USAMRICD-TR-18-14

In Vivo Evaluation of OxiClean™ as a Skin Decontamination Material following Topical Application of VX in Guinea Pigs

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November 2018

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an element of the

US Army Medical Research and Materiel Command

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
30-11-2017	Technical Report	January 2018 to March 2018

4. TITLE AND SUBTITLE

In Vivo Evaluation of OxiClean™ as a Skin Decontamination Material following Topical Application of VX in Guinea Pigs

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Koplovitz, I, Schulz, S, Morgan, J, Chen, P, Clarkson, E

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDR-AM
8350 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-TR-18-14

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Threat Reduction Agency
8725 John J. Kingman Road STOP 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

Funding: Defense Threat Reduction Agency, Joint Science and Technology Office

14. ABSTRACT

OxiClean™ is the brand name of a group of commonly used household products marketed for removal of stains from clothing and surfaces. Preliminary in vitro studies in a reaction vessel with one of the products, OxiClean™ Versatile Stain Remover (OVSR), showed a rapid, concentration-dependent destruction of VX and sulfur mustard. These results and the ready availability and low cost of OVSR suggested that this product might be useful for mass casualty skin decontamination (DC) following exposure to hazardous chemicals. The purpose of this investigation was to evaluate the in vivo effectiveness of OVSR as a skin DC material compared to water and soapy water following topical application of VX. Unanesthetized male guinea pigs were used as subjects. DC was performed 2 min after agent application with 1% or 14% solution (w/v) of OVSR, with a 1% solution (v/v) of Dawn™ dish detergent or with filtered tap water. DC was performed using a 3-step process. The VX LD50 estimates for water and Dawn™ detergent DC were 23- to 26-fold higher than the historic untreated LD50 of VX, respectively, while the LD50s for OVSR were shifted only by a maximum of 11-fold. The results suggest that OVSR should not be used for DC of skin exposed to hazardous chemicals.

15. SUBJECT TERMS

skin, decontamination, VX, guinea pigs, chemical agents, countermeasures, liquids, decontaminates

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Irwin Koplovitz
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	11	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-5342

Abstract

OxiClean™ is the brand name of a group of commonly used household products marketed for removal of stains from clothing and surfaces. Preliminary *in vitro* studies in a reaction vessel with one of the products, OxiClean™ Versatile Stain Remover (OVSR), showed a rapid, concentration-dependent destruction of VX and sulfur mustard. These results and the ready availability and low cost of OVSR suggested that this product might be useful for mass casualty skin decontamination (DC) following exposure to hazardous chemicals. The purpose of this investigation was to evaluate the *in vivo* effectiveness of OVSR as a skin DC material compared to water and soapy water following topical application of VX. Unanesthetized male guinea pigs were used as subjects. Animals were weighed, the fur on a flank was sheared with electric clippers, and a small exposure area was outlined with an indelible marker. Animals were randomly assigned to four DC groups. Neat liquid VX was applied to the exposure area using a positive displacement Hamilton digital syringe with attached blunt needle. DC was performed 2 min after agent application with 1% or 14% solution (w/v) of OVSR, with a 1% solution (v/v) of Dawn™ dish detergent or with filtered tap water. DC was performed using a 3-step process. First, the DC material was applied to the exposure area and left on the skin for 2 min; next the exposure area was rinsed with water and then dried. Fresh gauze applicators were used for each step. Following DC, the animals were monitored for signs of intoxication, and clinical assessments were taken at various times up to 24 hrs after exposure. Various doses of VX were used in each group to establish a dose-lethality relationship. Probit analysis was used to generate a VX dose-lethality curve with a slope and lethal dose estimates from 1-99% with 95% confidence limits for each DC material based on 24-hour responses. The VX LD_{50} estimates for water and Dawn™ detergent DC were 23- to 26-fold higher than the historic untreated LD_{50} of VX, respectively, while the LD_{50}s for OVSR were shifted only by a maximum of 11-fold. The results suggest that OVSR should not be used for DC of skin exposed to hazardous chemicals.

Introduction

OxiClean™ is the brand name of a group of commonly used household products marketed for removal of stains from clothing and surfaces. Preliminary *in vitro* studies in a reaction vessel with one of the products, OxiClean™ Versatile Stain Remover (OVSR), showed a rapid, concentration-dependent destruction of VX and sulfur mustard. These results and the ready availability and low cost of OVSR suggested that this product might be useful for skin decontamination (DC) following a mass casualty chemical exposure incident. The purpose of this investigation was to evaluate the *in vivo* effectiveness of OVSR as a skin DC material compared to two mass casualty DC materials, water and soapy water, following topical application of VX.

Methods

Animals and Husbandry:

Unanesthetized male guinea pigs [Hartley, Crl(HA)BR] ranging in weight from 300-500 gm were used as subjects. After arrival, the animals were maintained in quarantine for at least five days prior to use in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited animal care and use facility. On the morning of the experiment around 0800 hrs, animals were weighed, the fur on the left flank was carefully sheared with electric clippers, and excess loose fur was removed with a vacuum. A small exposure area was outlined with an indelible marker at approximately the same location on the left side of each animal midway between the spine and the ventral midline. After VX exposure and DC, animals were housed in individual cages with Iso-PAD™ enrichment bedding (Teklad) in a fume hood for the duration of the experiment (24 hours). Food and water were provided *ad libitum* after VX exposure and DC.

Experimental Procedures:

All exposure and DC procedures were conducted in the fume hood IAW USAMRICD SOPs. Neat liquid VX was applied to the exposure area using a positive displacement Hamilton digital syringe and attached blunt needle. Various challenge doses of VX were used in each group to establish a dose-lethality relationship using a stage-wise, adaptive dose design (Feder et al., 1991). Animals were randomly assigned to four DC groups. DC was performed 2 min after agent application with 1% or 14% solution (w/v) of OVSR, 1% solution (v/v) of Dawn™ dish detergent or “still” tap water. The OVSR concentrations were selected based on the *in vitro* results. The concentration of Dawn™ matches the soap & water concentration previously reported to be effective (Braue et al., 2011). DC was performed using a 3-step process to mimic the procedures that first responders might use following a mass casualty chemical exposure incident. In step 1, the DC material was applied to the exposure area and left on the skin for 2 min; in step 2 the exposure area was “rinsed” with water and then immediately dried in step 3. Gauze applicators were used to perform the DC procedures (Fig 1). The applicators in steps 1 and 2 were wetted with 9-10 ml of the respective DC solution. Application involved swiping the applicator in a head to tail direction over the exposure site 10 times in step 1, and 5 times in each of steps 2 (rinsing) and 3 (drying). Fresh applicators were used for each step on each animal. Following DC, the animals were monitored for signs of intoxication, and a clinical

assessment score from 0 to 5 was assigned at 2 hrs and 4 hrs after DC and 24 hrs after exposure. Criteria for scoring was as follows: 0= normal appearance and behavior; 1= slight lethargy and/or minor signs, but animal is upright and ambulates by itself; 2= noticeable lethargy and signs, but animal is upright and will ambulate if prodded; 3= animal is prostrate, has definite signs but is conscious and can support head; 4= animal is prostrate, unresponsive with or without pronounced signs; 5 = dead. A 0 to 5 (dead) was assigned to each animal at each observation time. Probit analysis was used to generate a VX dose-lethality curve with slope and lethal dose estimates from 1-99% with 95% confidence limits for each DC material based on 24 hr alive/dead responses. Protective ratios (PR) were estimated by dividing the VX LD_{50} in decontaminated animals by the historic (Clarkson unpublished data) VX LD_{50} in animals without DC.

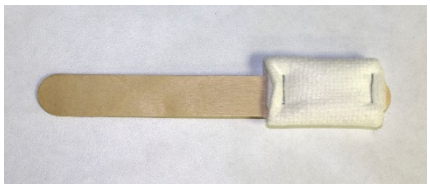

Figure 1: Applicator used to conduct DC procedures

Results

Figure 2 shows the probit-generated VX dose-lethality curves for each of the 4 DC treatment groups. A total of 22-29 animals were used in each group. The animals in the groups decontaminated with water, 1% Dawn™ detergent or 1% OVSR exhibited the full range of lethality responses from 0% to 100%, enabling the probit model to generate statistically valid VX dose-lethality curves. We were unable to establish a clear VX dose-lethality relationship in the animals decontaminated with 14% OVSR. While there were some survivors, animals challenged at the lowest VX dose (870 µg/kg) tested did not survive 24 hrs. To conserve animal use, no further resources were used to establish a dose-lethality relationship for the 14% OVSR, since additional animals would not have improved its effectiveness. The probit analysis curve for the 14% OVSR, represented by the dashed line, is only a rough approximation of the dose-lethality curve. Most animals surviving for 24 hrs across all DC treatment groups were normal appearing or displayed only minor clinical signs (Table 2).

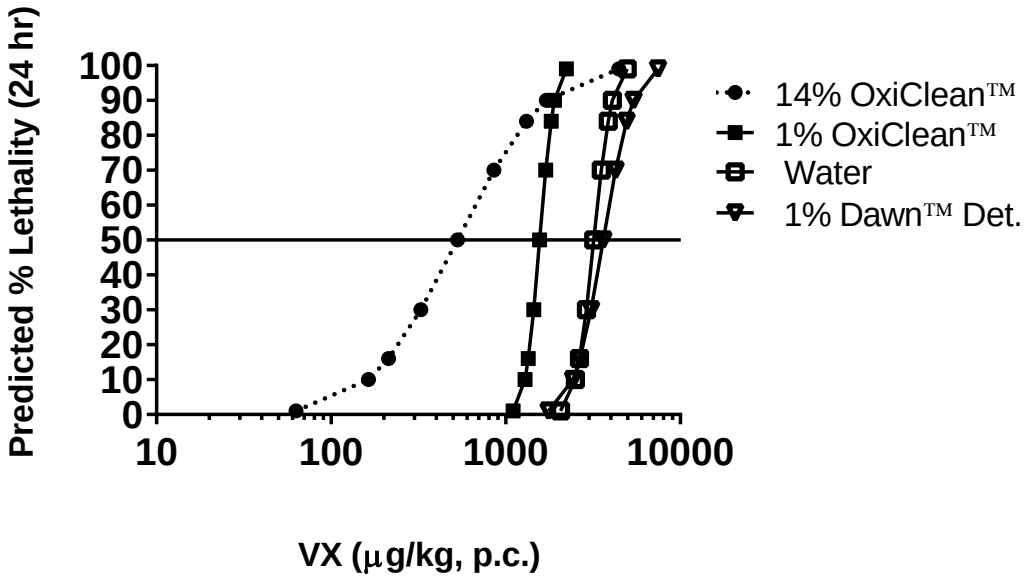

Figure 2: VX dose-lethality curves generated by probit analysis of 24 hr responses in guinea pigs decontaminated with OVSR, water or Dawn™ detergent 2 min after agent application. The curve for 14% OVSR (dotted line) is approximated (see results).

Table 1 summarizes the calculated LD_{50}s, 95% confidence intervals and slope estimates, and Table 2 lists individual animal responses. DC with water or Dawn™ detergent was significantly more effective than either concentration of OVSR, and the 14% solution of OVSR was less effective than the 1% solution. The LD_{50} estimates for water (3198 µg/kg) and Dawn™ detergent (3622 µg/kg) were 23- to 26-fold higher, respectively, than the historic untreated percutaneous LD_{50} (140 µg/kg) of VX, while the LD_{50} estimate for 1% OVSR was only 11-fold higher. The estimated LD_{50} for 14% OVSR was less than 6-fold higher.

Table 1: Efficacy of Skin Decontamination Performed 2 Minutes after Topical VX Application in Unanesthetized, Fur-Clipped Guinea Pigs

Decon	N	24 hr Probit Estimates			Estimated Protective Ratio[1]
		LD_{50}(µg/kg,pc)	95% CI	Slope	
Water	25	3198	2760 - 3706	12.1	23
1% Dawn™ Det.	29	3622	2926 - 4484	7.4	26
1% OVSR	22	1566	1391 - 1763	15.2	11
14% OVSR	23	<870	ND	2.5	<6

1. Protective Ratio = VX $LD_{50decon}$/VX $LD_{50untreated}$. Estimated using the historic USAMRICD untreated 24 LD_{50} value of 140 µg/kg, pc in the denominator.

Table 2: Individual Animal Responses in the Experimental Groups

Decon	Animal #	VX Challenge # LD_{50}s, pc	Clinical Assessment Score (hr)		
			2	4	24
Water	666	45.7	3	Dead	Dead
	684	45.7	Dead	Dead	Dead
	670	36.0	2	3	Dead
	673	36.0	1	4	Dead
	680	36.0	3	Dead	Dead
	708	29.8	1	3	Dead
	713	29.8	1	1	Dead
	722	29.8	0	2	Dead
	646	24.3	1	2	2
	664	24.3	1	1	1
	667	24.3	0	1	Dead
	677	24.3	1	3	Dead
	679	24.3	2	Dead	Dead
	715	24.3	0	0	0
	716	19.2	0	3	Dead
	718	19.2	0	0	2
	720	19.2	0	0	1
	652	15.4	0	0	0
	661	15.4	0	0	0
	674	15.4	1	0	0
	647	7.9	0	0	0
	650	7.9	0	0	0
	655	7.9	0	0	0
	649	3.9	0	0	0
	658	3.9	0	0	0

Decon	Animal #	VX Challenge # LD_{50}s, pc	Clinical Assessment Score (hr)		
			2	4	24
1% Dawn	625	45.7	1	2	Dead
	630	45.7	2	3	Dead
	725	29.8	0	0	0
	740	29.8	Dead	Dead	Dead
	626	26.4	2	3	Dead
	633	26.4	2	Dead	Dead
	732	26.4	Dead	Dead	Dead
	727	24.3	1	3	Dead
	738	24.3	0	0	4
	744	24.3	0	0	0
	709	21.6	0	0	1
	712	21.6	0	0	0
	724	21.6	0	1	1
	734	21.6	0	0	0
	688	19.2	0	0	0
	697	19.2	0	0	0
	704	19.2	0	0	0
	629	15.4	0	0	Dead
	638	15.4	0	0	0
	641	15.4	0	0	0
	687	15.4	0	0	0
	694	15.4	0	0	0
	706	15.4	0	0	0
	714	15.4	0	0	0
	719	15.4	1	1	0
	695	12.1	0	0	0
	699	12.1	0	0	0
	636	7.1	1	1	0
	640	7.1	1	0	0

Table 2: Individual animal Responses in the Experimental Groups (cont'd)

Decon	Animal #	VX Challenge # LD_{50}s, pc	Clinical Assessment Score (hr)		
			2	4	24
1% OxiClean	671	45.7	Dead	Dead	Dead
	678	45.7	Dead	Dead	Dead
	668	24.3	2	3	Dead
	682	24.3	1	2	Dead
	665	15.4	2	2	2
	675	15.4	2	Dead	Dead
	681	15.4	2	Dead	Dead
	689	12.1	0	0	Dead
	698	12.1	0	1	1
	705	12.1	1	Dead	Dead
	711	12.1	1	0	0
	717	12.1	1	Dead	Dead
	728	12.1	0	3	Dead
	685	9.6	0	0	0
	693	9.6	0	2	Dead
	726	9.6	0	0	0
	733	9.6	0	0	0
	743	9.6	0	0	1
	669	7.9	1	0	0
	676	7.9	0	0	0
	707	7.9	0	0	1
	723	7.9	0	0	0

Decon	Animal #	VX Challenge # LD_{50}s, pc	Clinical Assessment Score (hr)		
			2	4	24
14 % OxiClean	648	45.7	Dead	Dead	Dead
	657	45.7	Dead	Dead	Dead
	660	24.3	Dead	Dead	Dead
	651	24.3	2	Dead	Dead
	696	15.4	3	4	Dead
	656	15.4	2	Dead	Dead
	663	15.4	2	Dead	Dead
	645	15.4	1	3	Dead
	702	12.1	Dead	Dead	Dead
	692	12.1	3	3	Dead
	686	12.1	0	0	1
	690	9.6	3	Dead	Dead
	700	9.6	3	Dead	Dead
	736	9.6	1	2	Dead
	703	9.6	0	2	Dead
	729	8.6	4	Dead	Dead
	731	8.6	3	Dead	Dead
	739	8.6	1	2	Dead
	653	7.9	0	0	1
	662	7.9	0	0	0
	737	7.9	0	4	Dead
	735	6.2	2	Dead	Dead
	741	6.2	0	0	Dead

Discussion

OVSR was less effective than either plain water or a 1% solution of Dawn™ dish detergent as a skin decontaminate in preventing lethality following *in vivo* exposure to VX, and the higher the OVSR concentration the less effective it was. The reduced effectiveness of OVSR compared to water and Dawn™ dish detergent suggests that it alters the barrier function of the stratum corneum, enabling some VX to penetrate and get absorbed systemically. This potential "wash-in" effect occurred very quickly, since OVSR was left on the skin for only 2 min before removal by rinsing and drying the skin.

Plain water and Dawn™ dish detergent were similar in their decontamination effectiveness, increasing the LD_{50} of VX by 22- to 25-fold, respectively. The protection afforded by water and Dawn™ was likely due to dilution of the agent and physical removal by the DC procedure. In a previous study, Braue et al. (2011) reported a 17-fold increase in the 24 hr LD_{50} of VX with soapy water decontamination 2 min after topical agent administration.

The DC procedure used in this study attempted to mimic Department of Homeland Security (DHS) procedures that might be used by first responders to decontaminate casualties following a mass exposure incident. DHS procedures recommend washing the casualty with copious amounts of water or mild soap and water with a wash cloth (if available) for no more than 3 min. followed by rinsing with more water (following soap), and then drying with a towel (DHS/DHHS, 2014). Due to safety concerns to laboratory personnel and contamination of the fume hood by run off from a large amount of liquids, all three procedures in this study were performed using a gauze applicator saturated with the DC material (Fig 1). This applicator was similar to the one that was utilized previously to decontaminate animals with soapy water or dilute bleach (Braue et al., 2011).

Based on the results of this study, we conclude that OxiClean™ products should not be used for decontamination of skin exposed to hazardous chemicals. However, OxiClean™ products might be useful for decontaminating clothing/uniforms, inorganic materiel surfaces or mortuary remains.

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