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Respiratory Effects of Amifostine and DRDE-07: Probable Prophylactic Agents of Sulphur Mustard in Rats

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ABSTRACT

Amifostine (S-2[3-aminopropylamino]ethyl phosphorothioate) and one of its analogues, DRDE-07 (S-2[2-aminoethylamino]ethyl phenyl sulphide) are promising prophylactic agents for sulphur mustard (SM; a blistering agent) toxicity. When given orally, DRDE-07 was more effective than amifostine as a prophylactic agent against SM administered percutaneously. Various pharmacological and toxicological studies are required before the introduction of a chemical as a drug. The respiratory effects of amifostine and DRDE-07 were carried out in rats using a body plethysmograph fitted with a volumetric pressure transducer for sensing the respiratory flow signals. The signals were amplified, digitised, and stored on a personal computer for further analysis. After taking control recordings of respiratory signals, different doses (0.5 LD_{50}, 1.0 LD_{50} and 2.0 LD_{50}) of amifostine and DRDE-07 were administered orally (LD_{50} amifostine = 2262 mg/kg; DRDE-07 = 1599 mg/kg), and the respiratory changes were monitored for 4 h. Amifostine and DRDE-07 showed a uniform breathing pattern even in 2.0 LD_{50} dose. However, a significant dose-dependent decrease in respiratory frequency was observed following amifostine administration. DRDE-07 did not show any significant change. The tidal volume was not altered significantly both in amifostine and DRDE-07 administered animals. The study shows that DRDE-07, even in lethal doses, may not affect the respiration immediately, whereas, amifostine may decrease the respiratory frequency.

Keywords: Amifostine, DRDE-07, toxicity, respiratory frequency, tidal volume, prophylactic agents, sulphur mustard antidote, cytoprotective drug, sulphur mustard

1. INTRODUCTION

The nerve agents and blistering agents continue to be a threat as chemical warfare agents against the armed forces in spite of the control imposed by the Chemical Weapon Convention (CWC). The CWC prohibits the production, storage, transport, and use of chemicals on enemy forces[1]. One such chemical is sulphur mustard (SM), commonly known as mustard gas. It is included in the Schedule I of the CWC. Reports are available of its use in several instances before the CWC came into force[2-4]. State Parties, that have declared possessing SM, are in the process of destroying it. In spite of the CWC, the threat exists that SM can be used clandestinely during war or by terrorist organisations because of its simple method of preparation.

The chemical name of SM is "2,2'-dichloro diethyl sulphide", and it is an alkylating agent. SM

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causes serious blisters upon contact with human skin. In animal models, it is extremely lethal. Just one microlitre applied on the skin of a mouse or a rat may be lethal in a weeks time. SM forms sulphonium ion in the body and alkylates DNA, leading to DNA strand breaks and cell death[5-6]. Due to high electrophilic property of the sulphonium ion, SM binds to a variety of cellular macromolecules and death occurs due to multi-organ failure[7-8]. Eyes, skin, and respiratory tract are the principal target organs of SM toxicity[5,9-10].

Several antidotes have been reported for the systemic toxicity of SM in experimental animals, but none of these have been recommended so far[8,11-14]. SM is highly lipophilic and is absorbed quickly on contact with the skin. The effective method of reducing the toxicity of SM is by decontamination immediately after the contact[7]. The most commonly used decontaminant is Fuller's earth (a native form of aluminium silicate) that removes SM by adsorption, thereby reducing the toxicity[15]. Few chemical decontaminants for human use have also shown very good efficacy[16-17].

An effective prophylactic agent against SM is the need of the day, especially for personnel engaged in the destruction of SM and during inspection by the Organisation for Prohibition of Chemical Weapons (OPCW). From a series of aminothiols, two compounds, amifostine (S-2[3-aminopropylamino] ethyl phosphorothioate) and DRDE-07 (S-2[2-aminoethylamino]ethyl phenyl sulphide) gave very good protection as a prophylactic agent against SM[18-20]. When given orally, DRDE-07 was more effective than amifostine as a prophylactic agent against SM administered percutaneously[19,21]. Various pharmacological and toxicological studies are needed, before the introduction of a chemical as a drug. Several of these studies have been initiated and the effect of amifostine and DRDE-07 on respiratory variables in rats has been reported here.

2. MATERIALS

Randomly bred Wistar male rats (175-225 g, body weight) from DRDE Animal Facility were used. They were housed in polypropylene cages, 4 rats per cage) with dust-free rice husk as bedding material, and were provided with food (supplied by Amruth India Ltd) and water ad libitum. The care and upkeep of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. This study has the approval of the DRDE's Animal Ethical Committee.

Amifostine, (S-2[3-aminopropylamino]ethyl phosphorothioate) and DRDE-07, (S-2[2-aminoethylamino]ethyl phenyl sulphide) were synthesised in the chemistry laboratory. The compounds were characterised by elemental analysis, IR, ^1H NMR, and mass spectral analysis. The purity was assessed by thin layer chromatography. Amifostine and DRDE-07 were used as their hydrochlorides and these were water soluble. All other chemicals used were of analytical grade.

Amifostine : $NH_2–CH_2–CH_2–CH_2–NH–CH_2–CH_2–S–PO_3–H_2$

DRDE-07 : $NH_2–CH_2–CH_2–NH–CH_2–CH_2–S–C_6H_5$

3. METHODOLOGY

Four rats at a time were restrained in body plethysmographs for the recording of respiratory signals (Fig. 1). The glass plethysmographs (length 140 mm and dia 45 mm) can accommodate rats weighing between 150 g to 250 g. A volumetric pressure transducer (model PT5, Grass Instrument, USA) was used for sensing respiratory flow signals. A continuous air flow of 170 ml.min^{-1} was maintained into each body plethysmograph using a critical orifice (27 gauge needle). The signals from the individual transducers were amplified using universal amplifiers (Gould, USA). The amplified signals were digitised using an analog-to-digital converter (Metrabyte, Taunton, USA) and stored on a personal computer and analysed. The amplified signals were also fed into an oscillograph for recording of breathing pattern (WindoGraf, Gould, USA). A computer programme developed by the University of Pittsburgh, USA for monitoring of respiratory changes in small animals was used for recording of various respiratory variables[22].

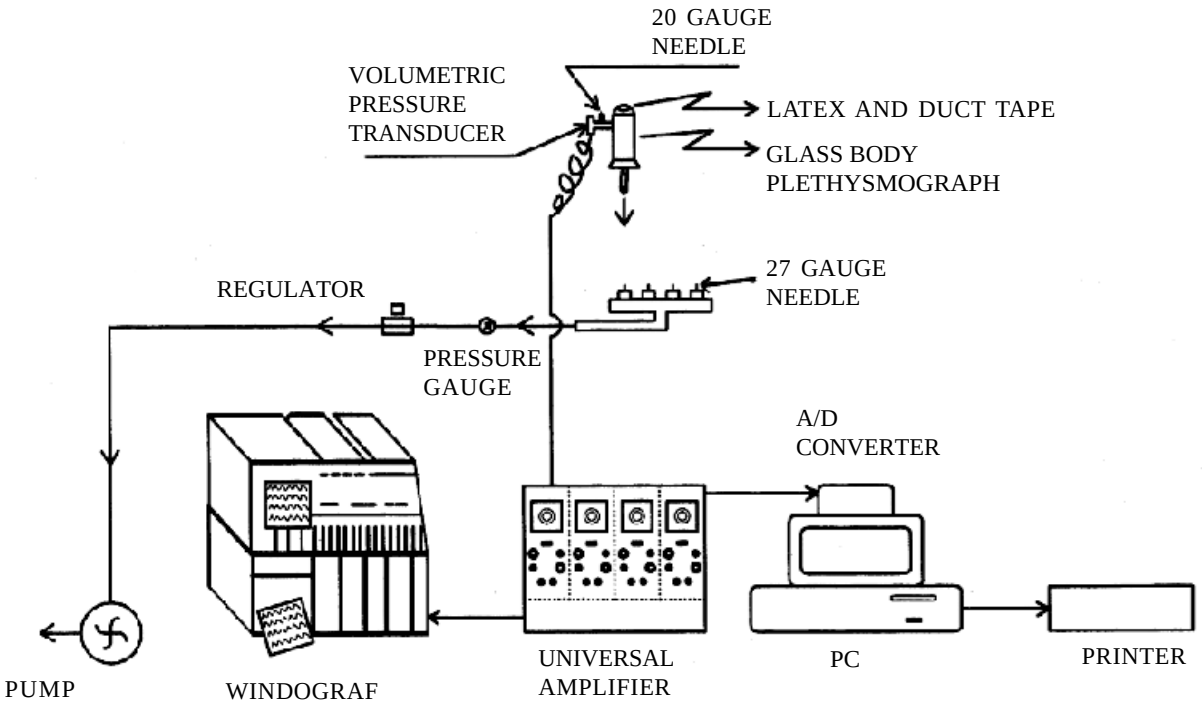

Figure 1. Schematic diagram of the respiratory analysis setup

The animals were acclimatised in the body plethysmographs for 30 min. After the acclimatisation, a control recording of respiratory variables was carried out for 30 min. Amifostine and DRDE-07 were given using an oral feeding cannula (20 gauge, Harvard Instruments, USA) and the respiratory variables were recorded for a period of 4 h after administration of the dose. Three doses of amifostine and DRDE-07 (0.5 LD_{50}, 1.0 LD_{50} and 2.0 LD_{50}) were given (oral LD_{50} amifostine = 2262 mg/kg; DRDE-07 = 1599 mg/kg). For each dose, four rats were used. Four rats served as control, and were administered saline only.

All the values obtained were converted as per cent of pre-drug administration values and expressed as mean ± standard error. The means were analysed by one way ANOVA, followed by Dunnett's test. SigmaStat (Jandel Scientific, San Rafael, USA) was used for all the statistical analysis. A probability of less than 0.05 was taken as statistically significant.

4. RESULTS

No rat died during the monitoring period of 4 h following 0.5 LD_{50} and 1.0 LD_{50} of amifostine and DRDE-07. Two rats died during the monitoring period following 2.0 LD_{50} of DRDE-07, but none in the amifostine group. But all the rats administered with 2.0 LD_{50} of amifostine and DRDE-07 died within 24 h. Three rats of amifostine and two rats of DRDE-07 in 1.0 LD_{50} group died within 24 h. All the rats given 0.5 LD_{50} of aminofostine and DRDE-07 survived.

The normal value for tidal volume was 0.260 ± 0.021 $ml.min^{-1}$ and respiratory frequency was 134 ± 5 min^{-1} (mean ± SEM; n = 28). Control animals (saline group) showed a uniform breathing pattern during the 4 h monitoring period (Fig. 2). A dose of 0.5 LD_{50} of amifostine and DRDE-07 also showed a uniform breathing pattern. A dose of 1.0 LD_{50} and 2.0 LD_{50} of amifostine showed a disturbed breathing pattern. There was not much change in the DRDE-07 groups. The breathing patterns of 1.0 LD_{50} amifostine and DRDE-07 are

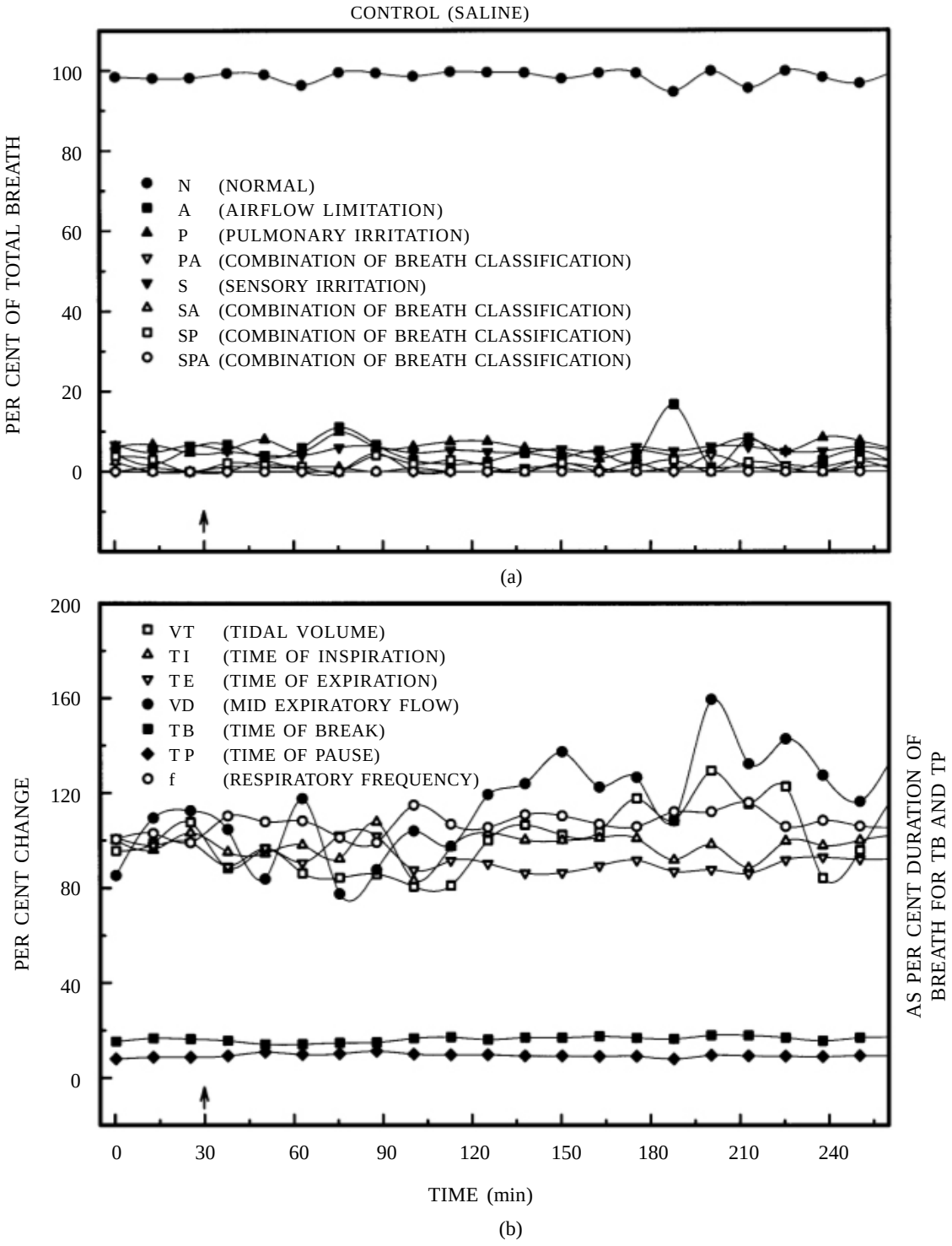

Figure 2. Time response analysis for breath classification and measured variables for a group of four rats: (a) before and (b) after oral administration of saline (control). The arrow indicates the time of administration.

shown in Figs 3 and 4. Tables 1 and 2 show the respiratory frequency and tidal volume of various doses of amifostine and DRDE-07 calculated from the online computer program. There was a significant dose-dependent and time-dependent decrease in the respiratory frequency following oral administration

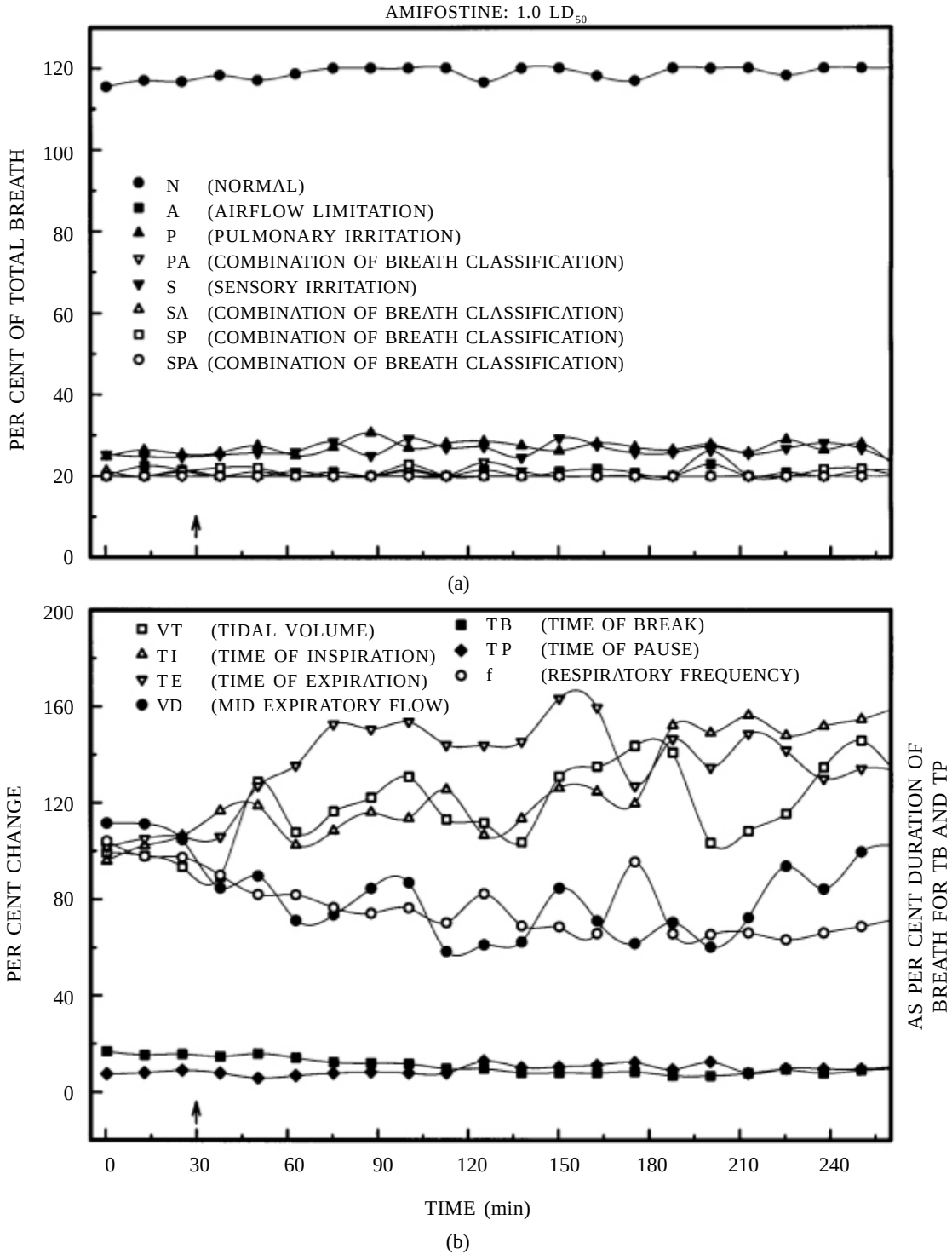

Figure 3. Time response analysis for breath classification and measured variables for a group of four rats: (a) before and (b) after oral administration of 1.0 LD_{50} of amifostine. The arrow indicates the time of administration.

of amifostine. But, DRDE-07 did not show any significant change. The tidal volume was not altered in amifostine and DRDE-07 administered rats.

5. DISCUSSION

Even after several decades of active research, a suitable antidote for the toxic effects of SM has

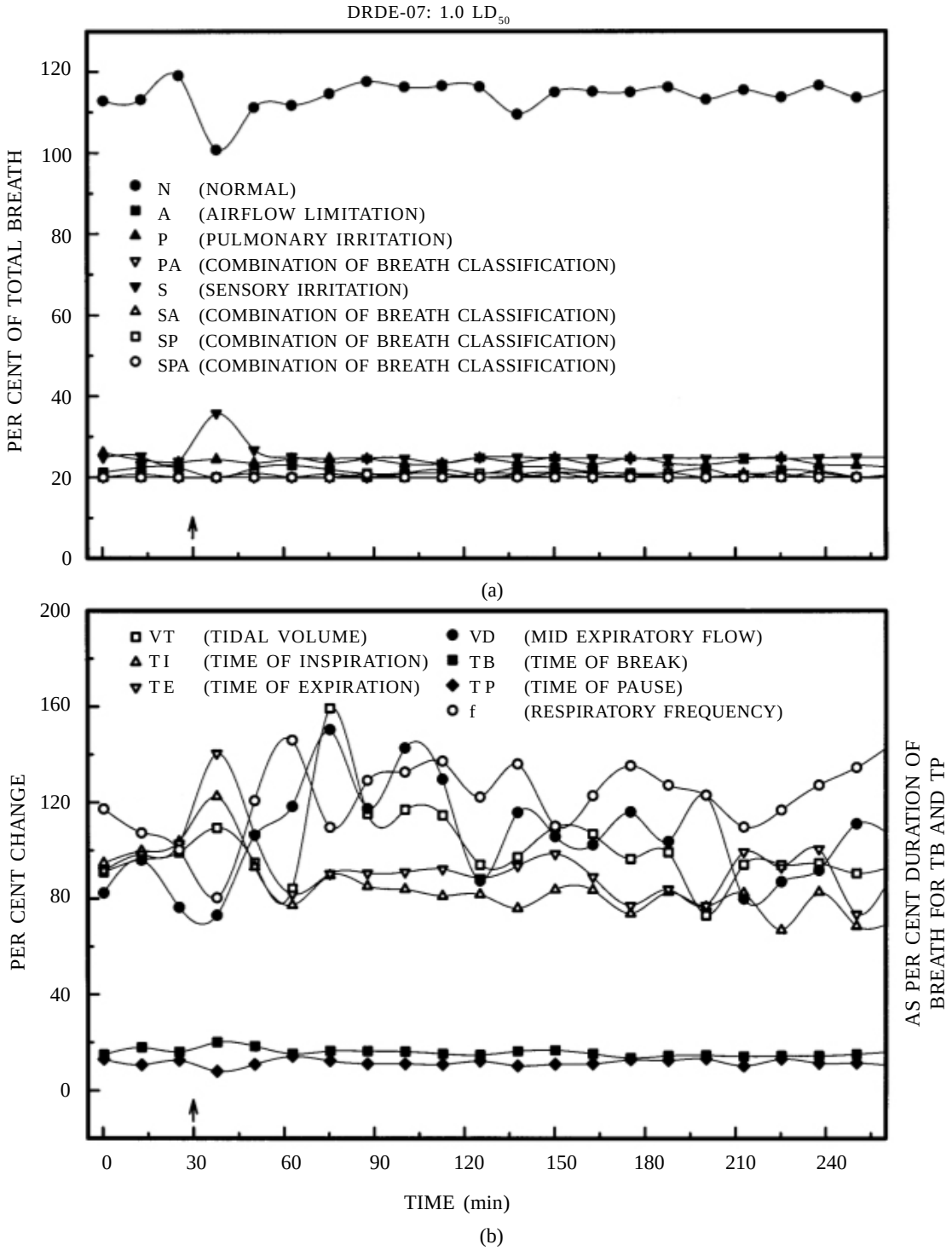

Figure 4. Time response analysis for breath classification and measured variables for a group of four rats: (a) before and (b) after oral administration of 1.0 LD_{50} of DRDE-07. The arrow indicates the time of administration.

not been developed. The present method of reducing the toxic effects of SM are by physical and chemical decontamination[7,16,17].

Amifostine is extensively studied as a cytoprotective drug[23]. Amifostine, when given prophylactically for chemotherapeutic agents like cisplatin and

Table 1. Effect of oral administration of amifostine and DRDE-07 on respiratory frequency in rats

Agent	Dose	Effect after			
		1 h	2 h	3 h	4 h
Control	Saline	105.9 ± 8.0	107.6 ± 5.3	109.0 ± 8.1	108.9 ± 9.8
Amifostine	0.5 LD_{50}	93.1 ± 1.9	86.0 ± 1.4	84.8 ± 3.1	83.1 ± 6.0*
Amifostine	1.0 LD_{50}	82.5 ± 2.6	74.8 ± 2.9	68.4 ± 1.7*	68.0 ± 2.5*
Amifostine	2.0 LD_{50}	82.4 ± 6.1	74.8 ± 2.9	55.8 ± 2.0*	52.9 ± 2.7*
DRDE-07	0.5 LD_{50}	93.4 ± 6.1	94.4 ± 5.4	91.3 ± 7.1	94.0 ± 6.2
DRDE-07	1.0 LD_{50}	98.7 ± 5.1	110.0 ± 10.2	111.9 ± 13.6	107.7 ± 9.5
DRDE-07#	2.0 LD_{50}	117.5	127.9	113.3	106.7
F	-	2.14	3.83	9.05	10.40
P	-	NS	< 0.05	< 0.001	< 0.001

Values are in per cent; mean ± SE (n = 4; #n = 2); * Statistically significant from control of the same time period

Table 2. Effect of oral administration of amifostine and DRDE-07 on tidal volume in rats

Agent	Dose	Effect after			
		1 h	2 h	3 h	4 h
Control	Saline	90.8 ± 7.7	99.1 ± 9.6	101.3 ± 23.2	117.5 ± 28.0
Amifostine	0.5 LD_{50}	91.0 ± 9.4	96.8 ± 15.6	105.0 ± 18.5	135.2 ± 23.3
Amifostine	1.0 LD_{50}	112.8 ± 12.2	121.5 ± 12.1	128.5 ± 14.7	127.2 ± 17.8
Amifostine	2.0 LD_{50}	126.4 ± 16.7	154.1 ± 31.6	162.2 ± 35.3	155.8 ± 27.2
DRDE-07	0.5 LD_{50}	104.7 ± 8.4	133.0 ± 14.7	118.4 ± 6.3	105.3 ± 21.2
DRDE-07	1.0 LD_{50}	109.5 ± 9.5	111.1 ± 18.6	102.5 ± 19.0	97.0 ± 20.0
DRDE-07#	2.0 LD_{50}	111.0	102.5	159.6	131.5
F	-	0.98	1.16	0.82	0.55
P	-	NS	NS	NS	NS

Values are in per cent; mean ± SE (n = 4; #n = 2); * Statistically significant from control of the same time period

cyclophosphamide, has been shown to protect selectively normal tissues without reducing the cytotoxic effects on the cancer cells[24-27]. Amifostine has also been shown to be effective against carbon tetrachloride-induced liver necrosis, and to protect tissues from the toxicities of radiation and alkylating agents, probably by scavenging the generated free radicals[25,28-30]. This triggered interest of the authors in amifostine and its analogues as a prophylactic agent against SM toxicity. Evaluation of these analogues revealed that DRDE-07 is a promising prophylactic agent for SM[18]. The initial studies by the authors revealed that intraperitoneal administration of amifostine was better than DRDE-07, but by the oral route, DRDE-07 showed very good protection against SM than amifostine[19,20].

The cytoprotective effect of amifostine is due to its free thiol metabolite that is formed by the action of the membrane-bound alkaline phosphatase[23,29]. Since DRDE-07 does not have a phosphate group, its further metabolism and mechanism of protection is not understood so far. It is expected that due to the presence of an aryl group in DRDE-07, the lipophilicity of the compound is increased with a

better bioavailability. Amifostine gave a protection of 9.5-fold compared to 27-fold protection of DRDE-07 in mouse model against SM toxicity. But in rat model, both amifostine and DRDE-07 gave about 2-fold to 3-fold protection[21].

Various pharmacological and toxicological data were generated on amifostine and DRDE-07. The earlier study by the authors revealed that oral administration of DRDE-07 induced a dose-dependent decrease in mean arterial blood pressure and the effects were pronounced at 1.0 LD_{50} and 2.0 LD_{50} doses[31]. Oral administration of amifostine also decreased mean arterial blood pressure at 1.0 LD_{50} and 2.0 LD_{50} doses (unpublished). The computer program developed for measuring respiratory variables of inhaled chemicals was used conveniently for the oral administration of amifostine and DRDE-07[22].

In the present study, a significant dose-dependent and time-dependent decrease in respiratory frequency were observed following oral administration of amifostine. But, no significant change was observed following oral administration of DRDE-07. The changes that were observed following oral administration of amifostine were also of slow onset. A variety of drugs that act as the central nervous system depressants, viz., general anaesthetics, opioid analgesics, sedatives, and hypnotics cause a depression of respiration, and respiratory stimulants like doxapram and nikethamide cause an increase in the respiratory frequency and tidal volume[32]. DRDE-07 did change the respiratory frequency, showing that it may not have any depressant or stimulant action on the central nervous system. The decrease in respiratory frequency shown by amifostine may be due to its central or peripheral action.

The present study shows that DRDE-07, even in lethal doses, may not affect the respiration immediately, whereas amifostine may decrease the respiratory frequency.

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REFERENCES

1. Krutzsch, W. & Trapp, R. (Eds). A commentary on the chemical weapons convention. Martinus Nijhoff Publishers, London, 1994. 543 p.

2. Smith, W.J. & Dunn, M.A. Medical defence against blistering chemical warfare agents. *Archive. Dermatol.*, 1991, **127**, 1207-13.

3. Eisenmenger, W.; Drasch, G.; Von Clarmann, M.; Kretschmer, E. & Roider, G. Clinical and morphological findings on mustard gas [bis(2-chloroethyl) sulphide] poisoning. *J. Forensic Sci.*, 1991, **36**, 1688-98.

4. Momeni, A.Z.; Enshaeih, S.; Meghdadi, M. & Amindjavaheri, M. Skin manifestations of mustard gas. A clinical study of 535 patients exposed to mustard gas. *Archive Dermatology*, 1992, **128**, 775-80.

5. Papirmeister, B.; Feister, A.J.; Robinson, S.I. & Ford, R.D. (Eds). Medical defence against mustard gas: Toxic mechanisms and pharmacological implications. CRC Press, Boca Raton, 1991. 359 p.

6. Lakshmana Rao, P.V.; Vijayaraghavan, R. & Bhaskar, A.S.B. Sulphur mustard induced DNA damage in mice after dermal and inhalation exposure. *Toxicology,* 1999, **139**, 39-51.

7. Somani, S.M. & Babu, S.R. Toxicodynamics of sulfur mustard. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 1989, **27**, 419-35.

8. Dacre, J.C. & Goldman, M. Toxicology and pharmacology of the chemical warfare agent sulfur mustard. *Pharmacological Reviews,* 1996, **48**, 290-26.

9. Pechura, C.M. & Rall, D.P. (Eds). Veterans at risk: The health effects of mustard gas and lewisite. National Academy Press, Washington DC, 1993. 428 p.

10. Vijayaraghavan, R. Modifications of breathing pattern induced by inhaled sulphur mustard in mice. *Arch. Toxicol.*, 1997, **71**, 157-64.

11. Callaway, S. & Pearce, K.A. Protection against systemic poisoning by mustard gas di(2-chloroethyl) sulphide by sodium thiosulphate and thiocit in albino rat. *Br. J. Pharmcol.*, 1958, **13**, 395-99.

12. Vojvodic, V.; Milosavljevic, Z.; Boskovic, B. & Bojanic, N. The protective effect of different drugs in rats poisoned by sulphur and nitrogen mustards. *Funda. Appl. Toxicol.*, 1985, **5**, S 160-68.

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

14. Kumar, O.; Sugendran, K. & Vijayaraghavan, R. Protective effect of various antioxidants on the toxicity of sulphur mustard administered to mice by inhalation or percutaneous routes. *Chemical. Biol. Int.*, 2001, **134**, 1-12.

15. Marrs, T.C.; Maynard, R.L. & Sidell, F.R. (Eds). Chemical warfare agents: Toxicology and treatment. John Wiley and Sons, Chichester, 1996. pp. 162-63.

16. Shih, M.L.; Korte, W.D.; Smith, J.R. & Szafraniec, L.L. Reactions of sulfides with S-330, a potential decontaminant of sulphur mustard in formulations. *J. Appl. Toxicol.*, 1999, **19**, S 83-88.

17. Vijayaraghavan, R.; Kumar, P.; Dubey, D.K. & Singh, R. Evaluation of CC2 as a decontaminant in various hydrophilic and lipophilic formulations against sulphur mustard. *Biomed. Environ. Sci.*, 2002, **15**, 25-35.

18. Joshi, U.; Raza, S.K.; Kumar, Pravin; Vijayaraghavan, R. & Jaiswal, D.K. A process for preparation of S-(o-aminoalkylamino) alkylaryl sulphide dihydrochloride. Indian patent filed, Patent Office, New Delhi, India, 1999.

19. Vijayaraghavan, R.; Kumar, P.; Joshi, U.; Raza, S.K.; Lakshmana Rao, P.V.; Malhotra, R.C. & Jaiswal, D.K. Prophylactic efficacy of amifostine and its analogues against sulphur mustard toxicity. *Toxicology*, 2001, **163**, 83-91.

20. Bhattacharya, R.; Rao, P.V.; Pant, S.C.; Kumar, P.; Tulsawani, R.K.; Pathak, U.; Kulkarni, A. & Vijayaraghavan, R. Protective effects of amifostine and its analogues on sulfur mustard toxicity in vitro and in vivo. *Toxicol. Appl. Pharmacol.*, 2001, **176**, 24-33.

21. Kumar, P.; Vijayaraghavan, R.; Kulkarni, A.; Pathak, U.; Raza, S.K. & Jaiswal, D.K. *In* vivo protection by amifostine and DRDE-07 against sulphur mustard toxicity. *Hum. Exp. Toxicol.*, 2002, **21**, 371-76.

22. Vijayaraghavan, R.; Thomson, R.; Schaper, M.; Lee Ann, B.; Stock, M.F.; Luo, J. & Alarie, Y. Computer-assisted recognition and quantification of sensory irritation, airway constriction and pulmonary irritation. *Archiv. Toxicol.*, 1994, **68**, 490-99.

23. Hospers, G.A.; Eisenhauer, E.A. & de Vries, E.G. The sulphhydryl containing compounds WR-2721 and glutathione as radioprotective and chemoprotective agents: A review, indications for use and prospects. *Br. J. Cancer*, 1999, **80**, 629-38.

24. Srivastava, A.; Nair, S.C.; Srivastava, V.M.; Balamurugan, A.N.; Jeyseelan, L.; Chandy, M. & Gunasekaran, S. Evaluation of uroprotective efficacy of amifostine against cyclophosphamide induced hemorrhagic cystitis. *Bone Marrow Transplant*, 1999, **23**, 463-67.

25. Links, M. & Lewis, C. Chemoprotectants: A review of their clinical pharmacological and therapeutic efficacy. *Drugs*, 1999, **57**, 293-08.

26. Castiglione, F.; Dalla Mola, A. & Porcile, G. Protection of normal tissues from radiation and cytotoxic therapy: The development of amifostine. *Tumori*, 1999, **85**, 85-91.

27. Wasserman, T. Radioprotective effects of amifostine. *Semin. Oncol.*, 1999, **26**, 89-94.

28. Valles, E.G.; de Castro, C.R. & Castro, J.A. Radioprotectors as late preventive agents against carbon tetrachloride-induced liver necrosis protection by 2-(3-aminopropylamino) ethylphosphorothioic acid (WR-2721). *Exp. Mol. Pathol.*, 1995, **63**, 101-09.

29. Spencer, C.M. & Goa, K.L. Amifostine: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential as a radioprotector and cytotoxic chemoprotector. *Drugs*, 1995, **50**, 1001-031.

30. Werner-Wasik, M. Future development of amifostine as a radioprotectant. *Semin. Oncol.*, 1999, **26**, 129-34.

31. Malviya, Vimal; Singh, Ram; Kumar, Deo; Pathak, U.; Kumar, Parvin; Jaiswal, D.K.; Mathur, R. & Vijayaraghavan, R. Cardio-respiratory effects of DRDE-07, a new prophylactic agent for sulphur mustard in anaesthetised rats. *Indian J. Pharmacol.*, 2004, **36**, 234-37.

32. Franz, D.N. Central nervous system stimulants. *In* The pharmacological basis of therapeutics, edited by A.G. Gilman; A.S. Goodman & A. Gilman. Ed. 6. MacMillan Publishing Co. Inc, New York, 1980. pp. 585-91.

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