The ability of the reactivators MINA and SwRI-80 to reduce toxic signs following nerve agent exposure in mice

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Introduction

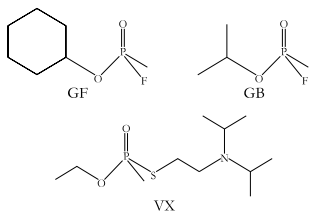
Acetylcholinesterase (AChE) is a highly catalytic enzyme that hydrolyses the neurotransmitter acetylcholine (ACh) into choline and acetate, terminating synaptic transmission. Under normal conditions, ACh activates glands, produces muscle contractions, and interacts with brain neurons involved with sleep, arousal, and memory. Nerve agents bind to the AChE active site and prevent AChE from breaking down ACh. The resulting excess ACh produces the signs of nerve agent intoxication: excessive salivation, bronchoconstriction, prolonged seizures and eventually death. Reactivator oximes bind to the nerve agent-inhibited AChE complex and biochemically remove part of the nerve agent, allowing AChE to return to its normal function. However, clinically used oximes have a quaternary structure and cannot penetrate the blood-brain barrier and thus cannot reactivate nerve agent-inhibited AChE in the brain. Novel tertiary oximes are being developed to penetrate the blood brain barrier and reactivate agent-inhibited AChE in the brain (Dail et al., 2019; Shih et al., 2012).

This study tested two novel tertiary oximes, monoisonitrosoacetone (MINA) and SwRI-80, on their effectiveness to reduce signs of sarin (GB), cyclosarin (GF), and VX nerve agent intoxication in two strains of carboxylesterase knock-out mice (Es1KO and KIKO). The use of these oximes in nerve agent therapies can provide safety measures to the military and civilians in the case of a nation-wide chemical attack.

Materials and Methods

Test subjects for this study consisted of ES1KO and KIKO strains of mice. Experimental groups were given a toxic dose subcutaneously of one of three nerve agents: GB, GF, or VX, structures of which are shown in Figure 1.

Figure 1 (right): The structures of the three nerve agents used in this study. GB and GF are non-persistent, while VX is considered persistent, meaning it does not easily degenerate or wash off (Sidell et al., 2013).



Fifteen minutes after nerve agent administration, the subjects were rated quantitatively from 1–9 by the degree of toxic signs they exhibited. The toxic signs were divided into categories—general motor signs (GMS), ear flick (EF), hypoactivity (H), general state (GS), and Straub tail (ST), which is categorized by a dorsiflexion of the tail in either a curled fashion over the animal or vertical to the body.

Table 1 shows the guide by which these toxic signs were scored.

Materials and Methods (cont.)

	0	1	2	3
GMS	Normal	Fasciculation	Tremors	Convulsions
EF	Absent	Present	--	--
H	Absent	Present	--	--
GS	Normal	Mildly Uncoordinated	Impaired Movement	Prostrated
ST	Absent	Present	--	--

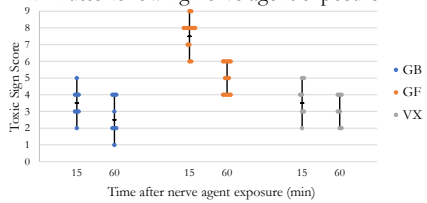
Table 1 (above): Ear flick, hypoactivity, and Straub tail signs were rated based on whether they were present in the test subject. General motor signs and general state signs were rated on a hierarchy of severity.

Toxic sign scores were recorded, and test subjects were then immediately given one of three treatment groups: a dose of MINA, a dose of SwRI-80 (both of which were given intraperitoneally), or no oxime. The mice were monitored for another 45 minutes, and toxic signs were again recorded at 60-minutes post-nerve agent exposure. Test subjects in the control group received no oxime dose (Shih et al., 2012). The two sets of toxic sign scores were graphed, and six Wilcoxon signed rank tests were conducted to determine if there were improvements in toxic signs within the treatment groups, the null hypothesis being there was no difference.

Results

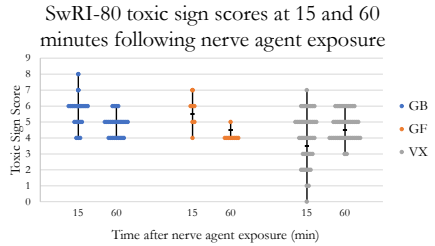
The 15- and 60-minute toxic sign scores for the MINA treatment groups were plotted in Graph 1.

Graph 1 (right): The 15- and 60-minute toxic sign scores for the MINA treatment group following GB, GF, and VX exposure. The medians, as well as the observed maximum and minimum values, for each set are marked.



The same process was repeated for the SwRI-80 treatment groups. The results were plotted in Graph 2. Six Wilcoxon signed rank tests were run on the data for each nerve agent with both oximes. For those treated with MINA, the tests yielded statistically significant reductions for the GF group ($W = 91.0$, $p = 0.002$), but not for the GB ($W = 18.5$, $p = 0.116$) or VX ($W = 10.0$, $p = 0.100$) groups. For those treated with SwRI-80, the tests yielded statistically significant reductions for the GB ($W = 179$, $p = 0.001$) and GF ($W = 36.0$, $p = 0.014$) groups, but not for the VX ($W = 202$, $p = 0.040$) group. These results confirmed the graphed data trends, revealing that SwRI-80 and MINA were partially effective in reducing signs of nerve agent intoxication.

Graph 2 (right): The 15- and 60-minute toxic sign scores for the SwRI-80 treatment group following GB, GF, and VX exposure. The medians, as well as the observed maximum and minimum values, for each set are marked.



Conclusions

The purpose of this study was to determine whether the oximes MINA and SwRI-80 were effective in mitigating or preventing signs of nerve agent intoxication in the central and peripheral nervous systems following exposure to the nerve agents GB, GF, or VX in ES1KO and KIKO mice. There was a significant reduction in 15- and 60-minute toxic sign scores when the mice were treated with MINA after exposure to GF, but not after exposure for GB or VX. There was also a significant reduction in the toxic sign scores between the 15- and 60-minute time intervals when the mice were treated with SwRI-80 after exposure to the GB and GF agents, but not the VX agent. These data show that MINA and SwRI-80 were partially effective in reducing the signs of nerve agent intoxication following GB, GF, or VX exposure, and that they may be able to be used alone or in conjunction with other treatments in nerve agent therapies. Further research into oxime effectiveness will aid in preventing nerve agent intoxication, protecting the military and civilians in chemical warfare.

References

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