MEDICAL PROPHYLAXIS TO MITIGATE CHEMICAL THREATS

Discovery Of Therapeutic Countermeasures Against Injuries Induced By Nerve Agents

Sofiya Micheva-Viteva Los Alamos National Laboratory **Emilia Solomon** B-TEK, LANL **Migun Shakya** B-GEN, LANL
Andreas Olsen Martinez B-GEN, LANL

Neurotoxins, including organophosphate chemical war agents (OP CWA) and GABA receptor inhibitors, trigger high neuronal action potential and excessive peripheral activity at the neuromuscular junction (NMJ) resulting in debilitating multi-symptom health problems ranging from muscle cramps and paralysis to coma and lethal cardiac arrest. Individuals surviving exposure to neurotoxic agents suffer chronic muscle weakness, peripheral neuropathy, and memory loss. The time of treatment with therapeutic agents is limited to the first 5 min of OP exposure. Afterwards, non-cholinergic and non-adrenergic responses are triggered and cause chronic neuropathy. While current medical countermeasures (MCM) to OP CWA intoxication are limited to inhibitors of acetylcholine receptors (ACHR) and regenerators of acetylcholinesterase (ACHE) enzymatic activity, we focused our MCM discovery efforts on naturally occurring cellular protection mechanism against cell damage caused by cholinergic crisis and toxic Ca2+ overload. We engineered complex tissue models of human neuromuscular junctions (hNMJ) for drug target discovery and validation studies. We performed unbiased global transcriptome analysis of neuronal, muscle, and NMJ tissue treated with various concentrations of neurotransmitter (Ach) and VX simulant (Paraoxon, POX). With this method, we uncovered core cellular regulatory mechanisms that are critical to neuronal and muscle function and which alternation during cholinergic crisis and OP intoxication could be, at least partially, reversed by FDA-approved therapeutics. We coupled our genomic screen with electrophysiological and fluorescent Ca2+-flux assays to validate the impact of the MCM on neuron protection and NMJ function recovery post nerve agent exposure. From nine FDA-approved therapeutics acting on different molecular targets, identified by our human in vitro NMJ discovery platform, we selected three lead compounds for validation in a mouse model of OP CWA intoxication. These FDA-approved therapeutics have proven to protect in vitro human and mouse NMJ tissue against Ca2+ overload and excitotoxicity triggered by the OP simulant of CWA. The re-purposed MCMs to nerve agent-induced cellular damage included a natural metabolite antagonizing multiple receptor functions (NMDA, A2ADR, AChR, and VDCC), an anti-seizure therapeutic, inhibiting high-voltage Ca2+ channels and neurotransmitter release, and an agonist to CaMKII and PKCe function that has been found to inhibit Cas3/9 function and to protect neurons from ROS-induced apoptosis while activating GABA and serotonin release.
Collectively, we have identified therapeutics acting on molecular targets downstream of nerve agent-induced dysregulation of ion flux and energy balance. Therefore, these FDA-approved drugs may have the potential to serve as broad-spectrum MCM to neurotoxic chemical agents and reduce the debilitating effect of CWA on the Warfighter’s health.

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