BIOTERRORISM

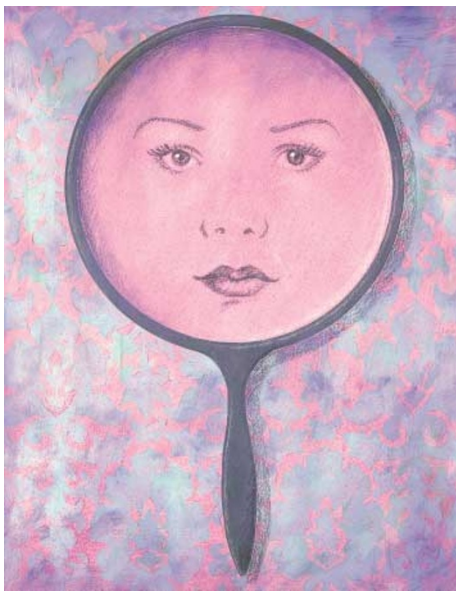
Paralyzing the two-faced BOTOX

Although it is fashionable to inject botulinum neurotoxins (BoNT) to reduce wrinkles, BoNTs are also among the most lethal biological substances to have been weaponized as a highly toxic aerosol form. Such a potential bioterrorist threat necessitates the development of therapeutic countermeasures against BoNTs. A collaborative effort by researchers at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the National Cancer Institute (NCI) and the University of Nebraska Medical Center (UNMC) reports the identification of several nonpeptidic small-molecule inhibitors of BoNT serotype A (BoNT/A) in the 10 October issue of *Biochemical and Biophysical Research Communications*.

A number of distinct BoNT serotypes (A–G) are produced by the spore-forming anaerobic bacteria *Clostridium botulinum*. BoNT is secreted as a holotoxin composed of two peptide chains linked by a disulphide bridge. The heavy chain (HC) binds to surface receptors on nerve terminals, which internalize the molecule via the formation of an endosome. Once inside the low-pH endosome, the light chain (LC) dissociates from the HC and is released into the cytosol, where it acts as a zinc metalloprotease and cleaves soluble NSF-attachment protein receptor (SNARE) proteins. Without functional SNARE complexes, the neurotransmitter acetylcholine is not released into neuromuscular junctions, leading to paralysis.

Translocation of the holotoxin and LC metalloprotease activity have been targets of previous research to identify peptide and small-molecule inhibitors. Several antimalarial compounds have been shown to interfere with translocation, probably because these agents raise the endosomal pH so that the LC cannot be released into the cytoplasm. A number of known protease inhibitors, such as captopril, lisinopril and enalapril, also inhibit LC protease activity, but only at mM concentrations.

In this study, Sina Bavari, Rick Gussio and colleagues identified small-molecule inhibitors of BoNT/A LC metalloprotease activity in the low M range. Initially, the NCI diversity set was screened and a number of compounds possessing inhibition at 20 M were identified. The diversity set contained 1,990 compounds (taken from the NCI's full repository) that were preselected to cover a wide range of conformational space, as well as pharmacophore diversity and structural rigidity. One of the most potent and therapeutically interesting inhibitors to be identified during this screen was michellamine B. Additional analyses of a series of bis (7-chloro-4-aminoquinoline) derivatives obtained from UNMC also resulted in the identification of several M-range inhibitors. Molecular modelling techniques involving conformational sampling and molecular docking studies revealed a common pharmacophore for the identified BoNT/A LC inhibitors. This pharmacophore will serve as a basis for directing future database-mining studies and synthetic medicinal chemistry efforts to identify and develop BoNT/A LC inhibitors with enhanced potency.



Melanie Brazil

References and links

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FURTHER READING Singh, B. R. Intimate details of the most poisonous poison. *Nature Struct. Biol.* **7**, 617–619 (2000) | Arnon, S. S. *et al.* Working Group on Civilian Biodefense. Botulinum toxin as a biological weapon: medical and public health management. *JAMA* **285**, 1059–1070 (2001) | Turton, K., Chaddock, J. A. & Acharya, K. R. Botulinum and tetanus neurotoxins: structure, function and therapeutic utility. *Trends Biochem. Sci.* **27**, 552–558 (2002).
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NCI Diversity Set: http://dtp.nci.nih.gov/branches/dscb/diversity_explanation.html