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ASSESSMENT OF NON-ABRADED WEAR OF SKIN EXPOSURE REDUCTION PASTE AGAINST CHEMICAL WARFARE AGENTS (SERPACWA)

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13. ABSTRACT *(Maximum 200 words)*
Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) is an FDA-approved topical skin protectant developed to complement the use of chemical protective clothing in preventing exposure to chemical/biological warfare agents. The question of how long the protective barrier would last under non-abraded conditions over 8 h (Test 1) or 16 h (Test 2) wear-time test periods was evaluated. The purpose of the non-abraded sites was to control for any change in efficacy of SERPACWA that might have occurred over the wear time when it was not abraded. We also report the efficacy of SERPACWA when it was challenged immediately after application to the skin. Six subjects were studied in a climate-controlled room (22°-24°C, 25%-30% rh). Four sites were marked on the volar surface of each forearm (2.4cm diameter). The two proximal sites were covered to protect these sites from being abraded. One arm was treated with SERPACWA (50 ml per test site) and the other remained untreated to serve as the control. Only one of the non-abraded sites received SERPACWA prior to the wear time. After 8 h or 16 h, SERPACWA was applied to the untreated site of the SERPACWA-treated arm. A 5 mMol (10 ml) dose of methyl nicotinate (Mnic) was used as the challenge agent to evaluate the efficacy of SERPACWA. Mnic was applied to each site and removed after 2 minutes. One of the distal sites on each arm remained unchallenged to control for the differences in basal blood flow over time. Laser Doppler Imaging (LDI; perfusion units="flux") and visual scoring (VS) were used to quantify cutaneous erythema. LDI scans and VS were performed prior to SERPACWA application and following a 15-minute post-Mnic challenge after Test 1 and Test 2. Analysis of variance of the LDI data from the non-abraded paired sites showed that SERPACWA-treated sites had mean skin blood flow measurements 190 and 165 flux units lower than SERPACWA-untreated sites for Test 1 and Test 2, respectively ($p<0.05$). There were no significant differences, when the 8 and 16 h SERPACWA-treated sites were compared with the sites immediately treated with SERPACWA and Mnic-challenged, and the unchallenged control sites. The VS data corroborated the LDI data.

14. SUBJECT TERMS
SERPACWA, methyl nicotinate (Mnic), topical skin protectant, cutaneous erythema, laser Doppler imaging

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EXECUTIVE SUMMARY

Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) is an FDA-approved topical skin protectant developed to complement the use of chemical protective clothing in preventing exposure to chemical/biological warfare agents. The closure sites of these garments are considered vulnerable areas for possible chemical exposure. SERPACWA may prevent chemical agents from making direct contact with the skin beneath these overgarment closures. In order to establish field readiness guidelines, specific questions related to optimal use of this product were addressed. The question of how long the protective barrier would last under abraded and non-abraded conditions over 8 h (Test 1) or 16 h (Test 2) wear-time test periods was evaluated in the third of four experiments conducted at the U.S. Army Research Institute of Environmental Medicine (USARIEM) in a cooperative research effort with the U.S. Army Medical Material Development Activity (USAMMDA). In this publication we report the outcome of SERPAWCA's durability when it is applied to the skin but protected from abrasion by chemical protective clothing. The purpose of the non-abraded sites was to control for any change in efficacy of SERPACWA that might have occurred over the wear time when it was not abraded. We also report the efficacy of SERPACWA when it was challenged immediately after application to the skin. (The results from the abraded test sites were reported elsewhere.) Six subjects were studied in a climate-controlled room (22°-24°C, 25%-30% rh). Four application sites were marked on the volar surface of each forearm (2.4cm diameter). The two proximal sites were covered during the wear tests to protect these sites from being abraded by garment wear. One arm was treated with SERPACWA (50 µl per test site) and the other remained untreated to serve as the control. Only one of the non-abraded sites received SERPACWA prior to the wear time. After 8 h (Test 1) or 16 h (Test 2), SERPACWA was applied to the untreated site of the SERPACWA-treated arm. A 5 mMol (10 µl) dose of methyl nicotinate (Mnic), a known non-immunological contact irritant, was used as the challenge agent to evaluate the efficacy of SERPACWA. The Mnic challenge was applied to each site and removed after 2 minutes. One of the distal sites on each arm remained unchallenged to control for the differences in basal blood flow over time. Laser Doppler Imaging (LDI; perfusion units="flux") and visual scoring (VS) were used to quantify basal skin blood flow and cutaneous erythema. LDI scans and VS were performed prior to SERPACWA application (baseline) and following a 15-minute post-Mnic challenge after Test 1 and Test 2. Analysis of variance of the LDI data from the non-abraded paired sites showed that SERPACWA-treated sites had mean skin blood flow measurements 190 and 165 flux units lower than SERPACWA-untreated sites for Test 1 and Test 2, respectively ($p<0.05$). There were no significant differences, when the 8 and 16 h SERPACWA-treated sites were compared with the sites immediately treated with SERPACWA and Mnic-challenged, and the unchallenged control sites. The VS data corroborated the LDI data. These results indicate that SERPACWA-treated skin, when protected from clothing wear, provides complete protection from the Mnic challenge for at least 16 h and that SERPACWA protects the skin immediately after application.

INTRODUCTION

SCIENTIFIC BACKGROUND

Chemical warfare agents (CWA) continue to be a major threat to U.S. war fighters and peacekeepers. CWA such as the blistering agent, sulfur mustard, and the nerve agents, soman and thickened soman, increase this threat because of the toxicity and lethality of these agents by percutaneous absorption. The most effective way to protect our soldiers from the effects of these agents is to prevent or limit their CWA exposure.

Chemical protective gear, including the jacket and trouser overgarments, mask, gloves, and boots, provide good protection, but the closure sites of this ensemble may be vulnerable to CWA exposure during wear. Topical Skin Protection (TSP), which is now called Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), has been proposed to complement the use of chemical protective clothing as protection against the percutaneous threat of chemical agents at the closure sites of these garments (4, 16). The mechanism of SERPACWA's protection quality is a function of its physicochemical characteristics. SERPACWA is non-reactive, non-wetting, and immiscible with nearly all other chemicals. SERPACWA may prevent CWA from making direct contact with the skin.

Studies of the efficacy of SERPACWA have historically utilized a topical surrogate substance with a potential offending agent such as an expected toxin or an allergen. Urushiol (poison ivy) extract has been used in clinical trials to evaluate other topical creams for protection (1) and by the U.S. Army to evaluate SERPACWA (1, 4, 16). The end point of protection was determined by observing evidence of agent penetration to skin. The observations included cutaneous erythema and vesiculation some days after the exposure. To date, SERPACWA has been shown to be an effective skin protectant against methyl nicotinate (Mnic) for up to an hour on sweating humans (4). The advantage of Mnic as a challenge to a skin protectant is the rapid skin response (non-immunologic contact reaction) manifested by cutaneous erythema that can be quantified by measuring basal skin blood flow via laser-Doppler Imaging (LDI) techniques. The end-point criterion for protection is determined by observing evidence of the non-immunologic contact reaction, such as cutaneous erythema, and an increase in flux after exposure to the challenge agent, Mnic.

Methyl nicotinate (methyl 3-pyridinecarboxylate), a lipid soluble ester of nicotinic acid produced by Sigma Aldrich Chemicals (St. Louis), is a well-studied contact irritant, which produces easily monitored, non-immunologic, immediate contact reactions. The non-immunologic contact reaction is due to increased prostaglandin, an inflammatory mediator released after penetration of Mnic through stratum corneum into the dermis (2, 3, 6, 7, 8, 13, 15, 17). Mnic concentrations from 0.1 to 150 mMol produce measurable reactions (2, 6, 7, 8, 14).

The LDI technique (Moor Instruments, England) provides a 2-dimensional pattern of cutaneous microcirculation. A low intensity laser beam is scanned across a tissue surface in a raster pattern using a moving mirror. Both large and small areas can be scanned, enabling the blood flow to be mapped and displayed via color-coded images. Quantification of the intensity and expansion of perfusion (beyond that which is detectable using standard clinical methods of evaluation) can be calculated by defining regions of interest. LDI provides a sensitive, accurate, reproducible, and noninvasive means of measuring changes in skin blood flow, as reported in the scientific literature (5, 6, 7, 9, 10, 13). The scanning technique is less variable than single-point laser technology, and offers the ability to evaluate several test sites simultaneously. The principles of operating the LDI scanner are no different than conventional laser-Doppler scanning technology, and a full technical description of the instrument has been published (11, 12).

MILITARY RELEVANCE

As described in the Scientific Background, SERPACWA was developed to supplement chemical protective clothing. The Food and Drug Administration (FDA) recently approved the New Drug Application (NDA #21-084) as a safe and effective topical product for use by soldiers to provide additional protection against CWA. However, the FDA and the Army Combat Developers requested additional studies of SERPACWA to ascertain conditions that optimize its effective use by service members, if ever needed during instances of chemical threat.

The U.S. Army Research Institute of Environmental Medicine agreed to a request from the U.S. Army Medical Materiel Development Activity to conduct a clinical study under Good Clinical Practice guidelines, with the objective of evaluating SERPACWA's effectiveness and durability in protecting the skin from a challenge agent. Within this protocol there were four experiments conducted concerning SERPACWA's effectiveness relative to application timing, skin preparation prior to application, length of effectiveness, and effectiveness of re-application. This report addresses SERPAWCA's durability when applied to the skin and tested immediately, and when protected from abrasion by the overgarments over an 8 and 16 h wear-time test period.

PURPOSE

The purpose of this experiment was to evaluate the durability of SERPACWA when applied to the skin but protected from abrasion by chemical protective clothing during 8 (Test 1) and 16 (Test 2) h wear-time test periods. The purpose of the non-abraded sites was to control for any change in efficacy of SERPACWA that might have occurred over the wear time when it was not abraded. A secondary purpose of this experiment was to determine the efficacy of SERPACWA when it was challenged immediately after application.

METHODS

TEST SUBJECTS

Six subjects (6 men – Test 1; 5 men and 1 woman – Test 2) between the ages of 18 and 20 volunteered to participate in each test after they were formally briefed on the design and risks of the study. The subjects (see Table 1 for characteristics) were enrolled in the study without exclusion for race, ethnicity, or gender. Subjects were nonsmokers; were prohibited from the use of any prescriptive or over-the-counter medications 2 days prior to the experiment; and refrained from alcohol intake 24 hours prior to the experiment. Subjects' volar forearm and wrists were free of any scars, tattoos, or skin disorders such as eczema, psoriasis, or sunburn that would interfere with the erythemic evaluation. Prior to participating, all subjects were medically cleared and tested for a normal erythemic response to a dilute solution of methyl nicotinate (10 µl of a 2.5 mMol Mnic aqueous solution) at a forearm skin site. Responders exhibited a measurable, non-immunologic contact reaction to the Mnic challenge as assessed by visual scoring (VS).

Table 1. Subject characteristics for Test 1 and Test 2.

Test	Subject	Gender	Age (yrs)	Ethnicity	Height (m)	Weight (kg)	Handedness/ Application arm
1	11	M	18	Hispanic	1.74	83.8	R/L
	12	M	20	Caucasian	1.80	92.8	L/L
	14	M	19	Caucasian	1.85	87.8	R/R
	15	M	19	African Am.	1.88	91.1	R/L
	16	M	20	Caucasian	1.70	73.5	R/L
	19	M	19	Caucasian	1.61	78.6	R/R
Mean			**19.2**		**1.76**	**84.6**	
SD			**0.8**		**0.10**	**7.5**	
2	11	M	18	Hispanic	1.74	83.8	R/L
	12	M	20	Caucasian	1.80	92.8	L/L
	13	F	18	African Am.	1.57	56.8	R/R
	14	M	19	Caucasian	1.85	87.8	R/R
	15	M	19	African Am.	1.88	91.1	R/L
	16	M	20	Caucasian	1.70	73.5	R/L
Mean			**19.0**		**1.76**	**81.0**	
SD			**0.9**		**0.11**	**13.7**	

ENVIRONMENTAL CONDITIONS

All experiments were performed in a climate controlled room set at normal room temperature (22° to 24°C, 25% to 30% rh).

EXPERIMENTAL DESIGN

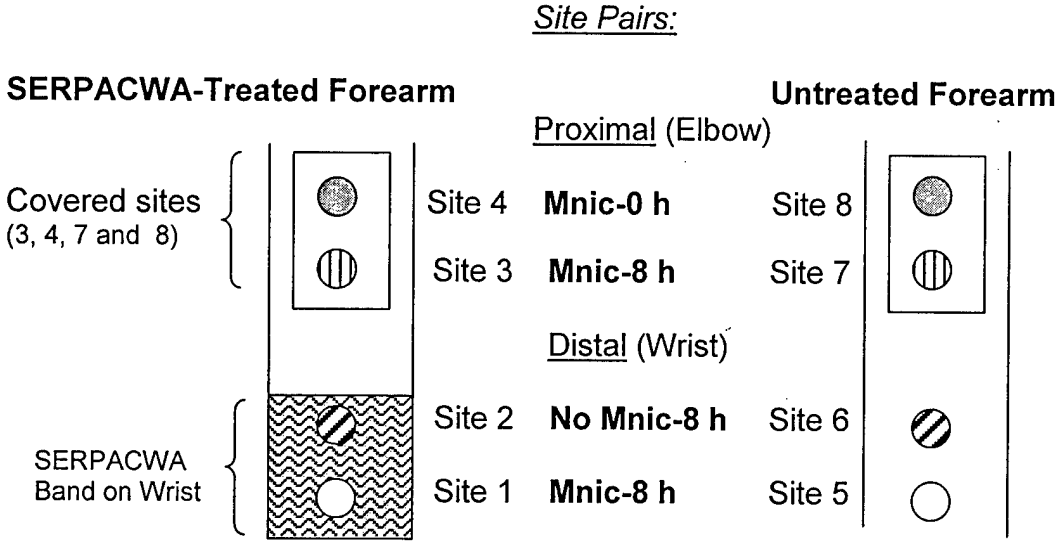
Each volunteer, once treated with SERPACWA on the volar surface of one arm, completed either an 8 or 16 h wear-time test period. The microvascular response of both forearm and wrist skin to a 5 mMol (10 µl) dose of aqueous Mnic was examined for evidence of SERPACWA barrier penetration.

TEST PROCEDURES

A methyl nicotinate stock solution (50 mMol in distilled water) was prepared from the crystalline solid each test day. Standard dilution techniques from a stock solution were used to prepare the 5 mMol Mnic challenge solution.

A black rectangular template was made for each subject to mark the test area on the volar surface of each arm. Four 2.4 cm diameter circular sites separated by 1 cm were identified and marked on the volar surface of each forearm (Figure 1). The distal sites (near the wrist) were used to evaluate the durability of SERPACWA under abraded conditions. The proximal two sites were covered with a plastic cover or metal perforated with small holes to protect the sites from friction of the garment during the wear time. These non-abraded sites were used to test wear time and immediate efficacy of SERPACWA. For each scan, subjects were seated and placed their forearms in a custom-made mold that positioned their hands in supination, with forearms and wrists close together beneath the LDI unit. The template was repositioned on the forearms prior to initiating the pre-SERPACWA control LDI scan, to provide a contrast for LDI flux graphic display. Subjects were required to wear laser protective goggles during all scans.

Figure 1. Diagram for Application Site Pairs
(This diagram represents an 8 h test)



SERPACWA was applied to three of the four sites on the randomly chosen SERPACWA-treated arm. Test sites #3, #4, #7, and #8 were covered during the wear test to protect these sites from being abraded by wear of the Battle Dress Uniform jacket and the chemical protective jacket. After SERPACWA application and donning of the chemical protective jacket, the subjects performed low intensity activities (cards, video games) and Common Task Training skills such as first aid, protection against nuclear/biological/chemical attack, while wearing the chemical protective jacket for the 8 and 16 h tests. After the set wear time was complete, SERPACWA was applied to the untreated site (either #3 or #4) of the SERPACWA-treated arm. The application the 5 mMol (10 µl) dose of Mnic was dispensed using an Eppendorf® Repeater® Pro pipette every 15 seconds to all contra-lateral pair sites except for either #1 and #5, or #2 and #6, which were randomly selected. The unchallenged sites controlled for differences in basal blood flow over time. After 2 minutes, the Mnic was removed by using a cotton swab to wick the droplets off each site. The post-Mnic challenge LDI scan was completed approximately 15 minutes after the Mnic was removed.

STATISTICAL ANALYSES

A one-way repeated measures analysis of variance was used to compare the post-Mnic challenge, as measured by LDI data and VS. To determine whether or not SERPACWA protected the skin against Mnic exposure, the non-abraded, SERPACWA-treated test site was compared to the non-abraded, SERPACWA-untreated test site for the specific wear times (8 or 16 h). To determine the degree of skin protection that the non-abraded, SERPACWA-treated test site provided, it was compared to the unchallenged control test site on the SERPACWA-treated arm. To determine whether SERPACWA application was effective immediately, SERPACWA was applied to the untreated site on the SERPACWA-treated arm following the 8 and 16 h test and challenged with Mnic immediately. After the Mnic was removed, the vasodilatory response was compared to those measured in 8 and 16 h non-abraded, SERPACWA-treated test sites. To determine the degree of skin protection for the immediately treated test site, it too was compared to the unchallenged control site on the SERPACWA-treated arm. The Mnic-only site served as a test of validity that the skin responded to the Mnic challenge. As reported in the Test Subject Selection section, all volunteers were screened for Mnic responsiveness; only responders were included in further testing. For all comparisons, significance was accepted at the 95% confidence level ($p<0.05$).

RESULTS

In order to determine the degree of protection SERPACWA provides following immediate application and over a prolonged period of protection from abrasion, we compared each test site with its contra-lateral site. Tables 2 and 3 illustrate the mean (±SD) skin perfusion and VS data for the four non-abraded forearm test site conditions. The data labeled LDI and VS PRE indicate there were no differences in either flux measurements or the VS among the sites prior to SERPACWA application for each test. However, 8 or 16 h later (LDI and VS POST), the sites treated with vs. without SERPACWA significantly ($P<0.05$) protected the skin from Mnic exposure. As evident in Figure 2, the percent change in vasodilatory response of the SERPACWA-untreated sites (NSC) to the Mnic challenge was 2.8 and 3.5 times greater than the SERPACWA-treated sites (SC) for Test 1 and Test 2, respectively. When the immediate SERPACWA-treated sites (SCI) were compared with SC following each wear-time test period, there was no statistical difference among the sites. The sites protected by SERPACWA immediately and over the 8 or 16 h tests had no significant difference in flux measurements compared to the unchallenged control site on the SERPACWA-treated arm. Flux values for the SERPACWA-treated control sites (SNC) are only shown in Tables 2 and 3. This observation indicates that skin protection was complete when challenged immediately with Mnic or after 8 or 16 h of non-abraded wear.

Table 2. Skin perfusion (LDI) and visual score (VS) data for each subject in Test 1.

LDI PRE					Control site
Subject #	SC8	NSC8	SCI	NSCI	SNC
11	56.7	68.1	31.0	44.2	49.3
12	80.2	109.8	104.1	57.6	76.6
14	54.9	84.5	68.6	85.4	56.9
15	49.9	38.7	37.1	34.1	43.7
16	82.7	66.7	75.3	67.9	71.6
19	63.1	69.1	93.0	82.9	52.8
Mean	64.6	72.8	68.2	62.0	58.5
SD	13.8	23.4	29.3	20.7	12.9

LDI POST					Control site
Subject #	SC8	NSC8	SCI	NSCI	SNC
11	39.4	181.4	73.8	179.0	47.6
12	73.0	275.4	108.7	309.1	144.5
14	69.2	249.4	50.8	251.9	61.1
15	35.2	254.7	30.6	192.9	20.7
16	155.1	266.1	64.3	256.3	58.9
19	52.7	335.8	107.5	314.6	70.3
Mean	70.8	260.5*	72.6	250.6**	67.2
SD	44.0	49.7	31.1	56.6	41.6

VS PRE					Control site
Subject #	SC8	NSC8	SCI	NSCI	SNC
11	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0
Mean	0.0	0.0	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0

VS POST					Control site
Subject #	SC8	NSC8	SCI	NSCI	SNC
11	0.0	1.5	0.0	1.5	0.0
12	0.0	1.5	0.0	2.0	0.0
14	0.5	1.5	0.0	1.5	0.0
15	0.0	1.0	0.0	1.0	0.0
16	1.0	2.5	0.0	2.5	0.0
19	0.0	2.5	0.0	2.0	0.0
Mean	0.3	1.8*	0.0	1.8**	0.0
SD	0.4	0.6	0.0	0.5	0.0

S=SERPACWA, NS=No SERPACWA, C=Challenged, I=Immediate, NC=No challenge. LDI and VS data were compiled from the PRE- and POST-SERPACWA scans. *P<0.05 vs. SERPACWA challenged after 8 h of wear. **P<0.05 vs. SERPACWA challenged following immediate application.

Table 3. Skin perfusion (LDI) and visual score (VS) data for each subject in Test 2.

LDI PRE					Control site
Subject #	SC16	NSC16	SCI	NSCI	SNC
11	34.0	47.4	33.7	50.4	40.1
12	87.2	71.5	99.9	81.9	79.1
13	23.9	25.2	28.5	26.4	35.2
14	47.0	52.4	49.4	78.0	68.1
15	36.2	36.5	33.6	37.5	41.1
16	58.4	57.8	55.3	56.2	62.6
Mean	**47.8**	**48.5**	**50.1**	**55.1**	**54.4**
SD	**22.6**	**16.2**	**26.5**	**21.9**	**17.9**

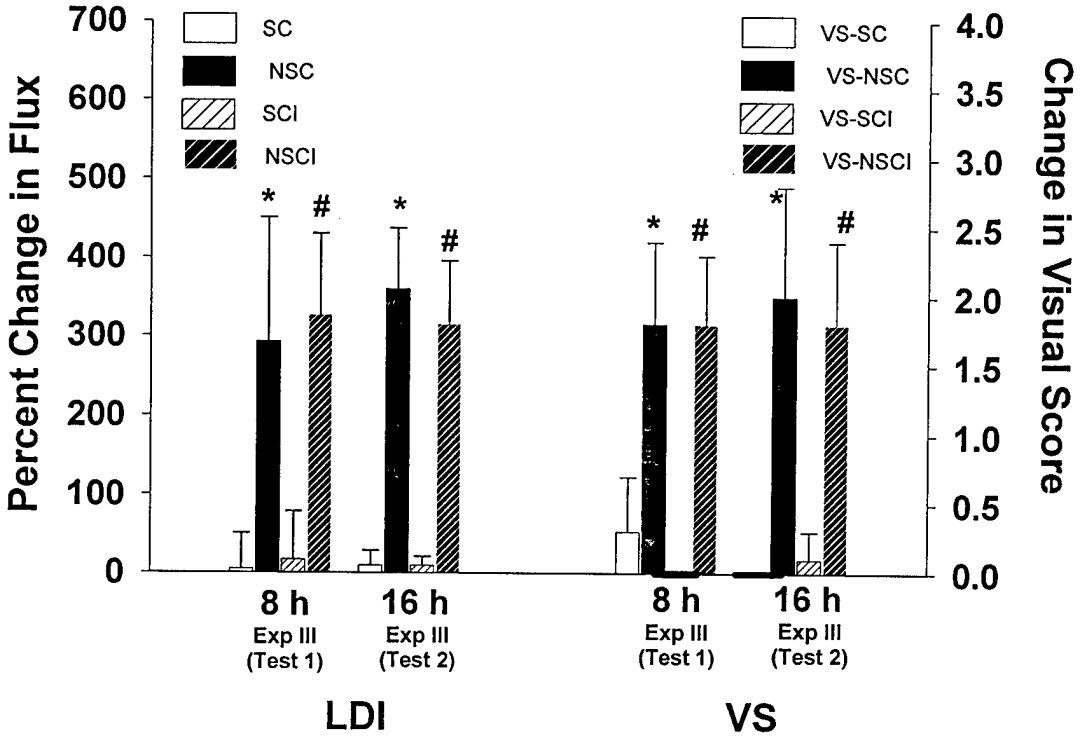
LDI POST					Control site
Subject #	SC16	NSC16	SCI	NSCI	SNC
11	35.3	214.4	40.4	216.1	63.9
12	74.8	278.7	89.6	319.8	74.6
13	30.6	128.8	31.5	114.7	35.8
14	60.1	192.2	54.3	207.9	53.5
15	43.9	210.6	40.7	180.2	52.1
16	52.6	267.2	58.3	274.5	61.6
Mean	**49.6**	**215.3***	**52.5**	**218.9****	**56.9**
SD	**16.4**	**54.3**	**20.7**	**71.8**	**13.1**

VS PRE					Control site
Subject #	SC16	NSC16	SCI	NSCI	SNC
11	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0
Mean	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
SD	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

VS POST					Control site
Subject #	SC16	NSC16	SCI	NSCI	SNC
11	0.0	1.0	+	1.0	0.0
12	0.0	1.5	0.0	1.5	0.0
13	0.0	2.5	0.0	2.5	0.0
14	0.0	1.5	0.5	1.5	0.0
15	0.0	2.5	0.0	2.0	0.0
16	0.0	3.0	0.0	2.5	0.0
Mean	**0.0**	**2.0***	**0.1**	**1.8****	**0.0**
SD	**0.0**	**0.8**	**0.2**	**0.6**	**0.0**

S=SERPACWA, NS=No SERPACWA, C=Challenged, I=Immediate, NC=No challenge. LDI and VS data were compiled from the PRE- and POST-SERPACWA scans. *P<0.05 vs. SERPACWA challenged after 16 h of wear. **P<0.05 vs. SERPACWA challenged following immediate application.

Figure 2. Percent change in flux for skin blood flow and change in visual score pre- to post-Mnic challenge



*P<0.05 vs. SERPACWA challenged post-8 and 16-h for flux and visual score.
#P<0.05 vs. SERPACWA immediately challenged for flux and visual score.

DISSCUSSION

The present study provides evidence that SERPACWA, when applied to the skin with an approximate thickness of only 0.1 mm, prohibited the penetration of a 5 mMol aqueous dose of methyl nicotinate following immediate application and after prolonged wear while protected from chemical protective overgarment abrasion. The data to support this statement are (1) no detectable vasodilatory response among the non-abraded SERPACWA-treated test sites, and (2) no apparent delay in providing immediate protection once applied to the skin.

Although there was substantial individual variation among the identified test sites, significant differences in both perfusion measurements and VS between SERPACWA-treated and SERPACWA-untreated sites were easily demonstrated with only 6 subjects per test group (Table 2 and 3). The results from both tests indicate that SERPACWA-treated skin, when protected from friction of clothing, provides complete protection from the Mnic challenge for at least 16 h, and SERPACWA protects the skin immediately after application

(Figure 2, $P<0.05$). To verify that SERPACWA completely protected the skin, flux measurements were compared to the unchallenged, SERPACWA-treated site (SNC – control site, Tables 2 and 3). There was no difference in skin perfusion among the SERPACWA-control site and the 8 and 16 h, non-abraded, SERPACWA-treated sites. Evidence of complete protection upon immediate application is based upon the finding of no difference in perfusion between the SERPACWA-immediately challenged site and the SNC site. (The results from the uncovered sites can be found elsewhere (13).)

CONCLUSIONS

The results of this study suggest that the use of SERPACWA is an effective means to increase the level of protection against chemical/biological agents. Upon immediate application, SERPACWA provided complete protection against the challenge agent. In the unlikely case that SERPACWA can be protected from skin abrasion, it provided complete protection for up to 16 h of wear.

RECOMMENDATION

In the event of a chemical/biological attack, the soldier's first line of defense is his chemical protective gear (mask, jacket and trouser overgarments, gloves, and boots). It is recommended that soldiers apply a film of SERPACWA (approximately 0.1 mm thick) to their skin at protective clothing closure sites to provide immediate additional protection against percutaneous threat agents.

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