Determining reactivation differences between DG-1-054 and DG-3-008 in African green monkey AChE

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Introduction

Acetylcholinesterase (AChE) is an enzyme present in many organisms that breaks down the neurotransmitter acetylcholine (ACh). While ACh plays an important role in brain and body function, excess levels can be dangerous. Organophosphorus nerve agents (OPNAs) are toxic man-made compounds that work by binding to the active site of AChE, thereby inhibiting it and preventing ACh from being hydrolyzed (Cadieux et. al, 2016). The resulting buildup of ACh can cause adverse effects such as seizures and eventually death. Because of this, it is necessary that treatments for exposure to OPNAs be effective.

Current treatment for OPNA includes the use of an oxime reactivator that removes the nerve agent from AChE's active site. In the past decade, non-oxime compounds have shown promising potential as potent reactivators of human AChE inhibited by a broad spectrum of OPNAs. Previously, these non-oximes (Figure 1 and Figure 2) have been tested on human AChE *in vitro*; however, effects of OPNAs and reactivators vary between species (Cadieux et. al, 2010).

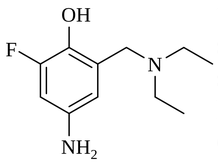


Figure 1 (Above): The structure of the non-oxime DG-1-054.

The purpose of this project was to evaluate the differences in reactivation potential of two compounds in African green monkey AChE, which is commonly used in lieu of human AChE during testing due to their many similarities, inhibited by a broad spectrum of OPNAs. The null hypothesis was that there is no significant difference between the reactivation potential of the reactivators DG-1-054 and DG-3-008. The alternate hypothesis was that there is a significant difference between the reactivation potential of DG-1-054 and DG-3-008.

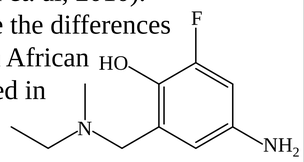


Figure 2 (Above): The structure of the non-oxime DG-3-008.

Materials and Methods

Buffer and stock solutions were made at the beginning of the procedure by adding 800 µL of 0.1M potassium phosphate buffer pH 7.4 to 11 Centri-Sep columns. These were then incubated for 30 minutes at room temperature (approximately 25 °C). After incubation, columns were checked for any bubbles, ensuring consistent sample flow.

The enzyme was inhibited by adding 5 µL of either saline or one of the following OPNAs: GB, GF, VX, or VR, by MRICD personnel. Each aliquot was then mixed and incubated at room temperature for approximately ten minutes. Each enzyme sample was processed through a size-exclusion Centri-Sep column to remove excess agent. Samples were then centrifuged and 2 µL of each were distributed to 1.5 mL Eppendorf tubes to be used in the assay. The compound being tested

Materials and Methods (continued)

was diluted to 20 µM in a reservoir holder. The substrate solution was prepared by adding 92.54 mg of acetylthiocholine (AtCh) and 253.66 mg of 5,5′-Dithiobis- (2-nitrobenzoicacid) (DTNB) to 200 mL of buffer. AtCh is an analog of and used in lieu of ACh. As AChE is reactivated, it breaks down AtCh, which then hydrolyzes DTNB. This interaction is measured by a spectrophotometer, which measures the absorbance of light as it passes through material. As DTNB is hydrolyzed, more light is absorbed. This change in light absorbance is what is measured.

The assay was performed by the Biomek Liquid Handler (Figure 3). Prior to starting the assay, a reservoir with 200 mL buffer, timepoint plates, and pipette tip boxes were placed in predetermined locations on the liquid handler. After diluting compound and enzyme, reactivation was initiated by adding the compound to enzyme. Aliquots were then removed, diluted into timepoint plates, and change in absorbance was measured by the FilterMax F5 Spectrophotometer for each aliquot collected over the course of approximately one hour.

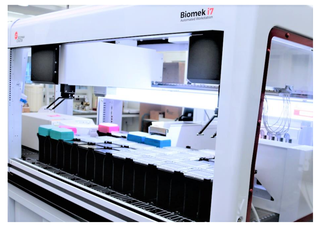
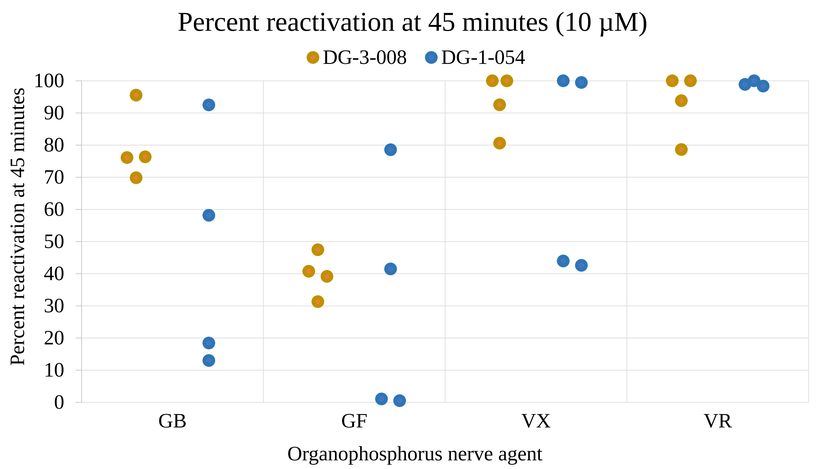


Figure 3: The Beckman-Coulter Biomek i7 Hybrid Handling robot.

Percent reactivation was calculated by dividing the activity of inhibited AChE by uninhibited AChE. Non-linear regression analysis was used to determine the observed rate of reactivation (k_{obs}), which was then used to calculate percent reactivation after 45 minutes of incubation.

Results



Graph 1: Percent reactivation of various OPNA-inhibited monkey AChE at 45 minutes incubation for 10 µM compound concentration. There are three data points for VR-inhibited AChE reactivated by DG-1-054.

Results (continued)

Agent	Mean and standard deviations	Test results	Mean difference in scores	95% Confidence interval
GB	**DG-3-008:** (M = 79.5, SD = 11.1) **DG-1-054:** (M = 45.5, SD = 37.2)	$t(6) = 1.75$ $p = .179$	33.9	−27.9 to 95.8
GF	**DG-3-008:** (M = 39.7, SD = 6.6) **DG-1-054:** (M = 30.4, SD = 37.4)	$t(6) = 0.49$ $p = .659$	9.3	−51.2 to 69.7
VX	**DG-3-008:** (M = 93.3, SD = 9.2) **DG-1-054:** (M = 71.5, SD = 32.6)	$t(6) = 1.29$ $p = .289$	21.8	−32.1 to 75.6
VR	**DG-3-008:** (M = 93.1, SD = 10.1) **DG-1-054:** (M = 99.1, SD = 0.9)	$t(6) = -1.18$ $p = .324$	−6.0	−22.1 to 10.2

Table 1: The results of two sample t-tests for GB-, GF-, VX-, and VR-inhibited AChE, none of which resulted in a significant difference in reactivation potential between the compounds.

Conclusion

The goal of the project, evaluating the differences in reactivation potential between DG-1-054 and DG-3-008, was achieved. Using the alpha level of 0.05, the null hypothesis was accepted for all nerve agents used. This suggests that there is no statistically significant difference between the reactivation potential of DG-3-008 and DG-1-054 against OPNA-inhibited African green monkey AChE. Researchers deciding between which compound is ideal for further testing can proceed with testing in tissues and *in vivo* to evaluate differences in efficacy between these compounds. Potential sources of error included using two different liquid handlers and two different samples of AChE across testing. This could have led to inconsistencies in data collection. The low sample sizes of this investigation should also be taken into consideration as it results in lower statistical power and reduced ability to detect a true difference in the data. Further research could involve conducting *ex vivo* and *in vivo* testing on the African green monkey and observing any possible differences in reactivation potential. This would provide a more accurate assessment of the reactivators' true potential in a therapeutic setting, as well as any side effects of the compounds.

References

Cadieux, C. L., Broomfield, C. A., Kirkpatrick, M. G., Kazanski, M. E., Lenz, D. E., & Cerasoli, D. M. (2010). Comparison of human and guinea pig acetylcholinesterase sequences and rates of oxime-assisted reactivation. *Chemico-Biological Interactions 187*(1–3), 229–233. https://doi.org/10.1016/j.cbi.2010.04.020

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