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NeuroBloc®/Myobloc®: Unique features and findings☆

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ABSTRACT

This review outlines factors that differentiate botulinum toxin serotypes and focuses on the unique features of the commercially available form of BoNT-B (i.e., Myobloc®/NeuroBloc®). A series of preclinical studies in Cynomolgus monkeys are reviewed. Each of these studies used electrophysiologic measures of changes in the compound muscle action potential (CMAP) following supramaximal nerve stimulation to evaluate the direct effects of the toxin in the injected muscle, as well as the spread of the effects to non-injected muscles. The results of 14 studies were summarized, including several that compared the effects of equivalent doses of BoNT-A and BoNT-B injected into muscles on the opposite side of the same monkey. There is clear evidence that when equivalent doses of BoNT-A and BoNT-B are assessed, there is greater spread to both nearby and remote non-injected muscles associated with BoNT-A. Similar studies in the mouse model demonstrated that high, but non-lethal, doses of BoNT-A unilaterally injected into the foot resulted in spread of the effects across the midline to the opposite non-treated foot, while there was no evidence of bilateral effects with equivalent unilateral injections of BoNT-B. Finally, this review summarizes a series of studies in the trapezius and gastrocnemius muscles of monkeys demonstrating that when doses producing equivalent initial effects of BoNT-A and BoNT-B are compared, the duration of effects and the time course of recovery are almost identical across toxins.

1. Unique features of botulinum toxins

The seven distinct botulinum neurotoxin (BoNT) serotypes (designated A, B, C, D, E, F and G) share many common features, including remarkable potency, a high affinity for cholinergic neurons and a zinc-dependent catalytic inactivation of one or more elements of the presynaptic SNARE complex (Dolly et al., 1984, 1994). Each of the serotypes prevents exocytosis of synaptic vesicles and the release of acetylcholine. The effective and expanding clinical use of BoNT therapy is largely related to the potency and specificity of this family of clostridia-derived neurotoxins, which allow the targeted administration of truly minute quantities of toxin to achieve clinically-relevant efficacy.

Although similar in action, the BoNT serotypes differ in a number of aspects that are of considerable importance for their potential clinical use. As implicit in the term "serotype", the BoNTs are antigenically distinct; a fact that allows the efficacious use of BoNT-B in patients with cervical dystonia that have become resistant to the effects of BoNT-A (Factor et al., 2005). There is currently no evidence of cross-reactivity of neutralizing antibodies to the various BoNT serotypes (Callaway, 2004). Access to specific neurons also differs across serotypes. BoNT acceptors involve a combination of polysialogangliosides and proteins exposed to the cell surface (Montecucco et al., 2004; Grumelli et al., 2005). Specific variants of these surface proteins determine the sensitivity of neurons to different serotypes of BoNT. For instance, synaptotagmins I

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and II have been shown to mediate the entry of BoNT-B, but not BoNT-A or G, into PC12 cells (Dong et al., 2004). Recent studies also suggest that the post-BoNT remodeling of the synapse and terminal sprouting necessary for recovery of function may also differ across serotypes (Meunier et al., 2003).

Each of the currently available commercial BoNT products (BoNT-A: Botox®, Dysport ®, Xeomin®; BoNT-B: NeuroBloc®/Myobloc®) has unique manufacturing processes which further differentiate their clinical use. The production of NeuroBloc®/Myobloc® was designed to produce a highly purified and stable liquid formulation containing uniform and intact type B complex (Callaway et al., 2001, 2002). Unlike commercial forms of BoNT-A, NeuroBloc®/Myobloc® is non-lyophilized. It is also not vacuum dried, so that the amount of denatured protein is minimized. In its injectable state, 65% of the neurotoxin present is in its nicked, and therefore active, dichain form (data on file: Solstice Neurosciences, Inc., Malvern, PA). Perhaps the most important unique feature of NeuroBloc®/Myobloc® is that it is manufactured in a liquid formulation with a pH of 5.6 (Callaway et al., 2002). As confirmed by high pressure liquid chromatography, this ensures that the toxin complex is intact and stable, with a single entity molecular weight of approximately 700 kDa. Thus, the toxin is injected as a uniform and intact complex, as opposed to partially dissociated subunits, and this may be one important biological characteristic that limits the spread of NeuroBloc®/Myobloc® to non-injected muscles (Schantz and Johnson, 1993; Arezzo et al., 2002).

For obvious reasons, the safety profile of the various forms of BoNT substantially impacts the clinical value of these products. BoNTs are among the most deadly substances known to man (Lamanns, 1969), which warrants vigilance concerning the potential spread of the toxin from the injection site (Kuehn, 2008). However, considering the number of patients and the range of conditions treated with BoNTs, there have been relatively few reports of serious adverse reactions (Coté et al., 2005; Birmingham et al., 2008). This safety profile underlies the substantial growth of the use of all forms of BoNT for the treatment of conditions characterized by excessive muscle contraction, such as dystonia, and the expansion of use of the toxin to treat conditions such as pain (Arezzo, 2002). We have known for more than two decades that, at therapeutic doses, the effects of the toxin are not confined to the injection site (Borodic et al., 1990). Spread of the toxin to nearby cholinergic neurons is most likely due to the diffusion of unbound toxin through extracellular space, driven by the concentration gradient and the dynamics of the injection; the muscle fascia is a relatively ineffective boundary (Shaari et al., 1991). A dose-dependent "spread" of the effects of BoNT has been confirmed in both preclinical and clinical models using a variety of techniques (e.g., Swartling et al., 2001; Arezzo et al., 2002). Remote "spread" (e.g., altered activity in the intrinsic muscles of the foot following injection in the neck) most likely involves vascular or lymphatic transport of the toxin (e.g., Girlanda et al., 1992; Ansved et al., 1997). A recent study by Antonucci et al. (2008) raises the additional possibility of long-distance retrograde transport of BoNT-A, which is an intriguing alternative explanation for the "remote" spread of the effects of BoNTs. Although elegant, the Antonucci study is preliminary and the results must be repeated. However, if confirmed, it is possible that once out of complex, BoNT could be transported along the alpha motor neuron to the ventral spinal cord. At that CNS site, the effects could be transcytosed, as has been suggested for the brain, to influence adjacent spinal motor neurons and to induce subsequent partial paralysis on non-injected muscles. The Antonucci study further suggests that there may be clear and substantial differences in the degree of retrograde transport across BoNT serotypes. The report documents evidence for retrograde transport of BoNT-A, but no such biological behavior for BoNT-E.

2. Unique preclinical studies in Old World monkeys

A unique aspect of the development of NeuroBloc®/Myobloc® was that, under a Food and Drug Administration (FDA, Rockville, MD, USA) IND, this agent was the subject of an extensive series of safety studies in Old World monkeys that explored both its direct effects in the injected muscle as well as the spread of its effects to non-injected muscles and to CNS sites. Old World monkeys are the closest feasible model to humans and are recognized as outstanding models for many conditions affecting the nervous system. The use of Cynomolgus monkeys avoids the limitations of the rodent models and the difficulty of dissecting the influence of the well documented species-specific sensitivities to various BoNT serotypes. Fourteen distinct studies were conducted, many of which compared the effects of NeuroBloc®/Myobloc® with those of Botox® when these agents were administered to identical contralateral muscles in the same monkey. These studies were originally supported by research grants from Athena Neurosciences, Elan Pharmaceuticals or Eisai Pharmaceuticals.

For each of these studies, BoNT-A was obtained from commercially available stocks (Botox®, Allergan Inc., Irvine, CA) supplied as lyophilized powder. To achieve desired doses, BoNT-A was reconstituted in 0.9% sodium chloride (NaCl) and diluted to a final concentration with 0.5 mg/mL human serum albumin in 0.9% NaCl. As per labeling instructions, BoNT-A was stored in a freezer at or below −7 °C. BoNT-B (NeuroBloc®/Myobloc®, Elan Pharmaceuticals Inc., South San Francisco, CA) was supplied as a clear liquid formulation, and was diluted with a solution consisting of 100 mM NaCl, 10 mM succinate, and 0.5 mg/mL human serum albumin. BoNT-B was stored refrigerated between 2 and 8 °C. Freshly prepared diluted toxin solutions were used for each dosing day.

All subjects were female Cynomolgus monkeys, which have a neuromuscular system remarkably similar to that of humans. Initial studies explored the "Hand Model" as depicted in Fig. 1a. In this model, the amplitude of the compound muscle action potential (CMAP) is first recorded from the abductor pollicis brevis (APB) muscle, bilaterally, following supramaximal stimulation of the median nerve at the wrist crease. The right and left side muscle is then injected with a specific dose of either BoNT-B or BoNT-A and the CMAP is measured again after a period of 15 days. In most studies, the different BoNTs were injected on