Disarming the botulinum neurotoxin

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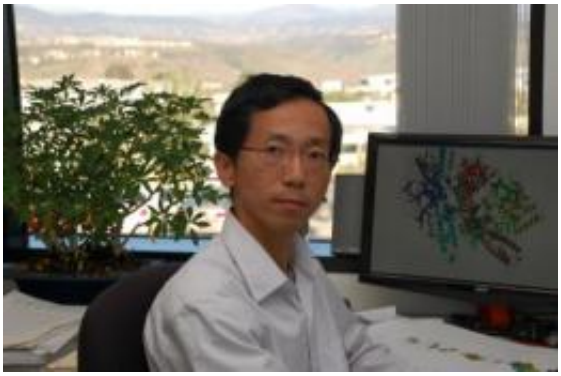

Rongsheng Jin, Ph.D., is an assistant professor at Sanford-Burnham Medical Research Institute. Credit: Sanford-Burnham Medical Research Institute

Researchers at Sanford-Burnham Medical Research Institute (Sanford-Burnham) and the Medical School of Hannover in Germany recently discovered how the botulinum neurotoxin, a potential bioterrorism agent, survives the hostile environment in the stomach on its journey through the human body. Their study, published February 24 in *Science*, reveals the first 3D structure of a neurotoxin together with its bodyguard, a protein made simultaneously in the same bacterium. The bodyguard keeps the toxin safe through the gut, then lets go as the toxin enters the bloodstream. This new information also reveals the toxin's weak spot—a point in the process that can be targeted with new therapeutics.

"Now that we better understand the structure of the bacterial machinery that was designed for highly efficient toxin protection and delivery, we

can see more clearly how to break it," said Rongsheng Jin, Ph.D., assistant professor in Sanford-Burnham's Del E. Webb Neuroscience, Aging and Stem Cell Research Center and senior author of the study.

The Janus-faced toxin

The botulinum neurotoxin is two-faced. On one side, it's the most poisonous substance known to man, causing botulism. Accidental botulinum neurotoxin poisoning is usually food-borne, but it's also considered a potential bioterrorism agent. On the other side, botulinum neurotoxin is also used an effective therapy and popular cosmetic, such as in BOTOX.

The neurotoxin accomplishes both the good and the bad using the same trick—paralyzing muscle cells by disrupting their connections with the nerves that tell them how and when to move. But before the neurotoxin can gain access to muscles and the neurons that control them, it must make a remarkable journey through the body—surviving the digestive enzymes and extreme acidic environment in the stomach, penetrating the small intestine, and entering the bloodstream.

Sneaking a peek at the neurotoxin and its bodyguard

This latest study on the botulinum neurotoxin was the result of a close collaboration between the Jin group and a research group at the Institute of Toxicology at the Medical School of Hannover, led by Andreas Rummel, Ph.D., an expert on clostridial neurotoxins. They used a technique called X-ray crystallography, which uses powerful X-ray beams to produce 3D images of proteins at the atomic level, to study a genetically inactivated, nontoxic version of the botulinum neurotoxin.

These experiments helped the team visualize the atomic structure of all

three parts of the toxin: 1) the region that recognizes neurons, 2) the enzyme that acts like a pair of scissors to cut human neural proteins and cause paralysis, and 3) the needle that punches holes to help deliver the enzyme to the nerve terminal. What's more, the researchers also captured the toxin's interaction with a second bacterial protein, called nontoxic nonhemagglutinin (NTNHA).

"We were surprised to see that NTNHA, which is not toxic, turned out to be remarkably similar to botulinum neurotoxin. It's composed of three parts, just like a copy of the toxin itself. These two proteins hug each other and interlock with what looks like a handshake," said Jin.

As the toxin moves through the body, NTNHA acts as its bodyguard, keeping it from being degraded when times are tough in the acidic stomach. However, as this study revealed, the toxin has a weak spot: when the toxin/NTNHA complex punches its way out of the small intestine, it's the change in pH that triggers a conformational change, breaks up the duo, and releases only the unprotected toxin into the bloodstream.

Towards prevention and therapy

According to Jin, this new knowledge about how the botulinum neurotoxin and NTNHA balance the need for strong binding and a timely release could be exploited to outsmart them.

"We now hope we might be able to fool the toxin and its bodyguard using a small molecule that sends the wrong signal—mimicking pH change, prematurely breaking up their protective embrace, and leaving the stomach's digestive enzymes and acid to do their job," he said. "We envision this type of therapy—either alone or in combination with other therapies currently in development—could be given preventively at a time when botulinum neurotoxin contamination becomes a public health

concern."

Moreover, this type of therapy could be designed for oral delivery, rather than injection, making it easier to treat large numbers of people during an outbreak. A similar strategy could be used to deliver other protein-based drugs that usually need to be injected. "Here, protein drugs could be linked to a botulinum neurotoxin fragment and protected with NTNHA. Then we could possibly take them by mouth," Jin said.

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