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Engineered Botulinum Neurotoxin With Extended Therapeutic Applications

MCW Case 1592

Stage of Development:
Proof-of-Concept In Vitro

Intellectual Property:
PCT Patent Application No. PCT/US2010/030875
US Patent 10,011,823
Filed April 13, 2010
(Pending/Issued Foreign and US Patents)

Inventors:

- Joseph Barbieri, Ph.D.
- Sheng Chen, Ph.D.

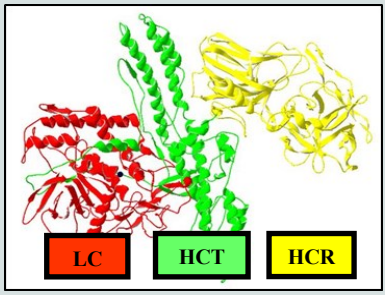
CONTACT:
KEVIN BOGGS
OFFICE OF TECHNOLOGY DEVELOPMENT

MEDICAL COLLEGE OF WISCONSIN
8701 Watertown Plank Road
Milwaukee, WI 53226

Phone: 414-955-4381
kpboggs@mcw.edu

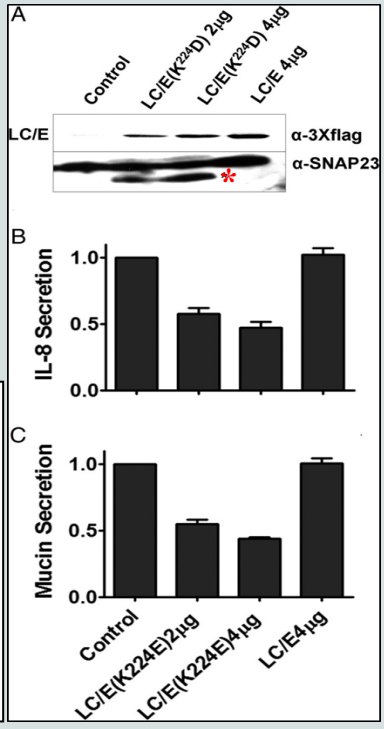
Clostridium botulinum bacteria produce a toxin (BoNT) that paralyzes muscles. BoNT enters peripheral cholinergic neurons where it cleaves neuron-specific SNARE proteins involved in secretion of the neurotransmitter acetylcholine into the neuromuscular junction. SNARE proteins are found in a variety of cell types and are involved in the secretion of proteins, hormones and other cellular products along with other cellular processes.

The temporary and reversible effects of BoNT has spurred the development of BoNT-based therapies for neuromuscular conditions. Moreover, favorable safety profiles of BoNT formulations have stimulated empirical and off-label use in a variety of conditions including cosmetic disorders.



The current invention is a novel BoNT-based therapeutic that targets the SNARE isoforms and inhibits their functions in non-neuronal cells. Like the wild-type molecule, our engineered BoNT has three (3) functional domains: C-terminal receptor binding domain (HCR (yellow)) that binds to the surface of the cell; an N-terminal zinc metalloprotease light chain (LC (red)), the catalytic (toxic) component; and a central translocation domain (HCT (green)) that delivers the catalytic LC into the cell. In our engineered toxin, amino acid residue 224 in the catalytic domain in the light chain has been changed from lysine to aspartic acid - i.e. "LC/E($K^{224}D$)".

Proof-of-Concept. LC/E($K^{224}D$) cleaves both non-neuronal SNAP23 and neuronal SNAP25, and inhibits mucin and IL-8 secretion by cells *in vitro*. This engineered BoNT could be used to reduce cellular secretions in asthma, cystic fibrosis, COPD, gastric acid reflux, inflammation/immune disorders and cancer.



Digitonin-treated HeLa cells were incubated with LC/E($K^{224}D$) or wildtype toxin LC/E. Cells were then exposed to TNF-α for 36 h. (*A*) Cell lysates were subjected to SDS/PAGE and LC/E expression and SNAP23 cleavage was measured by Western blot, using α-3Xflag antibody and α-SNAP23 antibody, respectively; [* indicates migration of the SNAP23 cleavage product.] ELIZA was used to measure IL-8 (*B*) and mucin (*C*) in the culture supernatants. From PNAS 2009;106:9180-9184.

Key Advantages:

- Catalytic domain cleaves human SNAP23 and SNAP 25
- Potentially targets several diseases
- Native BoNT structural domain/function relationships preserved
- Stable, soluble construct
- Binding domain can be modified for specific cell types as needed
- Currently produced in 5 mg amounts by batch culture - scalable