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Systematic analysis of botulinum neurotoxin type A immunogenicity in clinical studies

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ABSTRACT

Introduction: Controversy exists around the immunogenicity of the various formulations of botulinum neurotoxin type A (BoNT-A).

Methods: A systematic review of the literature (1968–2013) was performed, including review of materials from the FDA. Neutralizing antibody rates were calculated for overall BoNT-A and for each commercially available BoNT-A (abobotulinumtoxinA, incobotulinumtoxinA, onabotulinumtoxinA), and were compared by using the Chi-squared test. Two different onabotulinumtoxinA products were identified during the specified time-frame and classed as 'old' and 'new' versions.

Results: A total of 31 studies involving 5811 subjects met inclusion criteria and were analyzed. Therapeutic indications included: cervical dystonia, blepharospasm, spasticity, glabellar lines, hyperactive detrusor/sphincter dysfunction. The overall rate of development for neutralizing antibodies to BoNT-A was 2.1%. Individual rates were 1.4% for abobotulinumtoxinA, 0.8 to 1.1% for incobotulinumtoxinA, 7.2% for old onabotulinumtoxinA and 3.6% for new onabotulinumtoxinA. No significant differences were found between abobotulinumtoxinA and incobotulinumtoxinA rates (OR 1.82 [95%CI] [0.96–3.43], $p = 0.066$; OR 1.30 [0.69–2.46], $p = 0.415$). Rates of neutralizing antibodies were significantly lower with abobotulinumtoxinA and incobotulinumtoxinA versus either onabotulinumtoxinA formulations.

Conclusions: The overall neutralizing antibody rate for BoNT-A was low ($\leq 2.1\%$). The rate of developing neutralizing antibodies was similar between abobotulinumtoxinA and incobotulinumtoxinA, both significantly lower when compared with onabotulinumtoxinA.

1. Background

Botulinum neurotoxins are biopharmaceuticals widely used to treat a broad variety of clinical and cosmetic conditions involving muscle hyperactivity including various types of focal dystonia, post-stroke or cerebral palsy spasticity, hyperactive bladder, and glabellar lines (approved indications vary by country) [1]. Various botulinum neurotoxins of the type-A serotype (BoNT-A) have been available for almost 30 years and treatment with BoNT-A is considered safe and efficacious even when repeated treatment cycles are performed over many years [2–6].

Whereas primary non-responsiveness refers to a lack of response to treatment from the first application. The loss of a previously good clinical response to BoNT-A treatment (termed secondary non-responsiveness) is considered a key clinical concern and is often cited as the primary reason for switching patients to a botulinum neurotoxin type B preparation. Secondary non-responsiveness, was a common feature with the early preparation of 'old' Botox® (old-onabotulinumtoxinA; old-ONA) with up to 17% of patients being treated for cervical dystonia developing immunoresistance due to the development of neutralizing antibodies (NAb) [7]. This high prevalence was clearly a therapeutic issue and a more potent preparation of the same brand (onabotulinumtoxinA; ONA) was developed from a new toxin source in 1998 to contain less inactive toxin and thereby mitigate this problem [7,8].

Abbreviations: ABO, abobotulinumtoxinA; BoNT, botulinum neurotoxin; BoNT-A, botulinum neurotoxin type A; INCO, incobotulinumtoxinA; MDA, mouse diaphragm assay; MPA, mouse protection assay; NAb, neutralizing antibodies; ONA, onabotulinumtoxinA

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However, while its prevalence is much reduced, the issue of immunoresistance to BoNT-A has remained controversial and alternative BoNT-A preparations have been developed that do not contain complexing proteins with claims of reduced immunogenicity. In just the past 5 years, there has been a myriad of published papers on this issue, and clinicians are now faced with a conflicting (and therefore confusing) literature [8].

Since all commercially available BoNT preparations contain non-human proteins, they may act as antigens and cause antibody formation when injected. NAbs are antibodies which develop against the 150-kDa core BoNT (as opposed to non-neutralizing antibodies which develop against the hemagglutinin and non-toxin, non-hemagglutinin proteins that help stabilize and protect the core BoNT from environmental changes [9]). It is therefore important that the amount of inactive BoNT (that is initially produced by the Clostridium botulinum bacteria before protease cleavage) should be kept as low as possible [10]. It has been reported that BoNT products can be inactivated during the manufacturing process and that suboptimal storage between the time of manufacture and clinical use may also increase the amount of inactive toxin [11,12]. Similarly changes in the manufacturing process (e.g., method of isolation or type of excipients) can lead to variability in the structure of BoNT and even small changes can alter its immunogenicity [9,13]. Clinically, the risks of immunogenicity have been reported to be dose-dependent and the risks of developing NAbs have been shown to increase with higher cumulative doses [14,15], high frequency of treatment (injections given < 2 months apart) [15,16], and previous exposure to BoNT.

The main three commercially available BoNT-A preparations are Botox® (ONA; Allergan, Inc., Irvine, CA, USA), Dysport® (abobotulinumtoxinA (ABO), Ipsen Biopharm Ltd., Wrexham, UK) and Xeomin® (incobotulinumtoxinA (INCO) Merz Pharmaceuticals, Frankfurt am Main, Germany). Other available preparations do not yet have adequate data for comparison. This systematic review, performed by an immunologist, a BoNT expert and a clinician experienced in the use of BoNT-A for neurological conditions, aimed to systematically assess the rate of NAb development for each of the principal BoNT-A formulations by clinical indication.

2. Methods

2.1. Search strategy and selection criteria

A comprehensive search strategy using the PubMed, Biosis and EMBASE databases was designed to retrieve relevant clinical data from the published literature up to October 2013, and the literature review was performed in November 2013. The following search terms were pre-defined: botulinum, BoNT, BoNT-A, BoNTA, Botox, Dysport, Xeomin, onabot*, abobot* or incobot*, and neutralising antibodies or neutralizing antibodies or neutralizing antibody or neutralising antibody or immuno* or antibod* or antigen* or secondary non-responder or NAb or NAbs, and botulinum toxin database. In addition, because not all INCO studies supplied by the manufacturer to the US Food Drug Administration (FDA) have yet been published as full papers, it was decided to include data from the open-access FDA report on INCO [17]. All studies supplied in the ABO or ONA applications to the FDA appear to have been published in peer-reviewed journals (Fig. 1).

Two independent expert reviewers (an immunologist and a toxins expert) selected studies and extracted data. The analysis included all types of clinical studies, including clinical trials and observational studies (retrospective, prospective, cross-sectional) that have investigated or reported the frequency of NAbs to BoNT-A. Due to the significant differences in protein content and formulation, a distinction between 'old-ONA' (pre-1997 formulation) and ONA was made during data extraction [7]. Thus, four BoNT-A formulations (ABO, INCO, old-ONA and ONA) were included. Only full papers or FDA reports written in English or French were analyzed. Exclusion criteria for study selection included: a focus on BoNT-B, abstracts, case reports, reviews, meta-analysis, inclusion of only NAb-positive subjects. Exclusion criteria for data extraction included lack of/poor methodology, a focus on secondary non-responders, no investigation for antibodies.

2.2. Data extraction

The following information were extracted from each study: (1) study design; (2) number of subjects; (3) clinical indication; (4) BoNT formulation; (5) mean dose per treatment; (6) number of injections; (7) frequency of NAbs and (8) NAb detection method. In addition, the quality of identified studies was assessed in accordance with the methodology recommended by the EFNS Scientific Committee for the preparation of neurological guidelines [18]. In this assessment, the quality of the identified studies is classified into 4 categories (I–IV), where Class I refers to an adequately powered prospective, randomized, controlled clinical trial with masked outcome assessment in a representative population, and Class IV refers to evidence from uncontrolled studies (case series and expert opinion were excluded from the present review) [18].

2.3. Statistical analysis

BoNT-A NAb rates were compared by using the Chi-squared test; results were expressed as Odds Ratio (OR) and 95% confidence interval (CI). Due to uncertainty on which studies were included in the Xeomin® FDA report, two INCO populations were analyzed. These were designated: INCO-1 (based solely on the subject numbers included in the FDA report) and INCO-2 (based on subject numbers from the FDA report plus published studies). Statistical analyses were implemented in Statview v9.0 (SAS Institute, Cary, NC, USA). A P value of 0.05 or less was considered statistically significant for performed comparisons.

3. Results

The literature database search yielded 62 references; of these 17 did not meet assessment criteria for selection and 20 were selected but did not meet criteria for data extraction. Thus, 25 papers and 1 FDA report detailing the results of 31 studies involving 5811 subjects (assuming the larger INCO-2 population) were systematically evaluated (Table 1). The total population decreased to 5390 subjects when the more conservative INCO-1 population was analyzed.

Of the 31 studies assessing the immunogenicity of BoNT-A, 14 were studies in subjects with cervical dystonia, 8 were conducted in subjects with spasticity, 3 were conducted in subjects with blepharospasm, 3 were conducted in subjects with glabellar lines and 3 studies were conducted in subjects with bladder disorders (hyperactive detrusor or sphincter dysfunction). The quality of studies was generally poor with most data coming from retrospective or otherwise poorly designed studies.

In general, more data was available for ABO than ONA or INCO. Table 2 summarizes the dosing and number of BoNT-A injections per indication and BoNT product [7,19–42].

Most studies used functional laboratory tests to detect the presence of NAbs; studies generally either used the mouse protection assay (MPA, n = 13) or the mouse diaphragm assay (MDA, n = 12), the rest either used both assays or the method was not clearly reported. In addition, several studies reported using clinical assays, including unilateral brow injection, frontalis antibody test, ninhydrin sweat test and the extensor digitorum brevis test.

According to the available literature (including INCO-2 population), the overall NAb prevalence was 1.9% across all BoNT-A formulations. As expected, the prevalence of reported NAbs was higher for old-ONA (7.2%) versus the currently available INCO, ABO and ONA formulations (range 0.8–3.6%; Table 3). When the INCO data was analyzed using only data reported to the FDA (INC0-1 population, n = 1080 subjects),