Defence Science Journal, Vol 44, No 1, January 1994, pp 21-23

SHORT COMMUNICATION

Therapeutic Efficacy of Saline and Glucose-Saline against Dermally Applied Sulphur Mustard Intoxication in Mice

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ABSTRACT

A single dose of saline or glucose-saline (5 mg glucose/kg) offered similar protection to mice against sulphur mustard intoxication, the extent of survival being 83 per cent as against 33 per cent without treatment. All the animals were protected when the treatment was extended by another two consecutive days in the glucose-saline treated group. Both saline and glucose-saline treatments could ameliorate the haemoconcentration as well as normalise pO_2 and % oxygen saturation. The protection conferred is attributed to the probable replenishment of fluid loss.

1. INTRODUCTION

Sulphur mustard (HD) 2,2'-dichlorodiethyl sulphide, is a potent blistering agent. It was the major cause of casualties resulting from the use of chemicals in World War I. Its use in regional conflicts over the past 50 years, most recently in the Iran/Iraq conflict of the Middile East has been documented[1]. HD is an alkylating agent having antimitotic, mutagenic, carcinogenic and cytotoxic activities in addition to blister forming action[2].

The conventional treatment for HD poisoning involves physicochemical (decontamination) and medical (symptomatic therapy) measures. Earlier the beneficial effect of flavonoids as a therapeutic measure[3] against HD toxicity was reported. Besides, the therapeutic efficacies of a number of antidotes were evaluated against HD toxicity elsewhere and it was found to provide only limited protection[4,5]. In the present study, we have examined the efficacy of the treatment with normal saline or glucose-saline against dermally applied HD intoxication in mice. The *raison d'etre* for this approach is the resemblance of HD-induced skin pathology to that of thermal burns and to understand the protective action of saline or glucose-saline along with the blood acid–base status and blood levels of sodium and potassium. Three doses of glucose saline were tried, namely, 2.5, 5.0 and 10.0 mg/kg. Among these, 5.0 mg/kg was found to be the most effective dose and has, therefore, been taken to be the optimum dose.

2. MATERIALS AND METHODS

All the chemicals used were of analytical grade (E. Merck or BDH), while HD was synthesised in the Chemistry Division of this establishment; its purity as checked by gas chromatography, was found to be 98 per cent.

Male Swiss albino mice of 20-25 g body weight, bred and maintained in this Establishment, were used. The percutaneous LD_{50} of HD (in polyethylene glycol 300) was 154.7 mg/kg (95 per cent confidence limit; 136.3-175.5)[3]. HD (40 mg/ml) was prepared fresh by diluting in polyethylene glycol 300 and 75-100 μl volume was applied uniformly on the back of the mice on a circular area of 1.5 cm diameter, after closely clipping the hair[3,6]. For Groups 3 and 4, a single dose (5 ml/kg) of either saline or glucose-saline was administered intraperitoneally immediately after applying HD, while

Received 2 February 1993

in Groups 5 and 6, the saline or glucose-saline was administered on the second and third day also (total three doses). The mortalities of the animals were recorded till the 7th day post-treatment.

In the second study, the effect of dermally applied HD on blood haemoglobin (*Hb*), acid-base status and blood sodium and potassium levels at 1st, 3rd and 7th day of HD intoxication was elicited. Blood was drawn anaerobically from the orbital plexus of mild ether anaesthetised mice for the analysis of blood *Hb*, acid-base status and sodium-potassium levels using ABL 300 *KNa* acid-base laboratory equipment (Radiometer, Denmark). Finally, the protective effect of saline or glucose-saline treatment (a single dose immediately after HD application) on these parameters was studied on 3rd day of HD intoxication.

Statistical analysis of the data was done by Student's *t*-test and a probability of less than 0.05 was taken as significant.

3. RESULTS AND DISCUSSION

Following HD intoxication, the morbidity and mortality increased gradually over the first week. Basically, a symptomatic treatment has been resorted to in HD poisoning. HD injury was found to be healed in a similar manner, though at a slower rate than in the case of thermal burns[7]. It has been suggested that HD injury can be treated in the same way as thermal burns[7,8]. Accordingly, in the present study, we have examined the protective efficacy of saline or glucose-saline treatment against HD poisoning.

Table 1. Efficacy of saline or glucose-saline treatment on survival of mice intoxicated with sulphur mustard dermally.

Treatment	Day after treatment/% survival*						
	1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
HD alone	100	100	100	100	67	50	33
HD+saline† (single dose)	100	100	100	100	100	83	83
HD+glu-sal†100 (single dose)	100	100	100	100	83	83	
HD+saline (3 doses)	100	100	100	100	100	100	83
HD+glu-sal (3 doses)*	100	100	100	100	100	100	100

* [(No. survived/No. treated) × 100]; number of mice treated, n = 6 in each group † dose, 5 ml/kg.

The protection afforded by both saline and glucose-saline treatments against HD intoxication in mice is obvious from the data presented in Table 1. A single dose treatment of either saline or glucose-saline offered similar protection, the extent of survival increasing to 83 per cent as against 33 per cent survival

Table 2. Effect of dermally applied HD on blood haemoglobin (*Hb*), acid-base status, sodium and potassium levels at different periods after intoxication and the influence of saline or glucose-saline treatment on these parameters.

Group	*Hb* (g%)	*pH*	pO_2 (mm *Hg*)	% Sat	pCO_2 (mm Hg)	HCO_3 (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)
Control (Vehicle)	15.4 ±0.31	7.21 ±0.02	33.8 ±4.44	47.3 ±7.57	66.3 ±3.30	24.7 ±0.89	148 ±2.5	5.8 ±0.32
HD applied on								
1st day	18.2‡ ±0.32	7.18 ±0.01	42.3 ±1.93	63.0 ±3.22	59.8 ±1.96	21.6 ±0.68	147 ±1.5	6.8 ±0.21
3rd day	18.1‡ ±0.27	7.23 ±0.01	48.6‡ ±3.19	72.3‡ ±4.08	63.3 ±1.65	26.6 ±0.89	154 ±2.2	6.4 ±0.20
7th day	18.3‡ ±0:66	7.23 ±0.01	47.5‡ ±4.54	73.8‡ ±4.98	66.2 ±2.13	27.0 ±0.78	155 ±1.4	5.7 ±0.27
Treatment								
Saline, 3rd day	14.3 ±0.43	7.22 ±0.04	42.4 ±5.05	64.2 ±7.61	58.9 ±2.41	24.0 ±2.68	157 ±2.8	5.7 ±0.35
Glu-saline 3rd day	14.2 ±0.68	7.25 ±0.01	43.6 ±5.63	67.3 ±6.47	56.5 ±1.67	24.6 ±0.83	159 ±1.9	5.6 ±0.20

‡ Statistically significant as compared to control group.

without treatment. A higher dose (10 ml/kg) could not augment the protection (data not given). However, complete protection of animals was achieved by extending the treatment by another two consecutive days.

Dermally applied HD caused haemoconcentration and has not altered much the acid-base and electrolyte status of blood over 7 days, except for a significant increase in pO_2 and % oxygen saturation of *Hb* on 3rd and 7th day of HD intoxication (Table 2). This may be attributed to the possible increase in the oxygen affinity of *Hb* due to alkylation of amino-terminal, as *N*-carbamylation of amino-terminal of Hb was shown to increase oxygen affinity[9]. Saline or glucose-saline treatment could ameliorate the haemoconcentration as well as normalise pO_2 and % saturation (Table 2). However, the mechanism of reversal of the toxic effect by saline or glucose-saline is not clear as yet.

The results of the present study demonstrate the utility of saline and glucose-saline treatment in providing protection against HD poisoning in mice, probably through the replenishment of fluid loss and the electrolyte lost locally.

ACKNOWLEDGEMENTS

The authors are grateful to Dr RV Swamy, Director, Defence Research and Development Establishment, Gwalior, for his keen interest and helpful suggestions during the study. They also thank Dr RC Malhotra of Synthetic Chemistry Division for the supply of sulphur mustard.

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