Editorial: Increasing biomedical research of botulinum and its regulation

Bal Ram Singh

Botulinum Research Center,
University of Massachusetts Dartmouth,
Dartmouth, MA 02747, USA
E-mail: bsingh@umassd.edu

Biographical notes: Bal Ram Singh is a Professor of Chemistry and Biochemistry at the University of Massachusetts Dartmouth, and the Founding Director of the National Botulinum Research Centre at UMass Dartmouth, which has over three dozen national and international botulinum researchers associated with it. He has been working with structure and functions of botulinum neurotoxins for over 25 years, and is a leading Scientist in the field. He received his PhD in Chemistry from Texas Tech University, and post-doctoral training at the University of Wisconsin Madison.

Integration of basic and clinical aspects of botulinum neurotoxins received a shot in the arm with TOXINS 2012, and subsequent plans of holding this meeting every year. The first such meeting was held in Madison, WI, in 1993, organised by a basic researcher, Dr. Bibhuti Ranjan Das Gupta, who had spent over three decades researching biochemistry of botulinum neurotoxins, but had a vision to invite researchers working not only with botulinum neurotoxins but also those working with the targets of these toxins, i.e., nerves. These included both basic and clinical researchers using botulinum neurotoxins as tools and therapeutics. It is a welcome sign that the popularity of BoNT is increasing for research and clinical use, and the TOXINS 2012 meeting will be the third annual meeting on BoNT. The other two being the longest running Interagency Botulism Research Coordination Committee (IBRCC) celebrating its 50th year in 2013, and the Seventh Annual Dartmouth Botulinum Research Symposium, held every summer in Dartmouth, MA.

We are happy to carry the abstracts of the TOXINS 2012 in a special arrangement so that this issue of TBJ carrying the abstracts will have free public access. We hope to develop such relationships for future such meetings to document the discussions and promote further scholarly interactions.

As I have written before in these pages BoNT has come to represent a family of molecules with multifaceted implications – biothreat and food poison, fundamental science and tool of discovery, and clinical applications. Clinical applications of botulinum neurotoxin are ever increasing, as is the research on clinical applications. About 90% of the papers presented at the TOXINS 2012 were related to clinical applications.

Over 50 million individuals receive injections of therapeutic botulinum neurotoxin every year, alleviating neuromuscular disorders and cosmetic concerns. Nevertheless, the products have received the highest level of warning label – the black box – from US Food

and Drug Administration. Recent reports suggest that botulinum neurotoxin when injected even at therapeutic doses may trigger responses from central nervous system.

Additionally, botulinum neurotoxin is being used as a tool of neuroscience discovery, and its recombinants components are being redesigned for designer therapeutics.

A lot more research, including basic research on this molecule, the molecular composition of the botulinum neurotoxin complex, the changing genetic makeup of the organisms, and mechanism of action, needs to be carried out for safe and expanded use of botulinum related materials for therapeutics.

More scientific knowledge will also address a major concern of botulinum being used as a biothreat agent. But, this latter concern and associated regulatory issues are, in fact, dissuading more research with botulinum neurotoxins. Two major suppliers of botulinum neurotoxin reagents, Sigma and Waco, no longer supply such reagents for research. It is difficult to estimate how many researchers may have been disinclined to conduct research botulinum neurotoxins, despite the fact that 0.5 mg of toxin material is exempted from federal regulation in the USA.

With the latest guidelines, effective since April 4, 2013, classifying *Clostridium botulinum* and botulinum neurotoxins as tier 1 agents, the US federal guidelines have further tightened the noose around researchers conducting legitimate research with these organisms and reagents. Botulinum neurotoxins are biologically most effective molecules known, which at high concentrations (tens of nanogram) act as potent toxins whereas at low concentration (tens of picogram) act as very effective medicines.

The new classification of tier 1 for botulinum neurotoxins mostly introduces more security requirements, and rightly so, to safeguard the toxin against it falling in wrong hands. It actually even exempts animals injected with small quantity of toxins for bioassay purpose from being tagged as select agent, a term that triggers several regulations for its handling. The latest regulations also retain the exemption of up to 0.5 mg botulinum neurotoxin from select agent regulations.

Nevertheless, the biosafety regulations remain major concerns of researchers as well as those of the regulators. Although both *C. botulinum* and botulinum neurotoxins are classified as biosafety level 2 agents, the regulation refers to the Biosafety in Microbiological and Biomedical Laboratories (BMBL) for recommended practices.

According BMBL recommendation, “BSL-2 practices, containment equipment, and facilities are recommended for routine dilutions, titrations or diagnostic studies with materials known to contain or have the potential to contain BoNT. Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of toxin”.

These BSL2/BSL3 facilities requirements are similar to many of the infectious agents, such as anthrax, *Francisellatularensis*, *Yersinia pestis*, etc., which can naturally cause infection if aerosols are released. In case of *C. botulinum* infections are rare due to its anaerobic nature. There has, in fact, never been any report of accidental aerosol formation of *C. botulinum* or botulinum neurotoxins. There has been no reported case of botulinum neurotoxin accidentally causing botulism in laboratory conditions, except one case where aerosol experiments were being conducted.

Given the need of substantial experimental research needed for understanding several features of botulinum neurotoxin, and also its use as therapeutics, there should be efforts from the scientific community and regulatory agencies to develop special safety and security protocols for botulinum neurotoxins, which can address appropriate concerns of

safety and security while encouraging more researchers to be involved in botulinum research. One step that can be effectively used to alleviate many of the safety concerns is to develop an effective vaccine. Take an example of tetanus neurotoxin, which is equally toxic but is not a select agent because of the vaccination. In case of BoNT, although vaccination of general population is not advisable due to its use as a therapeutic, vaccination may be possible for at least researchers at risk while working with botulinum neurotoxins.

European agencies appear to have done better in managing safety and security issues involved in working with botulinum neurotoxins. Researchers there appear to have relaxed guidelines, which are followed but not inspected unlike USA. For example, European authorities even allow cloning and expression of native BoNT in *E. coli*, which is rare in the USA.

Another relative benign step that could at least psychologically alleviate the concern of many not that familiar with BoNT is to rename botulinum neurotoxin as botulinumneuromedicine (BoNEM), as suggested on these pages previously (Singh, 2009). For foot soldiers of the regulations, be local or federal, it makes huge difference what you call it.

References

Singh, B.R. (2009) 'Special editorial: Menace or the ultimate medicine – a case for botulinum neuromedicine', *The Botulinum J.*, Vol. 1, No. 3, pp.257–260.