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Median lethal dose determination for percutaneous exposure to soman and VX in guinea pigs and the effectiveness of decontamination with M291 SDK or SANDIA foam

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HIGHLIGHTS

- Determined the cutaneous median lethal dose of two chemical warfare agents.
- Demonstrated the M291 kit has limited effectiveness against cutaneous VX and soman.
- Demonstrated that SANDIA Foam is very effective against cutaneous VX and soman.

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ABSTRACT

Soman (GD) and VX are chemical warfare agents that can be absorbed through the skin. We determined the median lethal dose (MLD) for the cutaneous application of GD and VX in anesthetized haired guinea pigs and then tested the ability of a currently fielded decontamination kit, the M291 Skin Decontamination Kit (SDK), and decontaminating foam made by SANDIA Labs to decontaminate areas that have been exposed to cutaneous applications of GD and VX. The fur of guinea pigs was clipped on the left flank 24 h prior to exposure. Animals were anesthetized and 5 min later neat GD or neat VX was applied. The MLD for percutaneous exposure to GD was 11.6 mg/kg, and to VX it was 0.10 mg/kg. To test the ability of the M291 SDK, either GD or VX was applied and removed 1 min later with the pads of the M291 SDK clasped in a pair of forceps and wiped across the flank of the animal. The MLDs for GD and VX removed with the M291 SDK pads were 76.9 mg/kg and 0.87 mg/kg, respectively. When neat GD or neat VX was applied and removed 1 min later in the same manner with gauze soaked in SANDIA foam (MDF-100), the MLDs were 412 mg/kg and 10.4 mg/kg respectively. These data demonstrate that GD and VX are significantly less potent when applied cutaneously than previously reported for subcutaneous injections and indicate that improvement is needed on the limited protective ratio provided by the M291 SDK.

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1. Introduction

Nerve agents are among the most toxic of the known chemical agents. Nerve agents are organophosphonates that bind irreversibly to acetylcholinesterase (AChE) (Taylor et al., 1999) and to the bioscavenger butyrylcholinesterase (BChE) (Wolfe et al., 1992) in both the peripheral and central nervous system. AChE is responsible for terminating the action of the neurotransmitter acetylcholine (ACh) by hydrolysis. The inhibition of AChE by organophosphonates (chemical warfare agents) or organophosphates (insecticides), results in an excess of ACh and the over stimulation of muscarinic and nicotinic receptors. Characteristic signs of nerve agent poisoning and cholinergic overload include hypersecretion, respiratory distress and convulsions, which can lead to death.

The organophosphonates GD (soman, 1,2,2-tri-methylpropyl methylphosphonofluori-date) and VX (o-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate) have been widely studied by subcutaneous administration and are highly toxic. The major route of absorption of VX is via cutaneous exposure, and GD can be cutaneously absorbed in either its neat (undiluted with solvent) liquid state (Bide et al., 2005) or as thickened GD (Liu et al., 1999). However, the information currently available from subcutaneous administration may not be directly applicable to cutaneous exposure. In 1969, Fredriksson published a detailed study of the percutaneous absorption of sarin and two related analogs. He showed that there was a significant difference between the subcutaneous and percutaneous median lethal dose (MLD) values, and that after lethal percutaneous exposure, the time for the onset of signs and lethality could not be predicted

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by the subcutaneous MLD values. Fredriksson speculated that these variations could be the result of intradermal hydrolysis, inactivation by skin cholinesterases, absorption into subcutaneous fat, reaction within skin proteins, and evaporation of a significant fraction of sarin before absorption into the animals.

After cutaneous exposure to GD or VX, the rapid removal of agent from the surface of the skin could reduce its penetration into the general circulation and the resulting decrements of cholinergic toxicity, potentially preventing death. There is a limited window of opportunity for decontamination treatment following agent exposure. The signs of poisoning develop within minutes, and if decontamination is delayed, toxic levels of the nerve agents will likely be disseminated via the blood stream after the agent has been absorbed. Decontamination will prevent continued absorption of the agent, reducing the need for further medical management (Hamilton et al., 2004). Although physical removal of agent reduces the amount of agent penetrating the skin, physical removal alone does not provide sufficient protection. Bjarnason et al. (2008) exposed swine to 5×MLD of VX and decontaminated with gauze soaked with soapy water and found this ineffective in preventing lethality.

The M291 SDK was first issued to US forces in 1989, and although the Army has replaced it with Reactive Skin Decontamination Lotion (RSDL), the other services still have it in stock, and it could be used in the field. There are three main components incorporated into individual pouches: a fiber pad (six to a pouch), an absorbent activated charcoal, and a reactive resin, Ambergard XE-555 (Rohm and Haas, Philadelphia, PA), which is a black free-flowing powder. Each component serves a unique purpose. First, the cotton pad provides structural integrity for use on a finger. Second, the carbon incorporated into the pad absorbs organic material. Third, the ion-exchange resins bind chemical agents and very slowly detoxify them. The M291 SDK is used by simply wiping the contaminated skin and does not require significant training. However, the M291 SDK has some drawbacks, such as a black, offensive dust that can irritate the eye, thus limiting its use near the eyes, and can only be used around wounds, not directly on them (Gordon and Clarkson, 2009).

Efficacy of the M291 SDK has been evaluated in a number of animal models of organophosphonate poisoning. In an early report, rabbit skin was shaved (to mimic human skin without fur), and then exposed to GD or VX for 2 min. Decontamination with the M291 SDK yielded higher LD_{50}s (of more than 10- and 20-fold respectively) (Hurst, 1997), in comparison to animals not decontaminated. In another study, with rabbits under similar conditions, the penetration of VX was measured by red blood cell AChE inhibition. The M291 SDK increased the amount of VX required to inhibit AChE by 50% (Hurst, 1997).

Sandia National Laboratories has developed decontaminating foam products, which are licensed to Modec Inc., Denver, Colorado. MDF-100 is one of these products and is a solution stored in two parts (Gordon and Clarkson, 2009). Part A is a solution of 6.6% N,N,N,N^{11}, N^1-penta-methyl,-N^1-tallow alkyl 1,3-prop-anamine diammonium; 2.6% tallow pentamethyl propane quaternary ammonium compounds; 2.6% benzyl-C12-18 alkyl dimethyl; and 1% isopropyl alcohol. Part B is a solution of 8% hydrogen peroxide. Vigorously mixing the two parts results in a foam-like product that lasts for up to 30 min. When sprayed into a centrifuge tube and allowed to settle, a liquid level quickly forms (Fig. 1).

A competing decontaminating system not tested in these experiments is RSDL, developed by the Defence Research Establishment in Suffield, Canada. The RSDL solution is composed of 1.25 M potassium 2,3-butanedione monoximate in poly ethylene glycol monoethyl ethers of average molecular weight 550 Da ($MPEG_{550}$) with 10% water (pH 10.6). The RSDL solution is applied using pads of sponge-like plastic foam. The safety of this product was demonstrated, and in March 2003 it was approved by the FDA to remove or neutralize chemical warfare agents and T-2 fungal toxin from the skin (Gordon and Clarkson, 2009).

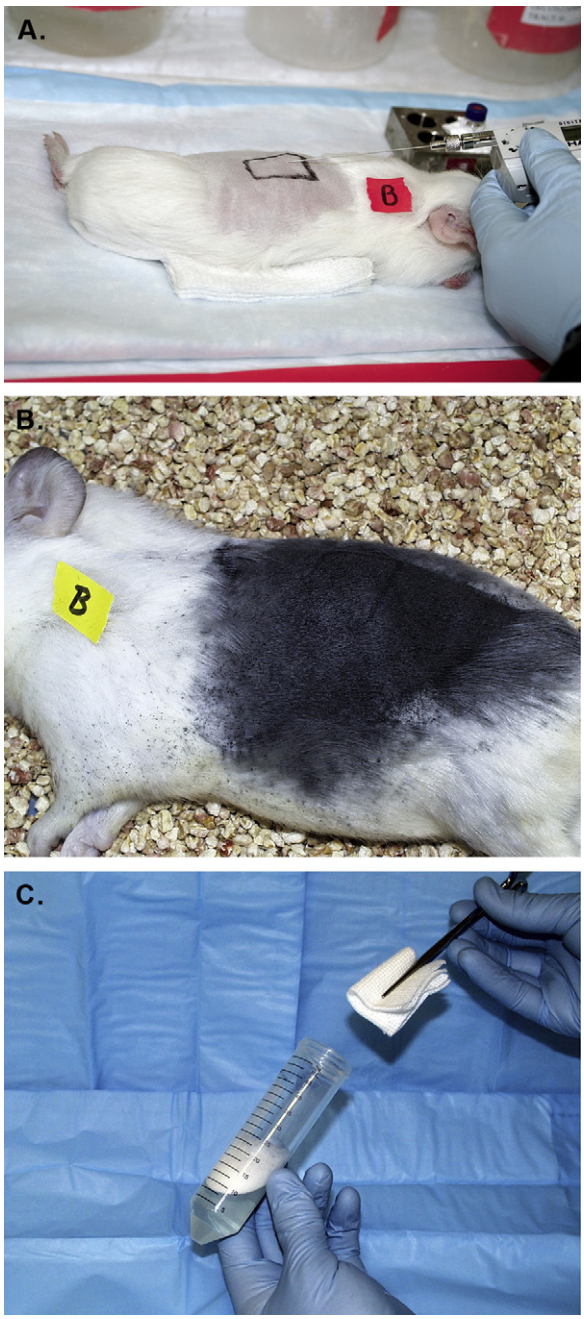

Fig. 1. Frame (A) shows an anesthetized guinea pig in lateral recumbency, which has had its fur clipped, with VX being applied by a 0.5-μl digital syringe. Visible behind the hand of the agent operator, the primary vial of VX is secured in an aluminum block. A square is drawn on the flank of the guinea pig; all VX and GD applications are done in the center of the square. Frame (B) shows an anesthetized guinea pig after agent application and decontamination with M291 SDK. The square centered on the point of agent application is visible, and the area decontaminated with M291 SDK is clearly greater than the area exposed to agent. Frame (C) shows 15 ml of SANDIA MDF-100 sprayed into a 50-ml centrifuge tube. The piece of absorbent gauze will fully absorb the 15 ml and be used to decontaminate an animal.

In this paper we seek to establish the MLD of GD and VX in our model, determine the efficacy of both the M291 SDK and SANDIA foam MDF-100 against GD and VX and compare them to published reports of RSDL efficacy.