

SHORT COMMUNICATION

## Amifostine: An Effective Prophylactic Agent against Sulphur Mustard Toxicity

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### ABSTRACT

Amifostine, S-2-(aminopropylamino) ethylphosphorothioate and two of its analogues have been evaluated as prophylactic agent against SM toxicity. The compounds were administered intraperitoneally (i.p.) at 0.2 LD<sub>50</sub> dose in mice 30 min prior to dermal application of SM. The protective efficacy was determined by observing the mortality for 14 days. The protection offered by amifostine was better than its analogues. Subsequent study on time-dependent protection, carried out with amifostine (0.2 LD<sub>50</sub>, i.p.) provided significant protection when the drug was administered as 30 min pre-treatment and simultaneous treatment against SM at 155 mg.kg<sup>-1</sup> dose (equal to 19-fold LD<sub>50</sub>). Furthermore, oral administration of amifostine (30 min pre-treatment) showed similar results. These findings suggest that amifostine is a promising prophylactic agent against SM toxicity.

**Keywords:** Amifostine, sulphur mustard, prophylactic agent, radioprotector, toxicity, alkylating agent

### 1. INTRODUCTION

Bis (2-chloroethyl) sulphide, known as sulphur mustard (SM), is a frequently used chemical warfare agent. The principal target organs of SM toxicity are eyes, skin and the respiratory tract<sup>1,2</sup>. SM is suggested to form sulphonium ion in cells which alkylates DNA, leading to strand breaks, and in turn cell death<sup>3</sup>. Several reports are available on its recent use<sup>4-6</sup>. Numerous compounds have been evaluated for their protective ability against the toxicity of SM in experimental animals<sup>1,7-9</sup>. The approaches, such as personal decontamination, prevention of alkylation, retrieval of SM-alkylated DNA and reversal of the detrimental biochemical events have shown little success from technical and/or practical point of view<sup>5</sup>. Therefore, there is need to search/test new compounds using various biological end points. The present work reports

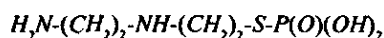
effectiveness of amifostine as a prophylactic agent against SM toxicity.

Despite the signing of the Chemical Weapons Convention (CWC) and its subsequent ratification by several countries, the possibility of SM being used clandestinely during warfare or by terrorist groups still exists as its preparation is quite easy. Moreover, risk of exposure during the inspection as well as destruction of stockpiled SM could not be ignored. Therefore, the findings of the present work may have applications in minimising the toxicity of SM.

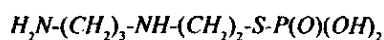
### 2. MATERIALS & METHODS

#### 2.1 Chemicals

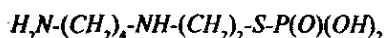
Amifostine and its analogues were synthesised according to the existing procedure<sup>10</sup> and characterised



(a)



(b)



(c)

### Structures of amifostine and its analogues

by their melting points, IR,  $^1H$ -NMR and mass spectroscopy (Table 1). SM was synthesised according to existing procedure<sup>11</sup> and was found to be more than 99 per cent pure by gas chromatographic (GC) analysis.

### 2.2 Animals

Randomly bred Swiss female mice (body weight: 25 g to 30 g) maintained in the animal house of the Establishment, were used for the study. They were housed in polypropylene cages on dust-free rice husk as the bedding material, and provided food (Amrut Ltd, India) and water *ad libitum*. This study has the approval of the Ethical Committee of the Establishment.

### 2.3 LD<sub>50</sub> Determination

The LD<sub>50</sub> of amifostine and its analogues, dissolved in water, were determined intraperitoneally (i.p.) or per orally (p.o.) and the animals were observed for mortality for 14 days. For the LD<sub>50</sub> determinations, 3 to 4 groups were used with each group consisting of 4 mice.

### 2.4 Determination of Protective Efficacy

Amifostine and its analogues were evaluated as prophylactic agent by administering these 30 min prior to SM application. The test compounds were administered (i.p.) at a dose of 0.2 LD<sub>50</sub>. Five microlitres of undiluted SM equal to 230 mg.kg<sup>-1</sup> was applied on the back of the mice using a micro pipette (this dose is 28 times LD<sub>50</sub> of SM; LD<sub>50</sub> = 8.1 mg.kg<sup>-1</sup>). The hair on the back of the mice were closely clipped using a pair of scissors, a day prior to SM application. The animals were observed for mortality for 14 days. For each compound, a group of 5 mice were used. A separate group of animals injected with distilled water and applied with SM served as control.

### 2.5 Time-dependent Efficacy of Amifostine

The time-dependent efficacy of amifostine was evaluated by administering it (i.p. or p.o.), either 30 min prior or simultaneously (0 min), or 60 min after application of SM. Amifostine was administered at a dose of 0.2 LD<sub>50</sub> and SM equal to 19 times LD<sub>50</sub> (155 mg.kg<sup>-1</sup>) diluted in PEG-300 was applied on the back of the mice. The animals were observed for mortality for 14 days. For each time, a group of 5 mice were used. A separate group of animals injected with distilled water and applied with SM served as control.

### 2.6 Statistical Analysis

The LD<sub>50</sub> was determined by the moving average method<sup>12</sup>. The protective efficacy was established

Table 1. Physico-chemical and spectral data of amifostine and its analogues

Compound No.	Yield (%)	Melting point (°C)	IR(KBr) (cm <sup>-1</sup> )	$^1H$ -NMR (D <sub>2</sub> O) δ	$^{31}P$ -NMR δ	MS (ESI) (M + H) <sup>+</sup>
a	80	143-44 (139-41)*	1107, 1067, 959, 846, 585	3.45 (2H, t, J = 7 Hz); 3.2 (6H, m)	16.23	201
b	80	160-61 (160-61)*	1111, 1080, 956, 898, 597	3.40 (2H, t, J = 7 Hz); 3.15 (4H, m); 2.95(2H, m); 2.12 (2H, m)	16.64	215
c	72	171-73 (Decomp. above 170)*	1154, 1091, 1060, 878, 562	3.45 (2H, t, J = 7 Hz); 3.04 (4H, m); 2.95(2H, m) 1.29 (4H, m)	16.57	229

\*Literature melting point

by Friedman's repeated measures ANOVA on ranks and compared with SM-treated group (control group) by Dunnett's method. For this cumulative percentage of death for each day over 14 days was computed using Sigma Stát (Jandel Sci. USA).

### 3. RESULTS & DISCUSSION

Chemically amifostine belongs to the class of S-( $\omega$ -aminoalkylamino) ethyl phosphorothioates [ $H_2N-(CH_2)_n-NH-(CH_2)_2-S-P(O)(OH)_2$ ; (b) amifostine:  $n = 3$ ]. The other two analogues prepared were ethyl [(a),  $n = 2$ ] and butyl [(c),  $n = 4$ ] derivatives. The LD<sub>50</sub> of amifostine and its analogues through i.p. route is given in Table 2. Among these compounds, (a) was found to be least toxic. Increase in the chain length from ethyl to butyl seems to increase the toxicity of this group of compounds.

Table 2. LD<sub>50</sub> of amifostine and its analogues in female mice\*

Test compound	LD <sub>50</sub> , mg.kg <sup>-1</sup>	
	i.p. route	p.o. route
a	1902 (1245-2904)	-
b	1131 (800-1600)	1049 (709-1552)
c	800 (490-1306)	-

\* LD<sub>50</sub> estimated by the moving average method (Gad & Weil, 1989). Figures in parenthesis represent confidence limit.

When a non-lethal dose of 0.2 LD<sub>50</sub> of amifostine and its two analogues was administered (i.p.) for evaluating their protective efficacy against SM lethality, amifostine was found to be more effective than its two analogues (Table 3). Further studies on prophylactic efficacy of amifostine through i.p. route showed that it can reduce the lethality when given 30 min pre-treatment or simultaneous treatment with SM. The results are shown in Table 4. Since the fact that the preferred route for administration of a drug is through p.o., the efficacy of amifostine through this route was also examined. The results showed that amifostine (30 min pre-treatment)

Table 3. Antidotal efficacy of amifostine and its analogues against sulphur mustard-induced lethality in mice\*

Compound No.	Lethality percentile after SM		
	25 <sup>th</sup>	50 <sup>th</sup> (median)	75 <sup>th</sup>
SM only	65	100	100
a	60	60**	75
b	20	20**	20
c	60	60**	60

\*Dose of the compounds for protective efficacy was 0.2 LD<sub>50</sub> given i.p. 30 min prior to SM; dose of undiluted SM was 230 mg.kg<sup>-1</sup> (LD<sub>50</sub> of SM=8.1 mg.kg<sup>-1</sup> by dermal route); for protection studies 5 mice per group were used. Median is the per cent of mice dying 7 days after SM application.

\*\*Statistically significant compared to sulphur mustard group by Friedman's repeated measures ANOVA followed by Dunnett's method;  $\chi^2 = 36.6$ ,  $df = 3$ ,  $p < 0.001$ .

administered (p.o.) could provide better protection against dermally applied SM (data not shown).

Amifostine is reported to undergo dephosphorylation to give its free-thiol compound (WR-1065) by membrane-bound alkaline phosphatase which quickly enters tissues and provides protection against alkylating agents and radiation<sup>13</sup>. When amifostine is given as a prophylactic agent with chemotherapeutic agents like cisplatin and cyclophosphamide it is shown to differentially protect normal tissues without reducing the effect of the anti-cancer agents on the cancer

Table 4. Time-dependent efficacy of amifostine against sulphur mustard-induced lethality in mice\*

Time of amifostine administration	Lethality percentile after SM		
	25 <sup>th</sup>	50 <sup>th</sup> (median)	75 <sup>th</sup>
SM only	20	80	100
30 min pre-treatment	00	20**	75
Simultaneous treatment	00	20**	100
60 min post-treatment	5	40	100

\*Dose of amifostine was 0.2 LD<sub>50</sub> given i.p.; dose of SM was 155 mg.kg<sup>-1</sup> (equal to 19 times LD<sub>50</sub> of SM) applied dermally; 5 mice per group were used, and median is the per cent of mice dying 7 days after SM application.

\*\*Statistically significant compared to sulphur mustard group by Friedman's repeated measures ANOVA followed by Dunnett's method;  $\chi^2 = 24.9$ ,  $df = 3$ ,  $p < 0.001$ .

cells<sup>14,15</sup>. Apart from this, amifostine can scavenge free radicals generated by radiation and by alkylating agents<sup>16</sup>. Hence, amifostine has been suggested to protect a broad range of normal tissues from the toxicities of alkylating agents and detrimental effects of radiation<sup>14,17,18</sup>. A similar mechanism could be envisaged for protection by amifostine against SM intoxication. It is also likely that amifostine or its dephosphorylated product, WR-1065 may react with SM<sup>3</sup> and thereby neutralise and reduce the levels of SM inside the cell, and in turn, result in protection. These findings suggest amifostine as a potential prophylactic agent against SM toxicity.

### ACKNOWLEDGEMENTS

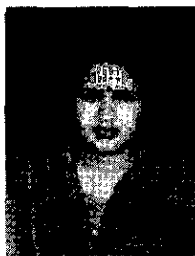
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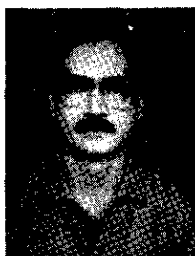
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