

Evaluation of *Clostridium botulinum* A5 neurotoxin actions in vivo and ex vivo at the mouse skeletal neuromuscular junction

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Clostridium botulinum neurotoxins (BoNTs), the most potent toxins known, are the cause of a worldwide life-threatening disease in humans and animals known as botulism. This disease usually manifests as descending symmetrical flaccid paralysis of skeletal muscles together with autonomic dysfunction. BoNTs include a large family of zinc metalloproteases that can be immunologically distinguished by homologous antitoxins into seven primary serotypes, designated A to G. Additionally, for BoNT/A, at least 8 subtypes (/A1-/A8) have been identified from gene sequence analysis. In France, several cases of human botulism due to BoNT/A5, /A6 or /A7 have been reported, though BoNT/A1 and /A2 are the prevalent forms of type-A botulism. The characterization of the *C. botulinum* strains involved in two cases of BoNT/A5-poisoning revealed that they possess the gene encoding for BoNT/A5 identical to the one previously reported. In the present study, we produced the BoNT/A5 crude complex (3 x 10⁷ LD₅₀/mg) from the *C. botulinum* A5 strain 126.07 [1], and studied *in vivo* and *ex vivo*, in murine models, the skeletal neuromuscular block caused by BoNT/A5, at different times after a single local toxin injection into the hind-limbs. The results show that the duration and degree of paralysis depended on the dose of BoNT/A5 and on the mouse strain studied. The transgenic Thy-1-YFP-16 black C57BL6 mice [2] were more sensitive to the action of the toxin than Swiss mice, as revealed by the digit abduction score (DAS) assay and by compound muscle action potential (CMAP) recordings from the same mouse *in vivo*, at different times after toxin injection. Functional and morphological *ex vivo* studies on muscles locally injected with BoNT/A5 *in vivo* reveal (a) the presence of axonal outgrowths (nodal and nerve terminal sprouts), (b) an extension of nicotinic acetylcholine receptor clusters, (c) a reduction of both muscle weight and muscle fiber cross sectional area, and (d) the prolonged atrophy of muscle fibers. Pre- and post-synaptic remodeling was completely abolished by an immune-purified rabbit polyclonal antibody directed against the BoNT/A1-Heavy chain (HcA1), when injected together with BoNT/A5. In conclusion, our results show that the actions of BoNT/A5 have many similarities to those of BoNT/A1 previously reported, although no direct comparison was performed in the present study. BoNT/A5 differed from BoNT/A1 by 5 identified amino acids in the Light chain domain (Lc) and by 32 amino acids in the Heavy chain domain (Hc). The fact that the antibody directed to the HcA1 completely prevented the lethal effect of BoNT/A5, as well as the block of the CMAP *in vivo*, and the morphological changes induced by the neurotoxin suggest that the antibody blocks the entry of BoNT/A5 into motor nerve terminals of the neuromuscular junction.

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[2] Feng G. et al. *Neuron.* 2000. 28(1), 41-51. doi: 10.1016/s0896-6273(00)00084-2.

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