DE-RISKED IMMUNOGENICITY FOR ENHANCED EFFICACY OF BOTULINUM TOXIN-BASED THERAPEUTICS

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Background: Immunogenicity can diminish therapeutic efficacy of natural and engineered botulinum toxins (BoNTs) and recombinant proteins based on BoNTs. De-immunization by T cell epitope modification is a strategy for reducing immunogenicity risk that blocks early steps in immune responses to prevent anti-drug antibody production. We set out to de-immunize a molecule composed of the light and heavy chains of BoNT/A (LH_N/A), a novel therapeutic platform for targeted secretion inhibition.

Methods: Immunoinformatic algorithms identified T cell epitope targets for epitope modification. Epitopes were assessed for human leukocyte antigen (HLA) class II DR binding and antigenicity using peripheral blood leukocytes from BoNT/A-exposed donors to confirm predictions and down-select de-immunization targets. LH_N/A variant proteins were produced in *E. coli* with site-specific changes computationally designed to reduce potential immunogenicity and preserve protein structure. Purified protein preparations were evaluated for light chain activity in cell-free and embryonic suprachiasmatic nucleus (eSCN) neuron SNAP-25 cleavage assays. Anti-drug antibody (ADA) and T cell responses were determined in human leukocyte antigen (HLA) transgenic mouse immunizations by bridging assay and interferon-gamma enzyme-linked immunospot assay (ELISpot), respectively.

Results: Computational T cell epitope mapping identified three H_N epitopes, all of which were confirmed to bind common HLA class II DR alleles and to elicit antigen-specific interferon-gamma release from cultured peripheral blood leukocytes obtained from BoNT/A-exposed donors (N=10). A single epitope shown to be immunoreactive in 90% of subjects was selected for modification. Two LH_N/A designs bearing single and double de-immunizing mutations positioned at alpha-helical sites with limited accessibility exhibited minimal tertiary structure perturbation in molecular dynamics simulations. Purified preparations of variant proteins were comparable to unmodified LH_N/A in aggregation state (95% monomeric) and SNAP-25 cleavage potency in both cell-free (pEC_{50}=670 pg/ml) and cell-based (~60% cleavage at 1 micromolar dose) assays. Relative to unmodified LH_N/A, the single mutation variant elicited reduced interferon-gamma responses to the target epitope and the whole protein in splenocytes from HLA DR4 transgenic mice and reduced serum ADA incidence and titer.

Conclusions: Epitope modification reduces LH_N/A T cell and antibody immunogenicity while maintaining enzymatic and cellular function. The engineered protein is a promising lead for de-immunized BoNT/A and targeted secretion inhibitors and demonstrates proof-of-concept for wider application to botulinum-based therapeutics.