Def Sci J, Vol 41, No 4, October 1991, pp 363-366

Study of the Efficacy of CC-2 and Fuller's Earth Combination as a Decontaminant against Sulphur Mustard (Mustard Gas) Dermal Intoxication in Mice

Pravin Kumar, U.S. Sharma and R. Vijayaraghavan

Defence Research and Development Establishment, Gwalior-474 002

ABSTRACT

Decontamination efficacy of Fuller's earth and CC-2 independently; and in different combinations was evaluated against toxicity of sulphur mustard applied percutaneously on mice. Maximum protection was obtained with Fuller's earth and CC-2 in a combination of 80:20 (w/w).

1. INTRODUCTION

Sulphur mustard (HD), 2,2'-dichlorodiethyl sulphide, commonly known as mustard gas is a blistering agent[1] and is frequently used as a chemical warfare agent[2]. It is one of the earliest known alkylating agents[3,4] and binds to DNA, proteins and cell membranes[2]. There is no specific antidote against HD intoxication; the treatment is only symptomatic[5,6]. The best method for minimising the injury is by decontamination as soon as HD comes in contact with the skin.

Fuller's earth (FE), largely consisting of montmorillonite, is recommended for removal of HD from the skin due to its high adsorptive capacity[7]. *N, N'*-dichloro-bis (2,4,6-trichlorophenyl) urea (CC-2)[8] has been used in the form of ointments[9], and also impregnated in cloth[10] for decontamination of HD.

In the present study FE (complying to BPC standard[11]), CC-2 and their various combinations (w/w ratios) were evaluated for their decontamination efficacy against HD, when applied on mouse skin.

Received 8 May 1990, revised 27 August 1990

2. MATERIALS AND METHODS

Male Swiss albino mice of 25-28 g body weight, bred and maintained in this establishment were used for the experiment. The hair was clipped closely on dorsal side, caudally. HD (98 per cent) was dissolved in olive oil and applied uniformly. The animals were observed for mortality till 7 days and LD_{50} was determined by Dixon's up and down method[12].

For decontamination experiments, the mice were given light anaesthesia with ether and 20 μl of pure HD was applied (equal to 6 LD_{50}) uniformly on the dorsal side. Exactly after 30 s the area was wiped with cotton only (control), or with the decontaminant or the decontaminant mixture (60 mg) on cotton. The decontamination was repeated once again at a gap of 15 s. The animals were given food (Lipton Feed, India) and water *ad libitum* and mortality was observed till 7 days. For preparing the decontaminant mixture, freshly recrystallised CC-2 was thoroughly mixed with FE in different w/w ratios. Statistical analysis of the data was done by χ^2-test.

3. RESULT AND DISCUSSION

Table 1 shows the protection given by the decontaminants and their combinations against HD intoxication in different groups of mice. In the control group, all the mice

Table 1. Efficacy of decontaminants against sulphur mustard applied on mice skin

Treatment	Control	FE only	CC-2 only	FE:CC-2 (w/w ratio) 90:10 (a)	80:20 (b)	70:30 (c)	50:50 (d)
Days after application				No. died/No. treated (% mortality)			
1.	20/20 (100)	4/20 (20)	5/20 (25)	0/6 (0)	0/25 (0)	0/6 (0)	0/6 (0)
2.	20/20 (100)	4/20 (20)	6/20 (30)	0/6 (0)	0/25 (0)	0/6 (0)	0/6 (0)
3.	20/20 (100)	5/20 (25)	6/20 (30)	0/6 (0)	0/25 (0)	0/6 (0)	0/6 (0)
4.	20/20 (100)	5/20 (25)	8/20 (40)	2/6 (33)	0/25 (0)	0/6 (0)	2/6 (33)
5.	20/20 (100)	10/20 (50)	10/20 (50)	3/6 (50)	0/25 (0)	1/6 (17)	3/6 (50)
6.	20/20 (100)	13/20 (65)	12/20 (60)	3/6 (50)	1/25 (4)	2/6 (33)	3/6 (50)
7.	20/20 (100)	14/20 (70)	13/20 (65)	4/6 (67)	1/25 (4)	3/6 (50)	4/6 (67)

HD applied was equal to 6 LD_{50}. Calculated LD_{50} of HD is 154.7 mg/kg by percutaneous route Decontamination was done 30 s after applying HD.

Statistical analysis on last day data by χ^2-test

b vs a : $\chi^2 = 9.80$; $P < 0.01$. b vs c : $\chi^2 = 5.48$; $P < 0.05$. b vs d : $\chi^2 = 9.80$; $P < 0.01$.

died within 24 hr after percutaneous application of 6 LD_{50} of HD. With FE and CC-2 used independently, mortality in seven days was 70 per cent and 65 per cent respectively, whereas it was only 4 per cent with a combination of FE and CC-2 in a w/w ratio of 80:20.

It is significant that the mice treated with a combination of FE and CC-2 showed no mortality till three days, whereas the mortality was 25 and 30 per cent with FE and CC-2 used independently for the same period of time.

The general principle of decontamination is to remove clothing and wash all the exposed areas with water. Removal of HD with water is contra-indicated as HD spreads and the blisters will be more (unpublished observation). FE has a high adsorptive capacity[7] and removes HD thereby reducing the toxicity. CC-2 reacts with HD instantaneously and the reaction is exothermic. The synergistic effect of the mixture of FE and CC-2 perhaps is due to the chemical interaction of CC-2 and HD[9] and the physical interaction of FE and HD. Moreover, as the reaction of CC-2 and HD is largely on the surface of FE, the heat developed does not injure the skin. The shelf life of a mixture of FE and CC-2, which is under investigation will throw more light on the proper ratio for this formulation.

ACKNOWLEDGEMENTS

The authors are grateful to Brig (Dr) KM Rao, Director, Defence Research and Development Establishment, Gwalior, Dr PK Ramachandaran and Dr RV Swamy for their encouragement and suggestions during the study.

REFERENCES

Frank, S.(Ed). Manual of military chemistry, Vol.1:Chemistry of chemical warfare agents. NTIS, Springfield, 1967. p. 108. AD 849866.

2. Somani, S.M. & Babu, S.R. Toxicodynamics of sulphur mustard. *Pharmacol. Therap. Toxicol.*, 1989, **27**, 419-35.
3. Papirmister, B.; Gross, C.L.; Meier, H.L.; Petrali, J.P. & Johnson, J.B. Molecular basis for mustard-induced vesication. *Fundam. Appl. Toxicol.*, 1985, **5**, S134-49.
4. Wheeler, G.P. Studies related to the mechanisms of action of cytotoxic alkylating agents. *Ann. Rev. Cancer Res.*, 1962, **22**, 651-88.
5. Vojvodic, V.; Milosavijevic, Z.; Boskovic, B. & Bojanaic, N. The protective effect of different drugs in rats poisoned by sulphur and nitrogen mustards. *Fundam. Appl. Toxicol.*, 1985, **5**, S160-68.
6. Willems, J.L. Clinical management of mustard gas casualties. *Ann. Medicinae Militaris*, 1989, **3**, 27-53.
7. McGraw-Hill encyclopedia of science and technology, Vol. 8. McGraw-Hill, New York, 1960. p. 587.

8 Chattaway, F.D. & Orton, K.J.P. *Die chloramino derivate des symmetrischen diphenylharnstoffes und deren umwandlungen. Berichte*, 1901, **34**, 1073-78.

9. Sulzberger, M.B.; Kanof, A. & Baer, R.L. Chloramide protection ointment. US patent 2, 630, 399. 3 March 1953.

10 Cowsar, D.R. Fabric containing microcapsules of chemical decontaminant encapsulated within semipermeable polymers. US patent 48,285. 29 February 1980. 15 p.

11 Council of the Pharmaceutical Society of Great Britain. British Pharmaceutical Codex. Pharmaceutical Press, London. 1954. p. 317.

12. Dixon, W.J. The up and down method for small samples. *Am. Statist. Assoc. J.*, 1965, **60**, 967-78.