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SHORT COMMUNICATION

EFFECTS OF TOPICALLY APPLIED SULPHUR MUSTARD ON TISSUE GLYCOGEN, BLOOD GLUCOSE, LACTATE AND PYRUVATE IN MICE

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Abstract: Bis-2-Chloroethyl sulphide, commonly known as sulphur mustard (SM) or mustard gas, an alkylating agent, is frequently used as a chemical warfare agent. Inhibition of glycolysis has been related to skin injury and cell death. The effects of SM on tissue glycogen, blood glucose, lactate/pyruvate ratio were investigated in the present study. After a single dermal application of 1.0 LD50 SM in mice, a significant hyperglycemia was observed at 24 hr post exposure. There was a corresponding decrease in liver glycogen content, with no alteration in glycogen content of brain, muscles and kidney. Blood pyruvate and lactate levels were not appreciably altered.

Key words: sulphur mustard glycogen glucose lactate pyruvate **mice**

INTRODUCTION

Bis (2–Chloroethyl) sulphide, commonly known as sulphur mustard (SM) or mustard gas, is an alkylating agent and frequently used as a chemical warfare agent (1). It is a potent blistering agent and is antimitotic, mutagenic, carcinogenic and cytotoxic (2). Dixon and Needham studied the effects of SM on different enzymes of skin and showed that there is a close relationship between vesication and inhibition of glycolysis and further suggested that inhibition of hexokinase plays an important role in SM systemic poisoning (3). Depletion of cellular NAD^+ by SM is believed to be the cause of inhibition of glycolysis and interference in the energy metabolism leading to cell death (3, 4). Even though SM was found to reach systemic circulation and various organs in humans after exposure (5), the systemic toxicity studies of SM are mostly lacking. Dermal application of SM induced lipid peroxidation in liver of mice has been reported (6). As inhibition of glycolysis by SM was shown to cause skin injury and cell death (3, 4), the present investigation was undertaken to study the effects of SM on tissue glycogen content, blood glucose and lactate/pyruvate ratio following dermal application.

METHODS

Sulphur mustard was synthesised in the Chemistry Division of Defence R & D Establishment and the purity was >95% as analysed by gas chromatography. All the chemicals used were of analytical grade (E. Merck or BDH or Sigma).

Twenty four male Swiss albino mice, bred in our laboratory, weighing between 20 and 30 g and maintained on a standard diet, were used for the experiments. A single dose of 1.0 LD50 SM, equivalent to 154.7 mg/kg, diluted in polyethylene glycol 300, was applied on the back side of mice after closely clipping the hair (6). Animal body weight was recorded daily. Six animals each were sacrificed after 24 hr, 3 and 7 days post exposure. Blood was collected in heparinized tubes from retro-orbital pluxus before killing the animals. Samples of liver, muscles, kidney and brain

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tissues were also taken for estimation of glycogen content. As controls, animals applied with only the vehicle were used.

Standard spectrophotometric methods were employed to estimate blood glucose (7), blood pyruvate and lactate (Sigma Diagnostic Kit, Procedure No. 726-UV/826-UV). The glycogen content in various tissues was estimated using standard method as described earlier (8).

Statistical analysis of the data obtained was done by Student's 't' test.

RESULTS

Table I provides the percent changes in body weight after dermal application of SM upto 7 days. There was a significant progressive reduction in body weight of mice treated with SM, compared to the control group. Table II shows the effects of SM on blood glucose and glycogen contents of various tissues after dermal intoxication. There was a significant hyperglycemia after 24 hr of SM intoxication, which subsequently returned to normal. There was a significant decrease in the liver glycogen content at 24 hr, which was maintained at 3rd day and 7th day post exposure. The glycogen content of brain, kidney and triceps muscles was not altered.

Dermal application of SM did not cause any appreciable change in blood pyruvate and lactate levels though a non-significant increase was observed in both the parameters on 3rd day without any alteration in lactate/pyruvate (L/P) ratio (Data not shown). A decrease in pyruvate, lactate and L/P ratio was observed on 7th day.

DISCUSSION

Although SM has been known for decades as a powerful chemical weapon to which mass population might be exposed, understanding of its systemic toxicity and mechanism of action is quite limited. Inhibition of various glycolytic and respiratory enzymes (2) and impaired glucose uptake (9) have been observed following exposure to SM. Results of present study indicate that topically applied SM does not affect the systemic carbohydrate metabolism appreciably. The significant elevation of blood glucose level and decrease in liver glycogen content at 24 hr post exposure (Table I) can be attributed to the general toxicant stress, as most of SM induced effects are delayed effects (8,10). The mild non-significant decrease in both blood pyruvate, lactate and L/P ratio was possibly due to delayed inhibitory effect of SM on glycolysis (2).

This study shows that topically applied SM does not affect systemic carbohydrate metabolism significantly.

TABLE I : Effect of dermally applied SM on body weight at different time intervals in mice.

Group	*Body weight (percent of initial)*						
	1	*2*	*3*	*4*	*5*	*6*	*7 days*
Control (vehicle)	103.4 ±0.95	106.3 ±1.28	109.3 ±1.17	113.1 ±0.99	115.2 ±1.37	117.7 ±1.47	119.1 ±1.80
SM (1.0 LD50)	93.5* ±1.40	83.7* ±0.73	77.3* ±1.84	69.4* ±2.34	63.9* ±3.04	62.1* ±0.36	57.1* ±1.33

The values are mean ± SE, n=6; *P < 0.001

TABLE II : Effect of dermally applied SM on blood glucose, and tissue glycogen at different time intervals in mice.

Group	*Blood*	*Tissues glyocogen (mg/g of wet tissue)*			
	glucose (mg/dl)	*Liver*	*Triceps*	*Kidney*	*Brain*
Control	84±4.71	38±2.96	4.6±0.83	2.7±0.36	1.9±0.27
SM (1 day)	132±8.12*	15±0.33*	3.6±0.89	2.0±0.33	2.0±0.38
SM (3 day)	82±10.72	12±3.52*	3.9±0.53	2.1±0.62	1.8±0.31
SM (7 day)	94±8.48	13±2.12*	5.0±0.63	2.4±0.58	1.7±0.40

The values are mean ± SE, n = 6; *P < 0.001

REFERENCES

1. Somani SM, Babu SR. Toxicodynamics of sulfur mustard. *Int J Clin Pharmacol Ther Toxicol* 1989; 27: 419-435.
2. Wheeler GP, Studies related to the mechanisms of action of cytotoxic alkylating agents. *Ann Rev Cancer Res* 1962; 22 : 651-688.
3. Dixon M, Needham DM. Biochemical research on chemical warfare agents. *Nature* 1946; 158 : 432-438.
4. Gross CL, Meier HL, Papirmeister B, Brinkley BF, Johnson JB. Sulphur mustard lowers nicotinamide adenine dinucleotide concentrations in human skin grafted to athymic nude mice. *Toxicol Appl Pharmacol* 1985; 81 : 85-90.
5. Drasch G, Kretschmer E, Kanert G, Von Meyer L. Concentration of mustard gas [Bis (B-chloroethyl) sulphide] in the tissues of victim of a vesicant exposure. *J Forensic Sci* 1987; 32 : 1788-1793.
6. Vijayaraghavan R, Sugendran K, Pant SC, Husain K, Malhotra RC. Dermal intoxication of mice with bis (2-chloroethyl) sulphide and the protective effect of flavonoids. *Toxicology* 1991; 69 : 35-42.
7. Hultman E. Rapid specific method for determination of aldosaccharides in body fluids. *Nature 1959 ; 183 : 108-109.*
8. Husain K, Matin MA. Changes in the level of glycogen and certain enzymes in malathion treated hyperglycemic rats. *Adv Bios* 1986; 5: 87-90.
9. Mol MAE, Van De Ruit AMBC, Kluivers AW. NAD^+ levels and glucose uptake of cultured human epidermal cells exposed to sulfur mustard. *Toxicol Appl Pharmacol* 1989; 98 : 159-165.
10. UriWormser. Toxicology of mustard gas. *Trends in Pharmacol Sci* 1991; 121: 164–167.