Editorial: Botulinum Neurotoxin detection

Kodumudi Venkat Venkateswaran

Radix BioSolutions,
111, Cooperative Way Suite 120,
Georgetown, TX 78681, USA
E-mail: drv@omniarraybt.com

Biographical notes: Kodumudi Venkat Venkateswaran an Independent Biotechnology Professional providing scientific advice to biotechnology companies in USA including Tetracore, Rockville, MD, and Radix Biosolutions, Georgetown, TX. He is also the Managing Director of Omni Array Biotechnology India (P) Ltd. at Coimbatore (India). His research interests include advanced detection of biothreat agents, technology development for drug discovery, molecular and personalized medicine. He received his PhD in Biochemistry/Immunology from Indian Institute of Science (IISc), Bangalore, India. He worked as scientist at Defense Research & Development Organisation in India for more than 10 years in the areas of microbiology and toxicology. He did Post-Doctoral Research at the University of California San Francisco and at the University of California Lawrence Livermore National Laboratory (LLNL), Livermore, CA. While working at LLNL his research projects included biodetection instrumentation and advanced diagnostic methods design, development and validation. He constantly serves as a reviewer for the National Institutes of Health in the Special Emphasis Panels.

Botulinum neurotoxin (BoNT) is one of the most poisonous substances known to mankind. Seven different serotypes and many subtypes of the neurotoxins are known and are produced by spore forming bacteria Clostridium botulinum. Because of the very high toxicity, in addition to being a serious public health risk, BoNTs also pose major threat as bioterrorism agent (Arnon et al., 2001). Rapid and sensitive detection of the BoNTs is an important first step to manage the harmful effects caused by the deadly botulinum toxins. BoNTs cause the clinically well-known picture of flaccid muscular paralysis. The BoNT-mediated proteolysis of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins inhibits the exocytosis of acetylcholine into neuromuscular junctions, leading to life-threatening flaccid paralysis.

The widely accepted and highly sensitive mouse bioassay for botulinum detection is considered as the gold standard test. Mouse bioassay provides a comprehensive way for testing the three different important steps of BoNT toxicity namely binding of toxin, its translocation and the endopeptidase activity. The major disadvantage with the mouse bioassay is the use of animals and requirement of longer time (~4 days) to get the results. Several in vitro detection methods using cell based, biochemical, and molecular methods as alternatives to mouse bioassay are under development and reviewed in the paper (Cai et al., 2007). Immunological methods using serotype specific antibodies for all seven

BoNT serotypes have become a valuable tool for the detection and identification of these toxins. Measurement of the endopeptidase activity of different BoNT serotypes using specific polypeptide/protein substrates provides the biochemical tool in characterising the toxicity. Use of fluorogenic peptide/protein substrates has made the endopeptidase tests very effective and high throughput for in vitro testing (Ruge et al., 2011). Mass spectrometric detection of endopeptidase activity has been shown to be highly sensitive and broadly applicable (Boyer et al., 2005, 2011). Molecular methods and real time quantitative Polymerase Chain Reaction (PCR) tests have become readily applicable rapid detection tools in botulinum neurotoxin research (Raphael et al., 2010; Hill et al., 2010).

Botulinum Research Center (BRC) at University of Massachusetts at Dartmouth (UMD) has been organising annual symposium for the past four years for highlighting the developments in the area of botulinum research. In conjunction with the UMD BRC annual symposia, workshops emphasising the practical aspects of botulinum toxin detection methods were organised on three occasions. Practical demonstrations of modern BoNT detection methods were held in addition to devoting time in panel discussions relating to the various aspects of BoNT identification and characterisation.

In an effort to provide the botulinum research community a few papers were solicited for publication in The Botulinum Journal. Susan Maslanka and colleagues from Centers for Disease Control, Atlanta have developed rapid, and sensitive Enzyme Linked Immunosorbent Assay (ELISA) for detecting BoNT A, B, E and F, the four main serotypes responsible for causing human sickness. They have demonstrated the usefulness of this in vitro test for rapid detection of the toxins from clinical samples and compared the performance with the gold standard mouse bioassay. This seminal work clearly demonstrates the power of a simple assay that is very effective and readily applicable for public health laboratories as a screening test.

One of the important aspects of production and characterisation of highly specific antibody reagent for BoNT immunoassays is presented in the research work by Ellen Goldman and colleagues at Naval Research Laboratory, Washington DC. This paper presents the development of single chain Llama antibodies to BoNT and their biochemical characterisation.

Concerted efforts by the botulinum research community are required to come out with an alternative test to mouse bioassay that can help in minimising the animal use. Availability of high quality biochemical and molecular reagents and inter-disciplinary collaborative research will surely provide the technology solution to this long-awaiting need of the botulinum research community in the near future.

References

Arnon, S.S., Schechter, R., Inglesby, T.V., Henderson, D.A., Bartlett, J.G., 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. and Tonat, K. (2001) 'Botulinum toxin as a biological weapon: medical and public health management', *JAMA*, Vol. 285, pp.1059–1070.

Boyer, A.E., Gallegos-Candela, M., Lins, R.C., Kuklenyik, Z., Woolfitt, A., Moura, H., Kalb, S., Quinn, C.P. and Barr, .JR. (2011) 'Quantitative mass spectrometry for bacterial protein toxins--a sensitive, specific, high-throughput tool for detection and diagnosis', *Molecules*, Vol. 16, pp.2391–2413.

Boyer, A.E., Moura, H., Woolfitt, A.R., Kalb, S.R., McWilliams, L.G., Pavlopoulos, A., Schmidt, J.G., Ashley, D.L. and Barr, J.R. (2005) 'From the mouse to the mass spectrometer: detection and differentiation of the endoproteinase activities of botulinum neurotoxins A-G by mass spectrometry', *Anal. Chem.*, Vol. 77, pp.3916–3924.

Cai, S., Singh, B.R. and Sharma, S. (2007) 'Botulism diagnostics: from clinical symptoms to in vitro assays', *Crit. Rev. Microbiol.*, Vol. 33, pp.109–125.

Hill, B.J., Skerry, J.C., Smith, T.J., Arnon, S.S. and Douek, D.C. (2010) 'Universal and specific quantitative detection of botulinum neurotoxin genes', *BMC Microbiol.*, Vol. 10, p.267.

Raphael, B.H., Joseph, L.A., McCroskey, L.M., Luquez, C. and Maslanka, S.E. (2010) 'Detection and differentiation of Clostridium botulinum type A strains using a focused DNA microarray', *Mol. Cell. Probes.*, Vol. 24, pp.146–153.

Ruge, D.R., Dunning, F.M., Piazza, T.M., Molles, B.E., Adler, M., Zeytin, F.N. and Tucker, W.C. (2011) 'Detection of six serotypes of botulinum neurotoxin using fluorogenic reporters', *Anal. Biochem.*, Vol. 411, pp.200–209.