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RSDL decontamination of human skin contaminated with the nerve agent VX

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HIGHLIGHTS

- Early initiated decontamination of VX with RSDL on human skin was efficient.
- RSDL-effectiveness was greatly reduced with extended OPC-exposure times.
- RSDL-efficacy was dependent on the chemical properties and concentrations of the OPCs.
- High pH and OPC-solubility were crucial for agent degradation by RSDL.

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ABSTRACT

Dermal exposure to low volatile organophosphorus compounds (OPC) may lead to penetration through the skin and uptake in the blood circulation. Skin decontamination of toxic OPCs, such as pesticides and chemical warfare nerve agents, might therefore be crucial for mitigating the systemic toxicity following dermal exposure. Reactive skin decontamination lotion (RSDL) has been shown to reduce toxic effects in animals dermally exposed to the nerve agent VX. In the present study, an *in vitro* flow-through diffusion cell was utilized to evaluate the efficacy of RSDL for decontamination of VX exposed to human epidermis. In particular, the impact of timing in the initiation of decontamination and agent dilution in water was studied. The impact of the lipophilic properties of VX in the RSDL decontamination was additionally addressed by comparing chemical degradation in RSDL and decontamination efficacy between the VX and the hydrophilic OPC triethyl phosphonoacetate (TEPA). The epidermal membrane was exposed to 20, 75 or 90% OPC diluted in deionized water and the decontamination was initiated 5, 10, 30, 60 or 120 min post-exposure.

Early decontamination of VX with RSDL, initiated 5–10 min after skin exposure, was very effective. Delayed decontamination initiated 30–60 min post-exposure was less effective but still the amount of penetrated agent was significantly reduced, while further delayed start of decontamination to 120 min resulted in very low efficacy. Comparing RSDL decontamination of VX with that of TEPA showed that the decontamination efficacy at high agent concentrations was higher for VX. The degradation mechanism of VX and TEPA during decontamination was dissected by ^{31}P NMR spectroscopy of the OPCs following reactions with RSDL and its three nucleophile components. The degradation rate was clearly associated with the high pH of the specific solution investigated; *i.e.* increased pH resulted in a more rapid degradation. In addition, the solubility of the OPC in RSDL also influenced the degradation rate since the degradation of VX was significantly faster when the NMR analysis was performed in the organic solvent acetonitrile compared to water.

In conclusion, we have applied the *in vitro* flow-through diffusion cell for evaluation of skin decontamination procedures of human epidermis exposed to OPCs. It was demonstrated that early decontamination is crucial for efficient mitigation of epidermal penetration of VX and that almost complete removal of the nerve agent from the skin surface is possible. Our data also indicate that the pH of RSDL together with the solubility of OPC in RSDL are of primary importance for the decontamination efficacy.

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1. Introduction

Organophosphorus compounds (OPC) are frequently used within the industry and agriculture (Kalkan et al., 2003; Yurumez et al., 2007). Organophosphorus pesticides and chemical warfare nerve agents are powerful inhibitors of acetylcholine esterase, the primary enzyme for regulation of acetylcholine (ACh) in nerve synapses. OPC-induced dysregulation of ACh results in uncontrolled activation of neuromuscular and central nervous functions leading to acute cholinergic toxicity. Following exposure to an OPC, the two most likely routes of entry into the body are through the skin or *via* the respiratory system. For low volatile nerve agents (*e.g.* VX) intoxication through contact exposure is expected to dominate leading to a primarily dermal uptake (Misik et al., 2015; Munro, 1994). For such agents skin decontamination is therefore considered to be crucial for mitigation of systemic uptake and intoxication (Bajgar, 2005; Thiermann et al., 2013; Yurumez et al., 2007).

Two principal methods have been applied for skin decontamination; firstly physical removal by washing with soap and water or by using absorbing powders such as the Fuller's Earth (Chan et al., 2013; Lademann et al., 2011; Taysse et al., 2007), and secondly decontamination involving chemical neutralization of the agent. The Reactive Skin Decontamination Lotion (RSDL) combines these two approaches by including a polyethylene glycol monomethyl ether (MPEG) for active desorption of the skin contaminant and chemical reactive oximes for neutralization of toxic nerve agents (Schwartz et al., 2012). Previous studies in animal models have demonstrated protective effects of skin decontamination following percutaneous exposure to VX and related OPCs, but data on decontamination efficacy on human skin is essentially lacking. In several animal *in vivo* studies, RSDL has been found to be more efficient against chemical warfare agents than other decontamination methods (Bjarnason et al., 2008; Braue et al., 2011a,b; Schwartz et al., 2012). However, results from previous studies on the ability of RSDL to prevent symptoms of intoxication, *e.g.* cholinesterase inhibition and clinical signs of cholinergic toxicity such as miosis and salivation, have been inconclusive, probably due to species differences in the physical skin barrier function and variation in experimental approaches (Bjarnason et al., 2008; Joosen et al., 2016, 2013; Taysse et al., 2007, 2011).

Apart from the composition of the decontamination solution, the practical procedure for the skin decontamination is also considered important for the efficacy. It is recognized from animal studies that early initiated decontamination, within a few minutes following exposure, is very effective. However, the evaluation of an efficient time span for delayed decontamination has provided inconsistent results (Braue et al., 2011a; Hamilton et al., 2004; Joosen et al., 2013; Rolland et al., 2013). Another concern is the enhanced skin penetration rate when highly concentrated agents are diluted in water as was recently shown by us to occur both for lipophilic and hydrophilic OPCs (Thors et al., 2016). Such penetration-enhancing effect may potentially reduce decontamination efficacy following addition of substantial amounts of water.

In the present study, the decontamination efficacy of RSDL on human skin following exposure to OPCs was evaluated utilizing an *in vitro* human epidermal membrane model consisting of a flow-through diffusion cell. By comparing RSDL decontamination of skin exposed to the nerve agent VX with the non-toxic OPC triethyl phosphonoacetate (TEPA), it was possible to address the contribution of the neutralizing component of RSDL and the impact of the hydrophilic/lipophilic properties of the OPCs on the decontamination efficacy. Furthermore, the mechanism by which RSDL and its individual components degrades the OPCs during decontamination was in detail investigated using NMR spectroscopy. Moreover, the impact of timing in the initiation of decontamination and the influence of agent dilution in water on the decontamination efficacy was considered.

2. Material and methods

2.1. Chemicals

O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX; >97% pure measured by NMR; CAS No. 50782-69-9) and Dekon139 (the potassium salt of the deprotonated diacetyl monoxime; Patent US5075297) were synthesised in house. Triethyl phosphonoacetate (TEPA; 98% pure; CAS No. 867-13-0) and diacetyl monoxime (DAM; ≥98% pure; CAS No. 57-71-6) were purchased from Sigma-Aldrich (St Louis, MO, USA). Reactive Skin Decontamination Lotion (RSDL) was obtained from Emergent BioSolutions (Gaithersburg, MD, US). The receptor solution contained a mixture of ethanol and deionized water (1:1; v:v).

2.2. Epidermal membrane preparation

Full-thickness human Caucasian skin from abdominal origin, was obtained from plastic surgery after informed consent of each patient. The full-thickness skin was cropped from subcutaneous fat and connective tissue and then stored at −80 °C. Following acclimatization at −20 °C for 3–4 days, skin was thawed in room temperature and small disks (0.64 cm^2) were punched out. Epidermis was excised from the underlying dermis by heat separation. Following immersion of skin disks in water at 60 °C for 2.5 min, epidermis was gently separated with forceps and the epidermal disks were hydrated for 18–24 h at +4 °C before use. The amount of appendages in the diffusion area of the epidermis disk was determined, disks exceeding one in the diffusion area were discarded to avoid excessive transappendageal diffusion. The cut off for excessive flux was a minimum of 1000-fold increased penetration rate in the first fraction collected. The separate experimental set-ups were performed with skin from the same donor patient. This study was approved by the Research Ethics Committee, Faculty of Medicine and Odontology, Umeå University, Umeå, Sweden (No. 03-161).

2.3. In vitro *skin penetration*

The *in vitro* skin penetration studies were performed as previously described (Thors et al., 2016). A flow-through diffusion cell designed in house according to the OECD guidelines was utilized throughout all experiments (OECD, 2004). The diffusion chamber was maintained at 32 °C (AccuBlock Digital Dry Bath; Labnet Int. Inc, Edison, NJ, USA) and the system was allowed to reach equilibrium of temperature and water balance for 30 min prior to experiments. Using a syringe pump (CMA 400; CMA Microdialysis, Kista, Sweden), the receptor solution was continuously pumped at a flow rate of 20 μl min^{-1}. The epidermis disk was mounted in the diffusion cell and a Teflon seal was positioned upon to seal off the receptor solution compartment. The nominal diffusion area was 0.13 cm^2 from the epidermal membrane to the receptor compartment. A zero sample of the receptor solution was collected before adding the donor solution. In experiments without decontamination, the donor solution including an infinite dose of OPC was added in a total volume of 25 μl, samples of the receptor solution were collected every ten minutes and kept at 10 °C in a fraction collector throughout the experiment (CMA 470 Refrigerated Fraction Collector; CMA Microdialysis, Kista, Sweden). The OPC concentrations evaluated were 20, 75 and 90% agent diluted in deionized water. Dilutions were freshly prepared in prior of each experiment. In order to ensure maximum recovery, a coverslip was placed on top of the Teflon seal following agent