Effect on Physiological Variables & Urinary Metabolites following a Single Dermal Application of Sulphur Mustard in Rats

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ABSTRACT

The effect of single dermal application of different doses of sulphur mustard (2,2'-dichlorodiethyl sulphide, SM), i.e. 5, 10 and 20 mg/kg body weight on some physiological variables, viz., body weight, intake of food and water, and excretion of excreta and urine was studied for one week. Levels of urinary metabolites, uric acid, urea, protein, creatine and creatinine were measured at 0, 1, 2, and 7 days in albino rats of Wister strain. A progressive dose-dependent fall in body weight was observed from day 1 onwards after SM application. Feed and water intake were reduced significantly. Excretion of urine and excreta was also affected adversely. The level of uric acid, a metabolite of purine bases, increased 24 hr post-exposure, in a time and dose-dependent manner. Excretion of urea decreased and the level of creatine increased drastically on 7th day. However, urinary excretions of protein and creatinine did not change. The study shows that single application of SM in rats induces a catabolic state.

1. INTRODUCTION

Sulphur mustard (2,2'-dichlorodiethyl sulphide, SM) is a potent vesicant and an alkylating agent[1]. It has been stockpiled for use as a chemical warfare agent in a number of countries and reports are available of its recent use[2]. Knowledge of the biological fate of SM is important to develop forensic methods to confirm and detect mustard poisoning in victims of chemical attacks. There are several reports[3-5] on distribution, metabolism and elimination of SM. Sulphur mustard reacts in an aqueous phase with compounds containing nucleophilic functional groups like amino, sulfhydryl, carboxylic and hydroxyl groups in proteins. The toxicity of SM has been assumed to result from interaction with one or more different cell constituents.

Sulphur mustard belongs to a class of agents which readily alkylate cellular DNA, RNA and proteins[6,7]. The DNA appears to be a critical target and alkylation of nucleic acid is an early molecular event in SM-induced toxicity. Alkylation of DNA by SM adducts occurs at N-7 position of guanine and at N-3 position of adenine[8]. Bifunctional adducts involve at N-7 position two adjacent guanine or two guanine groups situated on opposite strands[9], which leads to DNA strand break. The DNA breaks caused by mustard activate the chromosomal enzymes poly (ADP-ribose) polymerase. Activation of poly (ADP-ribose) polymerase consumes more available NAD^+ as its substrate to ADP ribosylate, a variety of nuclear proteins, which causes depletion of cellular NAD^+, inhibit glycolysis, interfere with energy metabolism and eventually cause cell death[10,11].

Several antidotes for SM have been screened, but they have found to give only marginal protection. Decontamination of SM from the skin

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is found to be the most efficient method for reducing toxicity.

In the present investigation we have studied the effect of single dermal application of SM to rats on physiological variables and urinary metabolites at different time intervals.

2. MATERIALS & METHODS

2.1 Chemicals

The purity of SM, synthesised in the Establishment, was > 95 per cent, as estimated by gas chromatography. All other chemicals used were of analytical grade (Sigma/Emerck/BDH/SRL).

2.2 Animals

Adult female albino rats of Wister strain, bred at the Defence Research & Development Establishment (DRDE), Gwalior, weighing 120-140 g were used. The rats were maintained on standard (AMRUT) rodent chow. Food and water were given *ad libitum*.

2.3 Determination of LD_{50}

The median lethal concentration (LD_{50}) with 95 per cent confidence limits of SM was determined by the method of Gad and Wiel[12]. For this, the hair on the back of the rat were closely clipped and SM was applied on the skin. The animals were observed for mortality till 14th day.

2.4 Treatment

The animals were divided into four groups of six each. The SM was diluted in PEG-300 and three different doses of SM, 5, 10 and 20 mg/kg body weight, were applied uniformly on the back of the rats on a circular area of 2 cm diameter, after closely clipping the hair of rats of groups 2, 3 and 4, respectively. In group 1 (control) animals, only 0.1 ml of PEG-300 was applied in the same manner.

2.5 Physiological Variables

The animals were kept individually 1 day prior to exposure in a metabolic cage. Body weight, feed intake, water intake, urine volume and excreta volume (solid matter) of animals of all the groups were recorded daily.

2.6 Biochemical Analysis

Urine samples were collected on 0, 1, 2, and 7 days of exposure and urine analysis was carried out for uric acid, urea, protein creatine and creatinine. Uric acid was determined by phosphotungstic acid method[13] and urea by diacetyl monoxime method[14]. Creatine and creatinine were estimated by picrate method[15] and protein by Folin's method[16].

2.7 Statistical Analysis

Repeated measures of ANOVA with Dunnett's multiple comparison were used to evaluate the differences between the control and experimental groups. For this, SigmaStat, Version 1.0 was used. A probability of less than 0.05 was taken as statistically significant.

3. RESULTS & DISCUSSION

LD_{50} values for dermal administration of SM to female rats are presented in Table 1. The results indicate that LD_{50} value decreases with increase in the observation period.

Dermal administration of SM to rats affects the physiological variables. SM-induced significant decrease in body weight in a time and dose-dependent manner (Fig. 1). Decrease in body

Table 1. LD_{50} values for sulphur mustard in rat

Period of observation	LD_{50} (mg/kg)	95 % confidence limit
1 day	320.00	203.51 - 503.18
3 days	40.00	22.98 - 69.63
7 days	12.60	5.57 - 28.52
14 days	5.95	3.27 - 10.82

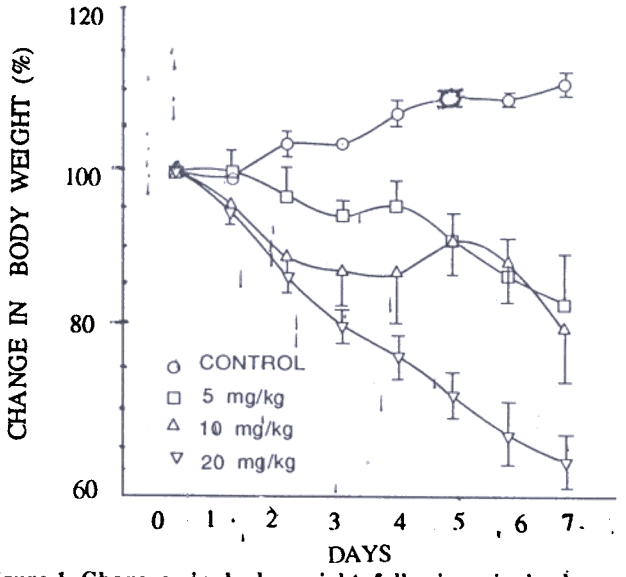

Figure 1. Changes in body weight following single dermal application of S M in female rat. Values are mean ± S E of 6 animals. Statistically significant by RM ANOVA. Body weight: Dose P < 0.001, day after exposure P < 0.001.

weight continued till death of the animals. Decrease in body weight was a consistent observation. Dose-dependent decrease in the body weight of mice after SM application was reported by Venkateswaran, *et al*[17]. This reduction was prominent in the group that was administered SM in higher doses. The reduction may be due to change in appetite and intestinal absorption, or to the arrest of cellular proliferation in gut epithelium, because SM is an antimitotic and cytostatic agent[17,18]. Food intake and excreta volume also decreased significantly 24 hr after SM administration at all dose levels. This decrease may be due to change in appetite or physical stress exerted by SM. Water intake and urine volume were also affected adversely (Fig. 2). There is decrease in food and water intake at all dose levels in comparison to the control animals. Lack of dose-dependent response with regard to food and water intake may be due to SM induced physiological changes at both low and high dose levels.

The results of the present study indicate that purine metabolism increases after SM exposure,

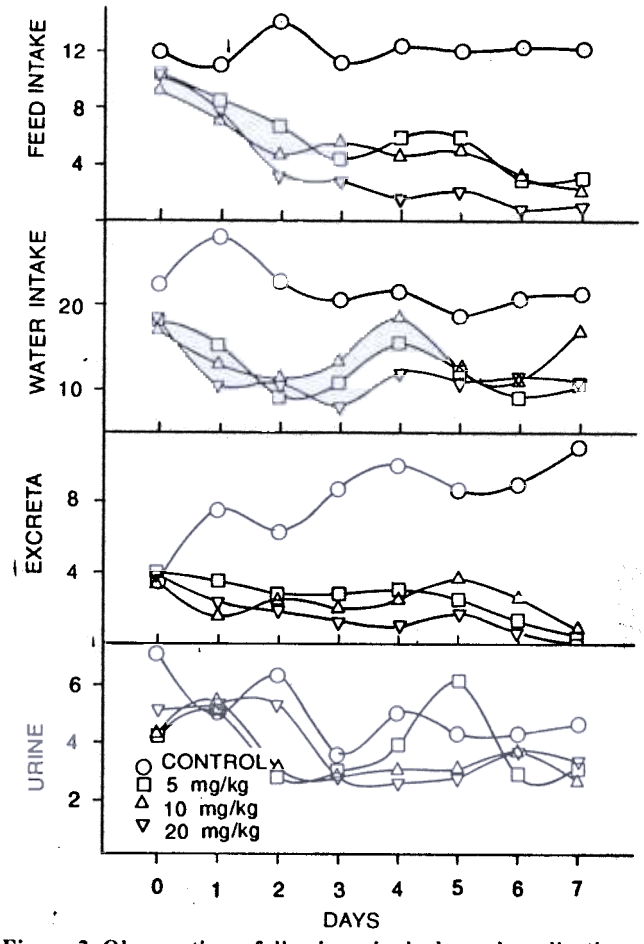

Figure 2. Observations following single dermal application of SM in female rat. Food intake and excreta expressed as g/24 hr and water intake and urine volumes as ml/24hr. Values are mean of 6 animals. Statistically significant by RM ANOVA. Food intake: Dose P < 0.001, Day after exposure P < 0.001. Excreta: Dose P < 0.01, Day after exposure P < 0.001. Water intake: Dose P < 0.001, day after exposure P < 0.001, Urine: Dose NS, day after exposure P < 0.01.

resulting in increased excretion of uric acid. Increased excretion of uric acid was observed 24 hr post-exposure and continued till 7th day in a dose and time-dependent manner (Fig. 3). Increase in uric acid level was due to increased apurination of DNA, as SM is a known DNA alkylating agent[1]. Bifunctional adducts are involved at N-7 position of two adjacent guanine groups or two guanine groups situated on opposite strands[9], which leads to apurination. Apurinated bases were first metabolised to hypoxanthine, then to xanthine and finally to uric acid. Increase in uric acid level was not associated with starvation. In starvation, uric acid level decreases[19]. There was a decrease in the

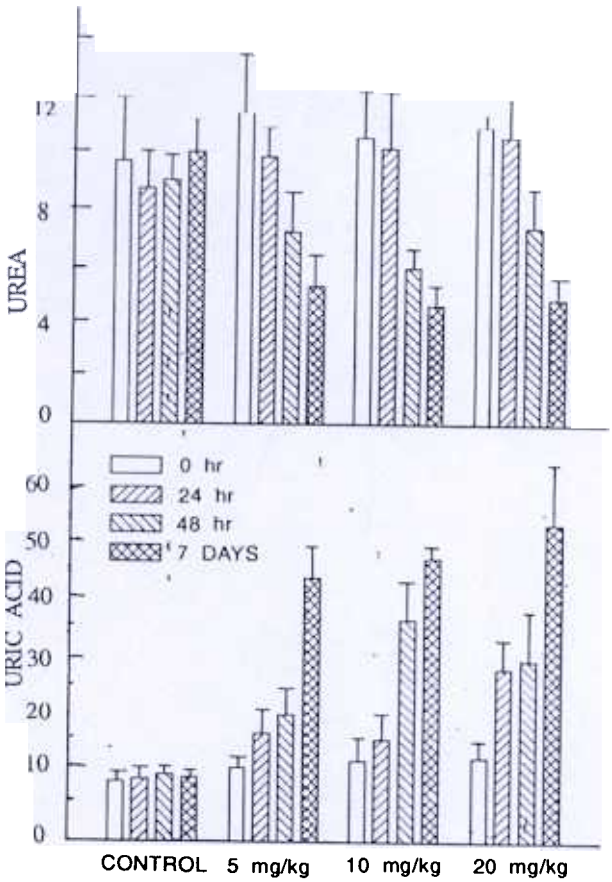

Figure 3. Effect of SM following single dermal application on urinary urea (g/24hr) and uric acid (mg/24hr). Values are mean ± SE of 6 animals. Statistically significant by RM ANOVA. Urea: Dose NS, day after exposure $P < 0.01$. Uric acid: Dose $P < 0.01$, day after exposure $P < 0.001$.

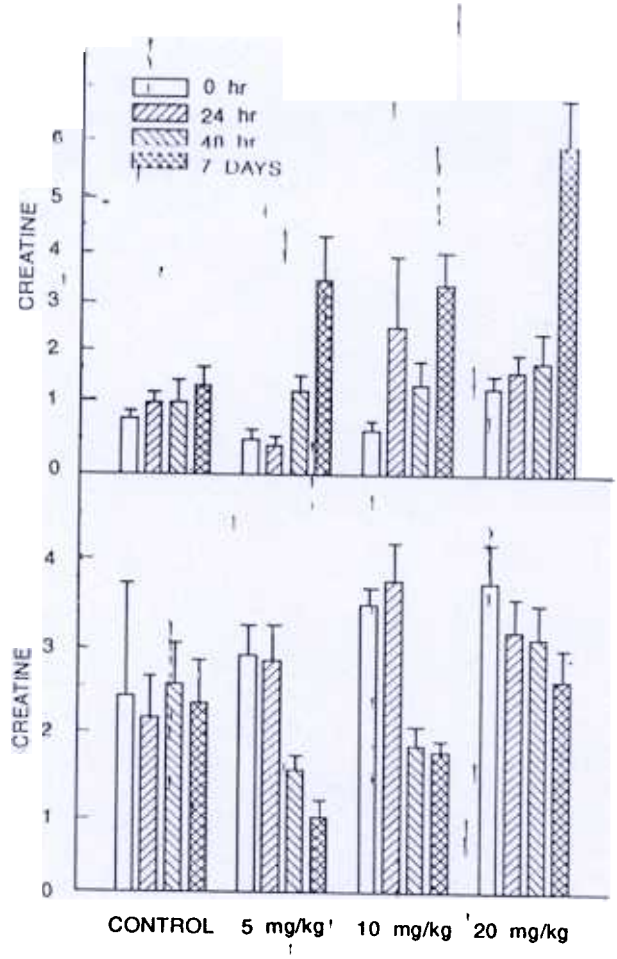

Figure 4. Effect of SM following single dermal application on urinary excretion of creatine (mg/24hr) and creatinine (mg/24hr). Values are mean ± SE of 6 animals. Statistically significant by RM ANOVA. Creatine: Dose NS, day after exposure $P < 0.001$. Creatinine: Dose NS, day after exposure $P < 0.05$.

level of urea (Fig. 3). Most of the ammonia formed by deamination of amino acids in the liver is converted to urea, which is excreted in urine[19]. Decreased urinary excretion may be due to pronounced kidney and liver disorders or decreased urea formation and decreased power of these organs to eliminate urea[19]. Excretion of urinary creatine (Fig. 4) increased significantly after 7th day of exposure. This increase may be associated with disintegration of muscular tissue or fasting, as food consumption is significantly reduced in these conditions. However, levels of creatinine (Fig. 4) and protein (data not given) were not affected. The result of the present study shows that a single dermal application of SM in rat induces a catabolic state.

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