

37. TOPICAL TREATMENT WITH IODINE REDUCES SKIN TOXICITY CAUSED BY SULFUR MUSTARD

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INTRODUCTION

Sulfur mustard (SM, mustard gas) is a potent blister agent employed as a chemical weapon in various conflicts during the 20th century (Mellor, et al. 1991). It is a potent alkylator and highly cytotoxic agent in both humans and animals (Dacre & Goldman 1996). Skin exposed to SM develops erythema followed by edema, vesicle and blister formation, ulceration, necrosis and desquamation (Smith, Graham, et al., 1995). It is accepted that the powerful alkylating activity of SM (Ludlum, et al. 1994) results from its conversion, in aqueous solution, to the highly electrophilic ethylene episulfonium derivative (Wormser 1991) which can be neutralized by nucleophilic agents. It has been proposed that protection against SM can be achieved by glutathione derivatives (Lindsay & Hambrook 1998). In spite of having some beneficial effects, these agents were not efficacious enough to be used as antidotes. Additional agents such as arginine analogs (Sawyer 1998), calcium channel blocker (Mazumder, et al. 1998), niacinamide (Meier & Johnson 1992) and its combination with promethazine and indomethacin (Yourick, et al. 1995) had a weak protective effect as post exposure treatment against SM in experimental animals although some of them were beneficial in *in vitro* and *in vivo* systems particularly as prophylactic treatment.

Iodine and iodophors like povidone iodine are widely used as antiseptic agents. Since their bacteria-killing effect stems from the oxidizing activity of iodine, our primary approach was to employ this characteristic to chemically neutralize SM by oxidation of its sulfur atom to form the less active sulfoxide form. Preliminary studies of our laboratory have shown that post-exposure treatment with povidone iodine protected against SM at interval of 10 min between exposure and treatment whereas at longer intervals the treatment was less effective (Wormser, et al. 1997). Our experience showed that the formulation of iodine plays a crucial role in its counter-irritating activity. Thus, in the present study we have developed a formulation capable of solving molecular iodine in an aqueous environment. The present study demonstrates the potent protective effect of the new iodine formulation against SM-induced skin lesions. The gross- and histopathology of guinea pig skin at different time intervals between SM exposure and iodine treatment were quantified.

MATERIALS AND METHODS

Backs of haired guinea pigs were shaved 24 hours prior the experiment. The animals were anesthetized by 30 mg/kg pentobarbital sodium ip. Backs were cleaned with wet soft white paper and let to dry out before the beginning of experiment. Six sites (three on each side) of each back were exposed to 1 μ L (1.2 mg) SM. One mL liquid iodine (Merck) preparation (otherwise indicated 2% in tetraglycol: water 1:1) was applied on three exposure sites of each animal while the other 3 SM-exposed sites of the animal remained untreated. Iodine was applied into wells that constructed by the following procedure. A plastic tube cover (inner diameter of 1.7 cm) was cut to form open-ended cylindrical well and a thin layer of commercial silicon sealing ointment was applied to one edge of the well. The well was then attached to the animal back so liquid inside the well did not leak out. Wells were constructed before exposure while SM was applied on the center of the well. Iodine was applied into the well 15 and 30 min after SM exposure. In additional series of experiments the well system was replaced by 3 layers of gauze pad (1.5x1.5cm) soaked in iodine solution (2% I₂ in tetraglycol) applied on site of exposure. Otherwise indicated the iodine preparation was left on the skin for 2 hours. In the end of the procedure the liquid iodine was sucked out and well was removed from the skin. In the end of experiment the animals were sacrificed by 100 mg/kg pentobarbital sodium ip. Their backs were photographed (together with a ruler) by Kodak 260 digital camera and gross pathology was assessed by ulceration area of each exposure site.

RESULTS AND DISCUSSION

Gross pathology evaluation of series of experiments revealed statistically significant reduction of 91% and 84% in the ulceration area at intervals of 15 and 30 min between exposure and treatment, respectively

(Table 1). The vehicle of iodine formulation had some protective effect (32% reduction) but was not statistically different from the exposed, untreated sites. It is also shown that iodine applied by the well system is more efficacious than by the gauze pad procedure (Fig. 1) presumably because the former has bigger reservoir of iodine than the latter.

Numerous iodine formulations were tested but non of them was proved to be as efficient as that containing TG iodine solvent. The superiority of the present iodine formulation stems from its ability to solve molecular iodine (I_2) in an aqueous environment. Molecular iodine (I_2) is practically water insoluble unless iodide (sodium or potassium salts) is present in the solution to form the water-soluble ion I_3^- . Molecular iodine can be dissolved in organic solvents such as ethanol or polyethyleneglycol-400 (PEG-400) but presence of water precipitates the iodine, thus iodine tincture (which contains ethyl alcohol and water) must also contain iodide to form I_3^- for proper dissolution. Experiments carried out in our laboratory showed that iodine tincture had much weaker protective effect than the iodine formulation described in the present study (data not shown). A possible explanation is that the negatively charged I_3^- poorly penetrates through biological membranes and barriers, thus, reducing its efficacy as counter-irritant. However, the TG-containing solvent system that solves I_2 without addition of iodide, thus keeping the molecular iodine in its non-charged form i.e. I_2 , might be more penetrable and stronger oxidizer than the negatively charged I_3^- , thus, would be more efficient in its counter-irritating activity. This explanation may also be applicable for the fact that the TG-containing formulation is superior over the iodide-containing formulations against thermal burns and in its bactericidal effect (data not shown). This assumption needs, of course, to be experimentally proved by physico-chemical and biochemical experiments.

Whatever is the reason for the activity of the iodine formulation, the main issue to be addressed concerns the mechanism of the protective action of iodine. The fact that iodine does not chemically inactivate SM was experimentally confirmed (data not shown) is evidence that the protective effect of iodine stems from epidermal/dermal processes affected by iodine. Moreover, the fact that post exposure treatment with iodine is effective also against thermal burns (Wormser 1998) further demonstrates that the antidotal activity of iodine results from cellular events occurring in the skin.

There is increasing evidence that exposure to irritants is associated with the trend of tissue to undergo programmed cell death, namely, apoptosis. Apoptotic cells were demonstrated in cultured keratinocytes (Rosenthal, et al. 1998), endothelial cells (Dabrowska, et al. 1996) and thymocytes (Hur, et al. 1998) after exposure to SM or its derivatives. In vivo studies have shown the appearance of apoptotic cells in SM-exposed skin of weanling pigs (Smith, et al. 1997). The apoptotic process composes a variety of biochemical reaction of which the activity of the cysteine proteinases, caspases, plays an important role (Asahi, et al. 1999). It is hypothesized that iodine exerts its protective activity by inhibition of apoptotic processes, namely, by oxidizing sulfhydryl group of either the active site of caspases or other functional proteins or peptides crucial for apoptosis. This hypothesis is currently under investigation.

Whatever the mechanism of iodine-induced protection is, the present study demonstrates the usefulness of the iodine formulation as a potent antidote against skin lesions caused by SM. It is proposed that this type of topical preparation can be used as a counterirritant at emergencies under both military and civilian circumstances.

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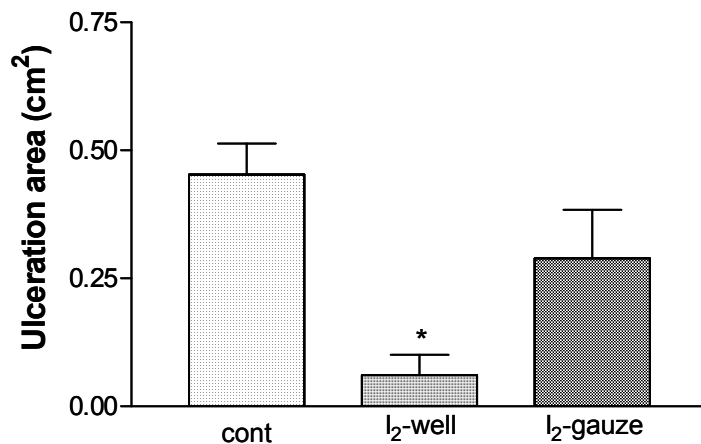
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KEYWORDS.

Mustard, sulfur mustard, blister, iodine

FIGURES AND TABLES

Figure 1. Effect of different routes of iodine application on SM skin toxicity.



Guinea pigs were exposed to 1.2 mg SM (1 μ l) and were topically treated with iodine using two procedures, the well system (n=9) and gauze pad (n=9) as described in Materials and Methods. Ulceration area was measured 2 days following exposure. Iodine was applied 15 min after SM exposure. Control indicates SM-exposed skin without iodine treatment (n=30) Results are expressed as mean \pm SE using the Kruskal-Wallis test and Dunnett's multiple comparison post test for statistical evaluation of the differences between the different routes of exposure and control group. * p<0.01

Table 1. Gross pathology effect of iodine against SM-skin toxicity.

Guinea pigs were exposed to 1.2 mg SM (1 μ L) and were topically treated with iodine as described in Materials and Methods. Ulceration area was measured 2 days following exposure. The time intervals between exposure and treatment were 15 (n=9) and 30 (n=18) min as indicated. Control indicates SM-exposed skin without iodine treatment (n=57) and Vehicle represents iodine vehicle (tetraglycol: water 1:1) applied 15 min after SM exposure (n=9). Results (expressed as percent of control) are mean \pm SE using the Kruskal-Wallis test and Dunnett's multiple comparison post test for statistical evaluation of the differences between the experimental groups.

*** p<0.001 at comparison between 15 and 30 min interval and cont.

p<0.05 at comparison between 15 min interval and vehicle.

Treatment	Ulceration area (% of control)	SE
Control	100	0
15 min	8.4 ***	0
30 min	15.4 ***	0
Vehicle	0	15.5