JOURNAL OF APPLIED TOXICOLOGY, VOL. 15(2), 133–138 (1995)

# Reduction of Erythema in Hairless Guinea Pigs after Cutaneous Sulfur Mustard Vapor Exposure by Pretreatment with Niacinamide, Promethazine and Indomethacin*

**Jeffrey J. Yourick,† Jeffrey S. Dawson and Larry W. Mitcheltree**
Applied Pharmacology and Comparative Pathology Branches, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425, USA

Key words: sulfur mustard; hairless guinea pig; niacinamide; promethazine; indomethacin.

**Erythema is the initial symptom that occurs after sulfur mustard (HD) cutaneous exposure. The time course of HD-induced erythema is similar to that observed after UV irradiation, which can be reduced by indomethacin. Sulfur mustard lethality is decreased by using promethazine, which is an antihistamine. Niacinamide can reduce microvesication after HD vapor exposure in hairless guinea pig (HGP) skin. The present study examines the effect of the combined administration of niacinamide, indomethacin and promethazine used alone or in all possible combinations on the degree of erythema and histopathologic skin damage after HD exposure in HGP. Niacinamide (750 mg $kg^{-1}$, i.p.), promethazine (12.5 mg $kg^{-1}$, i.m.) or indomethacin (4 mg $kg^{-1}$, p.o.) used singly or in combination was given as a 30-min pretreatment before an 8-min HD vapor cup skin exposure. Using a combination pretreatment of niacinamide, promethazine and indomethacin, erythema was reduced at 4 (91%) and 6 (55%) h, but not 24 h after HD. The incidence of histopathological skin changes (microvesicles, follicular involvement, epidermal necrosis, intracellular edema and pustular epidermatitis) 24 h after HD was not reduced. This study indicates that HD-induced erythema may result from several different mechanisms, including inflammation, histamine release and DNA damage. It is suggested that two phases of inflammation may occur: an early phase sensitive to antihistamines and non-steroidal antiinflammatory drugs and a late phase of extensive cell damage that was not sensitive to these drug pretreatments.**

## INTRODUCTION

Sulfur mustard (HD) exposure has been shown to cause erythema and microvesicle formation in hairless guinea pig skin.[1] In addition, erythema develops as early as 2 h while microvesicles do not appear until 16 h after HD vapor exposure in hairless guinea pig skin.[2]

The acute skin symptoms noted after HD liquid or vapor exposure in humans and animals follows a dose-dependent asymptomatic latent period of varying duration. Sulfur mustard skin lesions progress in three distinct symptomatic phases: erythema, blister formation and necrotic lesions. After a vapor cup exposure to human forearms, the typical progression of skin lesions was slight erythema (1 h), definite erythema (2–3 h), raised erythema and edema (8–12 h), pinhead vesicles (13–22 h) and coalesced blisters (16–48 h).[3]

The human sunburn reaction[4] and HD cutaneous exposure result in a similar time course of erythema and edema. One hour after UV irradiation, mast cell degranulation, perivenular edema, histamine release and erythema are evident. Before the appearance of erythema, prostaglandin $E_2$ ($PGE_2$) skin levels increase and remain elevated for 24 h. Ultraviolet-induced erythema has been proposed to result from different mechanisms,[5] which include direct injury to vascular endothelium,[6,7] release of inflammatory mediators ($PGE_2$, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$)[8] that diffuse from the epidermis to dermal vascular endothelium,[9,10] and release of mast cell vasoactive mediators (e.g. histamine).[11,12] In human skin exposed to UV irradiation it was found that endogenous release of histamine stimulates prostaglandin synthesis.[8] Ultraviolet-induced erythema is blocked by treatment with a non-steroidal antiinflammatory drug, indomethacin,[13–15] or antihistamines.[16]

A proposed mechanism of HD blister formation involves an initial alkylation of DNA followed sequentially by stimulation of DNA repair processes, alterations in cellular energy metabolism, increases in protease synthesis and release and separation at the epidermal–dermal junction.[17] Initial attempts to define the biochemical alterations leading to microvesication *in vivo* have focused on cellular $NAD^+$. The DNA repair enzyme, poly(ADP–ribose)polymerase, utilizes $NAD^+$ as a cofactor, and alkylating agent damage results in $NAD^+$ depletion.[18] Sulfur mustard exposure

* In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals', NIH Publication No. 85–23, revised 1985. The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

† Author to whom correspondence should be addressed, at: US Food and Drug Administration, Cosmetics Toxicology Branch HFS-128, 8301 Muirkirk Rd, Laurel, MD 20708, USA.

CCC 0260–437X/95/020133–06


*Received 18 July 1994*
*Accepted 18 July 1994*

of human keratinocytes,[19,20] human blood leukocytes,[21] human skin explants,[22] human skin grafts on athymic nude mice[23] or hairless guinea pig skin[24] results in cellular $NAD^+$ depletion.

Niacinamide is both a reversible inhibitor of poly(ADP–ribose)polymerase[25] and a substrate for $NAD^+$ synthesis.[26] Niacinamide pretreatment reduced microvesication, but erythema was not reduced or delayed.[2,24] Hence, niacinamide may not affect the acute vascular changes that occur as a result of HD exposure.

The role of inflammation in HD cutaneous injury has been reviewed recently.[27] The use of antiinflammatory compounds to treat HD exposures has been limited. Mast cell degranulation has been noted in human skin explants[28] and hairless guinea pig skin[29] exposed to HD. The antihistamine promethazine decreased the severity of HD rabbit skin lesions and was reported to increase survival of rats exposed to HD or nitrogen mustard.[30]

The present study was undertaken to determine whether the severity of HD-induced skin lesions can be reduced by administering, either alone or in combination, niacinamide, an antihistamine (promethazine) or a non-steroidal antiinflammatory compound (indomethacin).

## METHODS

### Animals

Male [Crl:IAF/HA(hr/hr)BR Vaf/Plus®, Charles River] hairless guinea pigs (*Cavia porcellus*), 300–400 g, were used in these experiments. Guinea pigs were quarantined on arrival and screened for evidence of disease before being released from quarantine. They were maintained under an AAALAC-accredited animal care and use program in polycarbonate plastic cages (Lab Products, Maywood, NJ) on hardwood chip contact bedding (Beta-Chip; Northeastern Products Corp., Warrensburg, NY). Animals were provided commercial guinea pig ration (Zeigler Bros., Gardners, PA) and tapwater *ad libitum*. Animal holding rooms were maintained at 21 ± 1°C with 50 ± 10% relative humidity using at least 10 complete changes per hour of 100% conditioned fresh air. The guinea pigs were on a 12-h light/dark full spectrum lighting cycle with no twilight.

### Chemicals

Niacinamide, promethazine and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO). Distilled 2,2′-dichlorodiethyl sulfide (HD; purity 97.6%) was obtained from the US Army Chemical and Biological Defense Command (Aberdeen Proving Ground, MD).

### Sulfur mustard exposures

Sulfur mustard exposures were performed as described previously.[24] Niacinamide (750 mg $kg^{-1}$, 3 ml $kg^{-1}$ in saline, i.p.), promethazine (12.5 mg $kg^{-1}$, 1 ml $kg^{-1}$ in saline, i.m.), indomethacin (4 mg $kg^{-1}$, 0.8 ml 100 $g^{-1}$ body wt. in 3% gum acacia in saline, p.o.) or saline (3 ml $kg^{-1}$, i.p.), used singly or in all possible combinations, was administered as a 30-min pretreatment. Animals were anaesthetized with a combination of ketamine hydrochloride (30 mg $kg^{-1}$, i.m.) and xylazine (6 mg $kg^{-1}$, i.m.) 15 min prior to HD exposure. Eight tape strips were attached to the back (four on each side of the dorsal midline) of each animal. Sulfur mustard (10 μl) was applied to a filter-paper disc attached to the inside top surface of a plastic disposable vial cap. The vapor cup was inverted onto the tape-strip and remained for 8 min. After HD exposure, the animals were placed in individual cages and remained in a fume-hood for 24 h. Each animal was euthanized singly in a halothane-saturated glass chamber.

### Erythema determination

Erythema was scored visually using a modified visual scoring scale from 0 to 4.[31]

### Histopathological analysis

Full-thickness skin punch biopsies were taken from the center of each exposure site after halothane euthanasia. Samples were placed in 10% neutral buffered formalin for a minimum of 24 h and then analyzed under light microscopy for intracellular edema, epidermal necrosis, pustular epidermatitis, follicular necrosis and microvesicles.

### Data analysis

Values reported are the mean ± SEM of groups from four to twelve animals. Significant differences between erythema group means were based on the Kruskal–Wallis test ($P < 0.05$) followed by the Mann–Whitney test (Fig. 1). Significant differences between the incidence of histopathological markers were determined using the Mann–Whitney test (Fig. 2).

## RESULTS

The effect of single and combination pretreatments on erythema after HD vapor exposure to hairless guinea pig skin is shown in Fig. 1. The combination pretreatment of niacinamide and promethazine significantly decreased the degree of erythema measured 4 h after HD by ca. 55%, while the triple combination of niacinamide, promethazine and indomethacin reduced erythema by 91% when compared with niacinamide or saline-pretreated animals (Fig. 1A). In addition, only the combination pretreatment of niacinamide, promethazine and indomethacin further decreased the erythema when compared with all dual pretreatment combinations. The combination pretreatment of niacinamide and promethazine and the triple combination pretreatment of niacinamide, promethazine and indomethacin still exhibited a reduction in erythema of ca. 50% 6 h after HD (Fig. 1B). However, at 24 h after HD

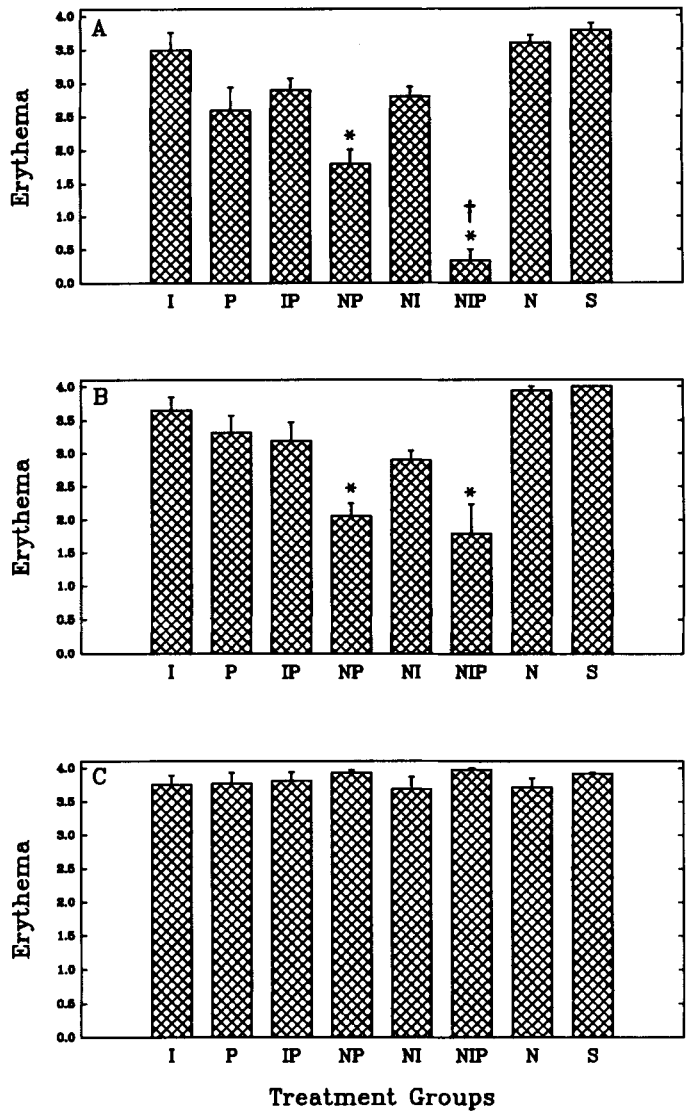


**Figure 1.** Effect of promethazine, indomethacin and niacinamide on erythema after sulfur mustard (HD) exposure. The control value for untreated skin is zero. Individual graphs represent 4 h after HD (A), 6 h after HD (B) and 24 h after HD (C). All drugs were administered as 30-min pretreatments: niacinamide (N, 750 mg $kg^{-1}$, 3 ml $kg^{-1}$ in saline, i.p.), promethazine (P; 12.5 mg $kg^{-1}$, 1 ml $kg^{-1}$ in saline, i.m.), indomethacin (I; 4 mg $kg^{-1}$, 0.8 ml 100 $g^{-1}$ body wt. in 3% gum acacia in saline, p.o.) or saline (S; 3 ml $kg^{-1}$, i.p.). Values are the mean ± SEM of four to twelve animals. * At least $P < 0.05$ from N and S, † $P < 0.01$ from NP.

no pretreatment combination offered any protection against erythema (Fig. 1C).

The effect of the administration of different pretreatment combinations on histopathological changes noted 24 h after HD exposure is shown in Fig. 2. A reduction in microvesicle formation (Fig. 2A) and follicular involvement (Fig. 2B) were noted with niacinamide pretreatment. No reduction in epidermal necrosis (Fig. 2C), intracellular edema (Fig. 2D) or pustular epidermatitis (Fig. 2E) was noted with niacinamide administration. No pretreatment regimen including promethazine or indomethacin resulted in reductions in histopathological skin changes 24 h after HD exposure (Fig. 2A–E). In fact, the use of niacinamide in combination with either promethazine or indomethacin resulted in the loss of protection afforded by niacinamide against microvesicle formation and follicular involvement.

## DISCUSSION

The pretreatment combination of niacinamide and promethazine reduced erythema after HD, while with the triple combination pretreatment including indomethacin even further reductions occurred. Neither niacinamide, promethazine nor indomethacin when used alone reduced erythema. Even though erythema was reduced up to 91% by the triple compound pretreatment after HD, skin injury (24 h) still occurred. No reduction in the incidence of any histopathological marker was noted.

Sulfur mustard has been reported to release histamine from skin. Histamine release and mast cell degranulation approximately doubled when full-thickness skin explants were exposed to HD.[32] In hairless guinea pig skin exposed to HD, the number of

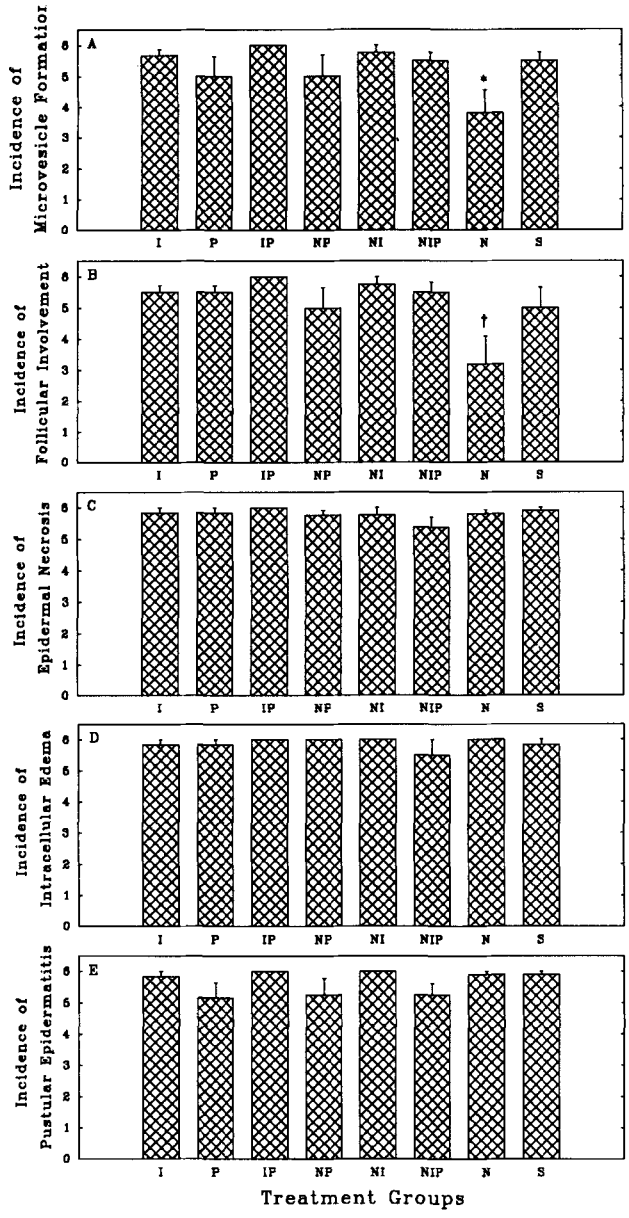


**Figure 2.** The incidence of specific histopathological markers 24 h after sulfur mustard (HD) vapor exposure in hairless guinea pig skin. Each panel represents the incidence of microvesicle formation (A), follicular necrosis (B), epidermal necrosis (C), intracellular edema (D) and pustular epidermatitis (E). See Fig. 1 legend for description of the drug pretreatment groups. Values are the mean ± SEM of four to twelve animals. Based on the Mann–Whitney (* $P < 0.01$; † $P = 0.053$) test, there was a difference from saline.

granulated mast cells and the cross-sectional area occupied by granules were decreased after exposure to HD.[29] Therefore, HD-induced mast cell degranulation and the subsequent histamine release may contribute to skin injury pathogenesis.

Skin vascular responses due to HD have been suggested to occur in two phases: an immediate and a delayed phase.[33] An early or 'immediate' phase occurring within 1 h was proposed to be due to direct endothelial cell membrane injury and vasoactive mediator release. During this acute-reversible phase, vascular leakage and a limited granulocyte infiltration occurred. The second or 'delayed' phase appeared 8 h after HD, and vascular leakage was attributed more to DNA alkylation-related cell death than to strictly vasoactive mediator release. This phase was characterized by basal epidermal cell death, generalized vascular leakage and extensive leukocyte infiltration.

The results from the present study indicate that the erythema associated with the immediate phase (direct endothelial damage and mediator release) is sensitive to antihistamine and niacinamide combination pretreatment. However, the delayed phase (epidermal cell death and generalized vascular leakage) of erythema/inflammation associated with tissue necrosis is ultimately unresponsive to antihistamine or antiinflammatory action. It is proposed that inflammation, as suggested by the present study, does not initiate the pathogenic mechanism of microvesicle formation, but rather may contribute to the exacerbation of microvesicle development.

Inflammatory mediators derived from activation of the prostaglandin cascade are also released by HD exposure. Prostaglandin $E_2$ ($PGE_2$) release increased in human skin explants topically exposed to HD.[32] It has been shown that $PGE_2$ and 6-keto-$PGF_{1\alpha}$ (a stable

metabolite of prostacyclin) are released from cultured human epidermal keratinocytes within 6 h after HD exposure (M.A.E. Mol; personal communication). The synthesis and release of these prostaglandin cascade mediators should be reduced by indomethacin, which is a cyclooxygenase inhibitor. In addition, the release of prostaglandins may be dependent, at least partially, on histamine. Exogenous histamine stimulates $PGE_2$ release from epidermal cell cultures, which could be blocked by antihistamines.[8] In the same study, human skin explants exposed to UV irradiation release $PGE_2$, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$, and this release was inhibited by antihistamines. It was suggested that histamine-stimulated prostaglandin release is a possible mechanism of irradiation-induced erythema. A similar erythema mechanism may be occurring with HD skin exposure. However, the present study showed that an antihistamine or cyclooxygenase inhibitor used singly or in combination could not prevent erythema. Only when niacinamide was used in combination with an antihistamine was there a reduction in erythema. It is possible that niacinamide is reducing the DNA damage component while the antihistamine is reducing histamine and prostaglandin release. Using a cyclooxygenase inhibitor alone may not intercede in the inflammatory cascade at a point early enough to reduce HD-induced erythema.

The current study investigated HD-induced erythema and whether combination pretreatments of an antihistamine, a non-steroidal antiinflammatory and niacinamide can reduce erythema and ultimately decrease skin injury. The combination of niacinamide, promethazine and indomethacin inhibited erythema but did not ultimately prevent skin injury. These results are consistent with two phases of skin injury after HD exposure. The first phase is an inflammatory phase of injury that is associated with vascular changes involving histamine and prostaglandin release. The second phase seems to be associated with more extensive tissue injury (cell necrosis and inflammation) that is beyond the point of treatment with antihistamines and/or antiinflammatory drugs without addressing the DNA alkylating component of HD injury.

## REFERENCES


1. M. M. Mershon, J. V. Wade, L. W. Mitcheltree, J. P. Petrali and E. H. Braue, The hairless guinea pig bioassay model for vesicant vapor exposures. *Fundam. Appl. Toxicol.* **15**, 622–630 (1990).
2. J. J. Yourick, J. S. Dawson, C. D. Benton, M. E. Craig and L. W. Mitcheltree, Pathogenesis of 2,2'-dichlorodiethyl sulfide in hairless guinea pigs. *Toxicology* **84**, 185–197 (1993).
3. B. Papirmeister, A. J. Feister, S. I. Robinson and R. D. Ford, *Medical Defense Against Mustard Gas*, pp. 14–21. CRC Press, Boston (1991).
4. B. A. Gilchrest, N. A. Soter, J. S. Stoff and M. A. Mihm, The human sunburn reaction: histologic and biochemical studies. *J. Am. Acad. Dermatol.* **5**, 411–422 (1981).
5. S. Shuster, The mechanism of ultraviolet erythema. *Br. J. Dermatol.* **106**, 235 (1982).
6. W. M. Sams and R. K. Winkelmann, The effect of ultraviolet light on cutaneous blood vessels. *J. Invest. Dermatol.* **53**, 79–83 (1969).
7. J. C. van der Luen, Theory of ultraviolet erythema. *Photochem. Photobiol.* **4**, 453–458 (1965).
8. A. P. Pentland, M. Mahoney, S. C. Jacobs and M. J. Holtzman, Enhanced prostaglandin synthesis after ultraviolet injury is mediated by endogenous histamine stimulation. *J. Clin. Invest.* **86**, 566–574 (1990).
9. B. E. Johnson, Ultraviolet radiation and lysosomes in skin. *Nature* **219**, 1258–1259 (1968).
10. B. E. Johnson and F. Daniels, Lysosomes and the reactions of skin to ultraviolet radiation. *J. Invest. Dermatol.* **53**, 85–94 (1969).
11. E. J. Valtonen, J. Janne and M. Simes, The effect of the erythemal reaction caused by ultraviolet irradiation on mast cell degranulation in the skin. *Acta Dermatol. Venereol.* **44**, 269–272 (1964).
12. R. A. Glover, C. S. Bailey, K. E. Barrett, S. I. Wasserman and I. Gigli, Histamine release from rodent and human mast cells induced by protoporphyrin and ultraviolet light: Studies of the mechanism of mast-cell activation in erythropoietic protoporphyria. *Br. J. Dermatol.* **122**, 501–512 (1990).
13. D. S. Snyder, Cutaneous effects of topical indomethacin, an inhibitor of prostaglandin synthesis, on UV-damaged skin. *J. Invest. Dermatol.* **64**, 322–325 (1975).
14. D. S. Snyder, Effect of topical indomethacin on UVR-induced redness and prostaglandin E levels in sunburned guinea pig skin. *Prostaglandins* **11**, 631–643 (1976).
15. D. S. Snyder and W. H. Eaglstein, Intradermal anti-prostaglandin agents and sunburn. *J. Invest. Dermatol.* **62**, 47–50 (1974).
16. L. J. A. Loewenthal, Triprolidine hydrochloride in the prevention of some solar dermatoses. *Br. J. Dermatol.* **75**, 254–256 (1963).
17. B. Papirmeister, C. L. Gross, H. L. Meier, J. P. Petrali and J. B. Johnson, Molecular basis for mustard-induced vesication. *Fundam. Appl. Toxicol.* **5**, S134–S149 (1985).
18. S. Shall, ADP-ribose in DNA repair, In *ADP-Ribosylation Reactions*, ed. by O. Hayashi and K. Ueda, pp. 477–520. Academic Press, New York (1982).
19. M. A. E. Mol, A. B. C. Van De Ruit and A. W. Kluivers, $NAD^+$ levels and glucose uptake of cultured human epidermal cells exposed to sulfur mustard. *Toxicol. Appl. Pharmacol.* **98**, 159–165 (1989).
20. W. J. Smith, C. L. Gross, P. Chan and H. L. Meier, The use of human epidermal keratinocytes in culture as a model for studying the biochemical mechanisms of sulfur mustard toxicity. *Cell. Biol. Toxicol.* **6**, 285–291 (1990).
21. H. L. Meier, C. L. Gross and B. Papirmeister, 2,2'-Dichlorodiethyl sulfide (sulfur mustard) decreases $NAD^+$ levels in human leukocytes. *Toxicol. Lett.* **39**, 109–122 (1987).
22. M. A. E. Mol, R. de Vries and A. W. Kluivers, Effects of nicotinamide on biochemical changes and microblistering induced by sulfur mustard in human skin organ cultures. *Toxicol. Appl. Pharmacol.* **107**, 439–449 (1991).
23. C. L. Gross, H. L. Meier, B. Papirmeister, F. B. Brinkley and J. B. Johnson, Sulfur mustard lowers nicotinamide adenine dinucleotide concentrations in human skin grafted to athymic nude mice. *Toxicol. Appl. Pharmacol.* **81**, 85–90 (1985).
24. J. J. Yourick, C. R. Clark and L. W. Mitcheltree, Niacinamide pretreatment reduces microvesicle formation in hairless guinea pigs cutaneously exposed to sulfur mustard. *Fundam. Appl. Toxicol.* **17**, 533–542 (1991).
25. M. R. Purnell and W. J. D. Whish, Novel inhibitors of poly (ADP-ribose) synthetase. *Biochem. J.* **185**, 775–777 (1980).
26. L.-F. H. Lin and L. M. Henderson, Pyridinium precursors of pyridine nucleotides in perfused rat kidney and in the testis. *J. Biol. Chem.* **247**, 8023–8030 (1972).
27. F. M. Cowan and C. A. Broomfield, Putative roles of inflammation in the dermatopathology of sulfur mustard. *Cell Biol. Toxicol.* **9**, 201–214 (1993).
28. A. M. Dannenberg, *Sulfur Mustard (SM) in Organ-Cultured*

*Human Skin: Markers of Injury and Inflammatory Mediators*, Contract No. DAMD17-87-C-7040, Final Report, AD #A227358. US Army Medical Research and Development Command, Fort Detrick, MD (1990).
29. J. S. Graham, M. A. Bryant and E. H. Braue, Effect of sulfur mustard on mast cells in hairless guinea pig skin. *Proc. 1993 Medical Defense Biosc. Rev.* (AD #A275667) **1**, 41–48 (1993).
30. V. Vojvodic, Z. Milosavljevic, B. Boskovic and N. Bojanic, The protective effect of different drugs in rats poisoned by sulfur and nitrogen mustards. *Fundam. Appl. Toxicol.* **5**, S160–S168 (1985).
31. J. H. Draize, Dermal toxicity. In *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, pp. 45–59. Association of Food and Drug Officials of the United States, Washington, DC (1959).
32. T. Rikimaru, M. Nakamura, T. Yano, G. Beck, G. S. Habicht, L. L. Rennie, M. Widra, C. A. Hirshman, M. C. Boulay, E. W. Spannhake, G. S. Lazarus, P. J. Pula and A. M. Dannenberg, Mediators initiating the inflammatory response released in organ culture by full-thickness human skin explants exposed to the irritant, sulfur mustard. *J. Invest. Dermatol.* **96**, 888–897 (1991).
33. R. F. Vogt, A. M. Dannenberg, B. H. Schofield, N. A. Hynes and B. Papirmeister, Pathogenesis of skin lesions caused by sulfur mustard. *Fundam. Appl. Toxicol.* **4**, S71–S83 (1984).