Evaluating the effect of species on the importance of enzyme-reactivator ratio

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Introduction

Organophosphorus (OP) nerve agents are highly toxic compounds that have been weaponized in chemical warfare in the past. Upon exposure, these substances inflict detrimental effects to the body. OP nerve agents will irreversibly bind to acetylcholinesterase (AChE) through covalent bond formation with the hydroxyl group on the active site serine residue upon entering the bloodstream. Widespread inhibition results in the neurotransmitter acetylcholine (ACh) no longer being broken down and accumulating rapidly at synaptic clefts, leading to a systemic cholinergic crisis. This clinically manifests as continued muscle contractions, seizures, and eventually death. The current fielded treatment for OP nerve agent exposure includes the administration of a symptomatic antimuscarinic antidote, an anticonvulsant drug, and a compound that removes OP nerve agent from inhibited AChE, known as a reactivator (Horn et al., 2018). Reactivators are able to cleave the covalent bond to the active site serine and restore AChE function. However, current fielded reactivators have notable limitations. Many are not effective across a broad-spectrum of OPs and are unable to cross the blood-brain barrier (Cadieux et al., 2016). This prompts the need for the study and discovery of new treatments for OP nerve agent intoxication. Novel oxime and non-oxime reactivator treatments have been explored in research due to this need. This study aims to evaluate the effect of the enzyme-reactivator ratio in the reactivation of nerve agent-inhibited AChE across various species in order to determine if the enzyme-reactivator ratio affects reactivation and whether it does so in a species-specific manner. If there is a species-specific difference in the reactivation mechanism, then that difference may be reactivator dependent. The results of this study will lead to further clarity on how to properly administer treatment to victims of OP nerve agent intoxication.

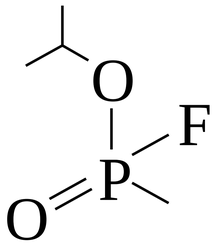


Figure 1 (above): The chemical structure of the OP nerve agent sarin (GB) is shown. This OP compound has been weaponized in chemical warfare and was utilized in this study to primarily serve as an inhibitor to both human and mouse AChE (Cadieux et al., 2016).

Materials and Methods

An assay that places emphasis on enzyme concentration, specifically in comparing the enzyme-reactivator ratio, was utilized within this project. In conducting the reactivation assay, two aliquots of recombinant human acetylcholinesterase (AChE) were incubated with the nerve agent sarin (GB) and saline (APO) to achieve ≥ 95% inhibition. Excess agent was removed by running the samples through CentriSep size-exclusion columns. Both the uninhibited and inhibited enzyme samples were added to their corresponding wells of a deep well plate. A 7:9 ratio series dilution of enzyme to potassium phosphate buffer was performed five times across the deep well plate with a buffer control built into each dilution, allowing for comparison of enzyme activity across multiple dilutions and to account for background activity, respectively.

Enzyme samples were then mixed with the reactivator 2PAM at 100 µM to initiate reactivation. Ellman's reagent (1 mM acetylthiocholine and 2 mM 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB)) was added to the enzyme and absorbance was read at 412 nm in a spectrophotometer for 1.5 minutes to characterize enzyme activity. Activity was measured for a total of nine time points to assess reactivation over the course of the experiment. The data was compiled and analyzed in SoftMax® Pro, Excel, and GraphPad Prism software in order to obtain the k_{obs} (observed rate of reaction) for each experiment.

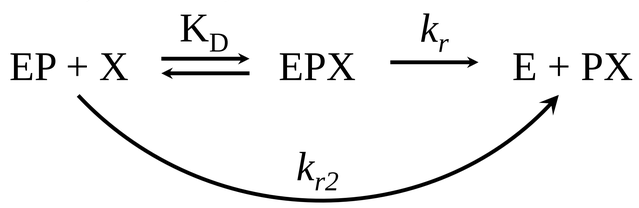
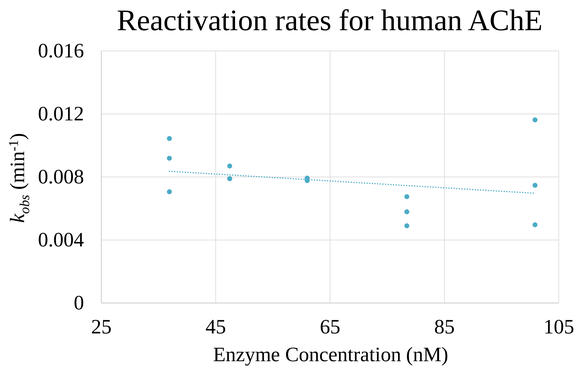


Figure 2 (above): The scheme of reactivation kinetics observed within this study is shown. K_D, k_r, and k_{r2} represent the reactivator binding constant, the reactivation rate, and the overall reactivation rate, respectively. The binding of a reactivator (X) to an enzyme-inhibitor compound (EP) is shown, with the reactivator, inhibitor, and enzyme forming a compound (EPX) before reactivation is performed and the inhibitor is removed (E + PX).

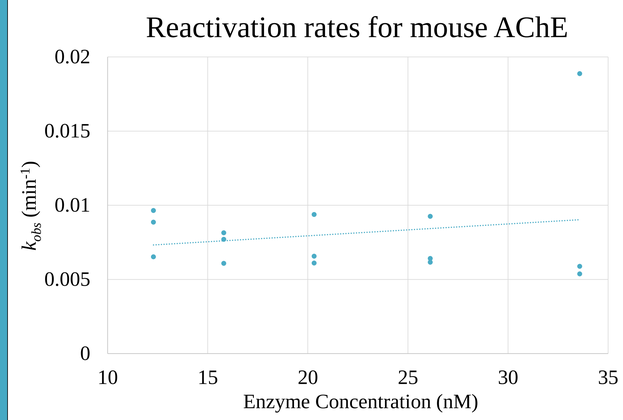
Results

The triplicate k_{obs} values for human and mouse AChE were averaged and fitted using linear regression analysis as seen in Graphs 1 & 2. The slopes of the lines of best fit were used to conduct an *F*-test to determine whether the slope of the line was significantly different from zero.



Graph 1 (left): Reactivation of human AChE. An *F*-test testing deviation of the slope of the line of best fit from zero yielded a *p*-value of 0.3787, suggesting no significant difference effect regarding enzyme-reactivator ratio on the reactivation of human AChE.

Results



Graph 2 (left): Reactivation of mouse AChE. An *F*-test testing deviation of the slope of the line of best fit from zero yielded a *p*-value of 0.0213, suggesting a significant difference effect on enzyme-reactivator ratio on the reactivation of mouse AChE.

Conclusion

The purpose of this study was to determine if the enzyme-reactivator ratio affects reactivation and whether it does so in a species-specific manner. In order to do so, an assay was conducted to compare the rates of reactivation of various concentrations of sarin-inhibited human and mouse AChE and obtain observed rate of reaction (k_{obs}). The results showed that as enzyme concentration changed, the observed rate of reaction changed for mouse AChE, but not for human AChE. Due to the significant difference in rate at which the observed rates of reaction changed between human and mouse AChE, the results further indicate that a species-specific mechanism of reactivation should be considered when developing and testing effective treatments to OP nerve agent intoxication. This assay can also be utilized to test non-oxime reactivators, which are assumed to act differently on inhibited AChE, as this assay provides insight into the mechanism of reactivation. Utilizing this method would help further contribute to the research regarding effective dosing and administration techniques to treat OP nerve agent intoxication.

References

Cadieux, C. L., Wang, H., Zhang, Y., Koenig, J. A., Shih, T. M., McDonough, J., Koh, J., & Cerasoli, D. M. (2016). Probing the activity of a non-oxime reactivator for acetylcholinesterase inhibited by organophosphorus nerve agents. *Chemico-Biological Interactions, 259*, 133–141. https://doi.org/10.1016/j.cbi.2016.04.002

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