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Editorial

Rising solutions for secondary treatment failure in patients on chronic botulinum neurotoxin therapy

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Long-term treatment with local injections of Botulinum neurotoxin type A (BoNT/A) and type B (BoNT/B) is thought to increase the likelihood for formation of neutralizing antibodies (NAbs), which may lead to a progressive decrease of the BoNT-related therapeutic effect and, thus in turn, to a secondary treatment failure (STF). STF is currently considered a relevant clinical challenge in the long-term management of patients under chronic BoNT treatments. NAbs specifically directed against the active neurotoxin part of the BoNT/A and BoNT/B complex have been indeed demonstrated in more than 50% of patients manifesting STF (Lange et al., 2009; Fabbri et al., 2016). More recently, Albrecht et al. (2019) have investigated the prevalence of NAbs directed against BoNT/A in a large cohort of chronically-treated patients affected by various neurologic conditions such as hemifacial spasm, blepharospasm, cervical dystonia, other types of dystonia and spasticity. The authors have demonstrated that 83 out of 596 patients (13.9%) chronically treated for 3–6 years with BoNT/A (predominantly patients with cervical dystonia and spasticity who received high doses of BoNT/A), have measurable NAbs possibly responsible for STF (Albrecht et al., 2019). Fully in line with previous clinical observations (Lange et al., 2009; Fabbri et al., 2016), Albrecht et al. (2019) also suggested that the total dose per injection, the interval between repetitive BoNT/A applications, the total number of injections, and the specific BoNT/A formulation (i.e., putative lower antigenicity of inco-BoNT/A than ona- and abo-BoNT/A) are the most relevant factors driving to formation of NAbs and possibly leading in turn, to STF (Lange et al., 2009; Fabbri et al., 2016; Albrecht et al., 2019). A possible clinical strategy to circumvent NAbs-related STF implies the injections of serotypes other than BoNT/A and BoNT/B able to avoid antibody cross-reactivity. Among the various BoNT serotypes, BoNT/D is characterized by a low degree of biological similarity with BoNT/A and BoNT/B, possibly resulting in different antigenicity and thus reducing the likelihood for NAbs formation.

This issue of *Clinical Neurophysiology* includes the neuropharmacological study of Kutschenko et al. (2019) who compared the biological activity of BoNT/D (recombinantly expressed in *E. coli*), and BoNT/A (inco-BoNT/A – Xeomin®) *ex vivo* in mice as well as *in vivo* in healthy humans. The *ex vivo* evaluation was achieved by means of the mouse phrenic nerve (MPN) hemidiaphragm assay, according to standardized procedures (the phrenic nerve was stimulated with 5–25 mA, at 1 Hz, with a 0.1 ms pulse duration while isometric hemidiaphragm contractions were simultaneously measured using a force transducer). The *ex vivo* evaluation included the calculation of dose-response curves for both BoNT/D and inco-BoNT/A. By contrast, the *in vivo* study consisted of 6 different doses of BoNT/D (280, 560, 1120, 2240, 4480, 8960 pg) and 3 different doses of inco-BoNT/A (20, 80 and 160 pg corresponding to 4, 16 and 32 Units, respectively), randomly injected in the Extensor Digitorum Brevis (EDB) muscles of 15 healthy volunteers. The pharmacological effect of BoNT on EDB muscles was calculated by means of standardized neurophysiological procedures (recordings of compound muscle action potential (CMAP) amplitude elicited by electric percutaneous supramaximal stimulation of the peroneal nerve, at the level of the ankle joint), and assessed in repetitive sessions (0–220 days after injection). Kutschenko et al. (2019) demonstrated that BoNT/D is characterized by a potent biological activity both *ex vivo* and *in vivo*. However, when comparing the dose-response curves calculated for BoNT/D and inco-BoNT/A, the biological activity of BoNT/D resulted about 3.7-fold lower than that of inco-BoNT/A. To achieve a comparable pharmacological effect, BoNT/D required a 110-fold higher protein dose than inco-BoNT/A. In addition, despite a similar maximal effect, the duration of action of BoNT/D was 50% shorter than that of inco-BoNT/A. Fully in line with the observations achieved in animals, the *in vivo* study in humans confirmed that compared to inco-BoNT/A, a 110-fold higher dose of BoNT/D is required for achieving a comparable reduction of the CMAP amplitudes recorded from EDB muscles. In conclusion, the authors demonstrated that the biological potency and duration of action of BoNT/D is inferior to that of BoNT/A (Kutschenko et al., 2019).

The seminal study of Kutschenko et al. (2019) provides a number of new and relevant information concerning the pharmacological properties of BoNT/D in animals as well as in healthy humans. Indeed, local injections of BoNT/D can block acetylcholine release in animal and in human motoneurons, dose-dependently. The pharmacological and neurophysiological findings here reported support the hypothesis that BoNT/D injections may be considered a potential alternative strategy in the clinical management of chronically treated patients affected by various neurological conditions and presenting STF with NAbs. When considering the observations of Kutschenko et al. (2019), however, a number of clinical,

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pharmacological and neurophysiological considerations should be taken into account. The need for relatively high BoNT/D doses in order to achieve a biological effect (110-fold higher protein doses than BoNT/A) explains the apparent inconsistency with the lack of effect previously reported by Eleopra et al. (2013). Since the risk of inducing NAbs also depends on the dose of proteins injected and the short-interval between the repetitive clinical applications, chronic BoNT/D treatments would in theory be associated with a high risk of developing further NAbs against BoNT/D. In addition, it is important to state that in about 50% of patients with STF, NAbs cannot be clearly demonstrated (Lange et al., 2009; Fabbri et al., 2016). Moreover, the presence of NAbs does not clearly correlate with the likelihood of developing STF. It is known that several antibodies bind to BoNT/A at molecular sites that are not related to the pharmacological activity of the toxin (e.g. antibodies specific to the protective hemagglutinin complex), thus being pathophysiologically not related to STF. Furthermore, patients with NAbs may still respond, at least partially, to the current BoNT/A therapy (Lange et al., 2009; Fabbri et al., 2016). Overall these considerations argue against a clear pathophysiological link between NAbs and STF. Conversely, it is likely that besides NAbs, STF also reflect additional factors including disease progression, changes in the pattern of muscle hyperactivity, inadequate muscle selection or injections, and finally even plasticity changes occurring at the level of the central nervous system (Trompetto et al., 2009; Lange et al., 2009; Fabbri et al., 2016; Albrecht et al., 2019). Hence, the specific contribution of all these factors to STF in patients with and without NAbs remains largely unclear.

In conclusion, by demonstrating both *ex vivo* and *in vivo* that BoNT/D has a potent biological activity, the study of Kutschenko et al. (2019), opens the way to future clinical trials aimed at assessing the clinical efficacy of BoNT/D in large cohorts of patients affected by various neurological disorders such as dystonia and spasticity. It would be also relevant to investigate whether chronically treated patients with BoNT/A, manifesting STF with Nabs, may become responsive again following a switch from BoNT/A to BoNT/D. Future studies suggesting clinical algorithms for the management of patients with STF, with and without NAbs, including clinical, neurophysiological and biochemical measures are warranted.

Conflict of interest statement

None of the authors have potential conflicts of interest to be disclosed.

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