A new wrinkle for botox: Research reveals how botulinum toxins affect neuron survival

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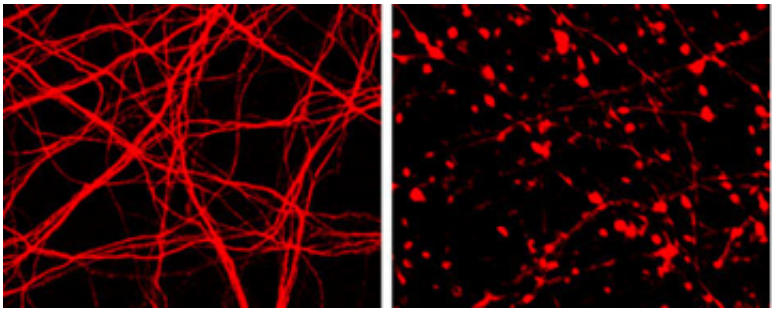

At left, a normal neuron. At right, neuron death induced by Botulinum type C toxin. Botox is type A. Credit: Dong Lab.

(Medical Xpress)—Botulinum toxins are feared as a food poison and bioterror threat, and for good reason. It takes only minute amounts of these bacterial toxins to block signals from nerve cells that control muscles. People die when the toxin paralyzes the muscles they need to breathe.

That is the deadly side of botulinum toxins and botulism disease, whose incidence has ebbed with the advent of safer food storage and processing. But one of the seven major botulinum toxin types, better known by its brand name Botox, is famously successful as a wrinkle remover.

Outside the cosmetic spa, botulinum toxins are now used to treat a

growing list of medical conditions. Injected into target regions of the body in extremely small quantities, they attenuate overactive neurons and relieve muscle spasms in cervical dystonia and arm tremors in multiple sclerosis. They can even halt excessive sweating and reduce migraine headaches. Type A—Botox—and type B are the only two types approved by the U.S. Food and Drug Administration for therapeutic and cosmetic uses, but other types are being explored as potential therapeutic toxins.

The effects of botulinum toxins usually last for up to a couple of months, so repeated injections are needed to maintain therapeutic effectiveness. This long-term exposure raises a critical question: In addition to producing temporary relief from overactive neurons, do botulinum toxins have the potential to cause irreversible nerve damage?

Now researchers from Harvard Medical School have investigated the effect that all seven botulinum toxins have on neuron survival. They have discovered that not all botulinum toxins are equally safe for neurons. Writing recently in *Nature Communications*, they reported that two of the seven botulinum toxins, type C and type E, induce degeneration of both cultured rodent neurons and human motor neurons derived from embryonic stem cells.

"Botulinum toxins are not expected to cause death of neurons if they just block signals between neurons and muscles, which is the well-established mode of action for this class of toxins," said Min Dong, HMS independent instructor in the Department of Microbiology and Immunobiology and the Division of Neuroscience at the New England Primate Research Center, and senior author of the paper. "It has been observed since as early as the 1980s that neurons die after exposure to type C toxin. The mechanism remains a mystery, but it raises a red flag as to whether botulinum toxins may affect additional neuronal functions."

Dong has been studying botulinum toxins for more than a decade, developing methods to detect the toxins in 2004 and identifying receptors for Botox in 2006, prior to establishing his lab at the New England Primate Research Center in 2009. Intrigued by the earlier observations on type C toxin, Dong and his postdoctoral fellow Lisheng Peng, first author of the study, designed assays to examine the effect of all seven botulinum toxins on neuron survival. Their study not only confirmed the previous observation on type C toxin, but it also revealed type E as the second toxin that can cause neuron death.

What could the mechanism be for the neuron death and why would it work only in types C and E, but not other botulinum toxins? Dong and his colleagues found that the answer lies in which host proteins are being attacked by each toxin type.

All botulinum toxins shut down signals from neurons to muscles by attacking a complex composed of three proteins inside neurons. It turns out that two of these three proteins, known as syntaxin 1 and SNAP-25, not only release signals from neurons, but also perform the essential housekeeping process of recycling the neuron's plasma membranes. Type C toxin attacks syntaxin 1, while type E toxin attacks SNAP-25. The resulting blockage of plasma membrane recycling leads to death of neurons.

In contrast to syntaxin 1 and SNAP-25, the third protein in the complex, known as synaptobrevin, is not involved in the recycling process. This explains why type B, D, F and G toxins, all of which attack synaptobrevin, do not cause neuron death.

The findings on Botox, or type A, are more complicated. Botox attacks SNAP-25, but it does not induce death of neurons. Clinical data have also demonstrated that Botox is safe for neuron survival in patients. Dong and his colleagues found out why: Type E cleaves a larger

fragment from SNAP-25 than type A, causing more extensive damage to the function of SNAP-25. In contrast, SNAP-25 cleaved by type A can still support the neuron's essential recycling of plasma membranes.

But the safety of type A toxin is not absolute, the scientists found. Once SNAP-25 has been cleaved by type A, its ability to tolerate additional mutations and defects in neurons is reduced.

"Botox is safe in general," Dong said, "but it can cause the death of neurons when we introduce mutations in SNAP-25 in our experiments. Whether this can occur in rare cases in patients needs to be studied further."

The discovery that syntaxin 1 and SNAP-25 are essential for neuron survival may also have implications for better understanding the neuron death process in neurodegenerative diseases, Dong said.

"Syntaxin 1 and SNAP-25 have been implicated in neurodegenerative diseases, and our data suggest that disruption of plasma membrane recycling might be a contributing factor."

As for clinical uses, caution is advised.

"We have to be careful about which type of botulinum toxin we can use in patients," Dong said. "Based on our findings, type C and type E toxins should not be used in humans."

More information: Peng, L. et al. Cytotoxicity of botulinum neurotoxins reveals a direct role of syntaxin 1 and SNAP-25 in neuron survival, *Nat Commun*. 2013 Feb 12;4:1472. doi: 10.1038/ncomms2462.

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