MEDICAL PROPHYLAXIS TO MITIGATE CHEMICAL THREATS

Preventing Neuronal Losses After Organophosphate Exposures By Curbing Neuroinflammation Via A Novel Nanoformulation Of Pralidoxime Designed To Cross The Blood-brain Barrier

Manish Moghe Georgetown University Medical Center **Joe Harford** SynerGene Therapeutics, inc. **Sang Soo Kim** SynerGene Therapeutics, Inc. & Georgetown University Medical Center **Miaoyin Guan** Georgetown University Medical Center **Antonina Rait** Georgetown University Medical Center **Kathleen Pirollo** Georgetown University Medical Center **Esther Chang** Georgetown University Medical Center

Background Information: Organophosphates inactivate acetylcholinesterase (AChE) in synapses of the central and peripheral nervous systems with consequent accumulation of acetylcholine producing severe toxicities and even death. Like ~98% of all drugs, pralidoxime (a.k.a. 2-PAM), the only FDA-approved reactivator of AChE, does not readily cross the blood-brain barrier (BBB). We have developed a novel formulation by encapsulating 2-PAM within a nanocomplex designed to cross the BBB via transferrin receptor-mediated transcytosis. This new nanomedicine (termed scL-2PAM based on the vehicle comprising a single chain Liposome) has proved to be superior to unencapsulated 2-PAM in terms of its ability to rescue mice from otherwise lethal exposures to paraoxon, a deadly organophosphate with anticholinesterase activity.

Purpose, Objective, Rationale & Methods: We sought to explore the mechanism of action of scL-2PAM as an organophosphate countermeasure in a mouse model via transcriptomic analyses using NanoString® methodology and to assess the impact on gene expression patterns of scL-2PAM treatments. The expressions of selected genes were further examined using quantitative polymerase chain reaction (qPCR) methods. Gene expression patterns were analyzed and correlated with changes in the brains of paraoxon-treated mice revealed by histology and immunohistochemistry. Such studies have the potential to identify new therapeutic targets for treating organophosphate poisonings.
Preliminary Results & Conclusions: Our transcriptomic analyses after paraoxon exposures revealed 18 differentially expressed genes (DEGs) that included FOS and CCL2, which have each been implicated in neuroinflammation. The OP-triggered elevations of brain FOS and CCL2 mRNAs were significantly more effectively prevented by scL-2PAM treatments than by unencapsulated 2-PAM, the currently used form of the reactivator. scL-2PAM represents the most recent application of our scL platform technology for drug delivery, which has shown good safety profiles in human trials involving investigational agents for cancers. Given that the scL nanodelivery system has already completed successful oncology trials and that scL-2PAM carries an already FDA-approved drug as its payload, our new nanomedicine should prove to be safe and effective if developed as a new countermeasure.

Impact to the JSTO Mission and the Joint Force: The National Toxicology Program defined "longer term effects" of sarin exposures as those present beyond 24 hours after exposure. In mice, we observed changes in microglial activation (neuroinflammation) at 24 hours after paraoxon exposures and blatant loss of neurons at 14 days post exposure. Although characterized as longer-term sequelae, the timeframe of these effects makes them highly relevant to the physical and cognitive capabilities of organophosphate-exposed warfighters that might impact their return to duty. Our new countermeasure, scL-2PAM, has the potential not only to improve survival after organophosphate exposures but also to ameliorate the neurological sequelae in warfighters who survive organophosphate exposures by curbing neuroinflammation and the consequent death of brain cells. Our transcriptomic studies also have the potential to reveal new therapeutic targets against organophosphate poisoning. If chemical reactivators with improved properties be synthesized in the future, these new compounds may also benefit from encapsulation within scL nanocomplexes to enhance their ability to cross the BBB.

Supported in part by the Joint Science and Technology Office of the Chemical and Biological Defense Program at the Defense Threat Reduction Agency of the US Department of Defense through a grant (HDTRA1-13-1-0049) and contract (HDTRA1-20-C-0045) to SynerGene Therapeutics, Inc. The content is solely the responsibility of the authors and does not necessarily reflect the official view of the United States Government.