Comparing the kinetic parameters of MMB-4 driven reactivation of recombinant AChE after inhibition by GF

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Introduction

This study distinguishes the efficiency of MMB-4, a reactivator oxime, when used as a countermeasure against recombinant acetylcholinesterase (AChE) from human, rat, guinea pig, green monkey, mouse, and pig when inhibited by the organophosphorus (OP) nerve agent cyclosarin (GF). GF intoxication prevents acetylcholine from being hydrolyzed. This project examines the performance of this specific reactivator methoxime (MMB-4) with a single OP nerve agent at differing concentrations. There has been extensive research regarding the effects of pralidoxime (2-PAM) when used as a countermeasure against exposure to OP nerve agents such as soman, tabun, and cyclosarin, giving it credibility as a "casual treatment of human OP poisoning," (Worek, Wille, Aurbek, Eyer, & Theirmann, 2010). The issue with using 2-PAM for the majority of nerve agent intoxication is that 2-PAM struggles to reach the central nervous system due to the blood brain barrier (BBB). MMB-4 in previous testing exhibited a high reactivity, however it was also noted that it had difficulty during the binding step (a low affinity toward the inhibited AChE) (Worek et al., 2010). It is proposed that there will be a difference in the overall performance of MMB-4 as species of AChE is varied.

Materials and Methods

To conduct reactivation assays, aliquots of recombinant AChE from several species were prepared according to inhibition status (Apo for non-inhibited, X for inhibited). The enzyme was incubated with GF for 10 minutes at room temperature to ensure a minimum of 95% inhibition. The samples were run through prepared Centrisep columns to remove excess agent and then centrifuged. The purified samples were diluted to a concentration with a functional activity level, then they were plated onto a deep well plate. Potassium phosphate buffer (KPO_4) at pH 7.0 was also plated across the deep well plate and each of the reaction plates for dilution. MMB-4 was diluted across and down the deep well plate to 12 different concentrations for testing. For each species, at least three successful assays were run, with 10 total timepoints: a time zero without the addition of MMB-4, and nine after exposure to MMB-4. Each run was analyzed using Microsoft Excel and Prism.

Materials and Methods (cont.)

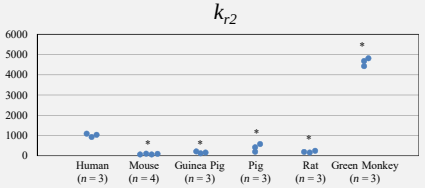
$$EP + X \underset{k_{r2}}{\overset{K_D}{\rightleftharpoons}} EPX \overset{k_r}{\rightleftharpoons} E + PX$$

Figure 1 (above): This figure displays the scheme of reactivation kinetics that represent the reaction during the assays.

The constants calculated were the reactivation rate (k_r), the dissociation constant (K_D), and the overall reactivation rate constant ((k_r / K_D) or k_{r2}). The reaction chemistry is displayed in Figure 1 where enzyme AChE is combined with reactivator MMB-4. The combination first undergoes a phase of binding (as represented by K_D), then it exists as a unit, and finally the reactivation occurs: the nerve agent GF is removed from the active site of AChE, returning natural functionality to the enzyme.

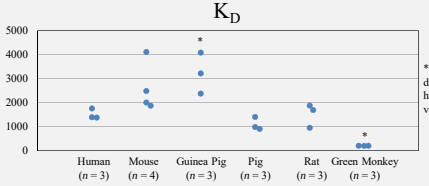
Results

Values of k_r, K_D, and k_{r2} were compared using two-sample *t*-tests for 95% confidence, and the values were plotted in Graphs 1, 2, and 3.



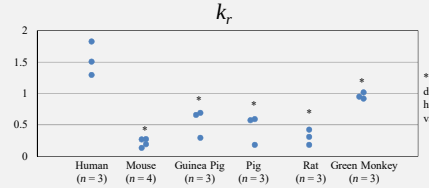
* = Significantly different from human AChE value ($p < 0.05$)

Graph 1 (left): This graph displays the values of k_{r2} ($min^{-1}M^{-1}$) per species and their significant differences when analyzed against human AChE.



* = Significantly different from human AChE value ($p < 0.05$)

Graph 2 (left): This graph displays the values of K_D (μM) per species and their significant differences when analyzed against human AChE.



* = Significantly different from human AChE value ($p < 0.05$)

Graph 3 (left): This graph displays the values of k_r (min^{-1}) per species and their significant differences when analyzed against human AChE.

Results (cont.)

Graph 1 displays the overall reactivation constants for each species. When compared to human AChE, every species was significantly different in value ($p < 0.05$). The K_D for each species was slightly less different as only green monkey and guinea pig AChE differed significantly from human AChE (Graph 2). Graph 3 displays that the k_r values for every species tested significantly differed from human AChE.

Conclusions

The purpose of this project was to determine how efficient the compound MMB-4 is at reactivating GF-inhibited recombinant AChE to aid in deciding which species would make the ideal clinical testing subject for the early stages of FDA approval. There were notable differences in the overall reactivation rate constants, dissociation constants, and reactivation rate constants between species, suggesting MMB-4's efficiency does vary between species, and that the mechanism of reaction impacts the overall reactivation. In most cases, the variation occurs during the reactivation phase, causing the overall reactivation values to also differ. This is a surprising result as it was expected that the reactivator itself should function in a similar way among all enzymes — no matter the species. This alteration in performance is due to minute differences in the sequence of amino acids that form the AChE. All of the species tested would not be considered an ideal model of human AChE for clinical trial, so the testing of more species' AChE will continue this project.

References

Worek, F., Wille, T., Aurbek, N., Eyer, P., & Thiermann, H. (2010). Reactivation of organophosphate-inhibited human, Cynomolgus monkey, swine and guinea pig acetylcholinesterase by MMB-4: A modified kinetic approach. *Toxicology and Applied Pharmacology,* 231–237.

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