Analysis of percutaneous VX toxicity within the conscious, freely moving Göttingen minipig

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Introduction

The low volatility of VX in juxtaposition with other chemical warfare nerve agents makes it primarily a dermal threat. Consequently, when determining the effects of VX, a percutaneous (pc) application provides perspective into the most likely route that this nerve agent will take to enter the body (Mumford, Price, & Wetherall, 2008). Moreover, it has been established that pig skin is both morphologically and histochemically similar to human skin (Mortensen, Brinek, & Lichtenberg, 1998), making the Göttingen minipig a viable candidate for pc VX study. The minipig also qualifies as a large model organism, thereby providing superior extrapolation to humans. This study had one primary goal: to define the time-dependent physiological effects of pc VX exposure within the conscious, unrestrained, unanaesthetized Göttingen minipig in order to improve understanding of VX and ultimately develop countermeasures with greater efficacy. Lethal systemic doses of VX were therefore used in order to increase the potential for toxicity and allow ascertainment of the temporal sequence of physiological changes caused by pc VX exposure.

Materials and Methods

**Animals:** The model organisms in this study were male Göttingen minipigs (n = 5), 4-5 months old and weighing 12.80 kg (± 0.3 SEM). The low volatility of VX in juxtaposition with other chemical warfare nerve agents makes it primarily a dermal threat. Consequently, when determining the effects of VX, a percutaneous (pc) application provides perspective into the most likely route that this nerve agent will take to enter the body (Mumford, Price, & Wetherall, 2008). Moreover, it has been established that pig skin is both morphologically and histochemically similar to human skin (Mortensen, Brinek, & Lichtenberg, 1998), making the Göttingen minipig a viable candidate for pc VX study. The minipig also qualifies as a large model organism, thereby providing superior extrapolation to humans. This study had one primary goal: to define the time-dependent physiological effects of pc VX exposure within the conscious, unrestrained, unanaesthetized Göttingen minipig in order to improve understanding of VX and ultimately develop countermeasures with greater efficacy. Lethal systemic doses of VX were therefore used in order to increase the potential for toxicity and allow ascertainment of the temporal sequence of physiological changes caused by pc VX exposure.

**Nerve Agent:** Neat VX was obtained from the chemical transfer facility and verified to be 91.5 wt % pure (± 0.9 S.D.) by 31P-NMR. All exposures in this study were completed within 1 month of the purity analysis.

**Surgery:** A more thorough description of surgical procedures for implantation of the external jugular vein is within Hulet et al., 2006. To assess percutaneous VX toxicity in the conscious guinea-pig. Journal of Applied Toxicology, 28, 694-702.

**Experimental Parameters:** Baseline electrophysiological measurements of 30-60 minutes were performed 3-4 days and 30 minutes prior to challenge. Whole blood samples were analyzed for cholinesterase activity and iSTAT-CG8+ cartridges were used to measure pH, pCO2, and K, with blood drawn at baseline and for every two hours until death.

**Statistics:** Pomonah Physiology Plus (P3) was utilized in order to analyze parameters. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity, pH, pCO2, and K were determined from whole blood samples, with B signifying baseline time and E signifying exposure time. SigmaStat was used to find average times to first sign, seizure, and death (n = 5).

**Materials and Methods (continued)**

Conclusions

This study provided supplementary understanding of VX while also supporting established antecedents. The regularity of times to symptoms suggested that the data was reproducible. Cholinesterase activity followed expected patterns and decreased over time, with AChE showing greater degeneration (Graph 1). Temperature showed an anticipated spike near the time of seizure, and consequently decreased (Graph 2). The data from pH and CO2 (Graph 5) revealed a strong correlation between the behavior of these parameters and the condition called acute respiratory acidosis. In this state, hypoventilation leads to an accumulation of CO2, clinically termed as hypercapnia; a chemical reaction then occurs between the blood and the excess CO2, leading to a drop in blood pH. Decrease in blood pH is medically connected with muscular seizures, diarrhea, nausea, and heart arrhythmias. Acidosis is typically linked with constant K levels. In this study, slight hyperkalemia was manifest at 2 hours following exposure, but unexpected decrease into hypokalemia was subsequently observed (Graph 6). This phenomenon must be studied further. The precipitous drop in pH and its subsequent effect on electrolyte balance may have worked in tandem with the cardiac rhythm changes caused by the direct cholinergic effect of percutaneous VX in order to put the heart at risk for arrhythmias and sudden cardiac arrest. The increase in QRS interval (Graph 3) coupled with the constancy of PR interval (Graph 4) indicated potential left bundle branch block, a serious cardiovascular impediment. Only five minipigs were used in this study, however, in order to provide greater experimental validity, supplementary assessment with additional data is necessary.

Bibliography


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