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

A Method for Simultaneous Evaluation of Blood Brain Barrier Transport and Therapeutic Functionality in Vvtro

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Exposure to organophosphorus (OP) compounds is of global security concern. OP exposure results in the inhibition of acetylcholinesterase (AChE) by phosphorylation of the active site serine, leading to the buildup of the neurotransmitter acetylcholine (ACh). Excess ACh causes cholinergic overstimulation followed by desensitization in the central, peripheral, and autonomic nervous systems. This can cause various symptoms including paralysis, seizures, respiratory failure, and death. The severity of OP poisoning has led to decades of research towards the development of effective antidotes to OP-inhibited AChE. Administration of atropine, diazepam, and oxime therapeutics is the current standard of care for OP-exposed patients. Unfortunately, the permanent positive charge on many oxime reactivators significantly limits blood brain barrier (BBB) permeability rendering any CNS damage from OP poisoning largely inaccessible. As such, there has been a thrust to develop therapeutics that can reactivate AChE and penetrate the BBB. With a significant increase in development of these therapeutics, there is also a need for a standard method for monitoring and comparing these important characteristics.

Towards this goal, Los Alamos National Laboratory has developed a new method to simultaneously evaluate the BBB permeability and enzymatic activity of candidate therapeutic molecules. This method couples two previously independent assays: (1) BBB permeability and (2) enzymatic therapeutic functionality. Test compounds are applied to the blood chamber of the BBB and incubated to test passage across the in vitro human tetracell BBB. This is the first time an in vitro BBB model has been adapted for this purpose. The brain chamber of the BBB model is supplemented with inhibited AChE. If the test therapeutics pass the BBB and retain their therapeutic functionality, they will immediately begin to reactivate AChE in the brain chamber. Aliquots are removed from the brain chamber at desired timepoints to quantify BBB passage and subsequent AChE reactivation. The reactivation assay is a secondary method that further validates BBB passage. This method will serve as a valuable down-selection tool when identifying top therapeutic candidates in the transition from in vitro to in vivo testing and can be adapted to investigate the therapeutic activity of any compound designed to cross the BBB.