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DOSE RESPONSE OF SULPHUR MUSTARD : BEHAVIORAL AND TOXIC SIGNS IN RATS

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Abstract : The present study elucidates the behavioral and toxic signs in rats following dermal application of sulphur mustard (SM). Graded doses of SM (0.10, 0.25, 0.50, 0.75 and 1.0 LD_{50}) were topically applied to male Wister rats. The body weight as well as behavioral/toxic signs and symptoms were recorded at 1, 2, 3, and 4th day after application of SM. Sulphur mustard consistently decreased body weights of rats in a dose and time dependent manner with maximum decrease on 3rd day post treatment. Sedation and diarrhea were significant in response to doses of SM intoxication in rats. It is concluded that the body weight, sedation and diarrhea may be used as a reliable parameter in evaluating SM intoxication. It is also suggested that hydration and hypertonic saline must be used as a rescue agent within 1–3 days after exposure to SM.

Key words : sulphur mustard, dermal intoxication, behaviour, signs, symptoms, rats

INTRODUCTION

Sulphur mustard (bis-2-chloroethyl sulphide) is a potent vesicant which has been used as a chemical warfare agent since World War I and recently during the Iran-Iraq conflict (1, 2). It is an alkylating agent and is mutagenic, carcinogenic and cytotoxic (1, 3, 4). It causes clinical symptoms after penetration through skin, inhalation, absorption from the eyes, or through ingestion of contaminated food.

Sulphur mustard is lipophilic in nature (5) and was found to reach systemic circulation and various organs in humans after exposure (6). The levels of mustard in various body tissues organs in humans after its exposure was found to be concentrated in brain and body fat (6). SM has been

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known to produce blisters on the skin in humans (7). However, systemic toxicity has also been documented in experimental animals (8–11), Elsayed et al (8) showed the translocation of mono-functional SM to the mouse brain after subcutaneous injection, thus causing oxidative stress. Recently, we have shown that sublethal dose of SM inhibted antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activity in blood cells (RBC, WBC and platelets) and body tissues (liver, spleen and brain) indicating potential oxidative stress to vital organs/tissues of rats (12). Effects of chronic exposure of low dose of mustard gas in humans have been shown to cause loss of taste and smell, nose bleed, sore throat, chest pain, wheezing and dyspnea. However, there is little information available on dose-response relationship to behaviour aspect. The present study deals with the behavioral/toxic clinical symptoms in rats exposed dermally to graded doses of SM at different time intervals.

METHODS

Sulphur mustard was synthesized in the Synthetic Chemistry Division of the Defence Research and Development Establishment, Gwalior and its purity was found to be >95% as analyzed by gas chromatography.

Adult male albino Wistar rats (105–120 gm) were randomly divided into six groups of 6 animals each. They were housed in polypropylene cages (3/cage) and dust free rice husk were used as bedding material. They were maintained at 25 ± 3°C and provided food (Lipton Feed, India) and water *ad libitum*. After closely clipping the hair (1 cm^2 area). SM was applied to the skin of rats as described earlier (12) and as follows :

Group I : Control (saline treated)

Group II : Sulphur mustard 19.6 mg/kg (0.1 LD_{50})

Group III : Sulphur mustard 49 mg/kg (0.25 LD_{50})

Group IV : Sulphur mustard 98 mg/kg (0.50 LD_{50})

Group V : Sulphur mustard 147 mg/kg (0.75 LD_{50})

Group VI : Sulphur mustard 196 mg/kg (1 LD_{50})

The LD_{50} value of SM in rats by dermal route is equivalent to 196 mg/kg (12). The animals were weighed daily between 1000–1100 hrs and their weights were recorded. The animals were carefully observed for various behavioral and toxic symptoms (diarrhea, sedation, righting reflex, lacrimation, stickiness of eyes, salivation, ulceration of mouth, reddening of skin, inflammation of fore limb and piloerection) by two independent investigators daily for a period of 4 days. The scoring for all the parameters was performed on a 5-point scale as normal (0), slight (1), moderate (2), severe (3) and very severe (4). The investigators alternated the handling so that individual subjects were not handled by the same person 2 days in a row. Gloves were worn during each handling session, and

care was taken by each investigator to handle each animal in a consistent fashion.

The data were analyzed statistically by Freidman's test, a two-way non-parametric analysis of variance (13). The source of variation with time has been controlled by analyzing the data for comparison between treatments within time periods and X^2 distribution has been used with K-1 degrees of freedom.

RESULTS

Results presented in Table I show the dose as well as time dependent changes in body weight of rats exposed to SM. The results indicate that SM consistently decreased body weight of rats in a dose and time dependent manner. The decrease in body weight was more pronounced at day 3, however, at day 4 the rats died at higher doses of SM (0.75 and 1.0 LD_{50})

The behavioral changes and toxic symptoms after dermal application of SM in rats are depicted in Table II. The results indicate that sedation and diarrhea were the only significant ($P<0.05$) responses of SM intoxication among the behavioral and toxic symptoms observed in rats. The severity of these symptoms were dose and time dependent. Among other prominent toxic symptoms exhibited by rats exposed to SM were stickiness of eye, inflammation of limbs, lacrimation, piloerection and salivation. The animals died at higher doses on day 4 after application of SM.

DISCUSSION

Sulphur mustard is a very persistent chemical agent that easily penetrate clothing and skin. It usually causes clinical symptoms in humans after the liquid penetrates the skin or the vapour is inhaled (1). However, there are great discrepancies in sensitivity of individuals to SM (14). Experimental studies in laboratory animals revealed skin lesions (blister formation) which are different from human skin blisters (9–12, 15). We observed skin lesions in rats 24 hrs after dermal application of

TABLE I : Effect of sulphur mustard (SM) on body weight in rats.

Treatment	*Body weight (% of initial)*			
	Day 1	*Day 2*	*Day 3*	*Day 4*
Control	103.5±1.2	107.2±2.1	110.0±3.3	115.2±3.9
SM 0.10 LD_{50}	101.2±2.7	101.1±1.7[a]	100.3±2.6[a]	89.5±3.1[ab]
SM 0.25 LD_{50}	100.7±2.3	99.2±0.8[a]	97.6±1.5[a]	93.1±1.8[a]
SM 0.50 LD_{50}	99.3±2.1	95.5±1.5[a]	91.6±2.0[ab]	86.3±1.9[ab]
SM 0.75 LD_{50}	96.2±2.6[a]	90.4±1.5[a]	85.0±2.2[ab]	dead
SM 1.0 LD_{50}	92.3±1.5[a]	84.7±1.6[ab]	75.6±1.9[ab]	dead

[a]=$P<0.05$ compared to control
[b]=$P<0.05$ compared to day 1.

TABLE II : Effect of dermally applied sulphur mustard on scores of behavioral and toxic symptoms in rats at different time intervals. Scoring System: 0 = Normal; 1=Slight; 2=Moderate; 3=Severe; 4=Very Severe. The data score is mean ± S.E. of 6 rats in each group. NS = Not significant.

Day	*Dose of Sulphur mustard (LD_{50})*						
	0.10	0.25	0.50	0.75	1.0	X^2	P
			DIARRHEA				
1	1.00 ± 0.00	1.17 ± 0.17	1.50 ± 0.22	1.67 ± 0.33	2.00 ± 0.36		
2	1.17 ± 0.17	1.50 ± 0.22	1.83 ± 0.31	2.00 ± 0.36	2.33 ± 0.21		
3	1.33 ± 0.21	1.67 ± 0.21	2.17 ± 0.31	2.83 ± 0.17	4.00 ± 0.00		
4	2.17 ± 0.17	2.33 ± 0.21	3.00 ± 0.00	Dead	Dead	38.54	<0.05
			SEDATION				
1	1.00 ± 0.00	1.33 ± 0.21	2.00 ± 0.00	2.33 ± 0.21	2.50 ± 0.22		
2	1.67 ± 0.21	1.83 ± 0.17	2.17 ± 0.17	2.67 ± 0.21	2.67 ± 0.21		
3	2.00 ± 0.00	2.17 ± 0.17	2.33 ± 0.21	3.00 ± 0.22	3.83 ± 0.17		
4	2.17 ± 0.17	2.33 ± 0.21	3.5 ± 0.00	Dead	Dead	44.11	<0.01
			RIGHTING REFLEX				
1	1.33 ± .21	1.33 ± 0.21	1.33 ± 0.21	1.67 ± 0.21	1.67 ± 0.21		
2	1.33 ± 0.21	1.17 ± 0.17	1.50 ± 0.22	2.00 ± 0.00	2.00 ± 0.00		
3	1.50 ± 0.22	1.50 ± 0.22	1.83 ± 0.17	2.17 ± 0.17	2.17 ± 0.17		
4	1.33 ± 0.21	1.50 ± 0.22	2.17 ± 0.17	Dead	Dead	11.68	NS
			LACRIMATION				
1	1.50 ± 0.22	1.83 ± 0.17	1.50 ± 0.22	1.83 ± 0.17	2.00 ± 0.26		
2	1.83 ± 0.17	2.00 ± 0.26	2.00 ± 0.26	2.17 ± 0.31	2.67 ± 0.21		
3	2.00 ± 0.00	2.17 ± 0.17	2.33 ± 0.21	3.33 ± 0.21	3.83 ± 0.17		
4	2.17 ± 0.17	2.33 ± 0.21	2.50 ± 0.22	Dead	Dead	22.35	NS
			STICKINESS OF EYES				
1	1.17 ± 0.17	1.33 ± 0.21	1.50 ± 0.22	1.83 ± 0.17	2.00 ± 0.26		
2	1.50 ± 0.22	1.67 ± 0.21	2.00 ± 0.26	2.17 ± 0.31	2.67 ± 0.21		
3	1.83 ± 0.31	2.17 ± 0.17	2.33 ± 0.21	3.33 ± 0.21	3.83 ± 0.17		
4	2.17 ± 0.17	2.33 ± 0.21	2.50 ± 0.22	Dead	Dead	28.65	NS
			SALIVATION				
1	1.50 ± 0.22	1.67 ± 0.21	1.67 ± 0.21	1.83 ± 0.17	2.00 ± 0.00		
2	1.83 ± 0.17	2.00 ± 0.00	2.17 ± 0.17	2.50 ± 0.22	2.67 ± 0.21		
3	2.17 ± 0.17	2.33 ± 0.21	2.50 ± 0.22	2.67 ± 0.21	2.83 ± 0.17		
4	2.50 ± 0.22	2.50 ± 0.22	2.50 ± 0.22	Dead	Dead	20.08	NS
			ULCERATION OF MOUTH				
1	----------	1.00 ± 0.00	1.17 ± 0.17	1.67 ± 0.21	1.83 ± 0.17		
2	1.33 ± 0.21	1.17 ± 0.17	1.17 ± 0.17	2.00 ± 0.00	1.67 ± 0.21		
3	1.33 ± 0.21	1.33 ± 0.21	1.50 ± 0.22	1.33 ± 0.33	2.17 ± 0.31		
4	1.55 ± 0.22	----------	2.00 ± 0.00	Dead	Dead	15.48	NS
			REDDENING OF SKIN				
1	1.67 ± 0.21	1.33 ± 0.21	1.33 ± 0.21	1.67 ± 0.21	2.17 ± 0.17		
2	1.50 ± 0.22	1.33 ± 0.21	1.17 ± 0.17	2.00 ± 0.00	1.67 ± 0.21		
3	1.67 ± 0.21	1.50 ± 0.22	1.83 ± 0.17	2.00 ± 0.26	1.83 ± 0.31		
4	1.67 ± 0.21	2.00 ± 0.00	2.16 ± 0.17	Dead	Dead	16.02	NS
			INFLAMMATION OF FORE LIMB				
1	1.33 ± 0.21	1.50 ± 0.22	1.83 ± 0.21	2.33 ± 0.21	2.50 ± 0.22		
2	1.67 ± 0.21	2.00 ± 0.26	2.33 ± 0.21	2.50 ± 0.22	2.67 ± 0.21		
3	1.83 ± 0.17	2.17 ± 0.17	2.67 ± 0.21	2.50 ± 0.22	2.83 ± 0.17		
4	2.17 ± 0.17	2.33 ± 0.21	2.33 ± 0.21	Dead	Dead	23.64	NS
			PILOERECTION				
1	1.00 ± 0.00	1.33 ± 0.21	1.50 ± 0.22	1.83 ± 0.17	----------		
2	1.17 ± 0.17	1.50 ± 0.22	2.00 ± 0.00	2.17 ± 0.17	2.00 ± 0.26		
3	1.33 ± 0.21	1.50 ± 0.22	2.17 ± 0.17	2.33 ± 0.21	2.33 ± 0.21		
4	1.50 ± 0.22	1.67 ± 0.21	1.83 ± 0.17	Dead	Dead	21.66	NS

SM which is consistent with the above reports. Exposure of humans to mustard gas vapour resulted in skin lesions which developed within 24 hr in majority of cases (16). The data of present study demonstrated the fall in body weight and appearance of behavioral and other toxic signs and symptoms in rats 24 hr after SM exposure at higher doses. Similar changes in body weight were also observed in mice at higher doses of SM (9–11). The progressive fall in body weight was observed in rats upto 4 days, but at high doses (0.75 and 1.0 LD_{50}) the rats died, whereas mice exposed to similar doses showed a significant fall in body weight 3 to 7 days post exposure (9, 11). These results indicate that rats are more sensitive to the lethality of SM as compared to mice. The fall in body weight may be due to increased catabolism (9), loss of body fluid due to diarrhea, and reduction in food and water intake (11) after exposure to SM. The most significant dose-dependent effect of SM on behavioral symptoms in rats was sedation indicating nervous system effect distal to the site of exposure. Previous studies have shown that SM translocated the mouse brain following single subcutaneous injection, thus causing oxidative stress to the nervous system (8). The residue of SM has also been reported to be concentrated in the brain of humans exposed to SM has also been reported to be concentrated in the brain of humans exposed to SM (6) delineating the perturbation of the nervous system. Evidence indicated a relationship between neuropsychiatric complications (mood and anxiety disorders), and traumatic stress disorders and mustard exposure to humans (17, 18). Delayed central nervous system effects of SM in Iranian patients have also been documented (7).

The other significant dose-dependent toxic symptoms of SM exposure observed in rats was diarrhea. The etiology of diarrhea in SM exposed rats may be due to either inactivation of brush border enzymes of the gastrointestinal tract or damage to the mucous membrane. Clinical prognosis of SM exposed Iranian patients revealed large stress ulcers of the duodenum with bleeding throughout the gastrointestinal tract, especially in the descending and sigmoid colon (18). Due to excessive diarrhea, dehydration occurs resulting in collapse. Therefore, the death of rats at higher doses of SM on day 4 post exposure in the present study may be the result from excessive dehydration leading to collapse.

To our knowledge this is the first report of its kind dealing with the severity and duration of the symptoms associated with mustard exposure. The symptoms were proportional to the dose and the duration of SM exposure. Based on these observations, it is suggested that hydration and hypertonic saline must be used as rescue measures against SM intoxication atleast 1–3 day post exposure.

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