REPURPOSING TO SPEED CHEMICAL AND BIOLOGICAL MEDICAL COUNTERMEASURE DISCOVERY AND DEVELOPMENT

Resurrecting The Dead: Recovery Of Organophosphorus Poisoned Acetylcholinesterase Using Quinone Methide Precursors

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The inhibition of acetylcholinesterase (AChE) by organophosphorus (OP) nerve agents and pesticides is reversible with the rapid treatment of reactivators, such as 2-pralidoxime (2-PAM). However, if treatment is not administered quickly or if the OP is particularly toxic, these reactivators are rendered useless. After inhibition, a subsequent dealkylation event can occur at the phosphylated, catalytic serine residue of AChE. Historically, this aged state of the enzyme has been recalcitrant to reactivation by pyridinium oximes, so in the literature, the inactive, aged AChE enzyme has been declared dead. Indeed, there are currently no FDA-approved treatments for the aged form of AChE, and it was considered irreversible until 2018. Our team has demonstrated the only compounds which are capable of reviving, or resurrecting, the OP-aged form of electric-eel AChE using quinone methide precursors (QMPs). Inspired by this initial discovery, a variety of QMPs have been synthesized and then tested in vitro against OP-aged forms of recombinant human AChE that resemble the erythrocyte (dimer), brain (tetramer), and readthrough (monomer) isoforms. A modified Ellman's assay, utilizing the artificial AChE substrate acetylthiocholine (ATC) and 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), was used to detect the reappearance of native AChE during these studies. After 24 hours, up to 90% of the aged enzyme was resurrected for each enzyme variant with select QMPs and also for specific OP compounds. In some cases, similar QMP frameworks are also an effective treatment for OP-inhibited AChE and can reactivate up to 70% of recombinant human (erythrocyte) AChE and 20% of the readthrough isoform. Although these QMPs are not as efficient with reactivation as 2-PAM, their lack of a permanent positive charge makes them more effective at crossing the blood-brain barrier. Such QMPs, possibly acting as both a reactivator and a resurrector, will help pave the way to new, more effective treatments for OP poisoning. In this presentation, we will present comparative in vitro assays with different variants of AChE as well as the resurrection efficiency with various QMPs in our structure-activity relationship library. We will also illustrate the protective efficacy of select QMPs from in vivo studies in a KIKO mouse model wherein the human AChE has replaced the mouse AChE (knock-in, KI) and the carboxylesterase has been knocked-out (KO). We will provide our results in the development of these QMP frameworks for the effective reactivation of OP-inhibited and resurrection of OP-aged AChE from our in vitro and in vivo studies.

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