

Surgical Treatment of Skin Lesions Induced by Sulfur Mustard ("Mustard Gas")-An Experimental Study in the Guinea Pig

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Abstract

Sulfur mustard has been known as a chemical warfare agent since 1915 and its biological effects, e.g. acting as an alkylating agent reacting with nucleophiles in proteins and nucleic acids, are well known. Still, there is today no specific antidote against or treatment of sulfur mustard induced cutaneous lesions. Recently, a technique of treating thermal burns with early surgical excision followed by skin grafting has been established. The present study was undertaken to evaluate, if a similar technique could be of value in the treatment of sulfur mustard lesions. Under anaesthesia, guinea pigs were exposed to sulfur mustard vapour, followed six hours later by excision of the exposed skin segment. In animals where the skin defect was primarily sutured, healing time was significantly shorter and increase in body mass greater compared to conservatively treated controls. No such differences were found if excision was followed by full thickness skin grafting. Further studies in other species are suggested to clarify the possible role of surgical excision of sulfur mustard skin lesions, followed by skin transplantation, in humans.

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Introduction

Sulfur mustard (SM) [bis(2-chloroethyl)sulphide], i.e. "mustard gas", was first synthesised around 1822¹ and for the first time launched as a chemical warfare agent on April 22, 1915, in the vicinity of the Belgian town of Ypres.² By the end of World War I SM had, together with the other chemical warfare agents, chlorine and phosgene, caused over 1 million deaths.³ Sulfur mustard was later used in the Ethiopian war from 1935-1936 and in China in 1938.⁴ No chemical warfare agents were deployed during World War II. However, from 1945-1948 altogether about 52 000 tons of SM-containing ordnance were dumped by the allied forces into the Baltic Sea near the Danish island Christiansö and the Swedish island Gotland. Each year fishermen in the Baltic, in 1984 as many as 23, as well as tourists, sustain skin injuries due to accidental contact with SM from these sources.⁵ In the Iraq/Iran war (1980-1988), Iraq used SM on at least seven occasions³ and in April 1988, Iraq used SM, as well as other chemical warfare agents, against Kurdish civilians.⁶

SM acts as a bifunctional alkylating agent, reacting with nucleophiles in a large variety of biologically important molecules, such as DNA, RNA and proteins. Although several of these reactions have been thoroughly documented,⁷ no antidote against or specific clinical treatment of SM-induced injuries are as yet available.⁸ Treatment of SM skin lesions has been recommended, after careful decontamination, to follow the same general rules as treatment of thermal burns, i.e. open treatment with the epidermis acting as a dressing, or application of moist occlusive bandages or silver sulphadiazine ointment.^{1,5} Healing time of SM-induced skin lesions, however, is approximately twice as long as that of comparable thermal burns.⁹ Healing is often complicated with secondary infection, hyperpigmentation and eventually atrophic or hypertrophic scar formation with deformity and possibly restriction of joint mobility.^{9,10}

In the last decade the advantage of early excision of the injured skin, immediately followed by skin grafting, has been increasingly recognised in patients with ther-

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mal burns.^{11,12} It was the purpose of the present study to evaluate, in an experimental animal model, whether a similar approach would be feasible in SM-induced cutaneous lesions.

Materials and Methods

Animals

Male guinea pigs ($n = 30$) with body mass (BM) 280 to 450 g were used. The conduct of the investigation conformed with the "Guide for the Care and Use of Laboratory Animals" (NIH publication #85-23, Bethesda, MD, USA, revised 1985) and was approved by the local ethical committee (Umeå, Sweden #A 65/92; A 49/93; A 77/94). Animals were housed two or three per cage in polycarbonate shoe-box cages in an animal facility that provided environmental temperature of $20 \pm 3^\circ\text{C}$; relative humidity of $50 \pm 20\%$ and artificial illumination with a photoperiod of 12 hours light/12 hours darkness. Cages contained bedding material (Beekay Bedding®, B & K Universal, N Humberside, UK). The animals were fed rodent diet pellets (Standard Diet®, B & K Universal, N Humberside, UK) with a supplement of breeder chow (Labfor K-18, Lactamin, Stockholm, Sweden). Tap water with ascorbic acid (200 mg/l) was provided *ad libitum*. Cages, chow and water bottles were autoclaved before use.

Eighteen to 24 hours prior to SM exposition, an approximately 5 cm² large skin area in either one or both flank(s) was shaved. Animals with visible skin lesions from shaving were excluded from the study. Animals were allocated to either of the following experimental groups:

- I: SM exposure + conservative treatment, i.e. open treatment with epidermis acting as a dressing ($n = 7$), *vide* Introduction;
- II: SM exposure + surgical excision and primary suture ($n = 8$);
- III: surgical excision and autografting without SM exposure ($n = 5$);
- IV: SM exposure + excision of exposed skin area followed by autografting ($n = 10$).

Anaesthesia

During SM vapour exposure animals were anaesthetised with enfluran (Ethrane®, Abbott Laboratories, North Chicago, Ill, USA), 1.5% to 4.5% in an air flow of approximately 2.5 l/min on an open spontaneous breathing system. To avoid hypothermia, they were placed on an electrically heated pad controlled by a rectal thermometer. This maintained rectal temperature at $37 \pm 0.5^\circ\text{C}$. For surgery, 1.5% to 4.5% enfluran in an N₂O-O₂-air mixture (2:2:1) was used.

SM Vapour Exposure Procedure

The skin was exposed to SM vapour in a flow through

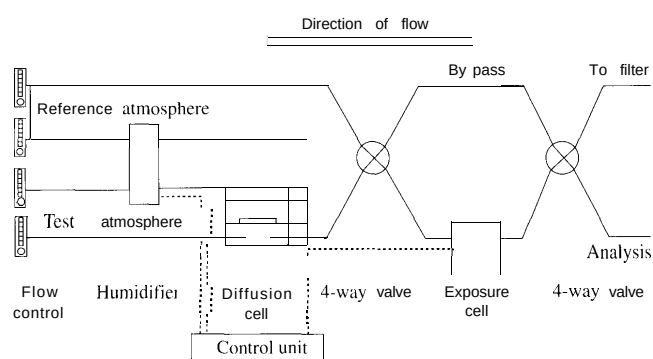


Fig. 1. Schematic representation of the exposure system. SM is deposited in the diffusion cell and the exposure tell is placed on the skin surface. Flow rate, humidity and temperature are controlled. By the 4-way valve, the atmosphere in the exposure cell can be switched from reference atmosphere to test atmosphere.

system (Fig. 1) designed at the Department of NBC Defence, Umeå, Sweden. Sulfur mustard vapour was generated in an electrically heated stainless steel diffusion cell similar in design to the one described by Aas et al.¹³

A polypropylene membrane was used as emission rate controlling separation between donor and flow through compartment of the cell. The donor compartment was loaded through a septum with 50 µl neat SM. Temperature of the cell was 45.5°C and flow rate 10 ml/min. An additional flow of air of 20 ml/min through a humidity control system was added to give a total flow of 30 ml/min through the exposure chamber. By a 4-way crossover valve the air flow can bypass the exposure chamber. By doing this, the SM vapour flow in the exposure chamber was replaced with a 30 ml/min flow of clean air with the same humidity. Via a second 4-way crossover valve the outlet from the exposure chamber or the bypass could be directed alternatively to the humidity sensor or a sampling port. All tubing was made of stainless steel or teflon and heated to 50°C to prevent any condensation.

Repeated testing and analysis by gas chromatography have shown that this system, after a equilibration period of 90 minutes following loading of the diffusion cell, can maintain a stable SM atmosphere for more than 6 hours. During the equilibration period, handling of the animals and a period of 5 minutes pre and post exposure the flow of SM vapour was directed to bypass the exposure chamber. Under the present condition the concentration of SM in the system was 1200 to 1300 mg/m³ and exposure time was 20 minutes giving an exposure dose (Ct) of approximately $2.5 \times 10^4 \text{ mg} \times \text{min}/\text{m}^3$. The relative humidity was kept at 10% and the temperature in the exposure chamber at 34°C .

The stainless steel skin exposure chamber was shaped like a symmetrical ellipsoid with pointed ends and curved to follow the shape of the animals' flank. The size of the chamber was $4.2 \times 1.3 \text{ cm}$ and

exposed surface area 5.0 cm². Sufficient sealing between the chamber and the skin was obtained by mounting the chamber with a slight pressure against the flank. For exposure the animals were placed on a heat bed in a 7.0 cm diameter plexi-glass tube which also served as an open breathing anaesthesia mask. The tube was partially cut open to give access to the flank of the animals to allow for the mounting of the exposure chamber.

Surgical Procedures

All surgical procedures were performed under anaesthesia and sterile conditions, using standard surgical techniques.

In the experimental group II, the SM-exposed skin area was excised 6 hours post exposure and the skin defect was immediately closed with 5/0 monofilament (Ethilon®, Ethicon, Norderstedt, Germany) or pseudomonofilament (Supramid®, B Braun Melsungen AG, Melsungen, Germany).

In group III, animals were subjected to excision of one skin segment, the form and size of SM-exposed areas, from both flanks. One of these segments was discarded and the other used as a full thickness skin autograft and thus sutured in place, using either suture material, covering the defect in the contralateral flank. The defect from skin graft harvesting was sutured immediately after excision.

In group IV, the SM-exposed area was excised 6 hours post exposure and a similar skin segment was harvested from the contralateral flank. The defect from harvesting was sutured and the skin segment transplanted as a full thickness skin autograft to the area of SM-exposed, excised skin.

Analgesia

All animals received buprenorphine (Temgesic®, Reckitt & Coleman, Hull, UK) 0.2 mg/ kg BM subcutaneously post surgery. This dose was iterated if judged necessary during the postoperative period.

Follow up

Animals were returned to the animal facility and checked daily. Body mass was registered on an electric scale (Brainweight® B 1500 D, Ohaus Scale Corp, Florham Park, NJ, USA). Healing process was recorded as well as general behaviour and well-being. Lesions were considered healed when all encrustations had disappeared, complete re-epithelialization had occurred and regrowth of fur had started. Sutures were removed when judged appropriate by standard surgical criteria.

Animals were sacrificed with an overdose of anaesthetics, followed by cardiac puncture, when complete healing of lesions had occurred.

Histology

Skin specimens from relevant areas were removed at surgery or after sacrifice and were immersion-fixed in 4% formalin, dehydrated in a graded series of ethanols and embedded in Histowax® (Histolab Products, Göteborg, Sweden). Sagittal sections (10 µm) were cut on a microtome (HM 350, Micron, Heidelberg, Germany), mounted on object slides, counterstained with haematoxylin-eosin and cover-slipped with Entellan® Neu (E Merck, Darmstadt, Germany). All slides were coded and examined using a light-field microscope (Microphot-FXA®, Nikon Corp, Tokyo, Japan). Microphotography of appropriate sections was performed on Kodak T-Max 100® black and white film for paper prints.

Statistics

The non-parametric Wilcoxon Rank Sum test was used. Statistical significance was considered at *P* values of ≤0.05.

Results

All animals survived SM exposure and surgery. One animal in group I developed cachexia at the time of complete healing; all other animals in all groups (I to IV) thrived and increased their BM. Within one hour of SM exposure, a skin erythema developed locally at the exposure site in all exposed animals. This erythema was even more pronounced at the time of surgery (6 hours post exposure). The clearly visible demarcation between exposed and unexposed skin together with a pen marking made at the end of exposure, made it possible to perform an exact excision of the exposed skin area.

In Group I the erythema became gradually less visible in 48 to 72 hours, and was eventually followed by development of a dry skin necrosis. Slowly, the area was re-epithelialized. Healing time was approximately three weeks (Table I).

In Group II uneventful healing *per primam* took place; the sutures could be removed in 8 to 10 days and complete healing was achieved in approximately two weeks (Table I).

In Group III the skin graft harvest site healed *per primam* and sutures were removed in 9 to 10 days. At the transplantation site, the transplant at first seemed to "take", but after 10 to 11 days, it was evident that the re-epithelialization took place predominantly as an in-growth of new epidermis, from the original wound edges, under the transplant, which thus during the later phase of the healing process served more like a dressing.

In Group IV the healing process was similar to the one described in Group III. In both groups, healing time of the transplanted site was approximately four weeks (Table I) and sutures could be removed in 9 to 11 days.

TABLE I: DIFFERENCE IN HEALING TIME AND BM INCREASE BETWEEN THE FOUR DIFFERENT EXPERIMENTAL GROUPS

	Group I	Group II	Group III	Group IV
Healing time, (days)				
Mean (range)	24 (19 - 30)	15 (14 - 16)***	30 (28 - 32)	30 (28 - 31)
BM increase (%) day 10				
Mean (range)	6 (0-10)-----***	16 (12 - 20)-----*-----	11 (6 - 16)-----**-----	8 (11 - 23)
BM increase (%) when healed				
Mean (range)	43 (17 - 64)	43 (22 - 66)-----*	59 (47 - 71)-----*	61 (40 - 76)

Statistical significances * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; Wilcoxon Rank Sum test. Note that in group IV, animals actually show BM decrease day 10 post SM exposure and surgery.

BM: body mass

Increase in BM at sacrifice, was approximately 43% in groups I and II and about 60% in groups III and IV (Table I). On day 10 (the approximate time of removal of sutures in surgical groups), the increase in BM was significantly higher in group II compared to other groups (Table I).

Histology

Histological sections from noninjured control skin areas (III & IV) showed a normal sagittal picture of hairy

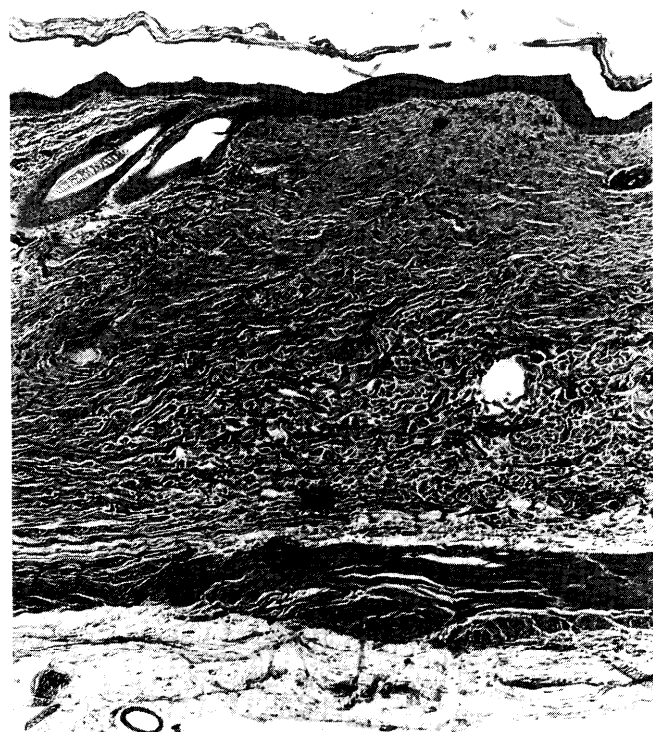


Fig. 2. Photomicrograph of a sagittal skin section from the primary sutured graft harvesting defect in a guinea pig of experimental group IV, showing a dermal scar covered by a thin epidermal layer. Magnification 64 x.

skin with a thin superficial layer of epidermal keratinized cells covering a considerable thicker dermis consisting of fibroblasts and containing numerous hair follicles, sweat and sebaceous glands as well as blood vessels. The hair follicles extended down to turn into hair bulbs in the hypodermis which mostly were formed of subcutaneous adipose tissues. Beneath the hypodermis was seen the underlying muscle tissue. Sections from the primary sutured graft harvesting defect (III & IV) showed a localised dermal scar covered by a thin epidermal layer (Fig. 2).

Sections of skin segments from excised and transplanted areas not previously subjected to SM exposure (III), showed after healing a normal sagittal picture of hairy skin.

The excised SM-exposed skin areas (IV) showed six hours after exposure a large number of inflammatory cells distributed in the loose connective tissue of the papillary layer of the upper dermis, as well as around the blood vessels of the *rete subpapillare* (Fig. 3). Sections from skin segments from these areas after excision and autografting, showed after healing a hypertrophied dermal scar consisting of densely packed fibroblasts which extended down to the fascia of the underlying muscles (Fig. 4).

Discussion

Although SM was used in the battlefield for the first time 80 years ago, the agent is by no means of historical interest only. It is estimated, that during the Iraq/Iran War from 1980 to 1988, the number of chemical casualties, predominantly caused by SM, was about 45 000; military personnel and civilians together.¹⁴ The possibility of Iraq using chemical agents, e.g. SM, during the Gulf War in 1991 was a real concern to the allied forces and the civilian populations in the Middle East. Thus, it is described, how women in Israel actually went through



Fig. 3. Photomicrograph of a sagittal section from a six-hour previously SM-exposed skin area. Note the large number of leukocytes distributed in the upper dermis, as well as around the blood vessels (arrows). Magnification 160 x.

delivery, during missile attacks, wearing a gas mask.” Even though the mortality from SM poisoning is low, 1% to 5%,¹⁴ its ability to incapacitate and impair fighting ability, still makes SM the “king of war gases” and a “chemical weapon of choice in modern tactical warfare”.¹⁶ Efforts have been made to find a specific treatment for or antidote against SM. Drugs possibly acting as SM scavengers, i.e. a combination of sodium thiosulfate, vitamin E and dexamethasone increased survival in rats exposed to three times the LD₅₀ dose of SM.¹⁷

In rabbits, local injection of cortisone after cutaneous SM exposure, reduced the skin lesion produced but did not reduce healing time.¹⁸ Obviously, these findings have not changed the strategy for treatment of SM-induced skin lesions in humans. Even after the most recent experience of treating SM victims of the Iraq/Iran war, conservative treatment, like the one in thermal burns, is still recommended.^{14,16,19,20} Surgical treatment is discussed only as debridement of necrotic tissue in severe injuries¹⁶ or in relation to management of combined injuries, i.e. conventional war wounds contaminated with chemical warfare agents.²¹ The purpose of the present investigation was to study, in an animal

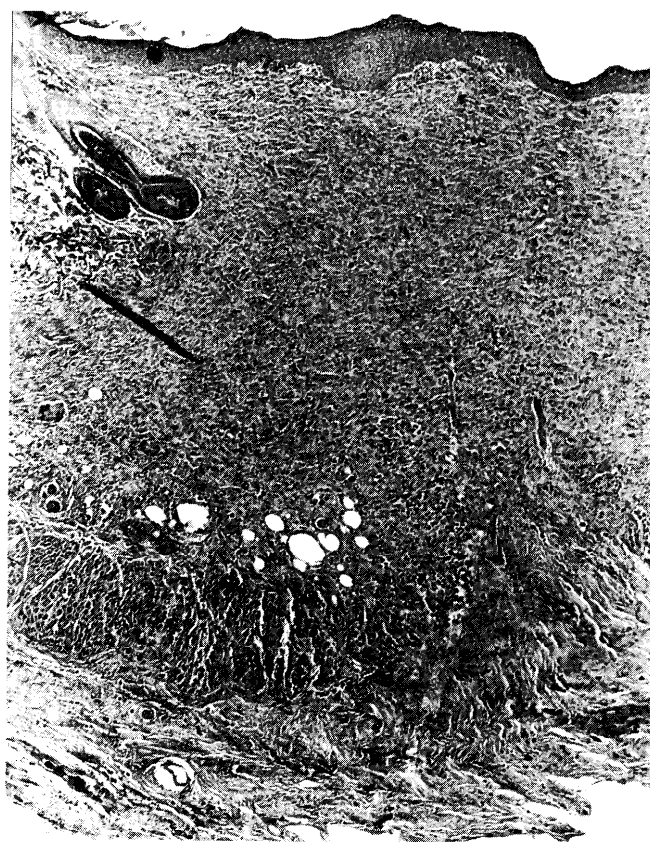


Fig. 4. Photomicrograph after healing of a sagittal section from a skin segment of a SM-exposed area treated with surgical excision and skin grafting. In the dermis a hypertrophied histological scar formation is seen, which extends down to the fascia of the underlying muscle. Magnification 64 x.

model, if modern surgical treatment of thermal burns, i.e. early excision of injured skin followed by skin grafting,^{11,12} could be of benefit in SM lesions. The guinea pig as well as a great variety of other species of laboratory animals have been used for assessment of SM-induced skin lesions and the majority of these species have not developed the blisters and bullae seen in man, with the possible exception of certain birds, frogs and the mammary glands of lactating dogs.²² Development of microblisters has been described in hairless guinea pigs.²³ In the present study, however, it was of interest to perform the surgical excision six hours after SM injury following the “six hour rule” for debridement of combat injuries.²⁴ In this period, it would be less likely for a human to develop blisters. This will take place after a latent period of 8 to 24 hours and more rapidly only after severe exposure.^{14,16} The dermal effects of SM have also been studied in *in vitro* systems such as human skin explants or epidermal keratinocytes in culture.^{25,26} The purpose of the present study to investigate the influence of surgical excision on healing time and general well being of the organism excluded, however, the use of *in vitro* models.

The standard exposure dose ($Ct = 2.5 \times 10^4 \text{ mg} \times \text{min} /$

m³) of SM vapour used in this study was well above what is known, as vapour, to induce erythema and blistering in humans.¹⁴ The exposure most likely leading to a severe cutaneous lesion is contact with liquid SM, either by direct exposure to droplets or aerosol dispersed from e.g. an artillery grenade, or by contact with contaminated surfaces. Concentrations of SM vapour high enough to inflict severe skin damage can occur close to the dispersal point, by evaporation from ground contamination at high ambient temperatures, and under clothing contaminated with liquid SM.

The experimental design with SM vapour exposure is not selected to mimic real conditions. It is used as a tool to administer an exact dose to defined areas. It also minimises risk for accidental exposure and greatly reduces any need for decontamination necessary for the safe handling of the animals.

Healing time was significantly shorter in group II compared to the other groups (Table I). There was no difference in healing time between groups I, III and IV. This could very well be explained by the fact that, in groups III and IV, the full thickness skin graft eventually did not "take", but served more like a dressing. Increase in BM can be looked upon as an indicator of the guinea pig's general well being. Animals in all groups increased their BM over time. At sacrifice (when lesions were already healed), there was no difference in BM increase between groups I & II and groups III & IV respectively. There was, however, a significantly higher BM increase in groups III & IV compared to groups I & II. This can be explained by the fact that healing time in the former groups was 1 to 2 weeks longer than in the latter, thus allowing these animals more time to increase BM before sacrifice. On day 10, i.e. approximate time of removal of sutures in all groups, however, BM increase in group II was significantly higher compared to the others (Table I).

Histological sections of lesions removed at surgery, showed an acute inflammatory response with pronounced leukocyte infiltration (Fig. 3), confirming what has been reported in acute SM skin lesions in rabbits and pigs²⁷ as well as in hairless guinea pigs.²⁸ Healing of skin graft harvest sites (Fig. 2) as well as transplant sites of unexposed (group III) animals was uncomplicated and resulted in a normal histological picture of hairy skin. In contrast, transplant sites of SM exposed animals (group IV) showed a hypertrophic scar formation (Fig. 4) extending down to underlying fascia. Possibly, there was some local SM effect on the healing process, comparable to the hypertrophic scar formation seen in humans. Direct cytotoxic effects or damage due to secondary inflammation as well as DNA damage may be of importance.²⁹

In this experimental model, surgical excision of the SM exposed area followed by primary suture was

beneficial, resulting in shorter healing time and greater BM increase, at least at the time of removal of sutures. The SM exposed area (5 cm²) was about 1.1% of total body surface area (TBSA). In a human, 1% of TBSA equals approximately the area of the palm of one hand. Even though it would be possible to treat such a small lesion with excision and full thickness skin grafting, split skin grafting would certainly be the method of choice. In thermal burns, areas up to 20% of TBSA are routinely treated in this manner.¹² In the guinea pig, the thin dermis precluded split skin grafting as a model, and obviously, full thickness skin grafting was not suitable due to the fact that the transplants served more like dressings instead of "taking" as grafts.

Further studies in other laboratory animal species, e.g. the pig, are suggested to determine the possible role of early excision and split skin grafting in human victims of SM-induced cutaneous lesions.

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