European Journal of Neurology 2011 doi:10.1111/j.1468-1331.2011.03559.x

REVIEW ARTICLE

Five-year experience with incobotulinumtoxinA (Xeomin®): the first botulinum toxin drug free of complexing proteins

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Keywords:
botulinum toxin type A, complexing proteins, incobotulinumtoxinA, long-term follow-up, therapeutic use

Received 28 January 2011
Accepted 31 March 2011

In 2005, incobotulinumtoxinA (Xeomin®), a new botulinum toxin (BT) type A drug without complexing proteins (CPs), became available. This paper reviews the specific features of Xeomin® and the experience gathered with it during the last 5 years. Compared with conventional BT drugs, Xeomin®'s extended shelf live and its simplified temperature restrictions indicate that CPs are not necessary for BT drug stability. Its reduced molecular size does not translate into diffusion differences, and its potency labelling is identical to that of onabotulinumtoxinA (Botox®). With a reduced content of inactivated botulinum neurotoxin, Xeomin® should have reduced antigenicity. Lack of CP's may further reduce antigenicity. Xeomin®'s therapeutic efficacy against cervical dystonia, blepharospasm and spasticity has been proven in large randomised, double-blind and placebo-controlled studies leading to registrations in many countries. Additional successful clinical use in axillary hyperhidrosis, hemifacial spasm, re-innervation synkinesias and hypersalivation as well as in dystonia and spasticity in extended doses and throughout extended observation periods has been documented meanwhile. Lack of reported cases of antibody-induced therapy failure (ABF), as to date, support the hypothesis of an improved antigenicity.

Introduction

Botulinum toxin (BT) has been used with remarkable success to treat various disorders caused by hyperactivity of muscles or of exocrine glands [1]. Its use to treat pain disorders is currently being explored [2].

Figure 1 shows the genealogy of BT drugs. In 1989, the first BT drug was registered as Oculinum®. Renamed Botox® ('old Botox®') in 1992, its formulation was changed in 2000 ('new Botox®'). In 2002, the name Botox Cosmetic®, and in 2005, the name Vistabel® was generated for its aesthetic indications. In 1991, the second BT drug was marketed as Dysport®. In 2009, it was renamed Reloxin® for aesthetic use. NeuroBloc® (named Myobloc® in the United States) was introduced in 2000. In 2005, Xeomin® (Merz Pharmaceuticals, Frankfurt/M, Germany) became available in Germany. It was licensed in 2009 for aesthetic indications under the name of Bocouture®. Also a BT type A drug, Xeomin® was the first BT drug in which the complexing proteins (CPs) had been removed. This paper wants to review the specific features of Xeomin® and the experience gathered with this drug during the last 5 years. The review is based upon the medical literature available in PubMed® (United States National Library of Medicine) until January 2011 and upon personal clinical experience of the author.

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Pharmacology

Structure

As shown in Fig. 2, BT drugs consist of the BT component and of excipients. The BT component is formed by botulinum neurotoxin (BNT, 150 kDa) and by nontoxic proteins also known as CPs [3]. In Xeomin®, the BT component is derived from the wild-type strain of *Clostridium botulinum* type A (ATCC 3502) [4]. CPs can be classified as non-haemagglutinating CPs (NHA-CP) and as haemagglutinating CPs (HA-CP). HA-CPs exist in the three different sizes HA1, HA2 and HA3. CPs and BNT form metastructures: BNT type A, B, C, D and G exists in a medium-size complex of BNT and NHA-CP (150 kDa + ~150 kDa = ~300 kDa) and in a large-size complex of BNT, NHA-CP and HA-CP (150 kDa + ~150 kDa + ~450 kDa = ~750 kDa). BNT type A also comes in a very large-size complex of 900 kDa. BNT types E and F form only a medium-size

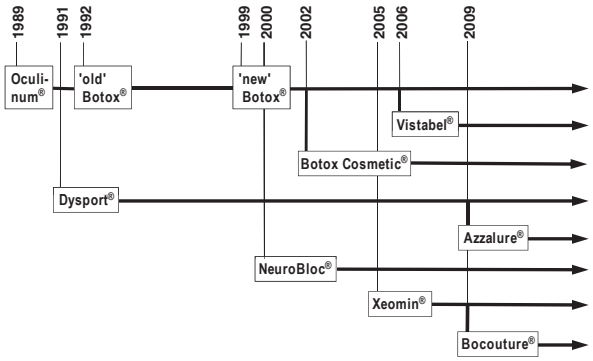
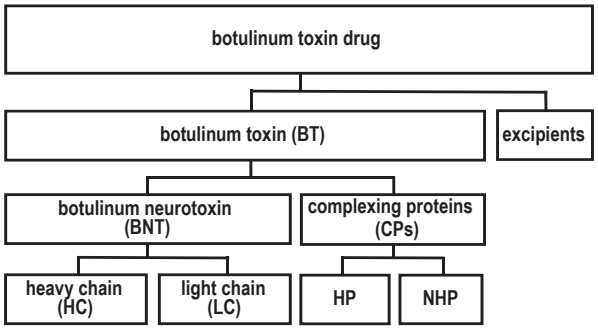


Figure 1 Genealogy of botulinum toxin drugs.



HP: Haemaglutinating proteins
NHP: Non-haemagglutinating proteins

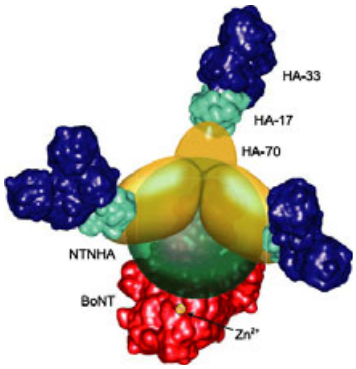
Figure 2 Structure of botulinum toxin drugs.

complex [4]. For BT type D, the exact subunit structure has been analysed (Fig. 3) [5]. Its large-size complex consists of one molecule BNT (150 kDa), one molecule NHA-CP (130 kDa), six molecules HA1 (198 kDa), three molecules HA2 (51 kDa) and three molecules HA2 (210 kDa) totalling 739 kDa [5]. The biological functions of the CPs will be discussed later.

Botulinum neurotoxin consists of the heavy amino acid chain (HC, 100 kDa) and of the light amino acid chain (LC, 50 kDa). HC binds BNT to the cholinergic nerve ending and translocates LC into the nerve cell. Intracellularly, LC cleaves the ***s***oluble ***N***-ethylmaleimide-**s**ensitive-factor ***a***ttachment ***re***ceptor (SNARE proteins) transporting the acetylcholine vesicle from the cell's endoplasmatic reticulum into the synaptic cleft, thus blocking the neuromuscular signal transmission.

Complexing proteins

Not much is known about the biological functions of the CPs. In a natural environment, they seem to protect and to stabilise BNT, e.g. when BT is ingested and exposed to low pH values and proteases within the stomach [6,7]. It was also suggested that CPs might



HA-33:	HA1 (haemagglutinating complexing protein 1, 33 kD)
HA-17:	HA2 (haemagglutinating complexing protein 2, 17 kD)
HA-70:	HA3 (haemagglutinating complexing protein 3, 70 kD)
NTNHA:	NHA (nonhaemagglutinating complexing protein, 150 kD)
BoNT:	BNT (botulinum neurotoxin)
Zn^{2+}:	Zn^{2+} dependent catalytic domain of botulinum neurotoxin

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Figure 3 Botulinum neurotoxin type D with its complexing protein subunits.

facilitate BT's target tissue binding [8] or its uptake and transcytosis in the gastrointestinal tract [9,10]. For BT drug stability, CPs do not seem to be necessary. Summaries of product characteristics (SPCs) indicate that CP-containing BT drugs have shelf lives of 2 years (Dysport®) or 3 years (Botox®, NeuroBloc®) and need to be stored at temperatures between 2°C and 8°C, whereas Xeomin® has a shelf life of 4 years and does not bear temperature restrictions [11]. At room temperature (25°C), all Xeomin® components including BNT, sucrose and human serum albumin as well as Xeomin®'s biological activity are stable for at least 48 months [11]. At 30°C, Xeomin® is stable for at least 18 months, at 40°C for at least 6 months and at 60°C for 1 month. At 80°C, Xeomin®'s biological activity decays after 5 days, whereas its proteolytic activity remains intact longer, indicating a higher temperature sensitivity of the binding and/or translocation domains than of the LC catalytic centre [11].

Although CPs are increasing the size of the BT component considerably, clinical as well as pre-clinical evidence suggests that CP-containing and CP-free BT drugs do not diffuse differently within the target tissues [12–14]. Reason for this is the rapid dissociation of the BT component into BNT and CPs immediately after injection, so that diffusion depends for both preparations upon the BNT size, which is identical in all products [15]. A recent study indicates that BNT–CP complexes may dissociate already within the therapeutic BT preparation itself [16]. Measurement of the compound muscle action potential of the abductor hallucis and the extensor digitorum brevis muscles does not show diffusion of Botox® and of Xeomin® from one

muscle to the other [17]. Measurement of the neural cell adhesion molecule (N-CAM) shows only a limited diffusion of Botox®, Dysport® and Xeomin® (dose conversion factor 1:4:1) into adjacent muscles, although the Dysport® diffusion was slightly larger than the Botox® or Xeomin® diffusion [18].

Antibodies may be formed against CPs in approximately 40% of patients receiving BT therapy [19]. As CPs are not relevant for the biological effects, and thus the therapeutic effects of BT drugs, CP antibodies, also called non-neutralising antibodies, are not interfering with the therapeutic efficacy of BT drugs. However, CPs may still play a role in antibody-induced failure of BT therapy (ABF). When BNT type C toxoid is applied to mice together with CPs, the BNT antigenicity is increased [20]. Further analysis shows that HA1 and HA3b are able to increase BNT antigenicity, whilst HA2 is not [20,21]. Interestingly, the immunological effect seems to be based upon an interleukin-6 increase in the target tissue, which, in return, is believed to raise the number of CD19 cells as producers of BNT antibodies. Other animal studies provided by the manufacturer of Xeomin® include forced stimulation studies on cynomolgus monkeys [22] and comparative studies between Botox®, Dysport® and Xeomin® in rabbits [23]. However, methodological problems including insufficient group size and lack of control groups limit the validity of these studies.

Immunology

Botulinum toxin therapy can occasionally fail because of formation of neutralising antibodies (ABF) [24,25]. Risk factors for ABF include the BT dose given at each injection series (single dose), time interval between injection series (interinjection interval) and injection series given within 2 or 3 weeks after the previous injection series (booster injections) as well as individual factors of the patient receiving BT therapy [26]. Recently, it became apparent that the immunological quality of the BT drug applied also modifies the ABF risk [27].

Botulinum neurotoxin contained in BT drugs may be present in a biologically active and in a biologically inactive form. Inactivation of BNT may arise from manufacturing and from prolonged storage of the BT drug. Although biologically inactive and not exerting therapeutic effects, inactivated BNT may still act as antigen. The specific biological activity (SBA) describes the biological activity given in mouse units (MU) per weight unit of total (i.e. active and inactivated) BNT given in nanograms (ng). The SBA, therefore, describes the immunological quality of BT drugs. High SBA is associated with low antigenicity and vice versa. The

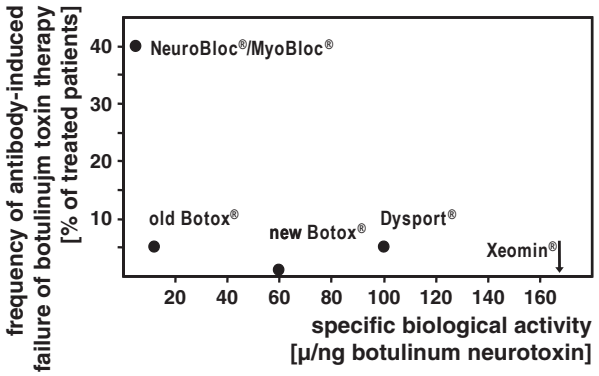

Figure 4 Correlation between the specific biological activity and the risk of antibody-induced therapy failure (ABF) in *de novo* patients treated with different botulinum toxin drugs for cervical dystonia [28,31,32].

SBA of NeuroBloc®/Myobloc® is 5 MU/ng, of Botox® 60 MU/ng, of Dysport® 100 MU/ng and of Xeomin® 167 MU/ng [27–30]. Figure 4 shows the correlation between the SBA and antigenicity. It is based on publications describing the frequency of ABF in patients treated with different BT drugs for cervical dystonia [28,31,32]. Whether the high antigenicity of the BT type B drug Neurobloc®/Myobloc® is only caused by its low SBA remains unclear.

Apart from a favourable SBA, separation of CP's may further reduce Xeomin®'s antigenicity. However, its actual ABF risk has not been established yet. Over the past 5 years, after Xeomin® became available, the author has used approximately 9500 vials without experiencing ABF, although each patient complaining of a reduced BT response received a complete work-up including electromyography (extensor digitorum brevis test, sternocleidomastoideus test) and BT-AB testing with the hemidiaphragm assay [19]. Similarly, no case of ABF in patients solely treated with Xeomin® has been published in the world literature so far. Recently, an interesting observation [33] supports the assumption of a reduced Xeomin® antigenicity: In this case study, a patient with severe cervical dystonia received Botox® for almost 3.9 years before developing ABF. Hemidiaphragm assay testing revealed a BT antibody titre of 7 mU/ml. After cessation of BT therapy for 5.1 years, the BT antibody titre had disappeared. When BT therapy was then re-initiated with identical treatment parameters as before, but with Xeomin® rather than with Botox®, neither ABF nor BT antibody formation occurred for currently up to 1.9 years.

Potency

The biological potency of BT drugs is measured in MU with 1 MU equal to the amount of BNT killing 50% of

a specified mouse population under controlled conditions. Although this definition is generally accepted, clinical experience showed obvious discrepancies between the potency labelling of different BT type A drugs as well as between BT drugs based upon different BT types. For BT type A drugs, it is not clear whether this discrepancy is caused by differences in the test procedures or whether simply Dysport®'s potency cannot be retrieved completely from the vial after reconstitution [34]. Xeomin®'s potency in comparison to Botox®'s potency has been discussed controversely. Whilst the manufacturer of Botox® claims a 20% reduced potency of Xeomin® against Botox® in an in-house mouse lethality assay [35], measurement of both drugs in the mouse lethality assay controlled by the registration authorities to release Xeomin® batches could not detect potency differences [36]. Clinical evidence arising from registration studies [12,13] as well as from independent studies including large number of patients and a wide range of indications and dosages [14] reveals also no differences in potency labelling between both drugs. Recently, a study comparing Botox® and Xeomin® in patients with blepharospasm showed a superior response to Botox® at 4 weeks post-treatment, but not at 8 and 16 weeks [37]. Lack of biological models for explaining this finding suggests further studies.

Clinical use

As of September 2010, Xeomin® was registered in Germany, Austria, Switzerland, France, Denmark, Norway, Sweden, Poland, Argentina, Brazil and the United States (in chronological order). In all of these countries Xeomin® is registered for the treatment of cervical dystonia and blepharospasm. In Germany, it is also registered for the treatment of arm spasticity after stroke. In Canada, Mexico, Germany, Argentina, Brazil, UK, France, Spain and Poland (in chronological order), Xeomin® holds registration for treatment of glabella lines.

Xeomin®'s registrations for cervical dystonia and blepharospasm are based upon two randomised, double-blind cross-over studies following a statistical non-inferiority design [12,13]. Based upon a 1:1 dose conversion ratio between Botox® and Xeomin®, 463 patients received 70–300 MU of either drug in the cervical dystonia study, whereas 256 patients received up to 35 MU/eye in the blepharospasm study. In both studies, there was no statistically significant difference between the patients' response to either drug, i.e. there was no difference with respect to the extent and duration of the therapeutic effect and with respect to type and frequency of adverse effects. Both studies, therefore, confirm identical potency labelling and identical diffusion properties of both drugs.

Xeomin®'s registration for spasticity is based upon a randomised, double-blind, placebo-controlled study on 148 patients with upper limb spasticity [38]. After treatment with a median dose of Xeomin®, 320 MU, muscle tone and disability were reduced to a statistically significant extent.

Xeomin® was in the meantime used for treatment of axillary hyperhidrosis and other forms of hyperhidrosis [14,39]. Long-term treatment has been monitored during an observation period of up to 3 years in various forms of dystonia (n = 91), various forms of spasticity (n = 84), hemifacial spasm and re-innervation synkinesias (n = 17), and in hypersalivation (n = 7) [14]. During this study, altogether 1050 injection series were applied, and maximal Xeomin® doses reached 840 MU. Even when applied in high doses, Xeomin® did not produce ABF. There were no diffusion differences detectable between Botox® and Xeomin®.

Outlook

Xeomin® shows improved SBA and improved stability compared with conventional BT dugs, whereas potency labelling and diffusion seems to be identical to Botox®. Xeomin®'s improved SBA suggests an improved antigenicity. Long-term use in large numbers of patients without any reported cases of ABF and an interesting case study support this hypothesis. However, robust clinical data are needed for confirmation of this hypothesis. Lack of CP's may further improve Xeomin®'s antigenicity. Here, too, animal experiments are needed to confirm this hypothesis. Further reduction in content of inactivated BNT and development of BT drugs with increased BNT receptor affinity may be a future challenge and may eventually allow to exploit the full potential of BT therapy without immunological restrictions.

Disclosure of conflict of interest

Over the past 3 years, the author has received honoraria for consultancies from Allergan, Solstice/Eisai, Ipsen, Merz and Syntaxin.

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