TOXIN MEDICAL COUNTERMEASURES - DEVELOPMENT OF NOVEL, BROAD-SPECTRUM COUNTERMEASURES FOR TOXIN EXPOSURE

Development of Functional Assays to Confirm Biological Activity of Select Conotoxins

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Conotoxins are a large, diverse group of neurotoxic peptides found in Conus genus cone snail venom. Each of the hundreds of species within Conus can produce hundreds of conotoxins for hunting prey and for defense. A few conotoxins are used as therapeutics, and a subset of α-conotoxins have been classified as Select Agents due to their toxicity and threat potential to humans. Since small alterations in structure can significantly impact the functionality of peptide toxins, it is imperative to confirm these compounds have retained their activity on biological receptors prior to use in studies.

Toxin potency can be measured through a variety of experimental techniques and model systems, including utilizing cell lines from various sources; however, these techniques, systems, and cell lines can have varying sensitivities and quality control metrics, leading to inconsistent potency measurements. Historical toxin potency experiments have focused on individual toxins, using limited replicates. This prevents comparability of potency between toxins, receptor subtypes, and experimental techniques.

To overcome these limitations, we have developed an automated workflow for patch clamp electrophysiology and Fluorometric Imaging Plate Reader (FLIPR) assays to assess toxin potency with the goal of guiding future animal studies, while also integrating quality control guidelines. Our workflow utilizes commercially validated reagents, including cell lines overexpressing receptors of interest, conotoxins, reference inhibitors, and assay reagents.

We have successfully utilized this workflow to collect novel comparative data on toxins that inhibit nicotinic acetylcholine receptor subtypes α3β4 (nAChR α3β4) and α1β1δε (nAChR α1β1δε), as well as voltage-gated calcium channel 2.2 (CaV 2.2). Using reference inhibitor dimethylphenylpiperazinium (DMPP) for both nAChR receptor subtypes, we have collected comparative toxicity data for α-conotoxins TxID and GI. Using reference inhibitor “CaV 2.2 Blocker 1,” we have collected comparative toxicity data for ω-conotoxin MVIIA.

Our workflow addresses significant gaps in the toxins field, enabling development of comparative toxin activity databases and improving quality standards, while also providing guidance for future animal studies and public health guidelines.