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Redox regulation of botulinum neurotoxin toxicity: therapeutic implications

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Abstract

Botulinum neurotoxin causes botulism, and the only effective antidote is the antitoxin. Botulinum neurotoxins are disulfide linked di-chain proteins encompassing a light chain Zn^{2+}-protease that is translocated by a heavy chain channel from the synaptic vesicle lumen into the neuronal cytosol where it acts. Protease release from the channel is required for toxicity. The Thioredoxin Reductase-Thioredoxin system cleaves the interchain disulfide, and its inhibition prevents neurotoxicity, and may provide novel strategies for chemoprophylaxis and therapy.

Keywords

botulism; protease; SNAREs; channel; chaperone; thioredoxin

Clostridium botulinum neurotoxin (BoNT) abrogates synaptic exocytosis and causes botulism, an acute and severe neuroparalytic disease characterized by symmetric descending flaccid paralysis [1,2]. Despite the wide recognition of BoNT as the most toxic protein known and as one of the most feared biological weapons of the 21st century, the only effective antidote is the antitoxin and there is an urgent need to identify small-molecule inhibitors (SMIs). Intoxication requires the endocytosed di-chain protein to undergo conformational changes in response to pH and redox gradients prevalent across synaptic vesicles (SVs; acidic and oxidizing in the vesicle lumen, and neutral and reducing in the cytosol) [3]. These conditions induce the formation of a protein-conducting channel by the heavy chain (HC) that translocates the light chain (LC) protease into the cytosol, colocalizing it with the substrate SNARE (Soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins [2]. Release of the LC at the completion of translocation entails reductive cleavage of the disulfide linkage to the HC [4]. How is this achieved? Pirazzini et al. [5] recently used a biochemical and pharmacological approach to provide compelling evidence that the Thioredoxin Reductase (TrxR)-Thioredoxin (Trx) system is present on the cytosolic surface of SVs, and catalyzes reduction of the LC-HC disulfide of BoNTs serotype A, C, and E (Box 1). Importantly, inhibitors of TrxR and Trx

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prevent neuronal intoxication by BoNT, attenuate its neuroparalytic action, and, when administered to mice prior to BoNT exposure, prolong time to death and decrease the death frequency. The implication is that the TrxR-Trx redox system is a target for pharmacological intervention.

Box 1

BoNT occurs in eight antigenic types denoted as serotypes A to H and designated using a "/" (e.g., BoNT/A). Substrate specific binding combined with sequence variations proximal to the active site determine serotype specificity [1,2].

BoNTs act at peripheral nerve terminals by a multistep mechanism involving binding, internalization, translocation across SVs, cytosolic traffic, and SNARE proteolysis [1,2]. Each step is a potential target for intervention. The LC executes the ultimate action of BoNTs on presynaptic terminals by cleaving the SNAREs. The amino-terminal half of HC is a protein translocase for its LC cargo driven by a proton gradient across SVs and a chaperone that promotes and maintains cargo unfolding during translocation. The carboxy-terminal half of HC (receptor-binding domain) contains two determinants of BoNT neurotropism: a ganglioside attachment site at the membrane and a protein receptor that dictates serotype specificity. The conserved LC–HC disulfide is a crucial determinant for cargo translocation and release, and therefore, is a *conditio sine qua non* for toxicity.

There is unprecedented interest in designing BoNT antidotes based on its modular structural and functional architecture. Design of antibotulinal agents targeted to block BoNT entry at the protein receptor-binding site constitutes a robust field encompassing therapeutic antibodies and competitive ligands [6]. Small-molecules, peptides, and peptidomimetics are intensely investigated as selective LC inhibitors [7]. Such strategy is focused on the cytosolic protease after its release from SVs. Accordingly, specific delivery to intoxicated neurons is a prerequisite for successful implementation. Targeting the translocation step is delicate as it involves design of SMIs directed to transient conformers of LC and HC during a dynamic process. Indeed, toosendanin, a traditional Chinese medicine that protects monkeys and mice from BoNT-induced death and preserves SNAP-25 (synaptosome-associated protein of 25 kDa -the substrate of BoNT/A, BoNT/E and BoNT/C) in spinal cord neurons, arrests translocation of LC/A and LC/E [8]. However, toosendanin is not an inhibitor after translocation, pointing to the complexity of designing a drug targeted to a protein-protein interface that is intrinsically ephemeral, as the LC unfolds and transits within a channel provided by its HC chaperone.

The identification of the TRxR-Trx system as the catalyst of the reductive cleavage of the LC-HC bond [5] is of paramount significance in the development of SMIs of BoNT. A widely held view considers the TRxR-Trx system as a critical regulator of redox signaling and cellular homeostasis [9,10]. Dysfunction of redox homeostasis is associated with devastating disorders from cancer to neurodegenerative diseases. It is, therefore, not surprising that numerous SMIs are intensely explored as potential means of intervention. Among these, Auranofin, a TRxR inhibitor, is in clinical use for rheumatoid arthritis. Additionally, PX-12 and Ebselen, inhibitors of Trx, are being investigated for cancer and

post-ischemia/stroke treatment [9]. Pirazzini et al. [5] demonstrate that these agents are BoNT inhibitors in diverse assays ranging from SNAP-25 cleavage in cerebellar neurons, to mouse death. Notably, these chemicals are inactive against the isolated LC, thereby highlighting the key role of the LC-HC disulfide in neurotoxicity.

The new findings pose interesting implications. Trx is ubiquitous and members of the Trx family are widely and densely expressed in the human brain [9,10]. The vital role of redox homeostasis may account for such redundancy and, in turn, imply that Trx inhibitors may exhibit low toxicity. At the root of their action is the conserved α/β Trx fold containing the active site motif CXXC that interchanges between reduced (sulfhydryl) and oxidized (disulfide) forms. The catalytic motif is exposed on a loop that connects the C-end of a β-strand with the N-end of an α-helix, rendering the redox centers accessible to the disulfides of target proteins [9,10]. TrxR, a flavoenzyme, in turn reduces the disulfide of Trx with electrons from NADPH [9,10]. The accessibility of the LC-HC disulfide at completion of translocation and LC exit from the HC channel is unknown. Nevertheless, given atomic resolution structures of numerous LCs (serotypes A to G) and Trxs affords opportunities to map detailed interactions between the folded LC (e.g., PDB 1XTG) and Trx (e.g., PDB 1F9M), and thereby discern unique structural elements that confer selective recognition of the LC-HC disulfide by Trx. An outcome of such strategy may consist of templates for inhibitor design. Pirazzini *et al* [5] examined several TrxR-Trx inhibitors; the survey though extensive is not exhaustive. Availability of sensitive assays amenable for high throughput screening of small-molecule libraries may open new paths to identify leads that may be active in the subnanomolar range, a step forward towards the development of antibotulinal agents based on Trx inhibitors. The TrxR-Trx system emerges, therefore, as a target for drug discovery to prevent and treat botulism, an urgent priority.

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