Review

Delayed complications of sulfur mustard poisoning in the skin and the immune system of Iranian veterans 16–20 years after exposure

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Abstract

Background Extensive cutaneous burns caused by alkylating chemical warfare agent sulfur mustard (SM) have been associated with the severe suppression of the immune system in humans. We aimed to study the association between late cutaneous and immunological complications of SM poisoning.

Methods Skin examination was performed on all SM-poisoned Iranian veterans in the province of Khorasan, Iran, who had significant clinical complications, and their SM intoxication was confirmed by toxicological analysis. Light microscopy was performed on eight skin biopsies. Blood cell counts, serum immunoglobulin and complement factor, as well as flow cytometric, analyses were performed on all the patients. The severity of cutaneous complications were classified into four grades and compared with hematological and immunological parameters, using Spearman's rank correlation test.

Results Forty male subjects, confirmed with SM poisoning 16–20 years earlier, were studied. The main objective findings were hyperpigmentation (55%), dry skin (40%), multiple cherry angiomas (37.5%), atrophy (27.5%), and hypopigmentation (25%). Histopathologic findings were nonspecific and compatible with hyperpigmented old atrophic scars. Except for the hematocrit and C4 levels, hematological and immunological parameters revealed no significant correlation with the severity grades of cutaneous complications.

Conclusion Sulfur mustard is an alkylating agent with prolonged adverse effects on both the skin and the immune system. Allthough skin is a major transporting system for SM's systemic absorption, there is probably no correlation between the severity of late cutaneous and immunological complications of SM poisoning.

Introduction

Sulfur mustard (SM) [bis-(2-chloroethyl) sulfide] was the most widely used vesicant chemical warfare agent in the past century. Originally employed as a weapon in WWI, it was responsible for greater than 80% of all the documented chemical casualties.[1,2] It was then used sporadically until 1983, when the Iraqi army employed it on a large scale against Iranian soldiers during the Iran-Iraq War (1983–88).[3]

Sulfur mustard is a colorless to amber oily liquid having the odor of onion, garlic, or mustard, hence its name.[4,5] It is absorbed by inhalation, through the skin, or through the gastrointestinal tract following consumption of contaminated food. After absorption, SM undergoes intramolecular cyclization to form an ethylene episulfonium ion intermediate. This,

in turn, reacts with and alkylates nucleic acids and proteins, resulting in impaired cell homeostasis and eventual cell death.[6,7]

The eyes, skin and the respiratory system are the three major targets of SM's local toxic effects. When absorbed in large amounts, it can also damage the rapidly proliferating cells of bone marrow and may cause leucopenia and severe suppression of the immune system.[8–10] The lipophilic nature of SM and the affinity of the skin for lipophilic substances make the skin a fairly efficient transporting system for this agent. Skin exposure, sufficient to cause severe vesication and skin necrosis, has been associated with systemic toxicity, at least during the early phases of exposure.[11,12] However, the relationship between late cutaneous and immunological complications of SM poisoning has not previously been reported.

The aim of this study was to evaluate the complications of SM poisoning in the skin and the immune system of severely intoxicated Iranian veterans and to determine the possible correlation between these complications.

Materials and Methods

Patients and study design
The Veteran Foundation provided us with the files of all chemically injured patients in the province of Khorasan, Iran. We reviewed the files and selected the patients who met the following criteria: (a) documented exposure to SM, as confirmed by toxicological studies on their urine and vesicular fluid during the war, and (b) significant clinical complications of SM poisoning in one or more of the major target organs (skin, eyes, and/or respiratory system). Patients with a known skin disease before exposure to SM and those with proven systemic illness were excluded. All the patients were initially admitted to Imam Reza University Teaching Hospital between 1983–88 and have undergone regular outpatient follow ups since then.

Forty-seven male subjects fulfilled the above criteria. Of these, 40 patients volunteered to participate in the study and signed the informed written consent document. After approval by the Medical Ethics Committee of the University, the patients were hospitalized in the Toxicology Ward of Imam Reza Hospital and underwent a thorough medical history interview and physical examination by an expert dermatologist on the research team.

Severity grading of cutaneous complications
The percentage of burned skin area on each patient was estimated using Wallace's "rule of nines." Patients without specific objective findings were classified grade 1 (no specific complication). Those with burn scars involving 1–18%, 19–36%, and > 36% of the total body surface area were classified grade 2 (mild), grade 3 (moderate), and grade 4 (severe) cutaneous complication, respectively.

Hematological and immunological procedures
Blood samples were taken using standard phlebotomy procedures. Hematocrit (HCT) and hemoglobin (Hb) levels, as well as total counts for white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs), were measured using the Technicon H1 autoanalyzer (Bayer Medical Systems, New York, NY). Percentages of lymphocytes, monocytes, and neutrophils, as well as CD3, CD4, CD8, CD19, and CD16+56 positive lymphocytes were determined by flow-cytometric analyses using the FACS Calibur cytometer (Becton Dickinson, San Jose, CA), equipped with Cellquest software (Largo, FL). The IgA, IgG, and IgM, serum immunoglobulin levels, as well as complement factors C3 and C4, were measured with SRID quantifications kits (Biogen, Mashhad, Iran). The serum IgE level was also determined using ELISA (Radims, Rome, Italy).

Cutaneous histopathological procedures
The dermatologist performed a skin biopsy on only eight patients having more distinct cutaneous lesions, namely hyperpigmented and atrophic scars. For light microscopy, tissues were fixed in a 10% phosphated-buffer (pH 7.0) formalin, then dehydrated and embedded in paraffin. Serial sections of 3-μm thickness were made and stained with Hematoxylin and Eosin.

Statistical analysis
All data were expressed as mean (± SD) unless otherwise indicated. Spearman's rank correlation test was used sequentially to compare the severity of cutaneous complications with the hematological and immunological parameters. The software SPSS vers 11.5 (SPSS Inc, Chicago, IL) was employed throughout, with the minimum level of significance set at $P = 0.05$ for all the comparisons.

Results

The age range of the patients was 32–76 years (43.8 ± 9.8). Exposure to SM occurred 16–20 years (18 ± 1.5) before the study. The main cutaneous symptoms reported were an itching without a burning sensation in 26 patients (65%), both an itching and burning sensation in eight patients (20%), and only a burning sensation in one patient (2.5%); five patients were symptom-free. Cutaneous symptoms were, generally, more severe during the colder seasons of the year.

Objective clinical findings
Objective skin findings were recorded in the following order: hyper-pigmentation (55%), erythematous papular rash (42.5%), dry skin (40%), multiple cherry angiomas (37.5%), atrophy (27.5%), hypo-pigmentation (25%), hair loss (10%), eczema (7.5%), depigmentation (5%), multiple melanocytic nevi (5%), and telangiectasis, tinea versicolor and hypertrophy (2.5% each). Most of the patients had a combination of two or three of these complications. The lesions were located on the genital areas and buttocks (47.5%), back (47.5%), front thorax and abdomen (45%), lower extremities (mainly inguinal; 45%), upper extremities (mainly axillary; 40%), and the head and neck (15%), as shown in Fig. 1.

While hyper- and hypo-pigmentations, as well as atrophy and hypertrophy, were the most common on the sites of healed skin blisters, other abnormal findings were observed on a more widespread distribution. Increased pigmentation was the most typical feature of previous blister sites. In nine patients (22.5%), this had the appearance of xeroderma pigmentosum, which is characterized by diffuse hyper-pigmented areas with superimposed hypo- and hyper-pigmented macules (Fig. 2).

A mean of 40.8 ± 38.1 cherry angiomas (range, 5–120) was found in 15 patients. Cherry angiomas in our patients had

Figure 1 The predilection of sulfur mustard to moist areas, such as the axilla

Figure 2 Diffuse hyperpigmentation with scattered hypopigmented macules on the back of an Iranian veteran, 16 years after exposure to sulfur mustard

developed mainly over the trunk and proximal extremities, which is the usual pattern observed in healthy non-exposed individuals. The number of cherry angiomas in the patients revealed no significant correlation with the patients' ages ($r = -0.022$, $P = 0.939$).

Of the 16 patients who suffered from moderate to severe dry skin, seven revealed a generalized pattern of dry skin. Seven patients had dry skin only on the extremities (mainly distal) and one patient had likewise on the trunk only. Associated findings of scaling and excoriation were also recorded in seven and four of these patients, respectively. None of these patients showed clinical manifestations of hypothyroidism or any other systemic illnesses which could cause dry skin. Four patients were diagnosed as having asthma, which is probably a late toxic sequel of SM inhalation.

Histopathological findings

Light microscopy of skin specimens in eight patients revealed atrophic scarring, characterized by marked epidermal atrophy, orthokeratotic hyperkeratosis and basal layer

hyperpigmentation. Abnormal findings within the dermis included perivascular mononuclear infiltrate scattered through the papillary dermis, melanophages in the upper dermis, nonspecific dermal fibrosis, and atrophy of dermal appendages, such as sebaceous glands, hair follicles and sweat glands.

Comparison of hematological and immunological parameters with the severity grades of cutaneous complications

As shown in Table 1, except for the negative significant correlation between HCT and the severity grades of cutaneous complications ($r = -0.389$, $P = 0.013$), none of the other hematological or flow cytometric parameters revealed a significant correlation with the severity grades of cutaneous complications.

A significant negative correlation was found between the serum C4 level and the severity grades of cutaneous complications ($r = -0.440$, $P = 0.005$). Other serum immunoglobulins

Table 1 Comparison of the severity grades of cutaneous complications with hematological and flow cytometric parameters in 40 Iranian Veterans 16–20 years after sulfur mustard poisoning

Parameters	Severity grades of cutaneous complications	
	r	*P*
WBC count	−0.015	0.926
RBC count	−0.049	0.763
Hb level	0.094	0.563
HCT (%)	−0.389	0.013*
PLT count	0.009	0.956
Lymph (%)	0.135	0.406
Mono (%)	0.164	0.312
Poly (%)	−0.172	0.289
CD3+ (%)	0.082	0.615
CD4+ (%)	0.266	0.097
CD8+ (%)	−0.181	0.263
CD19+ (%)	0.081	0.621
CD16+56+ (%)	−0.173	0.285

*Significant correlation between the parameter and the severity grades of the cutaneous complications.

Table 2 Comparison of severity grades of cutaneous complications with serum levels of immunoglobulins and complement factors in 40 Iranian Veterans 16–20 years after sulfur mustard poisoning

Parameters	Severity grades of cutaneous complications	
	r	*P*
C3 (mg/dl)	−0.225	0.162
C4 (mg/dl)	−0.44	0.005*
IgA (mg/dl)	0.075	0.646
IgG (mg/dl)	−0.093	0.570
IgM (mg/dl)	−0.051	0.755
IgE (IU)	−0.036	0.826

*Significant correlation between the parameter and the severity grades of the cutaneous complications.

and complement factors, however, had no significant correlation with the severity grades of cutaneous complications (Table 2).

Discussion

Sulfur mustard is an oily lipophilic agent that easily penetrates the skin within 3–5 min of contact. In human skin, the penetration rate of saturated vapor or liquid mustard is 1–4 μg/cm²/min. A large proportion of the SM that penetrates the skin is carried away by the circulation to provoke systemic injury. In general, 80% of the SM coming into contact with
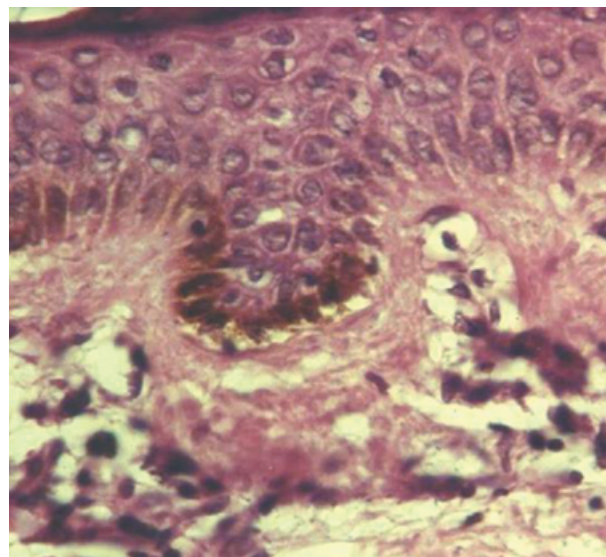

Figure 3 Light microscopy of hyperpigmented skin scar in an Iranian veteran, 18 years after exposure to sulfur mustard (H&E stain ×400). Marked hyperpigmentation of basal layer with peri-vascular mononuclear infiltrate through the papillary dermis
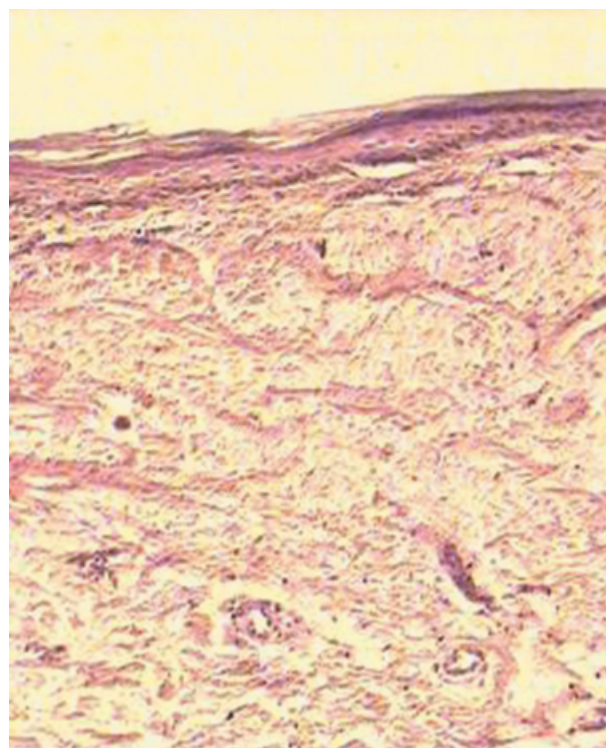

Figure 4 Light microscopy of atrophic skin scar in an Iranian veteran, 18 years after exposure to sulfur mustard (H&E stain ×400). Epidermal atrophy, severe dermal fibrosis and atrophy of the appendages

the skin will evaporate, and the remaining 20% will penetrate the skin. Of this amount, 12% reacts with components in the skin and approximately 8% is absorbed systemically.[11–13]

Injury resulting from systemic absorption of SM may be transitory or might lead to permanent damage of more sensitive proliferating tissues, such as bone marrow, the reticuloendothelial system, and the intestinal tract. Cutaneous exposure to large dosages of SM has been associated with marked leucopenia and severe suppression of the immune system during the early phases of exposure.[10] Zandieh et al. noted depression of cell-mediated immunity in chemically injured Iranian veterans 1–3 years after exposure to SM.[14] According to Hassan & Ebtekar, during the first week and up to the sixth month after SM exposure the percentage of patients with increased IgM, IgG, C_3, and C_4 serum levels were higher than in healthy controls. Even 8 years after contact, the percentage of patients with increased IgM and IgG was still significantly higher than that of the controls.[15] The impairment of natural killer cells has been reported several years after both occupational and battlefield exposure to SM. This is believed to be responsible for the increased risk of recurrent infections in these patients.[16,17]

Although a significant correlation between the severity of SM systemic toxicity and the extent of skin lesions was expected, we found no such correlation between cutaneous and immunological complications of SM poisoning in our patients. This might support Papirmeister et al.'s theory which proposed that SM damage to the cornea, mucous membranes, and the skin is generated by mechanisms other than DNA cross-linking. Intra-strand DNA cross-linking, which is probably responsible for the delayed toxic effects on rapidly proliferating tissues, such as bone marrow, produces a lethality at the lowest frequency of occurrence and at the lowest concentration of the agent. However, at a higher cellular exposure, mechanisms, such as NAD depletion and glutathione inactivation, become important and produce rapid cell death in a dose-dependent manner.[18,19] The absence of reliable data regarding the use of protective equipment by the patients at the time of the initial exposure to SM was a major limitation of this study in establishing a correlation between the severity of late cutaneous and immunological complications. In fact, systemic absorption of SM could be noticeably higher in the skin of patients' who were protected by a suit but had significant inhalational exposure as a result of not wearing a gas mask. Another potential confounding factor was the use of medications such as steroids by the patients. Steroids are known to have suppressive effects on the immune system and are commonly prescribed for SM-intoxicated patients who have severe respiratory complications. The negative correlation found between hematocrit and the severity of SM-induced cutaneous complications may represent chance fluctuation because there were no correlations with other erythrocytic indexes such as hemoglobin and RBC counts. The negative correlation with

C_4 level does not seem to be related to late cutaneous complications since complement factors are mainly changed during acute-phase responses.

Various late cutaneous complications, including hyper- and hypo-pigmentation, eczema, atrophy, and dry skin, have already been shown to have an association with SM exposure.[19,20] The relative risk of xerosis in a group of 500 SM-exposed Iranian veterans, who were compared with 500 age-matched non-exposed control group, was 24.3.[21] Among SM-exposed patients, there are also reports denoting a higher incidence of immunologically based skin diseases, such as psoriasis, discoid lupus erythematous, and vitiligo among SM-exposed patients.[22] In contrast to the normal population, disorders such as acne vulgaris, folliculitis, and tinea versicolor, however, are less frequent among SM-exposed patients. This is probably owing to the presence of chronic dry skin following cutaneous exposure to SM. Recurrent blisters and ulcerations have been reported in other studies,[22] but no such lesions were found in this study.

Injuries resulting in erythema and edema without vesicle formation are almost always followed by complete healing and no residual effects. Blistering and necrotic wounds, however, cause permanent residual effects.[21,23] Characteristically, the healed area looses its pigment, while the surrounding area of sublethaly damaged cells becomes hyper-pigmented.[20] Although atrophic scars are commonly found on the sites of healed SM burns, hypertrophic scars are considerably less frequent than thermal burns. Hyper-pigmentation, atrophy, and hypo-pigmentation were recorded in 55%, 27.5%, and 25% of our patients, respectively. Hypertrophy was found in only one patient (2.5%).

The severity of skin lesions from exposure to mustard gas is dependent upon the dose of the agent, humidity and the length of exposure.[11] Certain regions of the body, such as the genitalia, axilla, and buttocks, are more vulnerable to SM.[25,26] The skin in these sensitive areas is generally thinner and has more hair follicles which naturally facilitate SM's penetration. Furthermore, because of the film of moisture in its surface, hot sweaty skin aggravates the damage from SM's injurious vapor.

Interestingly, multiple cherry angiomas, which were under-represented in earlier studies,[27] are now found to be common. These were recorded in 37.5% of the patients with a mean of 40.8 ± 38.1 angiomas in each patient. In another study by Moradi & Aghaei,[28] a mean of 13.4 cherry angiomas (range, 4–31) was found in 36% of SM-exposed patients. Although the frequencies of multiple cherry angiomas in these two studies are estimated to be approximately greater than the frequency expected in the normal population, a comparison with a non-exposed control group is required for confirmation. Histopathologic changes in our patients were generally nonspecific and compatible with hyper-pigmented atrophic scars of skin.

Although some studies have suggested a causal association between acute exposure to SM and skin cancer,[29,30] we found no evidence of cutaneous malignancies in our subjects. Indeed, a quantitative risk assessment cannot be made with the available data. Observation periods, ranging from 35–45 years, may be required to produce meaningful data.

Regular patient follow ups has suggested that, except for dry skin and its associated itching sensation, other skin complications generally remain constant over the years. Dry skin and itching tend to intensify as patients become older and are regarded as the principal late skin complaints of these patients.

Acknowledgments

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