MedicalXpress

Novel antitoxin strategy developed using 'tagged binding agents'

February 25 2010

A study involving the world's deadliest substance has yielded a new strategy to clear toxins from the body—which may lead to more efficient strategies against toxins that may be used in a bioterrorist event, as well as snake bites, scorpion stings, and even some important chronic diseases.

A Tufts-led team developed the new strategy to deliver small binding agents that seek out Botulinum toxin molecules and bind to them at several points. The binding agents each contain a common "tag" that is recognized by a single, co-administered anti-tag antibody. Once the toxin molecule is surrounded by bound antibodies, it is flushed out of the system through the liver before it can poison the body.

Botulinum toxin, which causes botulism, is the most acutely poisonous substance known and is considered among the most dangerous bioterrorist threats. Studies have shown that one gram of the toxin, which is produced by a bacterium that lives in soil, could kill upwards of a million people. Although currently available antitoxins can be mass produced and delivered in the event of an outbreak, they are costly to develop, house and deliver—and have a short shelf-life.

The Tufts study, in collaboration with researchers at Thomas Jefferson University in Philadelphia, is published this month in the journal *Infection and Immunity* and was funded by the National Institutes of Allergy and Infectious Diseases (NIAID) and the New England Regional Center for Excellence (NERCE) for Biodefense and Emerging

Infectious Diseases.

"We've proven this approach to protect against Botulinum intoxication in mice and we hope this will lead to rapid development and deployment of many new anti-toxin therapies—for botulism and beyond," said Charles B. Shoemaker, PhD, professor of biomedical sciences at Tufts University's Cummings School of Veterinary Medicine and the study's corresponding author.

The new findings expand on a 2002 breakthrough at the University of California at San Francisco, where scientists combined three monoclonal antibodies against Botulinum toxin that attached to different parts of the toxin molecule. Including three different antibodies dramatically increased the potency compared to fewer antibodies and prevented intoxication even following high-dose exposure. However, developing, producing, and stockpiling three different monoclonal antibodies against each toxin type is very expensive.

Instead of using three antibodies, the Tufts approach uses three small binding agents to direct a single monoclonal antibody to multiple sites on the biomolecule being targeted for clearance. The type of binding agents used can be selected from many scaffolds developed for commercial therapeutic applications (e.g. nanobodies, aptamers, darpins, FN3, microbodies, etc). These binding agents can be rapidly identified and improved using modern technologies and generally have excellent commercial production and product shelf-life properties. The single anti-tag monoclonal antibody can also be selected to have optimal isotype and commercialization properties.

What's more, the binding agents can be produced with more than one tag, which enables them to direct more antibodies to the toxin—and synergistically improve target clearance from the body. Many binding agent scaffolds can be produced as functional multimers so that the

different binding agents could be produced as "beads on a string," leading to a single molecule that targets one, or even several, biomolecules for clearance from the body.

Using this approach, the researchers say, one would only need to create new binding agents, not new antibodies, to create a therapy to clear a toxin from the body—paving the way for new therapies that combat toxins ranging from animal venom to bioterrorist agents such as ricin. Tufts researchers are currently targeting Shiga toxin and C. difficile along with other types of Botulinum toxin. Future plans include targeting clearance of pathogenic cytokines that are implicated in inflammation and autoimmune diseases.

Treatment for botulism usually requires many weeks of intensive-care hospitalization, and exposure of even a small number of people would seriously disrupt health care delivery in any major city, studies have indicated. A vaccine has been developed, but widespread use is not currently being considered, the researchers say, since the likelihood of exposure is uncertain. Also, vaccination would block accepted treatments for a number of overactive muscle conditions, including dystonias, which respond to the toxin when administered in very small doses.

Provided by Tufts University