A Topical Skin Protectant against Chemical Warfare Agents

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Abstract

Background: Sulfur mustard and VX are potent chemical warfare agents that penetrate rapidly through the skin, causing severe prolonged injuries and sometimes death.

Objectives: To develop a topically applied pretreatment that will act as a barrier and prevent the absorption of these agents through the skin, reducing morbidity and saving life.

Methods: Several formulations were developed and tested in preclinical animal studies in pigs. The protecting cream was applied as a single application (0.5–1 ml/100 cm^2) prior to exposure (10 minutes to 12 hours) to sulfur mustard or VX. Assessment of sulfur mustard-induced skin damage was based on clinical and histologic evaluations. When tested against VX, clinical signs and blood cholinesterase activity were monitored. At the final stage of development, safety studies were conducted in animals and in human volunteers.

Results: The formulation that gave the best results, coded IB1 (under patent application), provided significant protection against a 1 hour exposure to sulfur mustard (droplets or vapor). All the pigs pretreated with IB1 cream survived a 1–4 hour challenge of 2xLD_{50} VX and did not exhibit any overt clinical signs. Protection was exhibited even when the cream was applied 12 hours (single application) prior to exposure. IB1 was found to be non-irritating in animals and humans. No adverse effects were found in a Phase I clinical study in young healthy volunteers when the cream was applied to around 20% of the skin surface (results presented elsewhere).

Conclusions: IB1 cream has been shown to be a safe and effective topical skin protectant against the chemical warfare agents sulfur mustard and VX.

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Sulfur mustard is a potent cutaneous vesicant that penetrates rapidly through the skin, causing severe and prolonged injuries. Since its first use in World War I and despite vast efforts, only limited success has been attained to find an effective treatment to prevent or reduce these lesions [1–3]. VX, O-ethyl-S-(2-diisopropylaminoethyl- methylphosphothiolate, a sulfur-containing organophosphate, is another percutaneous toxic compound and one of the most potent chemical warfare agents [4]. Due to its low volatility and persistence, VX is more effective as a skin penetrant and lethal contact agent than other nerve agents. A topically applied pretreatment that will act as a barrier may prevent the absorption of sulfur mustard and VX as well as their respective toxicity [5].

The "topical skin protectant" strategy has been adopted by the United States Army and by other countries, including Israel. The objective of the present study was to develop an efficient skin barrier to prevent the penetration of vesicants such as sulfur mustard and nerve agents (e.g., VX), through the skin. The requirements of such a preparation are that it is non-toxic and safe for human use, remains effective for a long time following application, causes minimal discomfort, and is compatible with the current decontamination doctrine.

Over the last few years we have tested a new generation of skin protectants consisting of hydrophilic creams (under patent application) that were applied to the skin prior to exposure. The preclinical experiments were conducted in pigs as an animal model since pig skin best mimics human skin [6].

The formulation, coded IB1, gave the best results in these animal studies and was later submitted for approval in Phase I clinical studies using human volunteers. Part of the preclinical data is presented in this report.

Methods

Animals

Young female pigs, a cross of Large White and Landrace (10–12 kg), supplied by the Institute of Animal Research, Kibbutz Lahav, Israel, were used in all the protection studies. All procedures involving animals were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

General experimental procedure

Prior to exposure, the hair on the back skin was removed by clipping and the whole area was divided into 8–12 fields (35 cm^2 each) and marked. These fields served for sulfur mustard exposure sites, with a different treatment on each site. For VX, being a systemic toxic agent, only one exposure site was used per animal. IB1 preparations were applied as a single application (0.5–1 ml/100 cm^2) prior to exposure. The efficacy of the formulation was tested following applications at different time intervals before exposure, ranging between 10 minutes and 12 hours.

Exposure to either sulfur mustard or VX was conducted in a laboratory-controlled environment and was terminated by decontamination with Fuller's earth powder or with a decontamination mixture that contains chloramine B as the active component, followed by rinsing with water.

Efficacy testing against sulfur mustard

Neat sulfur mustard, either as liquid droplets or as vapor, was applied topically onto the pig back for various exposure durations (10–60 min). Liquid sulfur mustard was applied as 0.2 or 1 µl droplets, three droplets at each exposure field. Sulfur mustard vapor was applied to the pig back using glass cups that were attached to the back. In these cups (25 mm diameter, 30 mm in

height) 5 µl of sulfur mustard were placed onto a filter paper disk within the top inner surface of the cup. Exposure was performed at room temperature and terminated by decontamination, as described. Each animal had its own unprotected exposed sites, adjacent to the sulfur mustard-treated skin areas. Experimental groups consisted of at least four animals each.

Assessment of skin damage was based on clinical and histologic evaluations. The extent of sulfur mustard-induced lesions was analyzed quantitatively 24 hours post-exposure by morphometric analysis of the damaged skin area (liquid sulfur mustard), or by the severity of the erythema response (sulfur mustard vapor) as measured by a colorimenter (Minolta chromatometer).

Efficacy testing against VX

Neat VX droplets (0.2 or 1 µl) were applied topically onto the back of the pig at a dose of 1.3 mg/kg for exposure durations of up to 5 hours. This dose represents twice the LD_{50} value at 24 hours exposure (1x LD_{50} = 0.65 mg/kg). Evaluation was based on a clinical follow-up of toxic signs (48 hours), mortality rate and activity of cholinesterase in whole blood samples [7]. Each experimental group consisted of three to five animals.

Safety preclinical testing

- *Skin irritation*. The primary skin irritation test for IB1 was carried out in rabbits and guinea pigs according to the Draize procedure [8] and included animals with intact (n=6) and abraded skin (n=6). Irritation and sensitivity tests were performed in human volunteers (n=50).
- *Toxicity*. A long-term toxicokinetic study was carried out in pigs by overdose application of IB1 on about 20% of skin surface, three times a day for 2 weeks. Evaluation included a clinical follow-up for 1 month, biochemical analysis of blood and urine, and pathologic evaluation of tissues (e.g., liver, kidney).

Results

Efficacy of IB1 against sulfur mustard

Exposure of pigs to sulfur mustard induced dose and time-dependent skin damage. Application of IB1 once, prior to sulfur mustard exposure, provided a significant protection against 0.2 and 1 µl droplets for exposure durations up to 1 hour, as demonstrated by the clinical outcome [Figure 1] and by the quantitative analysis of the area of the skin lesions [Figure 2].

The barrier properties of IB1 were immediate upon application and remained effective for at least 12 hours. No change was found in the efficacy of protection whether the cream was applied 10 minutes, 1 hour, 3 hours, 6 hours or 12 hours before exposure. Similar efficacy was obtained when tested against sulfur mustard vapor [Figure 3].

Efficacy of IB1 against VX

Control unprotected animals exposed to 1.3 mg/kg of VX (1 µl droplets) for 1 hour exhibited clinical symptoms of organophosphate poisoning, e.g., salivation and muscle fasciculation, starting at 6 hours after exposure. Inhibition of blood cholinesterase activity reached its peak value (60%) 8 hours after VX application. Under the

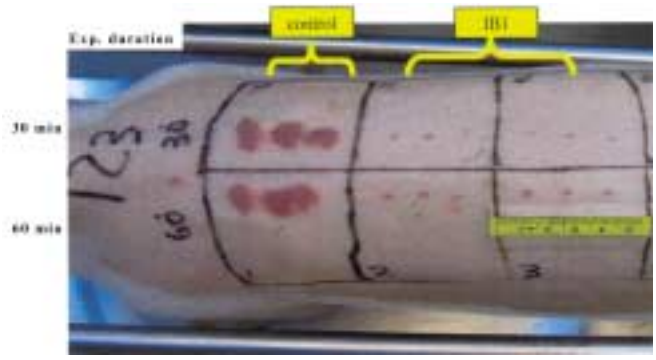

Figure 1. The efficacy of IB1 protection as evaluated in pigs 24 hours following exposure for 30 (upper panel) and 60 minutes (lower panel) to sulfur mustard (1 µl droplets). Note the minor damage to the treated skin compared to the severe lesions in the untreated control areas. IB1 was applied 1 hour before exposure. Similar results were obtained when IB1 was applied 6 hours before exposure.

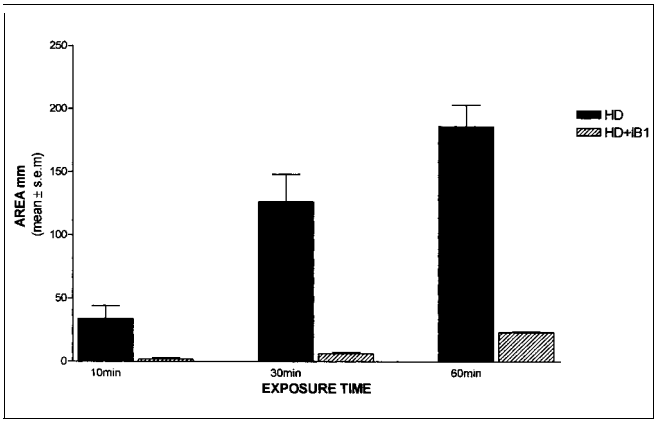

Figure 2. Quantitative analysis of the damaged skin area 24 hours following exposure to sulfur mustard (1 µl droplets) for 10–60 minutes. IB1 afforded about 90% protection (0% protection assigned to sulfur mustard controls) when applied either 1 or 6 hours before exposure.

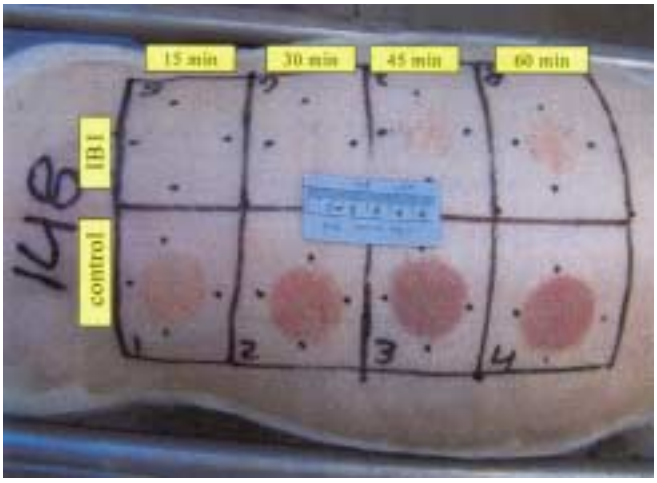

Figure 3. The efficacy of IB1 against sulfur mustard vapor exposure (15–60 minute exposure durations) in pigs. Note the severe erythema in untreated controls (lower panel) and the significant protection provided by IB1 (upper panel). IB1 was applied 1 hour before exposure.

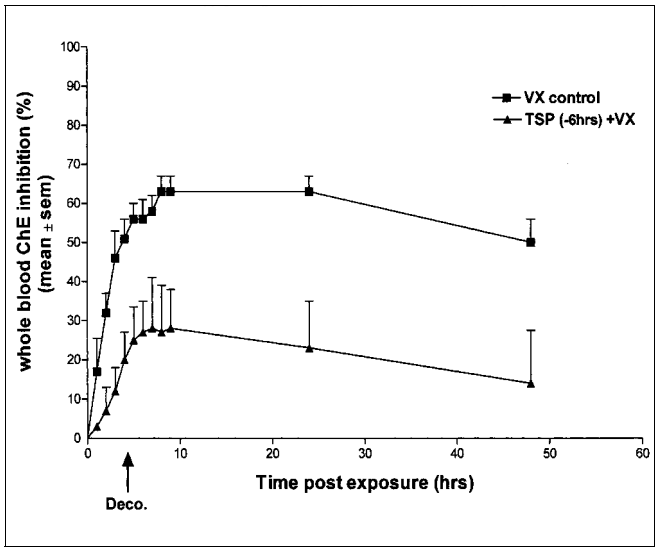

Figure 4. The effect of IB1 against VX (1.3 mg/kg) in pigs, expressed by the percent of inhibition in blood cholinesterase activity. IB1 was applied 6 hours before exposure. Note the more moderate slope of cholinesterase inhibition in IB1-treated animals, which led to the better clinical outcome observed in those animals.

same conditions, IB1-pretreated pigs did not exhibit any adverse clinical signs and cholinesterase inhibition increased to a maximal value of 25% only [Figure 4]. No change in efficacy was observed when IB1 was applied 12 hours (single application) prior to VX exposure.

When exposed to a prolonged challenge of VX (4 hours duration), most of the unprotected animals (>90%) died within 12–48 hours. In contrast, all the pigs pretreated with IB1 survived the 4 hour challenge with 1.3 mg/kg of VX and exhibited only minor short-term toxicity symptoms, if any.

Safety testing

- *Skin irritation*. IB1 was found to be non-irritating in guinea pigs and rabbits (score 0 for erythema, eschar and edema formation according to the Draize scale). Topical application of IB1 to human volunteers has not been associated with any acute skin irritation or with any allergic sensitization. The clinical results of the studies in human volunteers will be presented elsewhere.
- *Toxicity*. No adverse effects were found in a long-term toxicologic study conducted in pigs following treatment with IB1 for 2 weeks (three times a day). In addition, no detectable levels of IB1 ingredients were found in blood and urine samples during the experiment and during the subsequent 2 weeks.

Discussion

The data presented here indicate that a topical application of IB1 formed an effective skin barrier and significantly reduced the toxicity of chemical warfare agents – namely, sulfur mustard and VX.

The new generation of skin protectants developed in our laboratory is based on hydrophilic dermal preparations that are absorbed easily into the skin and interact with the stratum corneum layer, but do not yet reach the systemic circulation. Hindering the penetration through the skin provides the passive protection afforded by these formulations against percutaneous toxic agents. The barrier properties of IB1 are effective immediately following application and remain efficient for at least 12 hours (testing was not done for longer). This skin protectant is easily removed by water rinsing and is compatible with the current decontamination doctrine. The unique mechanism of action of the IB1 cream and its compatibility with the skin makes it superior to other available skin protectants.

IB1 contains ingredients approved for human use; it is non-toxic and safe for use, as shown by testing in animals and in humans. Its primary expected use is by application onto sensitive skin areas (armpits, groin, waist, wrists) in conjunction with physical protection (protective suit, face-mask and gloves). A quantity of around 10 ml is sufficient for one application over 20% of body surface.

In conclusion, IB1 is an effective skin protectant against chemical warfare agents and is safe for human use. In addition, its long shelf-life (data not shown) and low cost make it an ideal product for the protecting kit.

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