36. Xeomin® is stable without refrigeration: Complexing proteins are not required for stability of botulinum neurotoxin type A preparations

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Native botulinum toxin type A (BTX-A) is a high molecular weight complex of about 900 kDa, composed of the biologically active 150 kDa neurotoxin, several hemagglutinins, and other nontoxic proteins. Although these complexing proteins do not have any therapeutic effect, it has been speculated that they might be required for stability of botulinum neurotoxin type A preparations.

Xeomin® is a botulinum neurotoxin type A preparation, which, unlike other marketed BTX-A products, contains only the pure 150 kDa neurotoxin without complexing proteins. The stability of Xeomin® was assessed in comprehensive real-time and accelerated stability studies according to ICH guidelines. The study results showed no detrimental effects on the quality of Xeomin® after storage of up to 40 °C, and have accounted for its shelf-life of 3 years without the need for refrigeration.

In a further temperature stress study, samples of Xeomin® were stored above 40 °C. As in the ICH-conform stability studies, the samples were tested with fully validated or standardized pharmacopoeia analytical methods also used for release testing of Xeomin®. The results demonstrate that Xeomin® is not negatively affected by storage at temperatures between 40 and 60 °C for up to 1 month.

Altogether, these findings provide clear evidence that the complexing proteins in pharmaceutical preparations of botulinum neurotoxin A are not required for stability.

Keywords: Botulinum neurotoxin A; Stability; Complexing proteins; Xeomin®
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37. Secondary cleavage of a fluorescently labeled SNAP-25 substrate by Xeomin® drug product

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Objectives: To evaluate SNAP-25-type substrate cleavage product(s) generated by Botulinum neurotoxin Type-A (BoNT/A) and BoNT/A drug products utilizing a light-chain activity high-performance liquid chromatography (LCA-HPLC) method.

Methods: Samples were reacted with a fluorescently labeled substrate derived from the SNAP-25 sequence. Reaction products were subsequently separated by reversed-phase HPLC. Cleavage products were chromatographically identified by elution position using fluorescence detection.

Results: Xeomin® drug product samples produced two fragments rather than one. Identification of the secondary fragment confirmed that secondary cleavage occurs at a position on the substrate corresponding to SNAP-25 Arg198–Ala199 as opposed to the expected BoNT/A cleavage site (corresponding to SNAP-25 Gln197–Arg198). This is also the cleavage site for Trypsin and Type-C toxin. The secondary cleavage was not observed in any BOTOX® drug product samples, nor was it observed in 900 or 150 kDa bulk toxin samples under the experimental conditions employed. Only Xeomin® drug product generated the additional unexpected cleavage product. All of the Xeomin® lots analyzed exhibited this behavior.

Conclusions: Possible explanations include a contaminating trypsin-like protease, concurrent Type-C expression behavior, and/or damage to the 150 kDa toxin causing non-specific cleavage uncharacteristic of pristine Type-A toxin.

Keywords: Botulinum toxin; SNAP-25; Botox; Xeomin; Cleavage
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38. Small molecule therapeutic approaches for the treatment of botulinum neurotoxins A and B intoxication

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Botulinum neurotoxins (BoNT) are the etiological agents responsible for botulism, a disease characterized by peripheral neuromuscular blockade and a characteristic flaccid paralysis of humans. With the current warfare and terrorist activities around the world, potential bioterrorist weapons are of high-priority. Due to the long paralysis and necessity for intubations and mechanical respiration, the numbers of medical care units capable of proving supportive care for recovery are limited. Countermeasures are urgently needed, in this context; we have concentrated our research efforts upon ways to uncover small molecule inhibitors for BoNT/A and B as they can exhibit sustained intoxication. To accomplish this task we are engaged in a two-prong approach. The first is based on inhibiting the protease of BoNT using a series of small molecule inhibitors that ultimately have the correct characteristics for potential drug development. Our second tact examines Toosendanin a triterpenoid that is has been used in traditional Chinese medicine. We have preformed preliminary studies on the natural product toosendanin using a mouse model, and we will discuss the key features that are central to the antibotulismic properties of toosendanin, and provide insights into its mechanism of action.

Keywords: Protease Inhibitors; Therapeutics; Toosendanin; Botulinum neurotoxins A/B
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