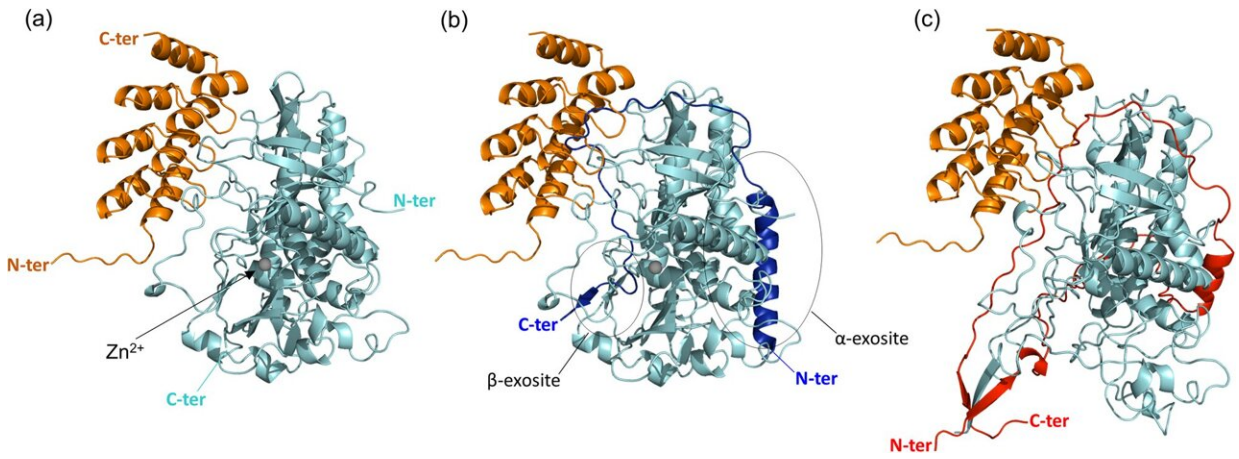
New possibilities for the medical use of botulinun toxin A1

December 18 2023, by Jan Berndorff



Crystal structure of the LC/A1-DARPin-F5 complex. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-44102-4

PSI researchers have discovered a surprising trick that could expand the possibilities for medical use of botulinun toxin A1, better known under the name Botox, as an active agent. They have developed antibody-like proteins that speed up the enzyme's effect on the transmission of nerve signals. This suggests that Botox might, for example, be able to relief pain more quickly than before.

The paper is published in the journal *Nature Communications*.

Botulinum neurotoxin A1, better known under the trademark Botox, is actually a nerve toxin produced by bacteria. It gained widespread public awareness through its use as a cosmetic aid. Many people have it injected into wrinkles to make them look younger.

The substance blocks signal transmission from nerves to muscles, thus relaxing them so that facial features appear smooth. What is less well known: Botox is also used very often in therapeutic medicine to treat conditions that can be traced back to cramping muscles or faulty nerve signals, including pains, spasms, bladder weakness, grinding of teeth, and misalignments, for example of the eyes. Botox is even used in treating stomach cancer, to block the vagus nerve and thus slow down tumor growth.

In any therapy, it is crucial to use this highly effective medicine in a very targeted manner with careful dosage, since Botox is the most potent natural nerve toxin of all, which can lead to dangerous paralysis in a clinical picture called botulism. Just one hundred nanograms or so administered intravenously can be enough to kill a person, because the toxin paralyzes the respiratory muscles, along with others.

Different types of botox

Botulinum neurotoxins are categorized in seven so-called serotype groups designated by the letters A through G. The Botox used in cosmetics comes from the first group. To be precise, it is designated subtype A1. It is known that three other serotypes—B, E, and F—can also lead to botulism in humans, with E and F acting significantly more rapidly but not as long as A and B.

The effect sets in after just hours and lasts a few weeks, which opens up important options in pain therapy and orthopedics, for example. Types C and D are effective in some animal species such as birds; to date, no

cases of botulism have been observed with type G.

The serotypes are mainly produced by different strains of the bacterium Clostridium botulinum. These microbes thrive anaerobically, that is, in the absence of oxygen, and are found mainly in the soil as well as marine and river sediments. If they get into food and are stored in airtight containers, as can be the case with preserved products, there is a risk of contamination with the toxin. Eating it can cause botulism. However, the disease occurs very rarely; in the past 10 years, there have only been one or two cases per year in Switzerland.

Surprising results

In a research project, a team led by Richard Kammerer of PSI's Laboratory for Biomolecular Research wanted to investigate whether it might be possible to influence the action of the toxin.

"For that we have, together with biochemist Andreas Plückthun from the University of Zurich, produced 25 so-called DARPins," Kammerer says. DARPins are small, artificially produced proteins that work similarly to antibodies. They are used in therapy and diagnosis as well as in fundamental medical research.

The idea was to find DARPins that selectively bind to the so-called catalytic domain of Botox serotype A1, the part of the enzyme that is responsible for its effect on the nerves, by cutting up certain proteins. The DARPins were expected to inhibit this function.

"In vitro—that is, on individual samples in the test tube—we have identified a suitable candidate that limits the function of the botulinum toxin," Kammerer reports.

Through studies at PSI's Swiss Light Source SLS, the researchers were

able to precisely observe the complex of DARPin and the catalytic domain, down to the molecular level, and to find out how the DARPin prevents cleavage.

But when the researchers also tested this DARPin in cell cultures, in collaboration with a team at the Institute for Biomedicine at the University of Padua in Italy, a completely different effect—opposite, in fact—suddenly became apparent: The toxic action of the Botox—the cleavage of proteins that are important for the nerves' signal transmission—took effect even more rapidly than usual.

"At first we thought we had done something wrong," says Oneda Leka, a postdoctoral researcher in the PSI Laboratory for Biomolecular Research and first author of the study. But further experiments confirmed the contradictory finding: Instead of decreasing, the toxic effect of the Botox enzyme accelerated.

Now the researchers repeated the experiments with real muscles, the diaphragms of mice. These remain intact for a long time in a nutrient solution and are a favored model for testing the effects of nerve toxins. Here too the results indicated that with the DARPin the paralyzing effect of the toxin set in more than twice as rapidly.

New options for Botox therapy

Now the big question was: Why is this so? The possible explanation is very complex biochemically. Simply put, it is that the DARPins actually destabilize the toxin in such a way that they are transported more rapidly into the interior of nerve cells. As a result, the toxin takes effect more quickly.

"For this reason, we think the DARPin could broaden the spectrum of possible uses of botulinum neurotoxin," says Oneda Leka.

Although the researchers did not perform any comparative tests within the framework of this study, it does appear that botulinum neurotoxin A1 with the DARPin works considerably faster than A1 without the antibodies. At the same time, the duration of the effect remains significantly longer than that of E and F.

So the addition of this DARPin provides an intermediate variant between serotype A and serotypes E and F. The result—unexpected as it was—opens up new possibilities for treating a variety of diseases. According to Richard Kammerer, "In pain medicine, an additive that speeds up the onset of the effect of a long-lasting, extremely effective drug could be of interest."

More information: Oneda Leka et al, A DARPin promotes faster onset of botulinum neurotoxin A1 action, *Nature Communications* (2023). DOI: 10.1038/s41467-023-44102-4

Provided by Paul Scherrer Institute