrative outcome data).

Classification of Recommendations

A= Established as effective, ineffective or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population. (Level A rating requires at least two consistent Class I studies).*

B= Probably effective, ineffective or harmful (or probably useful/predictive or not useful/predictive) for the given condition in the specified population. (Level B rating requires at least one Class I study or at least two consistent Class II studies).

C= Possibly effective, ineffective or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population. (Level C rating requires at least one Class II study or two consistent Class III studies).

U= Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven. (Studies not meeting criteria for Class I – Class III).

*In exceptional cases, one convincing Class I study may suffice for an "A" recommendation if 1) all criteria are met, 2) the magnitude of effect is large (relative rate improved outcome > 5 and the lower limit of the confidence interval is > 2).