MEDICAL PROPHYLAXIS TO MITIGATE CHEMICAL THREATS

In Vitro Assessment Of The Ferret Model For Nerve Agent Medical Countermeasure Evaluation

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Chemical warfare nerve agents (NAs), such as sarin (GB), soman (GD), VX, and the Novichok compounds, continue to be a threat to civilians and military personnel across the world. The current treatment for NA poisoning in the U.S. is administration of atropine, an acetylcholine receptor antagonist; 2-pyridine aldoxime methylchloride (2-PAM Cl), an oxime to reactivate acetylcholinesterase (AChE) that has been inhibited by the nerve agent; and an anticonvulsant. 2-PAM Cl is recognized as a poor reactivator for many NAs, and numerous countries (including the U.S.) have evaluated potential replacements. The FDA Animal Rule allows for appropriate studies in animals to evaluate medical countermeasures (MCMs) when well-controlled Phase 2 clinical efficacy studies are not feasible and/or ethical. The nonhuman primate (NHP) is the preferred large animal model for NA MCM evaluation. However, the supply of NHP species used for research has become exceedingly scarce in recent years, underscoring the need to identify alternative animal models. In animal model evaluation and selection, the response to both the challenge agent and the MCM must both be considered. Early evaluation of the ferret model as an alternative to the NHP has shown promise, although studies on NAs and oximes are lacking. To begin to fill this knowledge gap, we performed an in vitro evaluation of the following in blood collected from humans and ferrets: circulating levels of active AChE, butyrylcholinesterase (BChE), and carboxylesterase (CES), NA-induced inhibition, oxime-assisted reactivation, and aging in ferret vs. human AChE and BChE.

In contrast to the large variations in circulating enzyme activity between humans and many other non-primates, baseline levels of AChE, BChE, and CES enzyme activity in blood samples collected from healthy ferrets and humans (n=5 from each sex) were in a similar range. Approximately two-fold variation was observed between human vs. ferret BChE and CES, and human circulating AChE activity levels were ~4-fold higher than ferret. The inhibition rate constant for GB was also found to be in a similar range for human and ferret AChE and BChE: AChE human = 1.10 ± 0.21 × 107 M−1 min−1, ferret = 0.661 ± 0.069 × 107 M−1 min−1; BChE human = 0.695 ± 0.063 ×107 M−1 min−1, ferret = 1.53 ± 0.13 ×107 M−1 min−1. Preliminary reactivation results also indicate similarity between the species, and inhibition, reactivation, and aging experiments for paraoxon, GD, VX, and A-234 are ongoing. In summary, preliminary results of the analysis of basal blood activity levels of AChE, BchE, and CES as well as inhibition, reactivation and aging of AChE and BChE with GB and several oximes suggest greater similarity between humans and ferrets than many other non-primate laboratory species. At a time when the use of NHP models is becoming increasingly challenging due to high cost, ethical considerations, and poor availibility, the results of this study will be useful for evaluating the ferret as an alternative animal model for nerve agent MCM development.