Chemico-Biological Interactions 267 (2017) 48–56

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

The impact of skin decontamination on the time window for effective treatment of percutaneous VX exposure

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ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form
4 January 2016
Accepted 1 February 2016
Available online 5 February 2016

Keywords:
VX
Organophosphate
Percutaneous
Skin decontamination
Treatment
Nerve agent

ABSTRACT

The main goal of the present study was to obtain insight into depot formation and penetration following percutaneous VX poisoning, in order to identify an appropriate decontamination window that can enhance or support medical countermeasures.

The study was executed in two phases, using the hairless guinea pig as an animal model. In the first phase the effect of various decontamination regimens on levels of free VX in skin and plasma were studied as well as on blood cholinesterase levels. Animals were exposed to 0.5 mg/kg VX and were not decontaminated (control), decontaminated with RSDL once at 15 or 90 min after exposure or three times at 15, 25 and 35 (10-min interval) or 15, 45 and 75 min after exposure (30-min interval).

There was no significant effect of any of the decontamination regimens on the 6-h survival rate of the animals. However, all animals that had been decontaminated 15 min after exposure, showed a survival rate of more than 90%, compared to 50–60% in animals that were not decontaminated or decontaminated at 90 min after exposure.

In the second phase of the study, hairless guinea pigs were exposed to 1 mg/kg VX on the shoulder, followed either by decontamination with RSDL (10 min interval), conventional treatment on indication of clinical signs or a combination thereof. It appeared that a thorough, repeated decontamination alone could not save the majority of the animals. A 100% survival rate was observed in the group that received a combination of decontamination and treatment.

In conclusion, the effects of VX exposure could be influenced by various RSDL decontamination regimens. The results in freely moving animals showed that skin decontamination, although not fully effective in removing all VX from the skin and skin depot is crucial to support pharmacological intervention.

1. Introduction

In general, low volatile nerve agents like VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) pose a higher risk for dermal exposure over exposure via the respiratory route. Studies in anesthetized atropinized hairless guinea pigs have shown that maximum blood levels of VX are only reached several hours after dermal exposure to VX, followed by a slow elimination due to the highly persistent nature of the nerve agent [1]. Additionally, freely moving guinea pigs dermally exposed to VX required extensive continuous treatment to counteract the toxicological effects of VX entering the bloodstream from the undecontaminated skin. These findings urged the necessity of skin decontamination as an effective countermeasure to complement medical countermeasures. However, effective decontamination is hampered by a probable unawareness concerning the actual site and time of exposure. The primary indication of exposure might consist of systemic signs of toxicity, which are only present after progressive entering of agent into the bloodstream [2–4]. These findings have important implications for military or civilian first responders with respect to triage, decontamination approach, contact hazard and therapeutic drug regimens [5].

Although *in vitro* models are valuable to study interspecies differences [6], which can aid the understanding of differences in skin penetration between substances, *in vivo* experiments are necessary for translation to the human situation. *In vitro*, penetration rates

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http://dx.doi.org/10.1016/j.cbi.2016.02.001
0009-2797/

can be influenced by structural changes in the *in vitro* skin and the absence of blood flow [7,8]. Insight into depot formation and VX penetration into the blood stream following dermal exposure is needed to identify an appropriate decontamination window that can enhance or aid medical countermeasures and lead to improved understanding of the behavior of chemical warfare agents in the skin and uptake in the body.

To enable real-time monitoring of penetration of VX through the skin, *in vivo* skin microdialysis was applied in the anesthetized hairless guinea pig model to. This approach would allow monitoring of the effects of decontamination on the suspect skin depot of VX *in vivo* in relation to toxicokinetics and cholinesterase inhibition.

To investigate consequences of skin penetration following percutaneous exposure to VX, anesthetized hairless guinea pigs were used in which the toxicokinetics of VX, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition were measured over time in blood samples. In general, pig and mini pigs are considered highly suitable (large) animals models for dermal exposure, because of the similarities in skin composition [9]. As a small animal model, the hairless guinea pig skin closely reflects that of human skin *in vitro*, which justifies the use of this model to study toxicokinetics after VX exposure, both *in vivo* as well as *in vitro* [10,11].

Initial experiments were conducted to address the efficacy of fairly rapid decontamination at 15 min following exposure to a percutaneous dose of VX, and decontamination at 90 min after exposure. These time points were chosen as being realistic in a scenario where personal decontamination is readily available but not directly applied because of unawareness of exposure. The 90-min time point was chosen as the first time point at which clinical signs generally appear in the VX exposure scenario used [2,3]. Reactive Skin Decontamination Lotion (RSDL) was applied as personal decontaminant, as this is the fielded product in the Dutch Armed Forces.

Following this scenario, it was tested whether the efficacy obtained in the 15 min scenario would be improved by two additional application moments with two varying intervals, 10 and 30 min. This scenario was chosen because the initial experiments showed that residual agent was present in the skin, from which the VX poisoning progresses.

The use of anesthetized animals can mask effects of toxicity, therefore follow-up experiments were translated into a scenario with freely moving hairless guinea pigs, to include assessment of the consequences of dermal uptake by monitoring physiology and toxicodynamics. The objective of this part of the study was to address the time-resolved efficacy of skin decontamination and interactions with treatment protocols in use, combined with toxicokinetic and physiological data. The combination of experiments allowed the identification of individual contributions of decontamination and nerve agent antidotes to clinical outcome.

2. Methods

2.1. Experimental design

The research was executed in two phases.

In the initial phase, the effect of various decontamination regimens on levels of VX in the skin, VX plasma levels and blood cholinesterase was studied. To that end, five groups of animals were used. One group (n = 9) served as control, whereas in the other groups several RSDL application regiments were applied (n = 6/group, Fig. 1).

In the second phase, it was addressed to what extent decontamination at 15, 25 and 35 min and antidotes administered on indication of clinical signs (maximum three injections) would work complementary. Therefore, 4 groups of animals were used, as shown in Table 1.

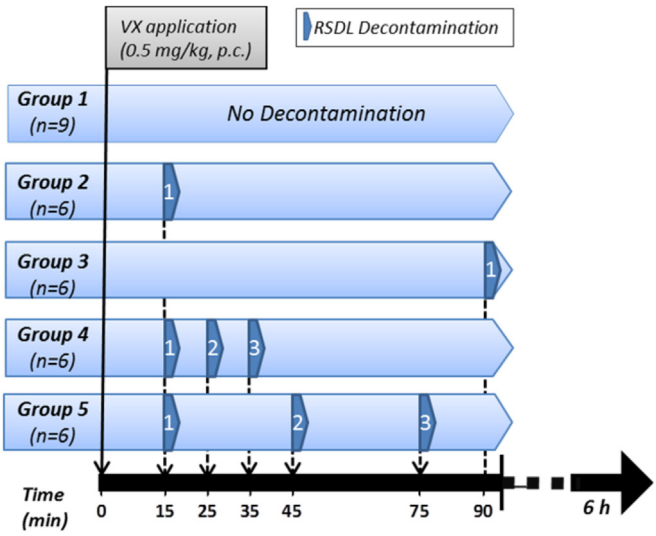

Fig. 1. Schematic overview of experimental groups for skin microdialysis experiments in hairless anesthetized guinea pigs. Decontamination with RSDL was performed at various time points using different time intervals. The maximum experiment duration was 6 h, decontamination was performed in the first 90 min.

2.2. Animals

Male hairless guinea pigs [Crl:IAF(HA)BR] (~500 g), obtained from Charles River Kingston (Stone Ridge, NY, USA) were used in the present study. Prior to the experiments they were housed with 2 animals per cage, and allowed to acclimate to standard conditions for two weeks. Room temperature was kept at 19–22 °C and relative humidity was maintained at 55–65% and lights were on from 7 a.m. to 7 p.m. Acidified water and standard guinea pig chow (Teklad global diet 2040, Harlan, Horst, The Netherlands) were available *ad libitum*. The experiments described received prior approval from the TNO Animal Ethical Committee. The protocols ensure that suffering is minimized in all cases. The first set of experiments was conducted under anesthesia, and the animals were euthanized after 6 h. In the second set of experiments animals were euthanized after 24 h or when the animals suffered from progressive severe signs of poisoning, such as bradycardia or apparent bronchoconstriction.

2.3. Chemicals

(±) VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl]methylphosphonothioate) and D_3-VX, were obtained from the stocks of TNO Defence, Security and Safety and were of purity ≥98% (determined with gas chromatography (GC)). The other chemicals used were of standard purity and purchased from renowned companies. RSDL was obtained from RSDecon (Bracco Solutions, Princeton, NJ, USA).

2.4. Phase I – in vivo skin microdialysis

Animals were weighed and anesthetized with 4% isoflurane in O_2. Anesthesia was maintained at 2–3% isoflurane and body

Table 1
Groups and groups sizes in efficacy experiment.

Group	Exposure	Decontamination	Treatment	Size (n)
1	VX	–	–	6
2	VX	3× RSDL	–	6
3	VX	–	3× Antidotes	6
4	VX	3× RSDL	3× Antidotes	6