Korean J. Toxicol.
Vol. 13, No. 1, 2 pp. 61-69, 1997

Protective Effect of Aminoglycosides and Their Combinations Against 2-Chloroethylethyl Sulfide Exposure

Yun-Bae Kim[a], Gyeung-Haeng Hur, Dae-Sung Choi, Sungho Shin, Seung-Hee Cha, Yong-Keun Park[b] and Dai-Eun Sok[c]

Biomedical Assessment Lab.(1-4-4), Agency for Defense Development, P.O. Box 35 Yuseong, Taejon 305-600,
[b]Department of Biology, Korea University, Seoul 131-701,
[c]College of Pharmacy, Chungnam National University, Taejon 305-764, KOREA
(Received December 23, 1996)
(Accepted March 20, 1997)

ABSTRACT : *Exposure of splenocytes to 2-chloroethylethyl sulfide (CEES) resulted in the cell death, and the cytotoxicity of CEES was prevented by inhibitors of lysosomal hydrolases. Therefore, it has been postulated that the cytotoxicity of CEES may be partially due to the lysosomal labilization. This study, based on this mechanism, was undertaken to determine whether aminoglycoside antibiotics as inhibitors of lysosomal phospholipases and their combinations with other lysosome stabilizers can be useful as a treatment to reduce the CEES toxicity in mice. 2-Chloroethylethyl sulfide (20 mg/kg body weight) was injected ip into female ICR mice, and candidate compounds were administered ip before or after the CEES challenge. Kanamycin (40 mg/kg body weight) as effective as deferoxamine (100 mg/kg body weight) enhanced the survival rate after 5 days of intoxication from 10% of control to 50 - 60%. The most effective was found to be the combination of kanamycin, cycloheximide, deferoxamine and dextrose showing an almost full protection against 2LD50 of CEES. Consistent with the protection of the CEES toxicity, the decrease of body weight in mice intoxicated with CEES was effectively prevented by kanamycin or its combinations. It is suggested that kanamycin or its combination (kanamycin, cycloheximide, deferoxamine and dextrose) would be one of effective antidotes against the CEES poisoning in mice.*

Key Words : *2-Chloroethylethyl sulfide (CEES), Aminoglycosides, Lysosome stabilization, Combinational therapy, Viability, Body weight*

1. INTRODUCTION

Although many reports on the biochemical mechanisms for the cytotoxicity of S-alkylating compounds such as 2-chloroethylethyl sulfide (CEES) and 2,2'-dichlorodiethyl sulfide in various types of cells have appeared, the informations concerning the effective detoxification *in vivo* are limited to a few (Sugendran *et al.*, 1994; Vijayaraghavan *et al.*, 1991; Vojvodic *et al.*, 1985).

In blood (Clayson *et al.*, 1993; Meier and Johnson, 1992) and spleen (Choi *et al.*, 1994) lymphocytes, nicotinamide, an inhibitor of poly(ADP-ribose) polymerase, had been observed to be effective in preventing the S-alkylating vesicants-induced cytotoxicity. However, a single administration of nicotinamide failed to decrease the toxicity in organ culture system (Mol *et al.*, 1991). Although a hypothesis of Ca^{2+}-mediated cytotoxicity in 2,2'-dichlorodiethyl sulfide (Hua *et al.*, 1993; Papirmeister *et al.*, 1991; Ray *et al.*, 1995) had been proposed, there are no reports on the successful protection with Ca^{2+} chelators in animal experiments. Meanwhile, it was suggested that oxidative-type damage, including lipid peroxidation and depletion of glutathione content, was responsible partially for the toxicity of 2,2'-dichlorodiethyl sulfide (Elsayed *et al.*, 1992 and 1989; Gentilhomme *et al.*, 1992; Vijayaraghavan *et al.*, 1991). In surport of the above suggestion, the survival time of animals poisoned by 2,2'-dichlorodiethyl sulfide was found to be prolonged by administration of either tocopherol or flavonoids (Vijayaraghavan *et al.*, 1991; Vojvodic *et al.*, 1985). In addition, cycloheximide, an

[a]To whom correspondence should be addressed.

inhibitor of protein synthesis, was observed to decrease the toxicity of nitro- gen mustard to some extent (Weissberg *et al.*, 1978).

Recently, an additional mechanism for CEES cytotoxicity was proposed: CEES can induce a lysosomal destabilization by presumably activating lysosomal cysteine-proteases or phospholipases (Choi *et al.*, 1995; Shin *et al.*, 1995). The CEES-induced cytotoxicity in splenocytes was successfully protected by lysosomal hydrolase inhibitors such as aminoglycosides, pepstatin and leupeptin (Sok *et al.*, 1995). Thus, the lysosomal hydrolase inhibitors or other lysosome stabilizers were expected to protect against the intoxication of animals by CEES.

These observations necessitate the investigation on the efficiency of aminoglycosides and their combinations with candidate drugs potentially capable to counteract toxic effects of CEES. This study was performed in relation to survival time, degree of protection and body weight loss. In the present study, it is proposed that aminoglycosides and their combinations would be beneficial for the treatment of poisoning by S-alkylating compounds such as CEES.

II. MATERIALS AND METHODS

1. Materials

2-Chloroethylethyl sulfide (CEES) was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). The other agents, including RPMI 1640 medium, were provided by Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fetal calf serum was from Difco Laboratories (Detroit, MI, U.S.A.).

2. Protection of splenocytes against CEES cytotoxicity

Splenocytes were prepared from the spleen of female ICR mice (body weight, 25-30 g, as described (Rosenberg and Lafrage-Frayssinet, 1973). Cultures were carried out at a cell density of 1×10^6/m*l* in RPMI 1640 medium supplemented with fetal calf serum (Choi *et al.*, 1995). After the incubation in a controlled environmental incubator (37°C and 5% CO_2) for the time indicated, an aliquot (100 μ*l*) of sample was taken and used for the determination of cell viability using trypan blue-exclusion method (Meier and Johnson, 1992). CEES (final conc., 1 mM) dissolved in a small volume of ethanol (final conc., 0.5%) was added directly to the culture medium containing splenocytes in either the absence or presence of each candidate compound which was added 5 min prior to CEES exposure. Culture tubes in control groups were given ethanol alone. Following exposure to CEES for 1 hr or 3 hr, the cell viability was determined as described above, and expressed as the percentage of the control without CEES. Results represent the mean±SE of five determinations. Tests of significance were performed using Dunnett's *t*-test (Gad and Weil, 1982), with $P<0.05$ as a criterion of difference.

3. Protection of mice against CEES intoxication

Female ICR mice (body weight, 20-25 g) were challenged with various doses (10-30 mg/kg body weight) of CEES. CEES was diluted in propylene glycol (4 m*l*/kg body weight) as a vehicle, and administered intraperitoneally into the lower part of left side of mouse abdomen (Balali-Mood, 1989; Field *et al.*, 1964). Candidate compounds or combinations of various doses, dissolved in physiological saline (10 m*l*/kg body weight), were administered into the other side of abdomen either before (20 min or immediately) or after (10 or 20 min) the CEES challenge as described in legends. Mice in control groups received the same volume of vehicles. The survival rate (%) and body weight of mice (10 - 15 mice per group) were measured every day during 5-day post-treatment period (Vojvodic *et al.*, 1985), and expressed as the percentile mean of control (vehicle-treated mice).

The experiments performed here were conducted according to the "Guide Principles in the Use of Animals in Toxicology" which had been adopted by the Society of Toxicology in 1989.

III. RESULTS

Earlier studies demonstrated that there may be

Table 1. Effect of candidate compounds on CEES-induced cytotoxicity in splenocytes. Splenocytes were incubated with each compound for 5 min prior to CEES exposure (1 mM), and further incubated for 1 hr or 3 hr. Cell viability was determined with trypan blue-exclusion method at the time indicated

Treatment	Concentration (μM)	Viability (% of control)
CEES alone (1 hr)		63.2±2.5
+Kanamycin	100	85.1±0.4*[a]
+Pepstatin	6	87.0±2.2*[a]
+Leupeptin	60	88.3±2.5*[a]
+Nicotinamide	1,000	85.3±2.9*[a]
+Deferoxamine	100	78.5±3.7*
+Trifluoperazine	0.5	74.1±5.2
+APMSF	20	57.1±4.7
CEES alone (3 hr)		39.8±4.1
+Cycloheximide	100	62.5±3.1*

*Significantly different from CEES alone ($P<0.05$).
[a]Please see references Choi *et al.* (1994) and Sok *et al.* (1995).

Table 2. Change in viability of mice after CEES intoxication. CEES (0-40 mg/kg body weight), diluted in propylene glycol (4 *ml*/kg body weight), was administered intraperitoneally, and the survival rate (%) was counted for 5 days

Compounds (mg/kg)	Day after treatment 0	1	2	3	4	5
CEES (0)	100	100	100	100	100	100
(10)	100	100	100	90	70	40
(20)	100	100	90	65	30	10
(30)	100	90	40	10	0	0
(40)	100	70	10	0	0	0

multiple mechanisms for the S-alkylating vesicants-induced cytotoxicity in lymphocytes (Choi *et al.*, 1994 and 1995; Clayson *et al.*, 1993; Papirmeister *et al.*, 1991). To see whether the prevention of CEES-induced cytotoxicity in organ cells may contribute to the protection of CEES toxicity *in vitro* tests, the protective action of various candidate compounds, which have been reported to reduce the cytotoxicity of CEES, was examined and compared.

First, when the effect of the individual compound against CEES-induced cytotoxicity in splenocytes was examined (Table 1), it was found that not only inhibitors of lysosomal hydrolases such as kanamycin, pepstatin and leupeptin (Sok *et al.*, 1995) but also an inhibitor of poly(ADP-ribose) polymerase, nicotinamide (Choi *et al.*, 1994 and 1995), enhanced the viability of cells exposed to 1 mM CEES for 1 hr from 63.2% to more than 85%. Meanwhile, deferoxamine and trifluoperazine exhibited a modest enhancement (74.1-78.5%). In a related experiment (unpublished data), however, the decrease of intracellular ATP in lymphocytes exposed to CEES for 1 hr was not prevented successfully by these compounds except nicotinamide which expressed an almost complete recovery (> 90%).

Based on the above results, we turned to the study on the *in vivo* protection against acute toxicity of CEES. LD50 value in intraperitoneal toxicity of CEES based on a 5-day mortality was estimated to be 10 mg/kg body weight (Table 2). In the repeated experiments, mice which survived after the period of observation of 5 days were found to be viable for at least 10 days (Vojvodic *et al.*, 1985). Table 3 shows that all drugs tested prolonged the survival time of mice administered by a single dose (20 mg/kg body weight, 2LD50) of CEES. Out of six singly-administered drugs, the best protective action against CEES toxicity was achieved by deferoxamine (Def, 100 mg/kg body weigtht) and kanamycin (KM, 40 mg/kg body weight), which showed a survivability of 60% and 50%, respectively on 5th day, compared to 10% of control. Among aminoglycoside antibiotics, kanamycin was more efficacious than gentamycin (GM), amicacin (AM) or tobramycin (TM). Cycloheximide (Cyh) at 1 mg/kg body weight exhibited a substantial protection, but its effect at 0.3 mg or 3 mg/kg body weight was meager. Also, dextrose demonstrated a similar protective action. Meanwhile, either nicotinamide or trifluoperazine was with a negligible effect (unpublished data). Even leupeptin or pepstatin, inhibitors of proteolytic enzymes, showed only a modest effect.

Next, the combination of two or three candidate drugs was tested for the protection against CEES toxicity. Simultaneous administration of two or three drugs was more effective than a single administration in the protective action against intoxication by 2LD50 of CEES. All the combinations at optimum doses, except the combination of kanamycin and deferoxamine which appeared to antagonize each other, augmented the survival

Table 3. Protective effect of candidate compounds and their combinations on the survival rate of mice intoxicated with CEES. Each compound or their combinations, dissolved in isotonic saline (10 ml/kg body weight), was administered 20 min before CEES intoxication (20 mg/kg body weight), and the survival rate was expressed the percentile mean of control as described in Materials and Methods section

Compounds (mg/kg)	Day after treatment					
	0	1	2	3	4	5
CEES alone (20)	100	100	90	65	30	10
+Kanamycin (KM)						
(10)	100	100	100	80	50	10
(20)	100	100	100	100	60	20
(40)	100	100	100	100	80	50
+Gentamycin (GM)						
(5)	100	100	100	100	70	20
(10)	100	100	100	100	65	25
(20)	100	100	100	100	60	40
(40)	100	100	100	60	45	10
+Amicacin (AM)						
(10)	100	100	100	75	45	10
(20)	100	100	100	100	70	25
(40)	100	100	100	100	60	40
+Tobramycin (TM)						
(1)	100	100	100	75	56	20
(2.5)	100	100	100	100	70	25
(5)	100	100	100	100	75	10
(10)	100	100	100	100	50	10
+Cycloheximide (Cyh)						
(0.3)	100	100	100	65	30	10
(1)	100	100	100	100	50	40
(3)	100	100	100	85	45	20
+Deferoxamine (Def)						
(20)	100	100	100	70	20	10
(50)	100	100	100	100	75	30
(100)	100	100	100	100	80	60
(200)	100	100	100	100	30	10
+Dextrose (Dex)						
(5)	100	100	100	70	40	20
(10)	100	100	100	100	75	45
(50)	100	100	100	70	55	35
(100)	100	100	100	70	40	20
(500)	100	100	100	85	65	50
(1000)	100	100	100	95	55	35
+ KM + Cyh						
(40) (1)	100	100	100	100	80	60
(3)	100	100	100	100	80	30
(10)	100	100	80	70	50	50
+ KM + Def						
(40) (100)	100	100	100	100	75	30
+ Cyh + Def						
(1) (100)	100	100	100	100	100	80
+ KM + Cyh + Def						
(40) (1) (10)	100	100	100	80	70	60
(20)	100	100	100	80	70	50
(50)	100	100	100	100	90	80
(100)	100	100	100	100	100	90
+ GM + Cyh + Def						
(20) (1) (100)	100	100	100	100	100	50
+ Dex + Def						
(500) (100)	100	100	100	100	80	60

Table 3. Continued.

Compounds (mg/kg)	Day after treatment					
	0	1	2	3	4	5
+ Dex + Def + KM						
(500) (100) (20)	100	100	100	100	80	40
(40)	100	100	100	100	100	60
+ Def + Cyh + KM + Dex						
(100) (1) (40) (5)	100	100	100	100	100	80
(10)	100	100	100	100	100	90
(50)	100	100	100	100	100	100
(100)	100	100	100	100	100	90
(500)	100	100	100	100	100	100

rate after 5 days of intoxication from 10% of control to≥60%. The most pronounced protection was achieved with a three component regime, kanamycin (40 mg/kg body weight), cycloheximide (1 mg/kg body weight) and deferoxamine (100 mg/kg body weight). It is noteworthy to observe that the addition of dextrose (Dex, 50-500 mg/kg body weight) to the combination of three components (KM+Cyh+Def) further enhanced the survivability to the level of full protection.

Based on the above results, we carried out a more detailed study on the protective effect of the best combination (kanamycin + cycloheximide + deferoxamine + detrose, KCDD). The dose (10, 20 or 30 mg/kg body weight) of CEES was varied, and the protective effect of KCDD recipe was evaluated. As shown in Fig. 1, the protective effect of KCDD differed according to the dose of CEES used. While CEES at a dose of 10 or 20 mg/kg body weight was successfully detoxified by KCDD, the poisoning by CEES of 30 mg/kg body weight was partially prevented. Thus, the dose of CEES to be detoxified by KCDD seems to be limited to between 2LD50 and 3LD50. In a further experiment, the effect of administration period on the protection by KCDD was investigated to see the mode by which the KCDD combination exerts the protective action against CEES. There was no remarkable difference between administrations 20 min before and after intoxication (data not shown). Thus, it appears that the protective action of KCDD may be due to the therapeutic action rather than prophylactic action such as scavenger effect of the KCDD combination.

In an attempt to further explain the antidotal action of the combination, KCDD, was examined in mice. The gradual loss of body weight in animals exposed to CEES differed according to the dose, and the maximal loss was commonly observed on the 1st day. When mice poisoned by CEES (20 mg/kg body weight) was treated with each candidate drug, the rate of body weight loss in drug-treated animals was much slower, compared to non-treated animals. Kanamycin and deferoxamine partially prevented the decrease in body weight (Fig. 2), while cycloheximide failed to prevent successfully the loss of body weight. In comparison, while de-

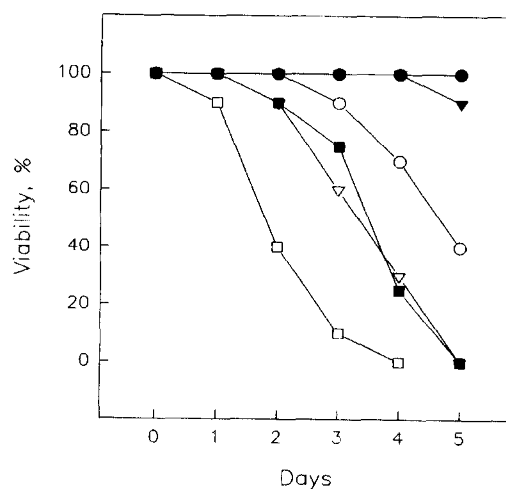

Fig. 1. Protective effect of the best combination of candidate compounds (KCDD) on the survival rate of mice intoxicated with various doses (10-30 mg/kg body weight) of CEES. ○, 10 mg/kg CEES alone; ●, KCDD+ 10 mg/kg CEES; ▽, 20 mg/kg CEES alone; ▼, KCDD+ 20 mg/kg CEES; □, 30 mg/kg CEES alone; ■, KCDD+ 30 mg/kg CEES. KCDD, kanamycin (40 mg/kg)+cycloheximide (1 mg/kg)+deferoxamine (100 mg/kg)+dextrose (500 mg/kg body weight). KCDD regime, dissolved in isotonic saline, was administered 20 min before CEES intoxication, and the survival rate was evaluated as described in Table 3 legend.

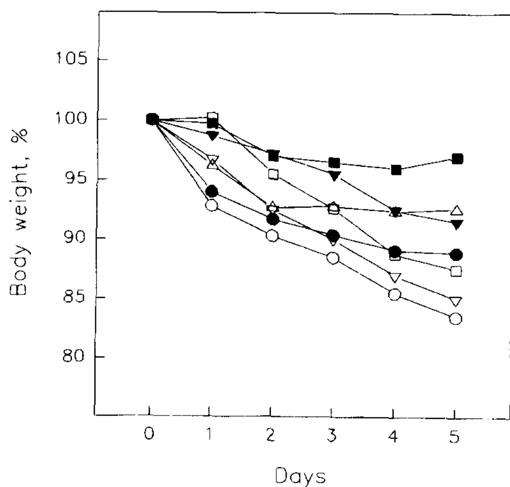

Fig. 2. Effect of various combinations of candidate compounds at their optimum doses on the body weight change of mice intoxicated with CEES (20 mg/kg body weight). ○, 20 mg/kg CEES alone; ●, KM; ▽, Def; ▼, Def+KM; □, Def+Cyh; ■, Def+Cyh+KM; △, Def+Cyh+GM. KM, kanamycin (40 mg/kg); Def, deferoxamine (100 mg/kg); Cyh, cycloheximide (1 mg/kg); GM, gentamycin (20 mg/kg body weight). Body weight was measured every day, and expressed as the percentile mean of that of initial day.

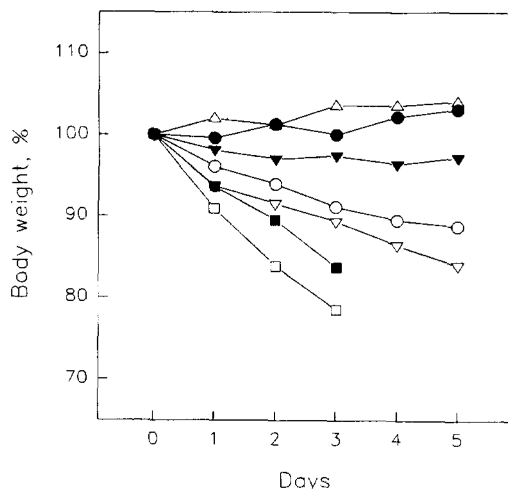

Fig. 3. Effect of the best combination of candidate compounds (KCDD) on the body weight change of mice intoxicated with various doses (10-30 mg/kg body weight) of CEES. △, vehicle (4 ml/kg propylene glycol); ○, 10 mg/kg CEES alone; ●, KCDD+10 mg/kg CEES; ▽, 20 mg/kg CEES alone; ▼, KCDD+20 mg/kg CEES; □, 30 mg/kg CEES alone; ■, KCDD+30 mg/kg CEES. KCDD was prepared as described in Fig. 1 legend.

feroxamine was more effective in the early phase of CEES intoxication, the effect of kanamycin appeared to be more remarkable in the later phase. The combination of deferoxamine and kanamycin enhanced the level of body weight from 83% of control to 92%, in contrast to 88% by the combination of deferoxamine and cycloheximide. The level achieved by the combination of three components, deferoxamine, cycloheximide and kanamycin, approximates 96%. The replacement of kanamycin by gentamycin (20 mg/kg body weight) in the combination was not favorable for the regain of body weight. Moreover, the inclusion of dextrose further enhanced the combinational effect of deferoxamine, cycloheximide and kanamycin. Thus, maximal weight gain was found with the combination of kanamycin, cycloheximide, deferoxamine and dextrose (Fig. 3), which was observed to be the best for the protection against CEES intoxication. However, the best combination failed to prevent the gradual loss of body weight in mice intoxicated with 3LD50 of CEES.

IV. DISCUSSION

The present study shows a favorable degree of protection achieved by several drugs in acute poisoning of mice by CEES. The drugs have been chosen on the basis of recent accomplishments in the cytotoxic mechanisms of sulfur mustards in organ cells; the activation of poly(ADP-ribose) polymerase (Papirmeister *et al.*, 1985), Ca^{2+}-mediated cytotoxicity (Hua *et al.*, 1993; Ray *et al.*, 1995) and radical-mediated lipid peroxidation (Elsayed *et al.*, 1992 and 1989; Vijayaraghavan *et al.*, 1991). Our recent observation, CEES-induced lysosomal labilization (Choi *et al.*, 1995; Shin *et al*,. 1995), adds to the above mechanisms.

Beneficial effect of kanamycin in the detoxification of CEES toxicity was manifested by significant reduction of lethal effects, and faster regaining of body weight loss in mice intoxicated by CEES. Moreover, the dose for effective detoxification of CEES is close to the range of the therapeutic dose for antibacterial effect. The effect of kanamycin might be partially due to the prevention of CEES-induced cytotoxicity in rapidly-dividing cells as demonstrated in the protection by lysosomal hydrolase inhibitors against CEES cytotoxicity in lymphocytes (Sok *et al.*, 1995). The primary mechanism for the effect of kanamycin may be the prevention of lysosome destabilization by in-

hibiting lysosomal phospholipases activated during the course of tissue injury by CEES, although other mechanisms are not excluded. Also, deferoxamine, a chelator of Fe^{3+} responsible for the formation of radicals causing lipid peroxidation, exhibited a significant protection in animal experiments, in support of previous results by Elsayed *et al.* (1992 and 1989) and Vijayaraghavan *et al.* (1991) who proposed the mustard-induced lipid peroxidation. The mobilization of Fe^{3+}, required for the lipid peroxidation-mediated cell killing, can be also induced by autophagic degradation of ferritin (Dean *et al.*, 1993; Laub *et al.*, 1985; Sakaida *et al.*, 1990), during which lysosomal phospholipids may be more labile to iron-catalyzed lipid peroxidation (Myers *et al.*, 1991; Weglicki *et al.*, 1984). Thus, some part of protection by deferoxamine might be due to the removal of Fe^{3+} mobilized following the autophagy accompanied by lysosomal labilization. This might explain partly why there seems to be an antagonism between kanamycin and deferoxamine in the detoxification of CEES toxicity.

In addition, cycloheximide, which showed a small effect to prevent the cytotoxicity of CEES in lymphocytes, remarkably antagonized the toxic action of CEES *in vivo* test at a dose of 1 mg/kg body weight, close to a concentration responsible for the inhibition of protein synthesis. Moreover, cycloheximide is known to be an inhibitor of autophagy (Kovacs *et al.*, 1981) related to lysosomal action. Thus, it is supposed that cycloheximide might show its effect both directly through autophagic inhibition and indirectly by interfering with the synthesis of proteins involved in cytotoxicity-inducing process. Recently, it was reported that dextrose-saline treatment was also effective for the protection of mice against 2,2'-dichlorodiethyl sulfide poisoning (Sugendran *et al.*, 1994) by replenishing the fluid loss. In our present study, dextrose, either alone or in combination with other drugs, showed a beneficial effect to some extent in reducing CEES toxicity.

Although nicotinamide was effective to protect lymphocytes against CEES cytotoxicity (Choi *et al.*, 1994; Clayson *et al.*, 1993), it failed to reduce the toxicity of CEES in animal experiments, suggesting that nicotinamide can prevent the cell death induced by decrease of intracellular ATP, but not the cell death by other causes. This suggestion might be consistent with our unpublished result that nicotinamide (1 mM), which prevented successfully the cytotoxicity of CEES in splenocytes during 1 hr incubation, failed to reduce the CEES cytotoxicity during the 24 hr exposure to CEES. Although corticosteroids such as dexamethasone had been used for the treatment of local and systemic effects produced by 2,2'-dichlorodiethyl sulfide (Vojvodic *et al.*, 1985), dexamethasone was ineffective to reduce the lethality in mice under conditions used. Rather, dexamethasone worsened the body weight loss of animals intoxicated with CEES (data not shown). A similar result was obtained in animals intoxicated with cyclophosphamide, another alkylating compound. Moreover, cyclophosphamide promoted the atrophy of thymus and spleen synergistically with dexamethasone (unpublished result). Even tocopherol, which expressed some protection in earlier observation (Vojvodic *et al.*, 1985), was ineffective to treat CEES poisoning. This discrepancy might be explained by the assumption that between 2,2'-dichlorodiethyl sulfide and CEES there are significant differences in physico-chemical properties such as vaporization, penetration through biomembranes and chemical reactivity.

The triple drug treatment regime (kanamycin, cycloheximide and deferoxamine) was one of the most effective in mice poisoned by CEES, protecting 90% of mice against 2LD50 of CEES. Furthermore, it is noteworthy to observe that KCDD regime expressed an almost full protection against 2LD50 CEES under the condition used. Consistent with its protective action against CEES toxicity, the KCDD regime, when singly administered before CEES intoxication, was remarkably effective in the prevention of body weight loss. It is possible that the effect of kanamycin on body weight was due to the water and salt retention following functional damage of kidneys. However, the possibility is less likely, since the effect of aminoglycosides in preventing the weight loss was greater with kanamycin than gentamycin which shows a greater nephrotoxicity. Moreover, the effect of kanamycin

was achievable with single dose. In addition, dextrose with a fluid-replenishing effect (Sugendran *et al.*, 1994) further potentiated the preventive effect of the triple regime containing kanamycin (kanamycin, cycloheximide and deferoxamine). Thus, it seems that aminoglycosides exert their protective action by mechanisms different from fluid retention. The above observations, taken together with the decrease in lethality, suggest that the regime KCDD would be promising as a kind of antidote against CEES poisoning.

ACKNOWLEDGEMENTS

The authors thank principal researcher Byung-Gon Han for constructive criticisms and Mr. Gyu-Baek Yeon for excellent technical assistance and animal care.

REFERENCES

Balali-Mood, M. (1989): Protective effects of N-acetylcysteine and sodium thiosulfate on sulfur mustard poisoning in mice *in Supplement to Proceedings of the Third International Symposium on Protection Against Chemical Warfare Agents* (National Defence Research Establishment, Ed.), (FOA, Umea), p. 201-210.

Choi, D.-S., Park, Y.-K., Kim, Y.-B., Hur, G.-H. and Sok, D.-E. (1994): Protective effect of poly(ADP-ribose) polymerase inhibitors on 2-chloroethylethyl sulfide-induced cytotoxicity in murine lymphocytes, *Korean Biochem. J.*, **27**, 544-549.

Choi, D.-S., Shin, S.-H., Kim, Y.-B., Cha, S.-H. and Sok, D.-E. (1995): An additional mechanism for the cytotoxicity of 2-chloroethylethyl sulfide in spleen lymphocytes; lysosomal labilization, *J. Biochem. Mol. Biol.*, **28**, 79-82.

Clayson, E., Kelly, S.A. and Meier, H.L. (1993): Effects of specific inhibitors of cellular functions on sulfur mustard-induced cell death, *Cell Biol. Toxicol.*, **9**, 165-175.

Dean, R.T., Gieseg, S. and Davies, M.J. (1993): Reactive species and their accumulation on radical-damaged proteins, *Trends Pharmacol. Sci.*, **18**, 437-441.

Elsayed, N.M., Omaye, S.T., Klain, G.J. and Korte, D. W., Jr. (1992): Free radical-mediated lung response to the monofunctional sulfur mustard butyl 2-chloroethyl sulfide after subcutaneous injection, *Toxicology*, **72**, 153-165.

Elsayed, N.M., Omaye, S.T., Klain, G.J., Inase, J.L., Hahlberg, E.T., Wheeler, C.R. and Korte, D.W., Jr. (1989): Responses of mouse brain to a single subcutaneous injection of the monofunctional sulfur mustard, butyl 2-chloroethyl sulfide (BCS), *Toxicology*, **58**, 11-20.

Field, J.B., Mireles, A., Dolendo, E.C. and Ershoff, B.H. (1964): Reversal of nitrogen mustard intoxication by anti-histamines, *Proc. Soc. Exp. Biol. Med.*, **115**, 1060-1062.

Gad, S.C. and Weil, C.S (1982): Statistics for toxicologists *in Principles and Methods of Toxicology* (Hayes, W., Ed.), (Raven Press, New York.), p. 273.

Gentilhomme, E., Neveux, Y., Hua, A., Thiriot, C., Faure, M. and Thivolet, J. (1992): *In vitro* toxicological lesions of bis(betachloroethyl)sulfide (BCES) on human epidermis reconstituted in culture. Morphological alterations and biochemical depletion of glutathione, *Toxicol. in Vitro*, **6**, 139-147.

Hua, A., Daniel, R., Jasseron, M.P. and Thiriot, C. (1993): Early cytotoxic effects induced by bis-chloroethyl sulphide (sulfur mustard): $[Ca^{2+}]_i$ rise and time-dependent inhibition of B77 fibroblast serum response, *J. Appl. Toxicol.*, **13**, 161-168.

Kovacs, A.L. and Seglen, P.O. (1981): Inhibition of hepatocyte protein degradation by methylaminopurines and inhibitors of protein synthesis, *Biochim. Biophys. Acta*, **676**, 213-220.

Laub, R., Schneider, Y.-J., Octave, J.-N., Trouet A. and Crichton, R.R. (1985). Cellular pharmacology of deferrioxamine B and derivatives in cultured rat hepatocytes in relation to iron mobilization, *Biochem. Pharmacol.*, **34**, 1175-1183.

Meier, H.L. and Johnson, J.B. (1992): The determination and prevention of cytotoxic effects induced in human lymphocytes by the alkylating agent 2,2'-dichlorodiethyl sulfide (sulfur mustard, HD), *Toxicol. Appl. Pharmacol.*, **113**, 234-239.

Mol, M.A.E., de Vries, R. and Kluivers, A.W. (1991): Effects of nicotinamide on biochemical changes and microblistering induced by sulfur mustard in human skin organ cultures, *Toxicol. Appl. Pharmacol.*, **107**, 439-449.

Myers, B.M., Prendergast, F.G., Holman, R., Kuntz, S. M. and LaRusso, N.F. (1991): Alterations in the structure, physicochemical properties, and pH of hepatocyte lysosomes in experimental iron overload, *J. Clin. Invest.*, **88**, 1207-1215.

Papirmeister, B., Feister, A.J., Robinson, S.I. and Ford, R.D. (1991): Medical Defense against Mustard Gas: Toxic Mechanisms and Pharmacological Implications (CRC Press, Boca Raton).

Papirmeister, B., Gross, C.L., Meier, H.L., Petrali, J.P. and Johnson, J.B. (1985): Molecular basis for mus-

tard-induced vesication, *Fund. Appl. Toxicol.*, **5**, S 134-S140.

Ray, R., Legere, R.H., Majerus, B.J. and Petrali, J.P. (1995): Sulfur mustard-induced increase in intracellular free calcium level and arachidonic acid release from cell membrane, *Toxicol. Appl. Pharmacol.*, **131**, 44-52.

Rosenberg, Y. and Lafrage-Frayssinet, C. (1973): Inhibitory effect of Fusarium T2-toxin on lymphoid DNA and protein synthesis, *Toxicol. Appl. Pharmacol.*, **70**, 283-288.

Sakaida, I., Kyle, M.E. and Farber, J.L. (1990): Autophagic degradation of protein generates a pool of ferric iron required for the killing of cultured hepatocytes by an oxidative stress, *Mol. Pharmacol.*, **37**, 435-442.

Shin, S., Choi, D.-S., Kim, Y.-B., Cha, S.-H. and Sok, D.-E. (1995): The release of arylsulfatase from liver lysosomes exposed to 2-chloroethylethyl sulfide, *Chem.-Biol. Interact.*, **97**, 229-238.

Sok, D.-E., Choi, D.-S., Park, Y.-K., Kim, Y.-B. and Cha, S.-H. (1995): Protection by lysosomal hydrolase inhibitors against cytotoxicity of 2-chloroethylethyl sulfide, *Fd Chem. Toxicol.*, **33**, 597-600.

Sugendran, K., Jeevaratnam, K., Vijayaraghavan, R. and Das Gupta, S. (1994): Therapeutic efficacy of saline and glucose-saline against dermally applied sulphur mustard intoxication in mice, *Def. Sci. J.*, **44**, 21-23.

Vojvodic, V., Milosavljevic, Z., Boskovic, B. and Bojanic, N. (1985): The protective effect of different drugs in rats poisoned by sulfur and nitrogen mustards, *Fund. Appl. Toxicol.*, **5**, S160-S168.

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.

Weglicki, W.B., Dickens, B.F. and Mak, I.T. (1984): Enhanced lysosomal phospholipid degradation and lysophospholipid production due to free radicals, *Biochem. Biophys. Res. Comm.*, **124**, 229-235.

Weissberg, J.B., Herion, J.C., Walker R.I. and Palmer, J.G. (1979): Effect of cycloheximide on the bone marrow toxicity of nitrogen mustard, *Cancer Res.*, **38**, 1523-1527.