proteinspotlight
> ONE MONTH, ONE PROTEIN <

Issue 19, February 2002
www.proteinspotlight.org

From sausages to wrinkles

Vivienne Baillie Gerritsen

It was a bout of sausage poisoning which led to the discovery of a protein now known as botulinum toxin. During the Napeolonic Wars, the Dukedom of Würtemberg in Stuttgart observed an increase in human deaths due to food intoxication. Smoked sausages seemed to be at the heart of the problem and the poison was subsequently termed sausage poison. It was the medical officer and poet Justinus Kerner (1786-1862) who first suspected poison of biological origin. The clinical symptoms added to his own experimental observations – he had fed bad sausage to various animals as well as to himself – led him to believe that the poison interfered with the motor and autonomous nervous signal transmission system. 'The nerve conduction is brought by the toxin into a condition in which its influence on the chemical process of life is interrupted.' Indeed, patients experienced a progressive paralysis from the cranium down which would end in death by suffocation following progressive difficulties in breathing. Kerner, however, did not know what the nature of the toxin was.

Justinus Kerner

Towards the end of the 19th century, Robert Koch (1843-1910) proved that biological organisms could be the cause of afflictions. And it was one of his former students – the Belgian microbiologist Emile Pierre van Ermengem – who identified the bacterium as well as the toxin which caused sausage poisoning. He accordingly called it *Bacillus botulinus*, from the latin botulus meaning sausage, though it was later renamed *Clostridium botulinum*. From then on, things went fast. The lethal toxin, botulinum toxin type A[1], was purified in the early 1920s and crystallised in the 1940s. It turned out to be a protein and its structure was studied in detail.

How does botox bring on this slow paralysis otherwise known as botulism? As Kerner predicted, botox meddles in the transmission of a nerve signal from a neuron to a neighbouring muscle cell. And it took over a century to understand how on the molecular level. Botulinum toxin is a dimer of a heavy and a light chain joined by one disulfide bridge. Once absorbed, it travels through the bloodstream to peripheral cholinergic synapses. There, the heavy chain binds irreversibly to specific receptors and the toxin is sucked into the neuron's cytosol via receptor-mediated endocytosis. The next step is to quit the endosome so that botulinum can deploy its toxic effect on the nerve ending. This may occur if the heavy chain forms a channel through which the light chain makes its getaway.

The light chain is a zinc endopeptidase. And it makes a beeline for SNAP-25, a protein essential for synaptic vesicle fusion. It splits SNAP-25 into two, and as a result the synaptic vesicle bulging with acetylcholine can no longer dock on the plasma membrane. Consequently, the neurotransmitter is not released into the synaptic cleft, the muscle fibre cannot contract, and muscle paralysis ensues.

[1] There are 7 different types of botulinum toxin (types A to G) of which botulinum toxin type A, also known as botox, is the most lethal.

Botulinum neurotoxin A has become part of biological warfare. It is the most lethal toxin known to date. One gram only of the crystalline toxin could kill a million people… Fortunately though, the constraints on concentrating and stabilising the toxin for aerosol dissemination for example are so great that such a disaster can probably be avoided. But the threat is there. During World War II, the United States of America produced over a million doses of antitoxin to counter the possible development of botox bioweapons by the Germans.

In this light, is it not ironic that the same lethal toxin has been used to overcome abnormal muscular afflictions since the 1960s? Justinus Kerner was the first to suggest that very small doses of the toxin could be used in pathologic hyperexcitability of the nervous system, i.e. muscle spasms. Blepharospasm is one example. People afflicted with blepharospasm blink incessantly to the extent of being unable to open their eyes at all. A small dose of botox relaxes the eye muscles, so that they can be opened or shut on command again. Unfortunately, the effect is temporary; the nerve endings regenerate, new synapses form and the muscles are back into business.

In the same way, for the past 30 years botox has been used to correct strabismus, relieve spasmodic torticollis and even hyperhidrosis (excessive sweating) by injecting minute doses of the toxin in the armpits, under the soles of feet or in the palms of hands, thus paralysing the sweat glands and checking the flow of sweat. The treatment for blepharospasm also led to a new technique of facial rejuvenation… Indeed, it did not take long before surgeons realised that botox injected close to the eye not only relaxes the muscles around the eyelids but also those which form wrinkles. A pinch of botox, and you have lost 20 years. What is more, patients who came for a dose of youth suffered much less from migraines, if they were subject to them.

A panacea? Botox must be injected in very small doses; larger doses could elicit an immune response to the toxin whose effects would then be neutralised. This raises an exciting prospect: the toxin could be used as an oral vector for vaccines. Botulinum toxin is particularly vigorous and can cross the gut and drop into the bloodstream unharmed, while most proteins are attacked by enzymes well before. Oral vaccines are tricky though. The immune system can be taught not to react to proteins absorbed through the gut and as a result antibodies against the vaccine are not made. The net result would be an actual weakening of the immune system. Botulinum toxin has certainly come a long way. Over 150 years ago, Kerner foresaw its therapeutic use but was still light years away from imagining its applications in warfare and rejuvenation.

Cross-references to Swiss-Prot

Botulinum neurotoxin type A, *Clostridium botulinum* : P10845

References

1. Becker-Wegerich P., Rauch L., Ruzicka T.
   Botulinum toxin A in the therapy of mimic facial lines
   Clin Exp Dermatol. 26:619-30(2001)
   PMID: 11696067

2. Arnon S. S., Schechter R., Inglesby T.V., Henderson D.A., Bartlett .JG., Ascher M.S., Eitzen E., Fine A.D., Hauer J., Layton M., Lillibridge S., Osterholm M.T., O'Toole T., Parker G., Perl T.M., Russell P.K., Swerdlow D.L., Tonat K.
   Botulinum toxin as a biological weapon: medical and public health management
   JAMA. 285:1059-1070(2001)
   PMID: 11209178

3. Botox, Botulinum Type A
   Excellent brief description of botulinum neurotoxin type A, how it works and what it is used for
   http://www.botox.com

Protein Spotlight (ISSN 1424-4721), http://www.proteinspotlight.org, is published by the Swiss-Prot group at the Swiss Institute of Bioinformatics (SIB).