J. Biosci., Vol. 20, Number 1, January 1995, pp 29–33. © Printed in India.

Studies on urine and tissues of rats, guineapigs and mice exposed to sulphur mustard using mass spectrometry

D N TRIPATHI*, K SUGENDRAN, R C MALHOTRA,
A BHATTACHARYA and S DAS GUPTA
Defence Research and Development Establishment, Gwalior 474 002, India

Ms received 11 March 1994; revised 22 August 1994

Abstract. Urine and tissues (brain, liver, kidney, fat and triceps muscles) from rodents (rats, guinea pigs and albino mice) treated with sulphur mustard percutaneously were examined for the presence of sulphur mustard and/or metobolites using electron impact direct inlet and GC-mass spectrometry. Sulphur mustard and thiodiglycol sulphoxide were not detected in these samples even after application of massive doses. However, thiodiglycol was identified in urine only.

Keywords. Sulphur mustard; mass spectrometry; urine; tissues; thiodiglycol, thiodiglycol sulphoxide.

1. Introduction

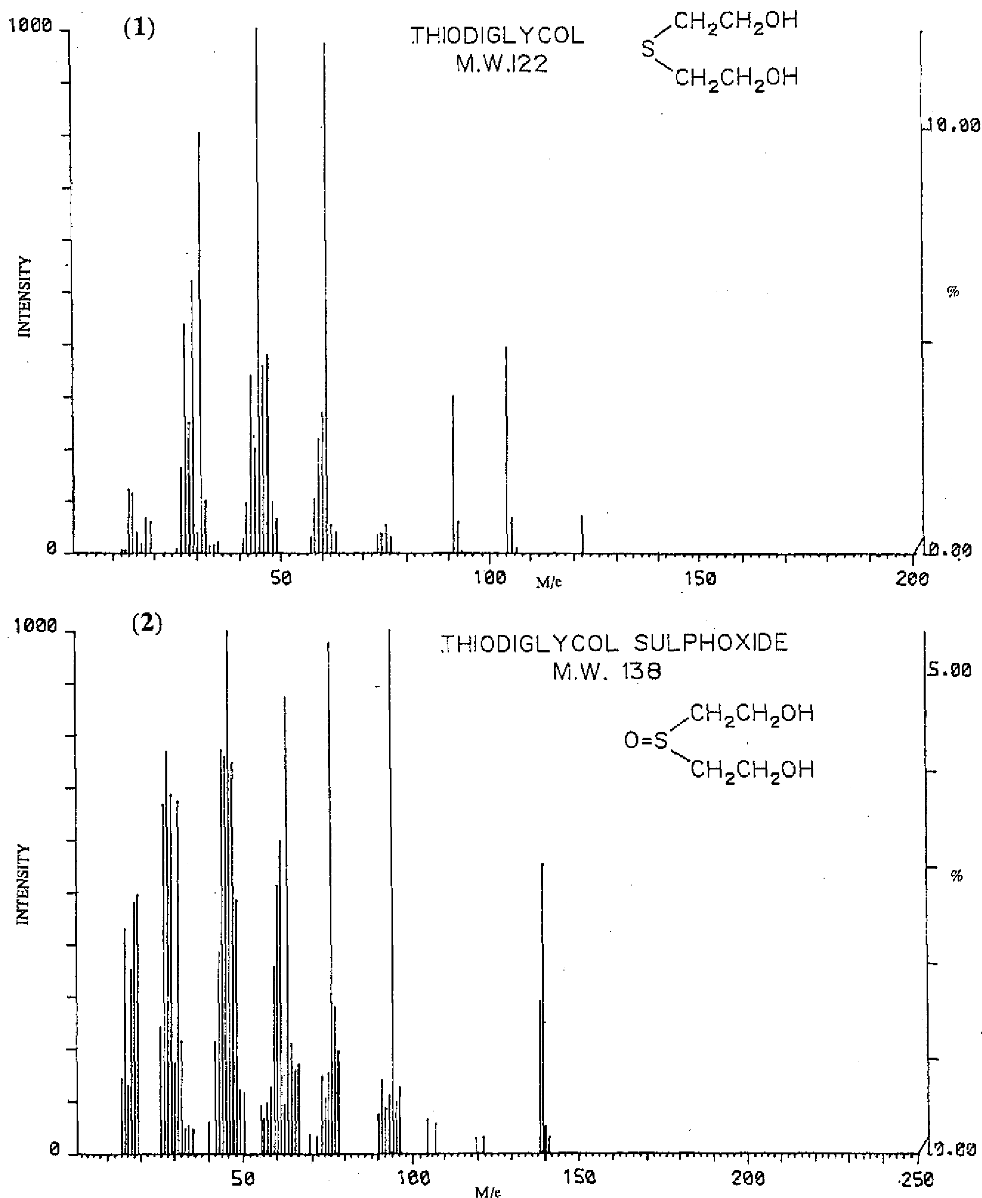
Sulphur mustard (SM) chemically known as 1,1'-thiobis (2-chloroethane) is a potent vesicant that produces extensive injuries at the site of exposure. It was first synthesized in 1822 and then used as a chemical weapon during World War I, and represents the oldest known alkylating agent. While there is an extensive literature on the toxicology and mechanism of action of SM (Somani and Babu 1989), there is a paucity of reliable information on its fate in biological systems. Renewed interest regarding its biological role appeared after the Iran-Iraq conflict in 1984. There were allegations by both countries that SM was used. Due to lack of standard procedures for early detection many investigators were prompted to develop analytical methods for detection of this compound. SM was analysed earlier in this laboratory using electron impact mass spectrometry (Tripathi *et al* 1984). Many workers have reported identification of SM and its metabolites by studying urine samples and tissues of SM exposed victims (Vycudilik 1985, 1987; Wils *et al* 1985, 1988; Drasch *et al* 1987). This paper reports results of analysis with mass spectrometry primarily to verify the presence of intact SM or its major metabolic products like thiodiglycol sulphoxide (Black and Read 1991; Black *et al* 1991) in urine and various tissues of rodents (mice, rats and guinea pigs) after percutaneous SM application.

2. Materials and methods

SM was synthesised in the DRDE and was 98% pure as checked by gas chromatography, IR, NMR, and GC-mass spectrometry.

[†]Corresponding author.

Thiodiglycol (E Merck) was obtained after distillation in our laboratory. Thiodiglycol sulphoxide was prepared by the direct reaction of thiodiglycol with concentrated nitric acid at room temperature. It is an exothermic reaction and the solution turns green. The green solution on dilution and neutralization gives a white precipitate of thiodiglycol sulphoxide. Purity characterization was done using HPLC, elemental analysis and spectroscopic methods. Mass spectral analysis of thiodiglycol and its sulphoxide was confirmed by comparing mass spectral data of pure compounds. Typical mass spectra are given in figures 1 and 2.



Figures 1 and 2. Mass spectrum of **(1)** thiodiglycol and **(2)** thiodiglycol sulphoxide.

2.1 *Animals*

Swiss male albino mice (20–25 g) were used on tissue distribution while male Wistar rats (150–200 g) and male English guinea pigs (1500–1800g) were used for studies in urine as well as tissue distribution of SM. All these animals were bred in our laboratory animal facility. Mice and rats were housed in polypropylene cages for collection of urine for 24 and 48 h. The animals (rats and guinea pigs) were kept individually in metabolic cages.

2.2 *Treatments*

Three different studies were carried out after determining the percutaneous LD_{50} of SM as described by Black *et al* (1991) and Vijayaraghavan *et al* (1991) *i.e.,* LD_{50} = 169·2 mg/kg for rats and 154·7 mg/kg for albino mice.

In the first study 6 male guinea pigs were used. The animals were randomly divided into 3 groups of 2 animals each. Group I served as control, while group II animals were treated with a 50 µl, 0·5 LD_{50} undiluted SM applied on a clipped area of 1·5cm diameter, group III animals were treated in a similar way except that the dose was 100 µl. After this the animals were kept individually in stainless steel metabolic cages. Urine samples were collected after 24 and 48 h, processed and extracted with diethyl ether for SM thiodiglycol in urine (Vycudilik 1987) dichloromethane for SM in tissues (Drasch *et al* 1987) and chloroform : methanol (50:50) for thiodiglycol sulphoxide both in tissues and urine (Black and Reed 1991). Similarly urine samples were collected from rats treated with 1 LD_{50} of SM and processed for analysis.

In the second study six male albino mice (Swiss) weighing 20–30 g were treated with 1 LD_{50} of SM while six animals were treated with the vehicle *i.e.*, polyethyleneglycol 300. Animals were sacrificed after 24 h post treatment. Samples of whole brain, liver, fat and triceps muscles were taken and extracted for identification of SM and its metabolites. Similarly 6 male albino rats weighing 150–200 g were treated with 1 LD_{50} of SM and after 24 h animals were sacrificed and the tissues mentioned above were taken and processed for mass spectral studies. The above study was also extended to guinea pigs in which 50 and 100 µl of undiluted SM was applied on a clipped area of 1·5 cm diameter. All the animals were sacrificed after 24 h post treatment. Samples of brain, liver, fat and triceps muscles were taken immediately and extracted.

In the third study a massive dose (4–6 LD_{50}) of SM was applied and the experiments were repeated as mentioned earlier. Surviving animals were sacrificed after 4 h.

2.3 *Recording of mass spectra*

A JEOL JMS DX-300 mass spectrometer coupled with JMAA–2000 data analysis system was used for the mass spectral studies. The mass spectra were recorded in EI Mode (70 ev) for direct inlet probe (temperature programmed). The temperature was programmed from 20° to 150°C with heating rale 64°C/min. The ion source chamber temperature was maintained at 80°C. For GC-mass spectrometric analysis helium (Matheson purity) was used as carrier gas with flow rate of 40 ml/min. The

injector, inlet and separator temperatures were kept at 150°, 200° and 200°C respectively. OV-17 packed column (5%) was used for this study. The column temperature was regulated from 20° to 200°C with heating rate of 8°C/min. For DI and GC-MS 1 μl of the sample was injected.

3. Results and discussion

Vycudilik (1987) and Drasch *et al* (1987) reported the finding of traces of mustard gas in urine and tissues of human victims. Black and Read (1991) obtained thiodiglycol sulphoxide as major metabolite in urine of SM exposed rodent. Therefore our own experiments were carried out to isolate sulphur mustard and its major metabolites from the rodents urine and various tissues. Total weights of tissues taken for this study were liver (3 g), kidney (4 g), triceps muscles (2g), brain (3 g) and volume of pooled urine was 20 ml. Electron impact (70 ev) mass spectrometry and GC/mass spectrometry showed neither a peak for SM at m/z 158 nor its characteristic fragmentation peaks in all the samples. However we could get peaks at m/z 122 ($C_4H_{10}O_2S$) due to thiodiglycol and m/z 105 (thiodiglycol –OH)$^+$ in urine sample. In all other tissues no significant peaks identifying SM or thiodiglycol, tihiodiglycol sulphoxide could be obtained. Absence of thiodiglycol sulphoxide was confirmed by the absence of molecular ion peak at m/z 138/139 in EI and m/z 139 in isobutane CI and its major fragment at m/z 94 ($MH–CH_4CH_2OH$)$^+$. However *in vitro* experiments with nascent urine spiked with 4 mg/ml of thiodiglycol sulphoxide (1 μl of this was introduced into direct inlet probe of mass spectrometer) gave the above mentioned peaks prominently. This leads to the conclusion that thiodiglycol sulphoxide in the tissues and urine samples metabolite study might be below this level. *In vivo* control samples were analysed parallel to each experiment for comparison.

In the past there were attempts to identify metabolites after SM intoxication (Davison and Rozman 1961; Roberts and Warwick 1963), Davison and Rozman (1961) suggested that the major urinary metabolite following intravenous administration of SM was unoxidized bis-conjugate of mustard with glutathione accounting for 45% of urinary metabolites while 15% was the hydrolysis product thiodiglycol.

Recently Black *et al* (1992) studied metabolism of SM after intraperitoneal administration to rat and found that the major excretion product arising from the hydrolysis of SM is thiodiglycol sulphoxide and not thiodiglycol. However, in our study after cutaneous application of mentioned doses of SM only urine sample gives presence of thiodiglycol. We were unable to detect SM or thiodiglycol sulphoxide both in urine and various tissues. This may be due to a route specific effect of SM administration.

Acknowledgements

We are grateful to Dr R Vaidyanathaswamy and Dr P K Ramachandran for encouragement and fruitful suggestions.

References

Black R M, Brewster K, Clark R J, Hambrook J L, Harrison J M and Howells D J 1992 Biological fate of sulphur mustard 1.1'thiobis(2-chlorocthane) isolation and identification of urinary metabolites

following intraperitoneal administration to rat; *Xenobiotica* **22** 405-418

Black R M and Read R W 1991 Methods for the analysis of thiodiglycol sulphoxide, a metabolite of sulphur mustard in urine using gas chromatography—mass spectrometry; *J. Chromatogr.* **558** 393-404

Black R M, Clarke R J and Read R W 1991 A method for the analysis of 1,1'-sulphony bis[2-(methylsulphinyl)ethane] and l-methylsulphinyl l-2[2-(methylthio)ethylsulphonyl] ethane, metabolites of sulphur mustard in urine using gas chromatography—mass spectrometry; *J. Chromatogr* **558** 405-414

Davison C and Rozman R S 1961 Metabolism of bis β-chloroethyl sulphide (sulphur mustard gas); *Biochem. Pharmacol.* **7** 65-68

Drasch G, Kretschoner E, Kauest G and Meyer L V 1987 Concentrations of mustard gas [Bis(2-chloroethyl)sulphide] in the tissues of a victims of a vesicant exposure; *J. Forensic Sci.* **32** 1788-1792

Roberts J J and Warmick G P 1963 Studies of the mode of action of alkylating agents VI. The metabolism of bis-2-chloroethyl sulphide (mustard gas) and related compounds; *Biochem. Pharmacol.* **12** 1326-1329

Somani S M and Babu S R 1989 Toxicodynamics of sulphur mustard; *Int. J. Clin, Pharmacol. Ther. Toxicol.* **27** 419-435

Tripathi D N, Bhattacharya A and Vaidyanathaswamy R 1984 Mass Spectral identification of bis-(2-chloroethyl)sulphide and related compounds; *Can. Soc. Forensic. Sci. J.* **17** 55-57

Vijayaraghavan R, Sugendran K, Pant S C, Husain K and Malhotra R C 1991 Dermal intoxication of mice with bis(2-chloroethyl) sulphide and the protective effect of flavonoids; *Toxicology* **69** 35—42

Vycudilik W 1985 Detection of mustard gas bis-(2-chloroethyl)sulphide in urine; *Forensic Sci. Int.* **28** 131-136

Vycudilik W 1987 Detection of bis-(2-chIoroethyl) sulphide (yperite) in urine by high resolution gas chromotography mass spectrometry; *Forensic Sci. Int.* **35** 67-71

Wils E R J, Hülst A G and Vanlaar J 1988 Analysis of thiodiglycol in urine of victims of a alleged attack with mustard gas, Part II; *J. Anal. Toxicol.* **12** 15-19

Wils E R J, Hülst A G, De Jong, Verweij and Boter H L 1985 Analysis of thiodiglycol in urine of victims of a alleged attack with mustard gas. Part I; *J. Anal. Toxicol.* **9** 254-257