

Introduction

Organophosphorus (OP) chemical compounds, such as tabun, sarin, soman, and VX, are highly toxic and commonly used in chemical warfare (McDonough, Hackley, Cross, Samson, & Nelson, 1983). They are referred to as cholinesterase inhibitors because of their ability to block an enzyme called acetylcholinesterase in the brain and body, thereby stopping the hydrolysis of the neurotransmitter acetylcholine and leading to the overstimulation of nerve cells, muscles and glands (McDonough, Jaax, Crowley, Mays, & Modrow, 1989). This causes a multitude of severe toxic signs such as hypersecretions, fasciculations, tremors, convulsions, respiratory distress, and prolonged seizures (*status epilepticus*). The prolonged seizures lead to subsequent rises in brain glutamate levels. Glutamate is an excitatory neurotransmitter that can cause nerve excitotoxicity when present in excessive levels that can lead to the development of neuropathology and severe neurological deficits (Zlotnik et al., 2006).

So far, the only options for military personnel that may be exposed to nerve agents are post-exposure therapy with atropine to block cholinergic overstimulation, an oxime such as 2-PAM to reactivate the inhibited enzyme, and an anticonvulsant to moderate seizures (Shih, Duniho, & McDonough, 2002). However, these drugs must be given immediately after exposure and in high doses to be maximally effective. This project tested an alternate method for mitigating the neurotoxic effects of nerve agents, by eliminating excess glutamate from the brain. It is hypothesized that a decrease of blood glutamate levels and an increase of the driving force for the brain-to-blood glutamate elimination can be achieved by infusing high amounts of the blood resident enzyme rat glutamate-oxaloacetate transaminase (rGOT) and the co-substrate oxaloacetate (OxAc) which act together to transform glutamate into 2-ketoglutarate. This treatment, applied after the onset of nerve agent seizures may significantly reduce the amount of brain glutamate and reduce or prevent the neuropathology (Gottlieb, Wang, & Teichberg, 2003; Zlotnik et al., 2006). This is what was tested in this project.

Methods and Materials

- Sprague-Dawley male rats with dealer-implanted jugular catheters served as subjects
- Animals were pretreated intraperitoneally (IP) with the oxime HI-6
- Thirty minutes later, the animals were challenged subcutaneously (SC) with 180 µg/kg soman
- One minute later, the animals were treated intramuscularly (IM) with 2.0 mg/kg atropine methyl nitrate (AMN)
- Animals were visually monitored for seizure onset
- Three minutes following seizure onset, the animals were administered by slow intravenous (IV) infusion (10 ml/kg over 30 min; a rate of 330 µl/kg/min) one of the combinations of OxAc + rGOT shown in Table 1
- Infusions were done using a TS-1B/W0109-1B syringe pumps allowing for precise infusion rates
- Animals were then treated IM with 10 mg/kg diazepam 40 minutes following seizure onset
- The animals were scored for presence and severity of toxic signs at 30 minutes, 1, 2, 4, and 24 hours after seizure onset
- At 24 hours, the animals were then perfused and the brain extracted
- Brains were processed for histopathology and sent to a pathologist for evaluation

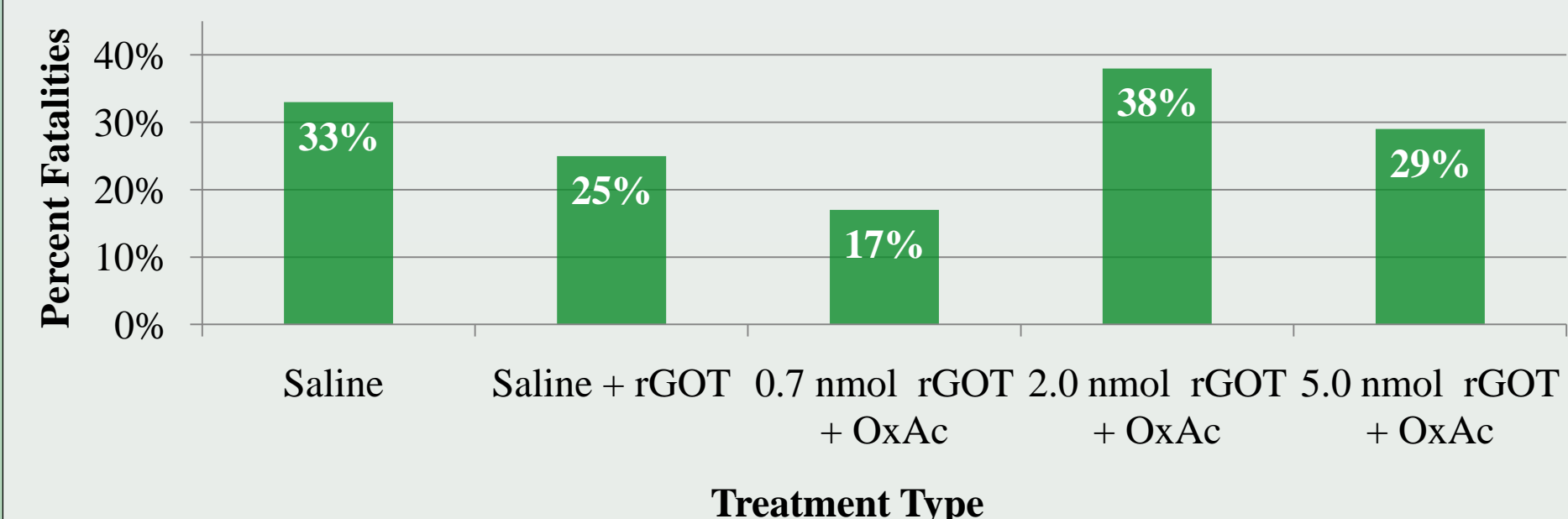
Results

One key piece of data collected was the average fatality rate of each treatment (Graph 1). Saline treatment vehicles acted as the control, averaging a fatality rate of 33%. Saline and OxAc treatments had a lower fatality rate of 25%, and 0.7 nmol rGOT and OxAc treatments had the lowest rate, with 17%. However, the other treatment types had an increase in the fatality rate. Another aspect studied was weight change in the animals before seizure onset and 24 hours following onset (Graph 2). The saline treatment vehicle had a weight change of 42.7g while the saline and rGOT treatment vehicle had a change of 38.3g. However, that was the lowest weight change seen when compared to the other treatment methods. The final piece of data collected on the animals was the toxic signs presented at 30 minutes and 1, 2, 4, and 24 hours post-seizure onset. However, the weight change and toxic sign data were subjected to Kruskal-Wallis One Way Analysis of Variance tests, both of which indicated that these differences between groups were not statistically significant.

| | Saline | OxAc | | | Total |
|---------------------|--------|-----------------|-----------------|------------------|-------|
| | | 25.0 µmol/ml/kg | 50.0 µmol/ml/kg | 250.0 µmol/ml/kg | |
| Saline | 13 | 13 | 13 | 13 | 52 |
| 0.7 nmol/ml/kg rGOT | 13 | 13 | 13 | 13 | 52 |
| 2.0 nmol/ml/kg rGOT | 13 | 13 | 13 | 13 | 52 |
| 5.0 nmol/ml/kg rGOT | 13 | 13 | 13 | 13 | 52 |

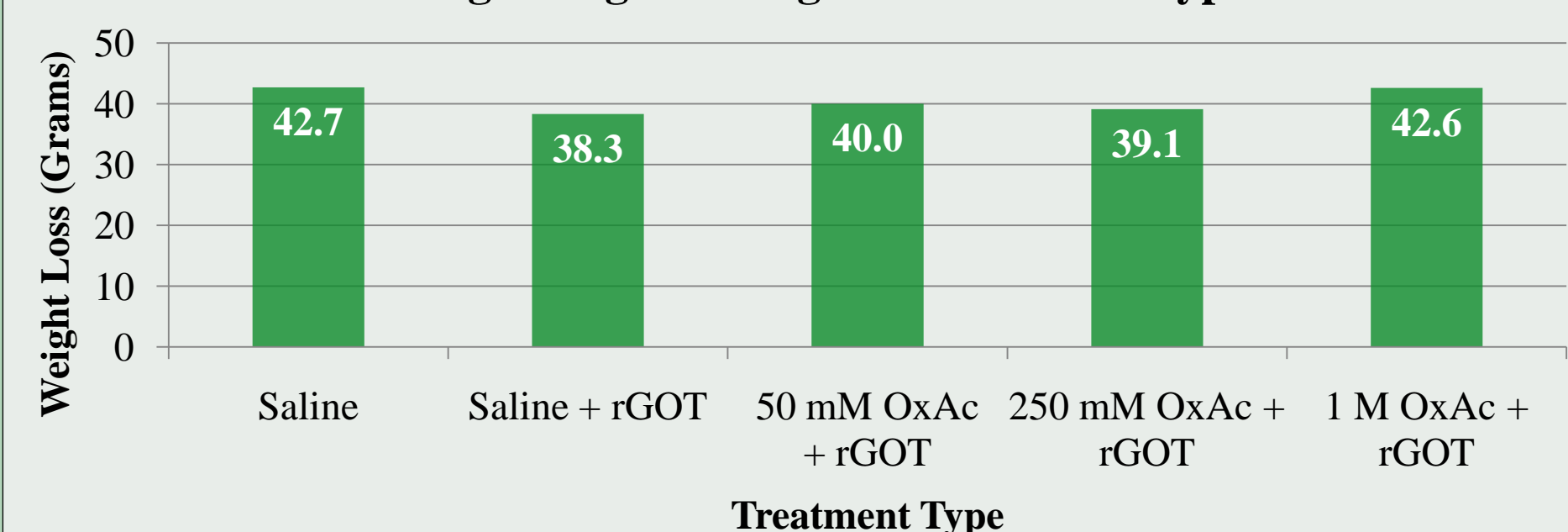
Table 1: Treatment vehicle combinations required for experimental procedure.

Percent Fatalities vs. Treatment Type



Graph 1: Average fatality rates for each treatment type.

Average Weight Change vs. Treatment Type



Graph 2: Average weight change observed in a 24 hour period for each treatment type.

Conclusion

The purpose of this project was to test the effectiveness of a blood glutamate scavenging system using a rGOT and OxAc treatment combination to reduce brain damage following nerve agent-induced seizures. The weight change and toxic signs data showed no statistically significant differences between the agent control and treated groups, so no definitive conclusions can be made based on these data. Still, it is possible to draw some conclusions when looking at the rate of mortality. When comparing the different treatments to the saline control, only the 0.7 nmol rGOT and OxAc treatment combinations resulted in a noticeable reduction in mortality. Other treatments, such as 2.0 nmol rGOT and OxAc combinations, had the opposite effects and resulted in a higher mortality rate.

These results are surprising since an increase in the concentration of the enzyme, rGOT, may have produced a detrimental effect. An increase in enzyme concentration should theoretically result in the greater conversion of substrates to products. In this case, higher levels of rGOT should have enhanced conversion of glutamate and OxAc to 2-ketoglutarate. However looking strictly at fatality rates may be not conclusive. Evaluation of potential differences in neuropathology is the key data needed to judge the effectiveness of this treatment.

Until the neuropathology data is fully available, it is premature to make any definitive conclusions about the effectiveness or failure of the tested treatment. Blood glutamate scavenging is a relatively new approach in providing neuroprotection against neurological injuries (Teichberg, Cohen-Kashi-Malina, Cooper, & Zlotnik, 2009). Treatments similar to the one tested in this study have been shown to reduce neural damage and consequent behavioral debilitation in rat models of closed head injury and stroke. Because of these successes, this approach may still prove effective against nerve agent-induced brain damage and warrants further consideration.

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