REPURPOSING TO SPEED CHEMICAL AND BIOLOGICAL MEDICAL COUNTERMEASURE DISCOVERY AND DEVELOPMENT

Towards A Pseudo-catalytic Bioscavenger: In Vitro Reactivation Of Organophosphorus-inhibited And Resurrection Of Organophosphorus-aged Butyrylcholinesterase By Quinone Methide Precursors

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Organophosphorus (OP) compounds are used as chemical warfare agents and pesticides, posing a serious threat because of their toxicity in cholinesterase inhibition. OP compounds inhibit acetylcholinesterase (AChE) by phosphylation of the catalytic serine residue, leading to accumulation of acetylcholine and to cholinergic crisis, often resulting in death. Butyrylcholinesterase (BChE), a similar cholinesterase, is inhibited by OP compounds. While BChE has no known essential biological function, it does function as a natural OP scavenger. BChE has been studied as a treatment option for OP poisoning from both an endogenous target as well as through injection of exogenous human or recombinant BChE, but always as a stochiometric bioscavenger. Thus, high doses of BChE are required which is expensive and inefficient. Moreover, upon OP inhibition of both AChE and BChE, there is a subsequent, spontaneous de-alkylation step which creates an aged form of the enzyme that is even more challenging to return to its native state. Current standard of care for OP intoxication includes various oximes, such as 2-PAM, which can reactivate the OP-inhibited form of AChE and BChE but offers no efficacy towards the aged form of either enzyme – for which reversal is termed "resurrection". Some OP compounds, such as soman, have aging half times on the scale of minutes, thus emphasizing a major limitation of currently approved reactivators. We present our evaluations of quinone methide precursors (QMPs) to reactivate OP-inhibited and to resurrect OP-aged BChE in order to create a pseudo-catalytic bioscavenger against OP exposure. As exogenous BChE is present in a high concentration in blood, a pseudo-catalytic BChE scavenger would provide substantial improvements for OP intoxication, and potentially as a small drug-like therapeutic. To test the in vitro viability of such QMPs, BChE is either inhibited or aged by an OP compound, and then incubated with a QMP for a given time before testing the recovered activity using a modified Ellman's assay. Previous research by our team presented the first reported in vitro demonstration in which the OP-aged form of AChE was resurrected to its native state by a QMP. We will present our results for a library of QMPs which are capable of very effective reactivation of OP-inhibited BChE and for a vast array of OPs including surrogates of VX, cyclosarin, tabun, and ethyl paraoxon. Furthermore, many of these QMPs reactivate a higher percentage of OP-inhibited BChE as compared to 2-PAM within 1 hour when used at a concentration of 250 μM (or lower). A separate set of QMPs can resurrect the methylphosphonate-aged form of BChE up to 10% within 24 hours. This will be the first report of resurrection of OP-aged BChE. These results suggest that providing a small drug-like molecule capable of reactivating OP-inhibited and resurrecting OP-aged BChE may expand the versatility of endogenous or exogenous BChE to act as a pseudo-catalytic bioscavenger for the warfighter.