

Development and validation of an in vitro assay for the potency determination of botulinum neurotoxins

Project team

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Project summary

Botulinum neurotoxins (BoNT), produced by bacteria of the species *Clostridium botulinum*, are a group of extremely potent protein toxins. They are the causative agents of botulism, a serious disease characterized by flaccid muscle paralysis in humans and animals. Due to this muscle-paralyzing activity, BoNT serotypes A and B are widely used in clinical and aesthetic medicine to treat a variety of disorders associated with local muscle overactivity, or to reduce facial wrinkles.

For each batch of BoNT/A and BoNT/B which has been produced for pharmaceutical purposes, an exact determination of potency is legally mandatory. The “gold standard” method for this determination is a toxicity (LD50) test in mice, which requires high numbers of test animals: In Germany alone, 48,000 mice were used for BoNT toxicity testing in the year 2015. Some approved alternative methods exist, but they are product-specific and patented, which strongly limits their applicability. A generally accepted and universally applicable in vitro alternative is not yet available.

We want to provide a reliable method for the in vitro determination of BoNT potency as widely applicable alternative to the mouse toxicity test. For this purpose, we have developed "Binding and Cleavage" (BINACLE) assays which reproduce the relevant mechanisms of action of BoNT/A and BoNT/B (i.e. binding to neurons and cleavage of neuronal proteins) in vitro. The assay principle is similar to the BINACLE assay that has previously been developed by our group for the detection of tetanus toxicity (see above).

We could show that the BINACLE assays are able to measure the biological activity of relevant pharmaceutical BoNT products with high sensitivity and specificity. At present, an international collaborative study is conducted to further characterize the applicability of the BINACLE assays for the potency determination of BoNT/A and BoNT/B products. The final aim of the project is an adoption of the methods by the European Pharmacopoeia in order to attain wide spread use instead of the distressing LD50-test.

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Awards

- In 2016, the project team was awarded an animal welfare research prize (Ursula M. Händel Tierschutzpreis) by the German Research Foundation (DFG).

- In 2017, the former project team member Emina Wild was awarded the "Langener Nachwuchswissenschaftspreis" as first author of a publication describing the BINACLE assay for the determination of BoNT/B activity [Toxicology in Vitro 34:97-105, 2016].