Pharmacological differences and clinical implications of various botulinum toxin preparations: a critical appraisal

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Summary

Three different type A botulinum neurotoxins (BoNT\textsubscript{As}) — onabotulinumtoxinA, abobotulinumtoxinA and incobotulinumtoxinA) — are currently marketed in Europe to treat several conditions. Differences between BoNT\textsubscript{A} preparations, which depend on their specific biotypes and manufacturing processes, lead to clinically relevant pharmacotherapeutic dissimilarities. All three available products are separately recognized and reviewed in American Academy of Neurology guidelines. The neurotoxin load/100U is likewise different among the different BoNT\textsubscript{As}, with the result that the specific potency of the 150kD BoNT\textsubscript{A} neurotoxin is calculated as 137 units/ng for onabotulinumtoxinA, 154 units/ng for abobotulinumtoxinA, and 227 units/ng for incobotulinumtoxinA. It is important for clinicians to have all three BoNT\textsubscript{As} available in order to choose the most suitable preparation for the specific indication in the single patient. Commercially available BoNT\textsubscript{As} must be recognized as different from one another, and therefore as non-interchangeable. The essential experience of the clinician is of the utmost importance in choosing the most appropriate treatment.

KEY WORDS: bio-equivalence, botulinum neurotoxin, commercial preparations, potency.

Introduction

Produced by \textit{Clostridium botulinum}, botulinum neurotoxins (BoNT\textsubscript{s}) are bacterial exotoxins that interfere with the exocytotic release of vesicular neurotransmitters. BoNT\textsubscript{s} are able to block cholinergic neuromuscular activity as well as sensory feedback to the central nervous system and, accordingly, act on both motor and sensory neurons depending on the target tissue (Kumar et al., 2016). Differential effects of BoNT\textsubscript{s} on excitatory and inhibitory neurons thus provide a unique therapeutic tool. At their site of action, BoNT\textsubscript{s} are cleaved into heavy and light chains by tissue proteinases. The heavy chain of each BoNT serotype binds to its specific neuronal ecto-receptor; this leads to its membrane translocation and endocytosis by intracellular synaptic vesicles. Instead, the light chain acts through different proteins of the soluble NSF (N-ethylmaleimide-sensitive fusion protein) attachment protein receptor (SNARE) complex (i.e., SNAP-25 for BoNT\textsubscript{A} and E, VAMP-2 for BoNT\textsubscript{B}, D, F and G, and both SNAP-25 and syntaxin-1a for BoNT\textsubscript{C}) to inhibit synaptic exocytosis, thus disabling neural transmission. While the action of BoNT in blocking the release of acetylcholine at the neuromuscular junction is well known, this action alone is likely inadequate to fully explain its apparent analgesic activity. Therefore, other receptors and transmitters are believed to be involved (Wheeler and Smith, 2013). For example, in bladder overactivity (OAB) it has been shown that botulinum toxin type A (BoNT\textsubscript{A}) reduces expression of the vanilloid receptor-1 (TRPV1) in primary dorsal root ganglion neurons, and also of purinergic receptor P2X3. Reduced TRPV1 expression correlates with reduced sensitivity, while the reduction of P2X3 expression correlates with reduced urgency (Apostolidis et al., 2005). Since they are able to reduce muscle contraction, BoNT\textsubscript{s} are useful in the treatment of overactive muscle conditions such as dynamic contraction. BoNT\textsubscript{A} was first successfully utilized 40 years ago in humans affected by strabismus (Scott, 1980). BoNT\textsubscript{A}, which is also the most potent BoNT, went on to be clinically developed during the 1980s, and the first BoNT\textsubscript{A}, onabotulinumtoxinA, was approved by the US Food and Drug Administration (FDA) in 1989 for the treatment of strabismus and blepharospasm associated with dystonia. Initially, clinical use of BoNT\textsubscript{A} preparations was based solely on their potent inhibitory effect on acetylcholine release at the neuromuscular junction, inducing local muscle relaxation. As clinical awareness of their potential applications has increased, their spectrum of clinical use has broadened considerably. At present, different BoNT\textsubscript{A} products are used to treat a range of conditions, including blepharospasm, hemifacial spasm, cervical dystonia, post-stroke upper and lower limb spasticity, dynamic equinus foot in children with cerebral palsy (CP), axillary hyperhidrosis, chronic migraine (CM), and urinary incontinence of both neurogenic and non-neurogenic origin. The three main BoNT\textsubscript{A} products currently marketed worldwide are onabotulinumtoxinA (Botox\textsuperscript{®}, Allergan Inc., Irvine, CA, USA), abobotulinumtoxinA (Dysport\textsuperscript{®}, Ipsen Ltd, Slough,
UK), and incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany). There exist important pharmacological differences between BoNTA preparations, which affect clinical activity, and the availability of different products has given rise to intense debate on dose equivalence and comparative effectiveness (Bry et al., 2014). This was acknowledged in 2009 by the FDA, which stated that “Potency units are specific to each single BoNT product and doses or biologic activity units cannot be compared or converted from one product to the other.”

In 2016, the American Academy of Neurology (AAN) updated its guidelines on the clinical use of BoNTs (Simpson et al., 2016), reviewing available products and assessing formulations for each indication separately. As a consequence, efficacy levels for different BoNT formulations were lower than if BoNTs were considered as a homogeneous category. Indeed, each BoNT product has its own indications and dosage, and all respective summaries of product characteristics (SmPCs) clearly state that BoNT units are specific for each product and not interchangeable. Furthermore, it has to be considered that biological drug manufacturing is a highly complex process, and it is now widely acknowledged that even minor changes in production may lead to significant variations in the final product that potentially affect safety and efficacy. This aspect has clearly emerged in recent years for biosimilars, whose similarity to the respective biological originators must be demonstrated through a comprehensive ‘comparability exercise’ (conducted at all manufacturing and development levels), and whose effective interchangeability is still debated (for example by the European Medicines Agency). In addition, the Italian State Council has recognized, with regard to biotechnology drugs, that each manufacturer’s product has a unique efficacy and safety profile, and there is no evidence of an acceptable level of replicability. Therefore, the similarity of a biosimilar to its originator molecule must be demonstrated on the basis of the principle of therapeutic equivalence.

We herein review the differences between BoNTA products and explore the reasons why they cannot be considered interchangeable.

Pharmacology

Structure

Botulinum toxins are a family of proteins that possess seven related but immunologically distinct serotypes. They are originally synthesized as a single inactive polypeptide chain of about 150 kilodalton (kDa); the polypeptide is activated upon cleavage by tissue proteinases, which results in the formation of an active di-chain molecule comprised of a 100 kDa heavy chain and a 50 kDa light chain, which are held together by a single disulfide bond. BoNT is made up of the botulinum (Bo) neurotoxin (NT) and non-toxic proteins called neurotoxin accessory proteins (NAPs). NAPs help to maintain the structure of the NT and greatly affect its overall size (Dressler and Benecke, 2007). There are seven BoNT serotypes (A, B, C, D, E, F and G), each including further subtypes; the A serotype includes four distinct subtypes. All the serotypes (except subtype C2) are neurotoxins and all inhibit acetylcholine release, although their intracellular target proteins, the specific characteristics of their actions, and their potencies vary substantially. Two BoNTs have been authorized for clinical use, serotypes A and B. BoNTA has historically been the most widely studied serotype for therapeutic purposes. OnabotulinumtoxinA and abobotulinumtoxinA contain neurotoxin protein complexes, although of different sizes, whereas incobotulinumtoxinA contains only the ~150 kDa neurotoxin, which is devoid of any NAPs (Bry et al., 2014). There are five BoNTA NAPs (4 different hemagglutinins and 1 non-hemagglutinin), which are synthesized by Clostridium botulinum and protect the neurotoxin from degradation (Chen et al., 1998).

Mechanism of action

The mechanism of action of BoNTAs, as reviewed by Foster et al. (2006), also implies the delivery and incorporation of integral membrane proteins into the plasma cell membrane. BoNTAs generally act at a pre-synaptic level by blocking the release of acetylcholine at the neuromuscular junction. They also block exocytosis of substances involved in pain, such as glutamate, glycine, substance P, calcitonin gene-related peptide (CGRP), noradrenaline, dopamine, and ATP. These aspects, in addition to the muscle relaxant and atrophic effect associated with BoNTs, can contribute to providing relief from some pain syndromes (Pirazzini et al., 2017). The heavy chain of each BoNT serotype binds to its neuronal ecto-acceptor. Acceptors are present on cholinergic and sensory neurons, and each BoNT serotype binds to a specific acceptor, and does not inhibit the binding of other serotypes to their acceptors. The different binding sites may be differentially distributed on cells, resulting in potential differences in their relative sensitivity to various serotypes.

Once bound to the acceptor, the neurotoxin is internalized by endocytosis. Following acidification in the endosome, the heavy chain forms a channel in the endosomal membrane and the disulfide bond holding the two chains together is reduced. Once freed, the light chain is translocated into the cytosol where it exerts its proteolytic activity on the protein component of the SNARE complex; internalization is maximal (in vitro at least) after about 90 minutes at 22°C (Black and Dolly, 1986). Inhibition of the release of SNARE complex regulates the fusion of synaptic vesicles with presynaptic membrane and the release of acetylcholine. In SNARE complex, synaptobrevin, a vesicle-associated membrane protein (VAMP), bonds with the membrane-associated protein syntaxin to bring about exocytosis. This association can be direct or mediated by other polypeptides such as SNAP-25 (25 kDa, synaptosomal-associated protein), NSF (N-ethyl-maleimide-sensitive factor) or alpha-, beta- and gamma-SNAP (soluble NSF attachment proteins) (Fig. 1) (Dickerson and Janda, 2006).

The different serotypes of the toxin have specific molecular targets. The A toxin catalyzes proteolysis of SNAP-25 between glu 197 and arg 198, while the B toxin catalyzes proteolysis of VAMP between glu 76 and phe 77. Serotypes D, F and G also act on VAMP, while serotypes C1 and E act on SNAP-25 (Setier, 2000). Proteolytic activity of these toxins inhibits the formation of
functional SNARE complexes, thus preventing adhesion of vesicles and their fusion with presynaptic membrane, and concomitant exocytosis of acetylcholine. Even though all seven serotypes have the same biological effect, namely inhibition of acetylcholine release, it should be highlighted that serotypes A and B have different molecular targets (SNAP-25 and VAMP, respectively). This may also help to explain the differences in clinical profiles of the various toxins, particularly in non-neuromuscular locations.

Manufacturing process of BoNTAs

A main reason why BoNTA products are not interchangeable is related to their biological nature. Secondary and tertiary structures are critical to the biological activity of BoNTAs and even small differences in the manufacturing process can alter their clinical profile. The structural and biochemical complexity of these products and their sensitivity to manufacturing methods have prompted manufacturers of biologicals to coin the phrase “the process is the product” (Schellekens, 2004). Schantz and Johnson (1992) were the first to describe the production and formulation of BoNTAs for clinical use. The manufacturing processes of BoNTAs, as well as the analytical specifications and methods for assessment of biological activity, are specific for each of the marketed products. Given that the manufacturing process (from the Clostridium strain to purification and biological assessments) is different from one manufacturer to another, the different BoNTA preparations (made using completely different processes) and their doses are not equivalent (Hellman and Torres-Russotto, 2015). Therefore, their benefits, side effects and treatment indications are not interchangeable.

Manufacturing of onabotulinumtoxinA

OnabotulinumtoxinA is produced by Schantz’s crystallization method (Schantz and Johnson, 1992) which leads to the formation of a 900 kDa homogeneous complex (Table I) formed by the isolated 150 kDa neurotoxin and NAPs (Hambleton, 1992, Inoue et al., 1996). Various strains of Clostridium produce protein complexes of different sizes, and all type A strains produce complexes of ~300 kDa, ~500 kDa and ~900 kDa (Odergren et al., 1998), while none produce the ~150 kDa peptide without NAPs. The NAPs in onabotulinumtoxinA include non-toxic hemagglutinins that stabilize the biological activity of the product in vivo and enable the complex to adhere to muscle tissue (Johnson and Br Mashaw, 2001). Finally, onabotulinumtoxinA is vacuum dried, i.e., the liquid is removed under reduced pressure without a freezing phase.
Manufacturing of abobotulinumtoxinA

AbobotulinumtoxinA is obtained by chromatographic purification as a product with an average molecular weight (MW) of 400 kDa (Table I), which, like onabotulinumtoxinA, also contains neurotoxin protein complexes (although these differ in size in the two products) (Inoue et al., 1996). AbobotulinumtoxinA is prepared by freeze-drying, another method that involves a freezing phase (Dressler et al., 2012).

Potency of BoNTs

The biological activity of the various toxins, i.e. their potency, is expressed in units of biological activity, and corresponds to the median lethal dose (LD50) obtained by intraperitoneal injection in mice (Odergren et al., 1998). As a replacement for this animal LD50 test, Allergan and Merz have implemented optimized cell-based potency assays (Fernandez-Salas et al., 2012; https://www.merz.com/blog/news/alternative-test-method-for-botulinum-neurotoxin-now-approved-in-europe/). However, each manufacturer uses its own lethality test and a unique product-specific reference standard for biological activity testing.

Similarly, the neurotoxin load (content in ng per100U) is different between the different BoNTAs (0.73 ng/100U for onabotulinumtoxinA, 0.65 ng/100U for abobotulinumtoxinA, 0.44 ng/100U for incobotulinumtoxinA) with the result that the specific potency of the 150kD BoNTA neurotoxin is calculated as 137 units/ng for onabotulinumtoxinA, 154 units/ng for abobotulinumtoxinA, and 227 units/ng for incobotulinumtoxinA (Frevet, 2010).

Therefore, biological activity units are specific to each BoNTA and not interchangeable. This was demonstrated in a study that evaluated incobotulinumtoxinA and onabotulinumtoxinA in the Allergan LD50 assay (Hunt and Clarke, 2009). When BoNTA was evaluated against the Allergan 100-unit standard, under Allergan assay conditions, the activity of incobotulinumtoxinA was less than 100 Allergan units (i.e., 69 to 78 units over three different tested lots). These results were replicated in other assays (Brown et al., 2013), confirming that, due to underlying product differences, assay conditions markedly influence potency measurements. In a mouse model, the potency of BoNT products in inducing hind limb paresis resulted in an estimated conversion ratio of incobotulinumtoxinA and onabotulinumtoxinA of between 1: 0.75 and 1: 0.5 (Kutschenko et al., 2016). This was confirmed by recent in vivo studies showing that, on a labeled unit-to-unit basis, onabotulinumtoxinA displayed greater biological activity and potency than incobotulinumtoxinA (Canty et al., 2017). Moreover, the mean digit abduction score (DAbS) of median effective dose (ED50) potency of incobotulinumtoxinA (ED50 range 7.0–10.2 U/kg) was significantly lower than that of onabotulinumtoxinA (ED50 range 4.4–6.4 U/kg), consistent with lower measured potencies in the LD50 assay for incobotulinumtoxinA (potency range 62-82 U) (Brown et al., 2013). The extent and duration of the biological effect of BoNT in humans have been tested using the frontalis test or the extensor digitorum brevis test for all BoNT products (Marion et al., 2016).

Pharmacological safety

Preclinical models (Aoki, 2002, Aoki et al., 2005, Foster et al., 2006) have indicated significant differences in the safety margins of various commercial BoNT preparations. The largest safety margin, calculated as the lethal dose/effective dose ratio (LD50/ED50), has been observed for onabotulinumtoxinA (19.8 ± 3.38), followed by abobotulinumtoxinA (10.3 ± 1.09), and then a type B preparation, rimabotulinumtoxinB (Myobloc®, 4.35 ± 0.39). Such differences may have important implications for current clinical use. The amplitude of the safety margins is superimposable on that of the diffusion margins, with the highest diffusion margin meaning the lowest migration outside target muscle; onabotulinumtoxinA has the highest margin (6.8), followed by abobotulinumtoxinA (1.0) and rimabotulinumtoxinB (0.4) (Aoki, 2002). In clinical practice, an even higher conversion ratio of 1.3 or more is most frequently used. Using such a conversion ratio, abobotulinumtoxinA has a more pronounced clinical effect, a longer effect duration and is associated with a higher incidence of side effects compared with incobotulinumtoxinA and onabotulinumtoxinA (Kutschenko et al., 2016).

These preclinical results are consistent with clinical reports for different BoNT preparations that document

Table I - Characteristics of commercially available BoNTAs (adapted from SIF).

<table>
<thead>
<tr>
<th></th>
<th>OnabotulinumtoxinA</th>
<th>AbobotulinumtoxinA</th>
<th>IncobotulinumtoxinA</th>
</tr>
</thead>
<tbody>
<tr>
<td>First approval</td>
<td>1989 (US)</td>
<td>1991 (UK)</td>
<td>2005 (Germany)</td>
</tr>
<tr>
<td>Production process</td>
<td>Crystallization</td>
<td>Chromatography</td>
<td>Chromatography</td>
</tr>
<tr>
<td>Stabilization process</td>
<td>Vacum dried</td>
<td>Lyophilization</td>
<td>Lyophilization</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>~900 kDa</td>
<td>~400 kDa</td>
<td>~150 kDa</td>
</tr>
<tr>
<td>Quantity of neurotoxin (ng protein/100 U)</td>
<td>~0.73ng/100 U</td>
<td>~0.65 ng/100 U</td>
<td>~0.44 ng/100 U</td>
</tr>
<tr>
<td>Excipient</td>
<td>NaCl</td>
<td>Lactose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Units/vial</td>
<td>100 U</td>
<td>500 U</td>
<td>100 U</td>
</tr>
<tr>
<td>Storage</td>
<td>36 months at 2-8°C</td>
<td>24 months at 2-8°C</td>
<td>48 months at &lt;25°C</td>
</tr>
<tr>
<td>Storage after reconstitution</td>
<td>Up to 24 h at 2-8°C</td>
<td>Up to 8 h at 2-8°C</td>
<td>Up to 24 h at 2-8°C</td>
</tr>
</tbody>
</table>
Differences between botulinum toxin preparations

Differences between botulinum toxin preparations (Bihari, 2005; Sampaio et al., 2004). In accordance with a fundamental principle of diffusion, BoNT protein complexes of higher MW will persist longer at the injection site and leak outside target muscle more slowly than those of lower MW. In reality, therapeutic indexes of the BoNTAs are ordered according to the MWs of the different BoNT complexes: the onabotulinumtoxinA preparation, containing the complex with the highest MW, has the highest therapeutic index among the BoNTAs (Aoki, 2001).

These results are important to counter the erroneous idea that different BoNT products can be converted by linear dose ratios. Animal models of muscle weakening have shown non-parallel dose-response curves, which indicate that dose ratio changes depend on the level of muscle-weakening efficacy or on specific mortality rates (Aoki, 2001). A more recent study that used DABs to compare the ED50 of different BoNTAs showed that a DABs of 2 could be obtained with 6 U of onabotulinumtoxinA and 10 U of incobotulinumtoxinA (Brown et al., 2013). Overall, onabotulinumtoxinA showed greater efficacy and longer effect duration than incobotulinumtoxinA at both high and low dosages in the DABs model (Brown et al., 2013).

According to the Italian Society of Pharmacology (SIF), in its 2013 document on BoNTs, all of the above-summarized data indicate that onabotulinumtoxinA shows the highest efficacy and safety margin compared with other BoNTAs, and in any case, no BoNTAs are either equivalent or interchangeable (SIF, 2013).

Clinical trials

Blepharospasm, hemifacial spasm and cervical dystonia

Comparative data on the efficacy and safety of available BoNTs are limited by the scope and size of the existing studies, the absence of uniform methodologies, and the lack of a suitable dose conversion between different BoNTA formulations. Several studies comparing onabotulinumtoxinA with abobotulinumtoxinA have attempted to determine a dose-conversion ratio that could render the products interchangeable. Ratios used in comparative studies with onabotulinumtoxinA and abobotulinumtoxinA are indicated in Table II.

In a study by Marion et al., 74 patients with blepharospasm (n=37) or hemifacial spasm (n=37), with good response to repeated cycles of abobotulinumtoxinA for

<table>
<thead>
<tr>
<th>Indication (Author)</th>
<th>OnabA: AbobA ratio</th>
<th>Relative incidence of adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blepharospasm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marion et al., 1995</td>
<td>1:3</td>
<td>NA</td>
</tr>
<tr>
<td>Nussgens and Roggenkamper, 1997;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roggenkamper et al., 2006</td>
<td>1:4</td>
<td>AbobA&gt;OnabA</td>
</tr>
<tr>
<td>Sampaio et al., 1997</td>
<td></td>
<td>AbobA&gt;OnabA</td>
</tr>
<tr>
<td>Bihari, 2005</td>
<td>1:4 – 1:5</td>
<td>NA</td>
</tr>
<tr>
<td>Bentivoglio et al., 2012</td>
<td>1:1 – 1:3.3</td>
<td>NA</td>
</tr>
<tr>
<td>Dodel et al., 1997</td>
<td>1:4 – 1:6</td>
<td>AbobA&gt;OnabA</td>
</tr>
<tr>
<td><strong>Cervical dystonia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naumann et al., 2003</td>
<td>1:5 – 1:6</td>
<td>AbobA=OnabA</td>
</tr>
<tr>
<td>Odergren et al., 1998</td>
<td>1:3</td>
<td>AbobA=OnabA</td>
</tr>
<tr>
<td>Ranoux et al., 2002</td>
<td>1:3 – 1:4</td>
<td>AbobA&gt;OnabA</td>
</tr>
<tr>
<td>Bihari, 2005</td>
<td>1:4 – 1:5</td>
<td>NA</td>
</tr>
<tr>
<td>Misra et al., 2012</td>
<td>3:1:1</td>
<td>AbobA&gt;OnabA</td>
</tr>
<tr>
<td>Rystedt et al, 2015</td>
<td>1:7:1</td>
<td>NA</td>
</tr>
<tr>
<td>Yun et al, 2015</td>
<td>2:5:1</td>
<td>AbobA=OnabA</td>
</tr>
<tr>
<td>Dodel et al., 1997</td>
<td>1:4 – 1:6</td>
<td>AbobA&gt;OnabA</td>
</tr>
<tr>
<td>Van den Bergh and Lison, 1998</td>
<td>1:2.5</td>
<td>AbobA=OnabA</td>
</tr>
<tr>
<td><strong>Hemifacial spasm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marion et al., 1995</td>
<td>1:3</td>
<td>NA</td>
</tr>
<tr>
<td>Bihari, 2005</td>
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<td>Van den Bergh and Lison, 1998</td>
<td>1:2.5</td>
<td>AbobA&gt;OnabA</td>
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<tr>
<td><strong>Spasticity</strong></td>
<td></td>
<td></td>
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<tr>
<td>Rasmussen, 2000</td>
<td>1:4</td>
<td>NA</td>
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<tr>
<td>Bhakta et al., 1996</td>
<td>1:4 – 1:5</td>
<td>NA</td>
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<tr>
<td>Hesse et al., 2012</td>
<td>1:5</td>
<td>NA</td>
</tr>
<tr>
<td>Keren-Capelovitch, et al, 2010</td>
<td>1:2.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: OnabA=OnabotulinumtoxinA; AbobA=AbobotulinumtoxinA; NA=not available/not applicable
at least 12 months, were administered increasing doses of onabotulinumtoxinA until a similar response to that obtained with abobotulinumtoxinA was observed. The dose ratio between onabotulinumtoxinA and abobotulinumtoxinA was 1:3 (Marion et al., 1995). Two additional double-blind studies of more than 300 patients with blepharospasm or hemifacial spasm compared onabotulinumtoxinA and abobotulinumtoxinA at a dose ratio of 1:4 and observed no significant differences in clinical efficacy or effect duration (Nussgens and Roggenkamper, 1997; Sampaio et al., 1997). However, in one of these two studies (Sampaio et al., 1997), the abobotulinumtoxinA group required more booster injections than the onabotulinumtoxinA one.

The REAL DOSE (Retrospective Evaluation of the Dose of Dysport and BOTOX in the Management of Cervical Dystonia and Blepharospasm) study, a multinational, observational, retrospective study, analyzed 114 patient records, 70 of patients with cervical dystonia and 44 of patients with blepharospasm (Marchetti et al., 2005). Ratios of mean dose for abobotulinumtoxinA to onabotulinumtoxinA ranged from a low of 2:1 to a high of 11:1; 31% of patients fell into the 5:1 to less than 6:1 group; 30% of patients had a mean ratio of 4:1 to less than 5:1; only 21% belonged to the 3:1 to less than 4:1 grouping.

The Authors concluded that the results are consistent with labeling for BoNTAs, stating that units of different preparations are not interchangeable and simple dose-conversion factors are not applicable. A retrospective analysis of patients suffering from blepharospasm and hemifacial spasm who received two treatments in consecutive sessions (i.e. switching one brand with the other), with overlapping clinical outcome, revealed a wide range of dose ratios. Only 51 treatment matches out of 2006 were found. The ratio for abobotulinumtoxinA to onabotulinumtoxinA was extremely variable, ranging from 1.2 to 13.3. According to the authors, this broad variability is due to the fact that “a true bioequivalence between the two BoNTAs might not exist for the intrinsic difference in their pharmacokinetic properties” (Bentivoglio et al., 2012).

A double-blind cross-over study lasting three consecutive periods, conducted in 54 patients with cervical dystonia, tested two dose ratios of abobotulinumtoxinA to onabotulinumtoxinA, i.e., 3:1 and 4:1 (Ranoux et al., 2002). In this study, abobotulinumtoxinA at both doses was superior to onabotulinumtoxinA, in terms of both symptom reduction and duration of effect, but accompanied by a higher incidence of AEs (36% and 33% in the 3:1 and 4:1 groups, respectively, vs 18% in the onabotulinumtoxinA group), mainly asthenia, dysphagia and dysphonia. More recently, a double-blind, randomized crossover trial evaluating abobotulinumtoxinA and onabotulinumtoxinA with two different dose conversion ratios (1:3 and 1:1.7) in patients with cervical dystonia did not show any differences over a short period, but the effect of onabotulinumtoxinA at the lower dose was reduced after 3 months (Rystedt et al., 2015). At week 12, a statistically significant difference in efficacy between abobotulinumtoxinA and onabotulinumtoxinA (1:3) was observed, suggesting a shorter duration of effect for the latter when this ratio (at low dose) was used. Two studies have compared onabotulinumtoxinA with incobotulinumtoxinA (Benecke et al., 2005; Roggenkamper et al., 2006), and both demonstrated similar efficacy and tolerability at a 1:1 dose ratio. AbobotulinumtoxinA was not inferior to onabotulinumtoxinA in patients with cervical dystonia at a conversion factor of 2.5:1 (Yun et al., 2015). All these data are summarized in the recent AAN guidelines (Simpson et al., 2016).

A direct comparison of onabotulinumtoxinA and incobotulinumtoxinA in the treatment of benign essential blepharospasm, performed using a split-face technique, showed no differences between the two products in either subjective or objective measures (Saad and Gourdeau, 2014). More recently, results of a TRUDOSE pilot study suggested that onabotulinumtoxinA and incobotulinumtoxinA for cervical dystonia and blepharospasm are not equivalent and do not support the 1:1 dose ratio. Average total dose ratios of incobotulinumtoxinA to onabotulinumtoxinA were 1.21 for cervical dystonia and 1.27 for blepharospasm. The distribution of the observed ratios (the majority being greater than 1:1) suggested that no single fixed dose ratio exists and dosing should be based on individual patient needs rather than on a specific dose ratio, as reported in the product labeling.

Another study comparing patient preferences between onabotulinumtoxinA and incobotulinumtoxinA in the treatment of benign essential blepharospasm reported that patients who preferred incobotulinumtoxinA over onabotulinumtoxinA believed it to be more effective, whereas those who preferred onabotulinumtoxinA concluded that it lasted longer. This study also demonstrated that patients who preferred incobotulinumtoxinA over onabotulinumtoxinA at a significantly shorter treatment interval (10.2 weeks vs 13.0 weeks) (Chundury et al., 2013). With regard to long-term treatments, Kollewe et al., (2015) compared efficacy and adverse effect data on 288 blepharospasm-affected patients treated with one of the three BoNTAs for at least eight consecutive treatments. Even though none of their findings revealed significant differences between BoNTAs, the effective dose of BoNT was significantly different between onabotulinumtoxinA (47±10U) and incobotulinumtoxinA (62±11U), and between onabotulinumtoxinA and abobotulinumtoxinA (120±35U). This resulted in a conversion ratio between onabotulinumtoxinA and incobotulinumtoxinA of 1:1.2.

An additional assessment comparing the efficacy and costs of onabotulinumtoxinA and incobotulinumtoxinA for the treatment of hemifacial spasm, blepharospasm and cervical dystonia was carried out by Juarez et al. (2011) in 393 patients. This study collected and analyzed one-year data after patients switched from onabotulinumtoxinA to incobotulinumtoxinA. It was found that the incobotulinumtoxinA treatment average doses were higher than the onabotulinumtoxinA treatment average doses, with increases of 9.6% for hemifacial spasm, 17.0% for blepharospasm, and 16.8% for cervical dystonia. In addition, the number of injections was increased by 25.9%, 36.3 and 6.5% for hemifacial spasm, blepharospasm and cervical dystonia, respectively. In comparison to the previous year of onabotulinumtoxinA treatment, total annual consumption of incobotulinumtoxinA in units was increased by 33.3, 41.7 and 25.8 for hemifacial spasm, blepharospasm, and cervical dystonia, respectively. These findings are consistent with previous studies that demonstrated higher potencies of onabotulinumtoxinA when assayed against incobotulinumtoxinA.
Spasticity

A 2009 European Consensus on the use of onabotulinumtoxinA and abobotulinumtoxinA in adult spasticity states that BoNTA “preparations are manufactured by different processes, have different formulations and potencies, which are determined by different biological assays. This results in potency differences of ‘units’ used for each preparation. Since there is no simple or accurate way of converting unit potency of one preparation to another, it is important that clinicians are familiar with the characteristics and dosages of each preparation and do not try to convert or extrapolate from one preparation to another”. They further state that, due to these considerations, “whichever preparation is used, dosages, dilutions and injections should be tailored to each individual patient” (Simpson et al., 2016). Moreover, in the AAN Practice Guidelines, evidence-based conclusions and recommendations were as follows: Upper Limb: onabotulinumtoxinA, onabotulinumtoxinA, incobotulinumtoxinA: level A; Lower Limb: abobotulinumtoxinA, onabotulinumtoxinA: level A; incobotulinumtoxinA: level U. There is insufficient evidence to indicate that any one of the BoNT formulations is superior to the others (Simpson et al., 2016).

From the SmPCs of onabotulinumtoxinA and incobotulinumtoxinA, notable differences emerge in the recommended doses of these two drugs for obtaining a therapeutic effect in post-stroke upper limb spasticity. The onabotulinumtoxinA: incobotulinumtoxinA ratio varies, ranging from 1:1.5 to 1:2.5 according to different treated hand muscles (SIF, 2013). At least two studies have demonstrated the consistent efficacy of onabotulinumtoxinA in reducing upper limb tone (Kaji et al., 2010; Marciniak et al., 2012). Disability Assessment Scale scores were better only in patients in whom improved limb position and dressing were chosen as principal treatment goals. With regard to lower limb spasticity, only onabotulinumtoxinA and abobotulinumtoxinA have this as an approved indication, while evidence is insufficient for incobotulinumtoxinA: level A; Lower Limb: abobotulinumtoxinA, onabotulinumtoxinA: level A; incobotulinumtoxinA: level U. There is insufficient evidence to indicate that any one of the BoNT formulations is superior to the others (Simpson et al., 2016).

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In conclusion, considering the high range of intra- and inter-patient variability, doses must be set for each BoNT preparation and for each patient. In studies examining active function, assessment is highly variable, and results are not comparable. In 23 studies on the efficacy and safety of BoNT for treatment of upper limb post-stroke spasticity, at least 25 different measures were used to assess the outcome, including a wide series of impairment measures (Kaku and Simpson, 2016). Most studies focused on reduction of muscle tone and demonstrated efficacy, but only a few measured changes in gait, velocity, functional abilities, or social participation. To compare the clinical efficacy and safety of different BoNT serotypes, more homogeneous studies are required.

Only onabotulinumtoxinA and abobotulinumtoxinA are indicated for children with cerebral palsy (CP). As in adult spasticity, a European Consensus on use of BoNT in children with CP has noted that all BoNT products are distinct (Heinen et al., 2010). It should also be highlighted that the term “unit” represents a different biological potency for each BoNT preparation. Individual dosages must be calculated independently for each BoNT preparation, and fixed-dose conversion factors are not applicable in the treatment of spasticity in children with CP (Heinen et al., 2010).

In general, BoNT treatment can be considered a safe and effective therapy for children with CP, especially in the hands of experienced injectors and for the majority of children (Strobl et al., 2015). This was also shown in results obtained in children with CP in a double-blind study comparing BoNT, placebo and physiotherapy (Ferrari et al., 2014). The 2010 AAN Guidelines also provide recommendations for BoNT treatment of spasticity in pediatric patient populations (Whelan and Delgado, 2010). In pediatric CP patients, BoNTA injections improved hemiparetic children more rapidly than those with bilateral palsy, and clinical effects lasted longer (Klochkova et al., 2013). In a double-blind, randomized sham-controlled trial, BoNTA therapy was effective in improving the facility of care and comfort for non-ambulant children with CP, with no increase in moderate and severe AEs compared to the sham group (Copeland et al., 2014). Another study showed that serial injections of BoNTA were effective and safe in children with spastic CP, significantly reducing muscle tone and improving gait at 3 and 6 months, with beneficial effects starting one week after injection (Wang and Gao, 2013). Thus, BoNTA is an important part of the multimodal management of children with CP that can support motor development and improve function when spasticity management in targeted muscle groups is clinically indicated (Strobl et al., 2015).
Neurogenic detrusor overactivity and overactive bladder

In the last 20 years, the potential therapeutic value of BoNTA in urology has also been a topic of great interest. However, it should be mentioned that, for urological indications as for other therapeutic uses, phase III evidence is currently available only for onabotulinumtoxinA. In four randomized worldwide phase III trials (two for neurogenic detrusor overactivity - NDO, and two for bladder overactivity - OAB) significant benefits for onabotulinumtoxinA versus placebo were demonstrated across the primary endpoints (reduction of urinary incontinence episodes) and a range of secondary endpoints, including measures of health-related quality of life. Considering these demonstrated benefits, the Guidelines of the European Association of Urology rated these treatments as grade A (level of evidence 1a), highlighting that only onabotulinumtoxinA is licensed in Europe to treat incontinence due to NDO and OAB in adults of either gender. They also added that (in agreement with the abovementioned guidelines and the guidelines for other therapeutic indications) other formulations of BoNT are not licensed for use in urgent urinary incontinence and that doses for onabotulinumtoxinA are not transposable to other BoNTA brands (Thuroff et al., 2011).

Chronic migraine

The mechanism of action of BoNTA in chronic migraine (CM) is still under debate, but it is suggested that inhibition of release of CGRP and substance P in the trigeminovascular system (Aurora and Brin, 2017) interferes with TRPV1 and TRPA1 receptors, usually more expressed in meningeal surfaces. Extracranial administration of BoNTA reduces their surface expression for up to 7 days (Zhang et al., 2016). The main study on the use of BoNTAs in chronic migraine is the Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) trial, which evaluated, for a 24-week period, the safety and efficacy of onabotulinumtoxinA in 1384 adult patients suffering from CM (Dodick et al., 2010).

On the basis of these and many other results, the AAN 2016 Guidelines specify that onabotulinumtoxinA is effective for headache in CM and should be offered in order to increase headache-free days (Level A) (Simpson et al., 2016). These recommendations are also in line with those of the Italian Society for the Study of Headache, which recommends the use of onabotulinumtoxinA in CM, specifying that, given its demonstrated efficacy in treating symptoms and correlated disabilities and in improving quality of life (Sarchielli et al., 2012), the available data support its use. Multiple treatments with onabotulinumtoxinA are well tolerated, and AEs seem to decrease with the number of treatment cycles (Diener et al., 2014). At present, onabotulinumtoxinA is the only drug that has been approved by US and European regulatory authorities for the prophylaxis of CM.

In 2016 an Italian Consensus Conference published evidence and recommendations on the treatment of headache and other nociceptive and mixed pain conditions in neurorehabilitation. The main recommendation relevant to CM is to follow Italian and European guidelines on headache disorders, particularly with regard to the use of BoNTA in CM and in refractory trigeminal neuralgia (Picelli, 2016).

Axillary hyperhidrosis

An evidence-based review conducted by a Therapeutics and Technology Assessment Subcommittee of the AAN concluded that BoNTs may be recommended as a treatment option for patients with axillary hyperhidrosis (Level A) (Naumann et al., 2003). A small self-controlled study, comparing the efficacy of onabotulinumtoxinA and rimabotulinumtoxinB in subjects with bilateral axillary hyperhidrosis treated with rimabotulinumtoxinB in one axilla and onabotulinumtoxinA in the other, showed that all subjects except one reported an excellent improvement in hyperhidrosis in both axillae. No patient reported residual hyperhidrosis on clinical examination. There was also no difference in the duration of hyperhidrosis improvement (Dressler et al., 2002). OnabotulinumtoxinA is the only BoNT with an approved indication for axillary hyperhidrosis.

Therapeutic indications

There are significant differences between BoNT products in terms of therapeutic indications in Italy (Table III).

Table III - Therapeutic indications for BoNTA products approved in Italy.

<table>
<thead>
<tr>
<th>Indication</th>
<th>OnabotulinumtoxinA</th>
<th>AbobotulinumtoxinA</th>
<th>IncobotulinumtoxinA</th>
</tr>
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<tbody>
<tr>
<td>Cervical dystonia</td>
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<tr>
<td>Blepharospasm</td>
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<tr>
<td>Hemifacial spasm</td>
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<tr>
<td>Dynamic equinus foot deformity</td>
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<td>Upper limb spasticity associated with stroke in adults</td>
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</tr>
<tr>
<td>Lower limb spasticity associated with stroke in adults</td>
<td></td>
<td></td>
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<tr>
<td>Axillary hyperhidrosis</td>
<td></td>
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<tr>
<td>Prophylaxis of chronic migraine</td>
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<tr>
<td>Neurogenic detrusor overactivity*</td>
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<tr>
<td>Idiopathic overactive bladder§</td>
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</tbody>
</table>

*With urinary incontinence due to subcervical spinal cord injury (traumatic or non-traumatic), or multiple sclerosis; §With symptoms of urinary incontinence, urgency and frequency; °Of a predominantly rotational form (spasmodic torticollis)
OnabotulinumtoxinA has the highest number of approved indications.Clinicians should keep in mind that the use of a drug is authorized only within the approved indications and use of a specific product in a non-approved indication is considered off-label even though that indication has been approved for another BoNT product (SIF, 2013). The approved indications for the different BoNTs further underline the lack of interchangeability among different products.

**Tolerability profile of BoNTAs**

The difference in AEs emerging in comparative trials has been discussed above. Post-marketing data, including data in the World Health Organization database, indicate a lower incidence of AEs associated with onabotulinumtoxinA treatment. Table II shows the incidence of AEs as emerging from comparative studies of onabotulinumtoxinA versus abobotulinumtoxinA. Dysphagia is the AE most frequently reported after abobotulinumtoxinA administration. Other common AEs include dizziness, eyelid ptosis, visual disturbances, mouth dryness; all are reported with higher frequency in patients treated with abobotulinumtoxinA compared with those treated with onabotulinumtoxinA (SIF, 2013). Clinical safety profiles are reported in the respective product labels subdivided by indication. Common AEs are related to the injection procedure, including injection site reactions (inflammation, tenderness, swelling, erythema), hypoesthesia, paraesthesia, localized infection, bleeding and/or bruising. Local muscle weakness is the expected pharmacological action of BoNT in muscle tissue. However, weakness of muscles adjacent to or remote from the injection site has been reported, probably related to the issue of "toxin spread" from the injection area, as discussed above.

**Immunogenicity**

Neutralizing antibodies to BoNTAs may develop during treatment, but rates are currently low thanks to progressive refinements in manufacturing [≤1% of people treated with BoNTAs (Brin et al., 2008; Naumann et al., 2013)]. However, a direct correlation between neutralizing antibodies and clinical response cannot be established, and the relationship is not fully understood (Brin et al., 2014). Antibodies may also develop against NAPs, but these are 'non-neutralizing', i.e., they do not impair clinical activity (Joshi et al., 2011). It is hypothesized from preclinical studies that NAPs may exert a protective role by physically hiding the NT portion from the immune system, thus eliciting neutralizing antibody production (Chen et al., 1997).

**Concluding remarks**

Analysis of the relevant data allows several practical conclusions to be drawn. There are important pharmacological differences between BoNT preparations related to both their manufacture and their formulation. Different injection techniques in various areas have led to different results because the effects also depend on the injection volume and the addition of protective substances such as albumin, which alter the potency and duration of action. Such differences affect clinical aspects such as dose, duration and efficacy. Studies on dose equivalence and comparative effectiveness have shown that dose units are different for each BoNT preparation and are not interchangeable from one product to another. The latest AAN Guidelines can be considered an important step towards differentiation of different BoNT products, and mean that future BoNTs will have to have a well-defined identity. Moreover, the expanding clinical use of BoNTAs has created new challenges for physicians and a need for adequate efficacy and safety data for each new therapeutic indication, thus making non-interchangeability an increasingly important issue. It must be considered that switching from an established effective dose of one BoNTA product to another product poses the issue of dose ratio and the other product cannot be expected to necessarily provide the same clinical outcome. In conclusion, it is important for clinicians to have all the different BoNTAs at their disposal in order to have the possibility of choosing the most suitable preparation for each indication and for each patient. However, for all the reasons discussed herein, the various commercially available BoNTAs must all be considered fundamentally different, since they are all biological ‘originators’, and as such they are non-interchangeable. Clinician experience is essential and of the utmost importance in choosing the most appropriate treatment (Alam, 2012).

**References**


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Differences between botulinum toxin preparations


