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Protective Effect of Povidone Iodine Ointment against Skin Lesions Induced by Chemical and Thermal Stimuli

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Mustard gas (sulfur mustard, HD) is a powerful vesicant employed as a chemical weapon. The present study demonstrates the effect of povidone iodine (PI) ointment against skin toxicity caused by HD. Gross and histopathological examinations showed that application of PI 20 min or less following exposure to the vesicant resulted in marked skin protection. The shorter, interval between exposure and treatment, the better was the protection achieved. Povidone iodine was also effective against other mustards, such as carboxybutylchloroethyl sulfide (CBCS) and mechlorethamine. The fact that PI protected the skin against agents that cannot be oxidized, such as iodoacetic acid, divinylsulfone and cantharidine, indicated that the antidotal effect of PI was unrelated to oxidation of the nitrogen and sulfur atoms of the mustards. Furthermore, NMR spectroscopy of CBCS treated with iodine did not show oxidation of the sulfur atom. Clinical experience with patients after accidential heat burns (mostly of grade I) has shown that topical application of PI ointment immediately after the stimulus significantly reduced, and often prevented, skin lesions. Apart from being a safe and widely used disinfectant, PI ointment is recommended as an efficient protective agent against skin toxicity caused by hazardous chemicals and by heat stimuli.

INTRODUCTION

Mustard gas (sulfur mustard, HD) is a highly potent alkylator[1] and severely cytotoxic vesicant in humans and animals.[2–4] Owing to its lypophilic properties, it rapidly penetrates the skin.[5,6] Topical application of HD may cause erythema, which appears within hours of exposure followed by oedema, blistering and ulceration.[7,8] There is an urgent need for an efficient pharmacological antidote against mustard gas toxicity. The employment of mustard gas in several conflicts in this century[9–11] and by terrorist groups further emphasizes the need for antidotal preparation against this vesicant. Several attempts to find protecting agents have been made[12–15] but their activity was too weak to be used as pharmacological protectants against HD.

Povidone iodine (PI) ointment is widely used as an antiseptic agent and for the treatment of thermal burns.[16,20] Because both chemical and thermal burns share common pathological features, such as erythema, oedema and blisters, we have tested the protective effect of PI ointment against skin injuries caused by HD and other mustard and non-mustard alkylators. We have characterized and quantified the pathological changes after exposure to the irritants and have demonstrated the beneficial effect of PI ointment.

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METHODS

Syntheses of HD and carboxybutylchloroethyl sulfide (CBCS; $Cl\text{-}CH_2\text{-}CH_2\text{-}S\text{-}(CH_2)_4\text{-}COOH$) and animal intoxication studies were carried out as described previously.[17,18] Briefly, HD (1 μl) was applied on the back of a preshaved male guinea pig that was anaesthetized by intraperitoneal injection of 30 mg kg^{-1} pentobarbital sodium. Up to eight applications were made per guinea pig, each on the centre of a 0.7 × 0.7 cm square (outlined with black pencil). At indicated time intervals (see Fig. 1) following HD exposure, a thin layer of 40 mg of 10% PI was applied on the entire square area. Four days after treatment, the animals were killed by ether anaesthesia and skin samples were removed and preserved in 4% buffered formol saline. Tissues were sectioned at 4–5 μm and stained with haematoxylin and eosine (H&E) for histological evaluation.

Similar procedures were used with other vesicants. The CBCS (neat liquid) was applied at a dose of 1 μl per application site. Mechlorethamine hydrochloride was dissolved in ethanol (50 mg ml^{-1}) and 10 μl

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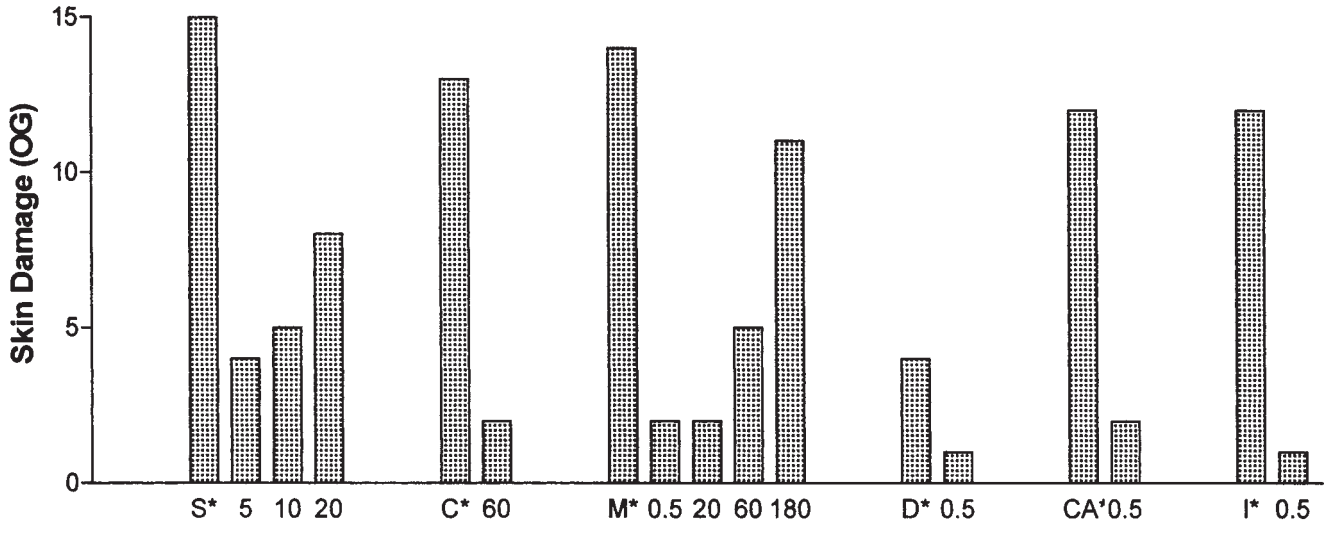

Figure 1. Skin histopathology of mustards and non-mustard vesicants and the protective effect of PI ointment. Backs of animals were exposed to the vesicants 24 h after shaving. Mechlorethamine (M), cantharidine (CA) and iodoacetate (I) were applied at doses of 0.5, 1.4 and 0.93 mg per application site, respectively. The HD (S), CBCS (C) and divinyl sulfone (D) were applied at doses of 1 μl per application site. Quantitative histopathological evaluation, marked as overall grade (OG), took into consideration the size and deepness of the lesion and the various components of tissue response (i.e. epidermal acanthosis, necrosis, exfoliation and leucocyte cell infiltration; dermal necrosis and leucocyte infiltration). Each of the parameters was scored using four grades (0, no change; 1, slight change; 2, moderate change; 3, marked change). For each skin sample, OG expresses the sum of grades of the various components of histological tissue reactivity. The irritants were tested at least three times with and without the protectant. *Application of the irritant only. The numbers indicate the time intervals (min) between application of the vesicant and treatment with PI; 0.5 indicates that PI was applied within 0.5 min following vesicant exposure.

(500 μg) was applied per site. The guinea pigs were also topically exposed to 1 mg of cantharidine (5 μl of a solution of 1 mg 5 μl^{-1} dimethylformamide), 5 μmol of iodoacetic acid (5 μl of 1 N in ethanol) and 1 μL of divinyl sulfone per application site. With cantharidine, male rats were used for the experiments instead of guinea pigs. Povidone iodine ointment (40 mg) was applied following exposure as described in the previous paragraph for HD. The experiments were terminated two days after exposures. All irritants were tested in triplicate with and without the protectant.

RESULTS AND DISCUSSION

Figure 1 shows that the severity of skin lesions is related to the interval of time between HD exposure and PI treatment. The results are presented in terms of 'overall grade' (OG) values, which measure the degree of skin damage (see Fig. 1 caption). Application of PI up to 10 min following HD exposure still conferred a high degree of protection. A longer interval such as 20 min between exposure and treatment resulted in a lower degree of protection, but it was still good enough for PI to be used as an HD antidote.

Povidone iodine was also effective against other mustard derivatives, such as the monofunctional sulfur mustard CBCS and the difunctional nitrogen mustard mechlorethamine (Fig. 1). Significant protection was observed against CBCS (OG = 2) after a 60-min interval whereas a similar degree of protection was observed when the skin was treated within 20 min after mechlorethamine exposure. Longer intervals of 60 and 180 min between nitrogen mustard exposure and PI ointment application were also effective but to a lesser extent, namely, OG values were 5 and 11, respectively, whereas without PI treatment OG = 14 (Fig. 1). The skin toxicity of non-mustard alkylators such as iodoacetate and cantharidine was strongly inhibited when PI was applied immediately after the toxic agents (OG = 2 and OG = 0, respectively, compared with OG = 12 without PI; Fig. 1). Divinyl sulfone reactivity was also blocked by PI treatment but the effect was not as dramatic as with the previous irritants because of the relatively low toxicity (OG = 4) in our experimental system. As a control, other types of commercial preparations containing tretinoin 0.05% and tocopherol did not prevent mechlorethamine- and divinyl-sulfone-induced skin toxicity when applied immediately after intoxication (not shown).

The presently used army decontaminating powder (Fuller's Earth) is efficient when applied shortly after exposure because of a rapid penetration of HD into the skin (personal communication). Our results demonstrate the protection conferred by PI ointment against skin ulceration caused by HD and other mustard derivatives. The fact that PI treatment *after* mustard exposure showed such beneficial results gives this antidote practical importance in the treatment of skin burns caused by alkylators. In spite of the success in finding this counterirritant, the mechanism of the protecting action of PI remains to be clarified. The possibility of oxidation by iodine of the sulfur and nitrogen in HD, CBCS and mechlorethamine, respectively, to yield the inactive sulfoxide[19] or nitrogen oxide forms, respectively, should be ruled out because the toxicity of other compounds that are not subjected to oxidation, such as iodoacetic acid and cantharidine, was also prevented by PI ointment. In addition, analysis by NMR spectroscopy of CBCS treated with iodine did not indicate oxidation of the sulfur (data not shown). Another

explanation that the ointment is operating by a physical decontaminating mechanism, such as dilution of the vesicant, may also be ruled out because other preparations (commercial tocopherol and tretinoin creams) did not protect the skin against the ulcerative effects of mechlorethamine and divinylsulfone (data not shown).

Although animals showed a positive response to iodine, its clinical significance for humans has to be elucidated. Controlled studies showed that application of HD on human skin resulted in erythema, oedema and blister,[21,22] whereas the guinea pig skin, as in other animal models, develops erythema and oedema but without macroblisters; however, epidermal microblisters are formed after HD exposure in the guinea pig model (recent unpublished results). The extrapolation from animals to humans is still a problem that remains to be solved; nevertheless, our data on the protective effect of iodine against thermal burns in humans may indicate that this counterirritant has similar effects on both species.

Our experience with patients after accidental heat burns (mostly of grade I; caused by hot water or oil and hot steam) has shown that topical application of PI ointment immediately after the stimulus dramatically reduced the degree of skin lesions.[20] As with chemical burns, the longer the interval between the stimulus and treatment, the weaker was the protection achieved. However, owing to the rapid reaction of skin to heat stimulus, these intervals are shorter than those observed with thermal burns. It is planned to conduct a controlled human study for testing the efficiency of PI against thermal burns.

In order to establish the mechanism of PI protection it is necessary to test separately the protective effect of each component of the ointment in order to find the active agent, or the active combination of components, that produces the beneficial effect. This is highly important, not only for understanding the molecular mechanism of PI-induced protection, but mainly because this will lead to clarification of the pathological processes occurring during the first stages of burns caused by chemicals, and also by thermal stimuli, and thus to improved post-burn protective agents.

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