Different reactivation rates of organophosphorus-inhibited human acetylcholinesterase and mouse acetylcholinesterase

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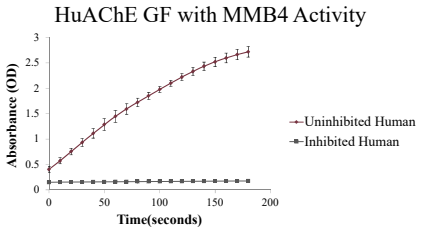
Introduction

Organophosphorus (OP) nerve agents are a threat to the military and civilians, as shown by the recent use of nerve agents as weapons in the Middle East. The most promising approach to treatment focuses on discovering reactivators that can restore function to OP-inhibited acetylcholinesterase (AChE), which is the toxic target of OPs. Past research has shown flaws in determining efficacy of reactivators due to differences in reactivation rates between the inhibited AChE of different species. Past data has mainly looked at the differences between human and guinea pig AChE reactivation, because guinea pigs are the most commonly used animal model for research into OP countermeasures (Worek, Reiter, Eyer, & Szinincz, 2002). However, guinea pigs have been found to be poor predictors of the efficacy of reactivators in humans. For this reason, recent efforts have focused on using mice rather than guinea pigs, but the functional differences between human and mouse AChE are not well defined. This research will attempt to determine if functional differences exist between human and mouse AChE with respect to the efficacy of reactivators.

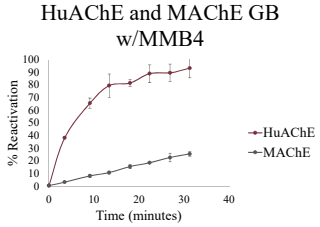
Materials and Methods

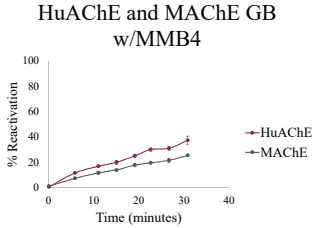
Recombinant human and mouse AChE enzyme were incubated with inhibitor (Sarin (GB) or Cyclosarin (GF)) for 10 minutes at room temperature to achieve ≥ 95% inhibition. The enzyme was applied to a CentriSep size exclusion column to remove any excess inhibitor. The process was performed in parallel with a control sample of enzyme which is exposed to 1 mM KPO_4 buffer with 1 mg/ml bovine serum albumin at pH 7.0. Different AChE activity was found using a modified Ellman assay with 1 mM acetylthiocholine and 2 mM dithiobisnitrobenzoic acid (DTNB). The uninhibited and inhibited samples was exposed to reactivator (2-PAM and MMB4) at a final concentration of 1 mM. Aliquots of each sample were removed at several time points, diluted twenty-fold and measured for activity against AtCh (Graph 1). Each time point incorporated the uninhibited enzyme sample as a positive control to compare the reactivation of the inhibited sample to and determine the percent reactivation. The calculated reactivation at each time point was plotted against the time of the measurement and fit using a non-linear regression (Graph 2 and Graph 3) analysis to determine the half-time of reactivation ($t_{1/2}$) and the observed rate of reactivation (k_r). Using the nonlinear regression the reactivation percentage was calculated five minutes into the reactivation. Student *t*-tests were used to assess the statistical significance of differences between samples ($\alpha = 0.05$).

Results

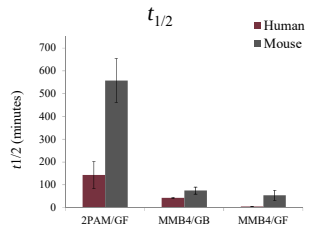


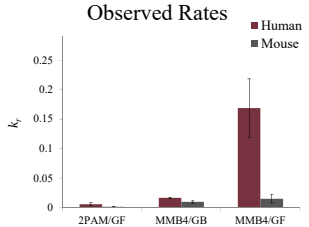
Graph 1: Example of raw data for a specific time point. Each point is the absorbance(OD) for a specific time. The vertical error bars are the standard deviations between three replicates in the plate.



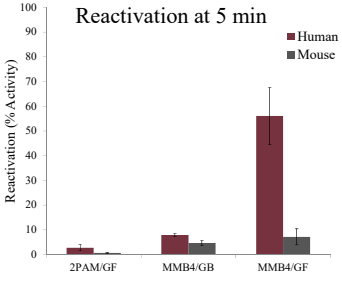


Graph 2 (left) and Graph 3 (right): Examples of nonlinear fit of activity by comparing the uninhibited sample to the inhibited sample for eight time points. The error bars are standard deviations between the three replicates in the plate





Graph 4 (left) and Graph 5 (right): The difference between the time to reach 50% reactivation and the difference between the rate(k_r) at which the reactivation reaction proceeds. The error bars are the standard deviation between three different trials.



Graph 6: The difference between reactivation percentage of human and mouse AChE with different reactivators a 5%. The error bars are the standard deviation between three different trials.

Results (cont.)

Several parameters ($t_{½}$, k_r, and percent reactivation at five minutes) (Graph 4, Graph 5, Graph 6) for each reactivation condition were compared in this study. For all conditions except human AChE with GF and MMB4, the calculated $t_{½}$ values fell well outside the experimental time course, which introduced large variability into the estimation of this value. Similarly, the k_r values for the same conditions were so small that significant comparisons are difficult. In contrast, the percent reactivation at five minutes for all of the conditions allowed for easy comparison of the conditions, with 2PAM/GF and MMB4/GB having no significant differences between human and mouse AChE. When MMB4 was used to reactivate AChE after inhibition with GF, the percent reactivation at five minutes was ~eight-fold higher for human AChE compared with mouse AChE at the same time point.

Conclusion

Currently, in vivo testing of OP inhibition and reactivation of AChE are conducted in guinea pigs rather than mice because guinea pigs have low serum carboxylesterase (CaE). High levels of serum CaE, like those found in mice, make an organism less susceptible to OP intoxication. Unfortunately, past research has shown that guinea pig AChE is sufficiently different from human AChE to cause altered reactivation rates with reactivators after OP inhibition (Cadieux et al., 2010). The mouse genome has been mapped and can be genetically altered, allowing generation of novel animal models. Efforts are now underway to characterize mice that lack serum CaE and also express human AChE rather than mouse AChE. The data presented here support the hypothesis that mouse and human AChE have functional differences (causing different reactivation rates). The statistical tests show significant differences between the mouse and human AChE for MMB4 with GF. The data also indicate that AChE reactivation on red blood cells exposed to GF and then to MMB4 may be useful as a phenotypic test for use with the novel genetically modified mice.

References

Cadieux, L., Broomfield, C., Kirkpatrick, M., Kazanski, M., Lenz, D., & Cerasoli, D. (2010). Comparison of human and guinea pig acetylcholinesterase sequences and rates of oxime-assisted reactivation. *Chemico-Biological Interactions, 187*(1-3), 229-233

Worek, F., Reiter, G., Eyer, P., & Szinincz, L (2002). Reactivation kinetics of acetylcholinesterase from different species inhibited by highly toxic organophosphates. *Archives of Toxicology, 76*(9), 523-529.