Evaluating the effect of species on reactivation of OPNA-inhibited AChE by reactivator DG-1-054

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Introduction

The effects of exposure to organophosphorus nerve agents (OPNAs) pose a deadly risk to the Warfighter, resulting in the need for effective treatment options. Nerve agents inhibit the active site of the enzyme acetylcholinesterase (AChE) by forming a covalent bond. The blockage of the active site causes a build-up of the neurotransmitter acetylcholine (ACh), in the synaptic cleft, which causes bradycardia, seizures, difficulty breathing, and death (Cadieux et al., 2016). Reactivators are used to directly treat the cause of this inhibition and restore AChE activity (Luo et al., 2007). Currently, the only Food and Drug Administration approved reactivator, 2-pralidoxime (2-PAM), is an oxime that is traditionally delivered in an auto-injector. However, 2-PAM displays limited reactivation potential against a broad spectrum of OPNAs and is unable to cross the blood brain barrier (Shih et al., 2011), in part due to the positive charge. As a result, the potential efficacy of this treatment is limited and the search for a more effective reactivator is ongoing. A non-oxime reactivator, 4-amino-α-diethylamino-*o*-cresol (ADOC) was found to have a promising potential for reactivation (Cadieux et al., 2016).

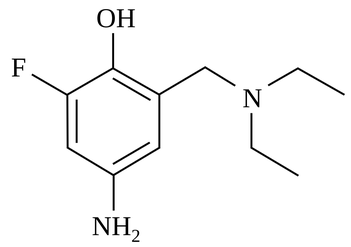

Figure 1 (above): This is the structure of DG-1-054, an ADOC analog (the reactivator). The benzene ring has an amine (NH_2), fluorine (F), and hydroxide (OH) group.

The purpose of this project was to determine if there was a species-specific difference in percent reactivation at 20 minutes of OPNA-inhibited mouse and human AChE by DG-1-054 (Figure 1). The null hypothesis was that there was no difference in the percent reactivation at 20 minutes between the OPNA-inhibited AChE species.

Materials and Methods

Before the enzyme and compound were prepared, the buffer and stock solutions were made. In each of the Centri-Sep columns, 800 µL of 0.1 M potassium phosphate (KPO_4) buffer at pH 7.4 were added. One hundred and fifty mL of substrate was made from premade concentrated stocks of acetylthiocholine iodine (AtCh), 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), and KPO_4 buffer.

The enzyme was prepared by adding either saline or an OPNA to mouse or recombinant human AChE and incubated for 10 minutes. The OPNAs utilized in this project were tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX, and VR. Enzyme samples were run through a size-exclusion Centri-Sep column to get rid of excess agent and further diluted into KPO_4 buffer by the liquid handling robot (*Biomek FX^P Beckman-Coulter*).

The compound (DG-1-054) was diluted to 20 µM from a 50 mM stock solution.

The remainder of the assay was run on the *Biomek FX^P* based on pre-programed instructions. Enzyme samples were mixed with DG-1-054 to initiate reactivation. Aliquots were then removed at various time points by the *Biomek* and diluted 25-fold. The activity against AtCh at 405 nm was measured with the FilterMax F5 Multimode Microplate Reader. Figure 2 is the robot after an assay was finished.

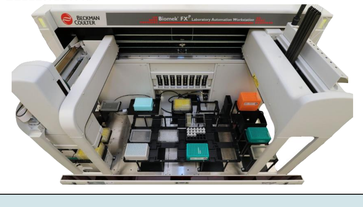

Figure 2 (right): This is the *Biomek*. There are two arms to the robot which pipette and pick up plates to move them. Cadieux, L. (2021). *Biomek FX^P Beckman-Coulter* [Photograph].

Results

The percent reactivation of mouse and human AChE by DG-1-054 at the highest tested concentration (10 µM) at 20 minutes was calculated using non-linear regression for the triplicate and used for all further statistical analysis. Statistical tests could not be run for GA- and GD-inhibited AChE due to no variability in reactivation. Graph 1 and Table 1 display the distribution in percent reactivation at 20 minutes for OPNA-inhibited mouse and human AChE.

Graph 1 (above): Percent reactivation at 20 minutes of GB-, GF-, VX-, and VR-inhibited mouse and human AChE. The results from each of the two-sample *t*-test with a 95% confidence interval found that there was a species-specific difference between VX- ($t(2) = -7.41$, $p = .018$) and VR- ($t(3) = -12.2$, $p = .001$) inhibited mouse and human AChE (* $p < .05$). While there was not a statistically significant difference between GB- ($t(2) = -2.70$, $p = .114$) and GF- ($t(2) = -2.81$, $p = .107$) inhibited mouse and human AChE, a functional difference has been observed.

Results

Reactivation at 20 minutes (% Control) ($M \pm SD$)		
Agent	Mouse	Human
GA	$3.55 \times 10^{-13} \pm 0$	$3.55 \times 10^{-13} \pm 0$
GB	7.14 ± 3.96	37.1 ± 18.8
GD	$3.55 \times 10^{-13} \pm 0$	$3.55 \times 10^{-13} \pm 0$
GF	0.050 ± 0.090	17.0 ± 10.5
VX	15.0 ± 2.00	76.8 ± 14.3
VR	17.2 ± 10.0	97.0 ± 5.24

Table 1 (left): Mean percent reactivation at 20 minutes by DG-1-054 at 10 µM of mouse and human AChE broken down by OPNA. The error associated with GB and GF inhibited AChE could contribute to the lack of statistical significance despite notable functional differences.

Conclusions

The purpose of this project was to determine if there was a significant difference between percent reactivation at 20 minutes for mouse and human AChE by DG-1-054. It was concluded that there was a significant difference in percent reactivation at 20 minutes for VX- and VR-inhibited mouse and human AChE at 10 µM. This supports the alternative hypothesis meaning that there is a species-specific difference. Scientists would have to be cautious in extrapolating data from mice to humans. The data shows that reactivation of mouse AChE is not equivalent to reactivation of human AChE, indicating that the mouse enzyme is a poor model for human systems.

Despite there being little room for human error, the robot could have made errors such as being inconsistent with the amount of liquid being pipetted. This would have caused variation as a confounding variable. Furthermore, the enzyme could have degraded prior to being used, which would have led to less percent reactivation. For future projects, other species of AChE could be tested with DG-1-054 to see if a species can accurately represent human AChE, or mouse and human AChE could be tested with other non-oxime reactivators.

References

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