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Cysteamire, Isothiouronium 誘導體 및 이들과 Phosphorothioate와의 混合物들의 放射線 防護效果

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Radioprotective Effects of Cysteamine, Isothiouronium Derivatives and Their Combinations with a Phosphorothioate

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Abstract

Cysteamine(Mercaptoethylamine, MEA), S,2-Aminoethylisothiouronium Bromide. HBr (AET), and S-(2-Aminoethyl) Dihydrogen Phosphorothioate (WR-638) were prepared and radioprotective effects of these chemicals and their combinations against ^{60}Co γ-ray radiation were studied in irradiated ICR mice. Intraperitonially administered chemicals or their combinations resulted drug deaths in cases of MEA, AET, and their combinations, whereas there were a slight or no drug death in cases of WR-638 and the combination of AET with WR-638. All tested chemicals and their combinations yielded radioprotective effects against ^{60}Co γ-ray radiation. An additive radioprotection effect was observed in case of the combination of AET with WR-638.

I. INTRODUCTION

Chemicals summarized in the table. 1 and others had been known in listeratures[1] as the potent chemical radioprotectants against ionizing radiation, but these chemicals had suffered from their toxic effectshin vivo, which had prohibited them from being applied clinically as the prophylactic agent for a radiation injury. In case of the WR-series of phosphorothioate structures, the toxic effect was expected to be very mild enough to be applied clinically and extensive investigations for proving the feasibility of these chemicals for a practical application had been accumulated during the period of past ten years[2]. Lately, the clinical application at a hospital in the United States has been reported with a good result[3]. The phosphorothioate group in the molecule of this series of compounds seemed to be dephosphoryated in vivo by the action of an enzyme and this route of the biological reaction has been considered to result the less toxicity.[4]

In some recent literatures[5,6] an additive radioprotective effect was observed for the mixture of aforementioned chemicals with no additive toxic effects, which seemed to be a promising approach to effect an increased radioprotective effect with less amount of chemicals of toxicity.

In this paper, both drug toxicities and radioprotective effects of known chemical radioprotectants were closely studied in the irradiated ICR mice administering chemicals of the author's preparation. Particular emphasis was laid on the evaluation of the drug toxicity and the radioprotective effect of the prepared compound in vivo. Further attempts were also envisaged to observe the additive radioprotective effect of the binary mixture of the prepared compounds with a phosphorothioate which has been known as the less toxic radioprotectant as described above.

Table 1. Chemical Radioprotectants against Ionizing Radiation.

Classification	Relevant Chemical Structure
Cysteines	$HS-CH_2-\underset{\displaystyle NH_2}{\underset{\vert}{CH}}\ COOH$
Cysteamines	$HS-CH_2-CH_2-NH_2$ and their derivatives.
AET	$H_2N-CH_2-CH_2-S-\underset{\displaystyle NH}{\overset{\displaystyle \Vert}{C}}NH_2$
WR-Series	$H_2N(CH_2)_nNH(CH_2)_mSPO_3H_2$ (n=3, m=2, WR-2721) (n=2, m=0, WR-638)
Disulfides	$S-CH_2CH_2-NH_2$ $S-CH_2CH_2-NH_2$ (Capsytamine)
Others	$H_2N(CH_2)_n-\underset{\displaystyle NH_2}{\underset{\vert}{CH}}-CH_2SPO_3H_2$

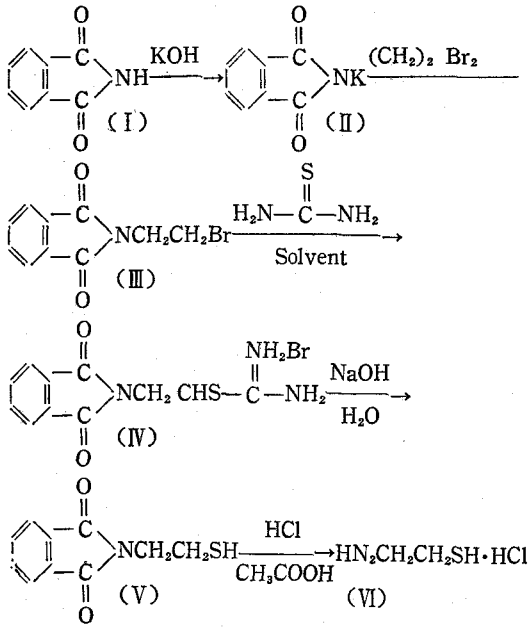
II. EXPERIMENTAL

1. Chemicals

The following chemical compounds were prepared at this laboratory according to the synthetic schemes shown in the Fig.1: Mercaptoethylamine hydrochloride (MEA), S. 2-Aminoethylisothiouronium Bromide HBr (AET), and Sodiumhydrogen S-(2-Aminoethyl) Phosphorothioate. (WR-638) Detailed data on the preparative process and constants were reported elsewhere in the author's publication.[7]

Fig. 1. Schems of Preparing Chemical Radioprotectants.

1. Cysteamine (MEA)



2. S, 2-Aminoethylisothiuronium Bromide HBr.(AET)

$$H_2NCH_2CH_2OH \xrightarrow[H_2O]{HBr} H_2NCH_2CH_2Br\cdot HBr \quad (VII)$$

$$H_2NCH_2CH_2Br\cdot HBr \xrightarrow[\text{Solvent}]{H_2N-\overset{\displaystyle S}{\overset{\displaystyle \Vert}{C}}-NH_2} H_2N-CH_2-CH_2-\underset{\displaystyle NH_2}{\overset{\displaystyle \Vert}{S}}-C-NH_2\cdot HBr \quad (VIII)$$

3. Sodiumhydrogen S-(2-aminoethyl)phosphorothioate (WR-638)

$$PCl_3 + S \xrightarrow{AlCl_3} PSCl_3$$

$$PSCl_3 + NaOH \xrightarrow{H_2O} Na_3SPO_3\cdot H_2O \xrightarrow{EtOH\cdot\triangle} Na_3SPO_3$$

$$NH_2CH_2CH_2OH \xrightarrow[H_2O]{HBr} NH_2CH_2CH_2Br\cdot HBr \quad (VII)$$

$$NH_2CH_2CH_2Br\cdot HBr \xrightarrow[C_2H_5OH\ DMF]{Na_3SPO_3} H_2NCH_2CH_2SPO_3HNa \quad (IX)$$

2. Methods

A. Lethal Radiation Dose.

ICR male mice, 4 and 8 weeks old and weighing 25 to 35g., were used throughout. The conditions of irradiation were: ^{60}Co γ-Ray (AEC, Theratrom-780, Source Strength; 4000 Ci.), exposure rate of 100 R/min., and the distance from the source of 80 cm. Mice in a group of 10, in partitioned Lucite

cages, were exposed to γ-rays in the range of 700 to 1200 R. The γ-ray dose measured in air with Capintec Digital Dosimeter-192 during each exposure. The groups of the mice were housed per cage and given free access to food and water after irradiation. The basic design of the experiment is shown in the table.2, with a listing of the total number of mice in each group and the number that survived within 30 days of treatment. Survival percentage of the treated mice was calculated as shown in the table. 2.

Table 2. Lethal Dose of the Irradiated Mice[a] under ^{60}Co γ-Ray Radiation.

Radiation Dose[b] (R)	Numbers of Animal Irradiated	Survival[c] Percent.(%)	Remarks
700	10	100	
800	10	60	
900	10	30	
1,100	10	0	
1,200	10	0	

a. ICR strain. 4-8 weeks old weighing 25-35 g.
b. Dose Rate; 100R/Min.
c. Survival percentage was examined at the end of 30 days after being irradiated.

B. Radioprotective Effect.

ICR both male and female mice, 4 and 8 weeks old and weighing 25 to 35g., were used throughout. The following prepared chemicals were given alone or as a binary mixture: MEA, AET, and WR-638. Solutions of these chemicals or their binary mixtures in physiological saline solution were prepared adjusting such drug concentration as each 0.2ml. portion of the solution would contain the amount of the drug dose listed in tables 3. and 4.

The solutions were neutralized to pH 7.0-7.5 before use and they were administered in the mice intraperitonially 15 minutes prior to the irradiation. The conditions of irradiation and postirradiation treatment of the mice were the same as described in A. Total radiation dose in these experiments were 950 rad. The basic design of the experiment is shown in tables 3 and 4, with a listing of the total number of mice in each group, the number that survived within 3 days of treatment(checking drug deaths) and the number that survived in 10, 15, 20, 25, and 30 days of treatment (checking radiation deaths), respectively. Results were summarized in tables 3 and 4.

C. Additive Radioprotective Effect.

ICR male mice, 4 and 8 weeks old and weighing 25 to 35 g. were used throughout. The basic experimental conditions with regards to radiation conditions, drug treatment, and post irradiation works were the same as described in B. except that

Table 3. Radioprotective Effect of Prepared Chemicals and Their Combinations in the Irradiated Male Mice[a].

Chemicals or Combinations	Drug Dose[b] (mg./25g. of weight)	Numbers of Animal Irradiated[c]	Numbers of Survived Mice Days after being irradiated 3	10	15	20	25	30	Remarks
Control	0.2 ml. of Saline Sol.	10	10	10	0	0	0	0	
MEA	5[6]	10	9	9	9	9	9	9	
AET	8[6]	10	8	8	8	8	8	8	
WR-638	8	10	10	10	5	0	0	0	
MEA and AET	5+8	10	4	4	4	4	4	4	
MEA and WR-638	5+8	10	7	7	7	7	7	7	
AET and WR-638	8+8	10	10	10	10	10	10	10	

a. ICR mice. 4-8 weeks old weighing 25-35g.
b. Administered intraperitoneally 15 minutes prior to the irradiation.
c. Radiation Dose; 950 rad. [2][3] of ^{60}Co r-Ray. Dose Rate; 100 rad./min. Total body was irradiated.

Table 4. Radioprotective Effect of Prepared Chemicals and Their Combinations in the Irradiated Female Mice[a]

Chemicals or Combinations	Drug Dose[b] (mg./25g. of weight)	Numbers of Animal Irradiated[c]	Numbers of Survived Mice Days after being irradiated						Remarks
			3	10	15	20	25	30	
Control	0.2ml. of Saline Sol.	10	10	9	3	0	0	0	
MEA	5[6)]	10	6	6	5	5	5	5	
AET	8[6)]	10	5	5	5	5	5	5	
WR-638	8	10	10	10	2	1	0	0	
MEA and AET	5+8	10	3	3	3	3	3	3	
MEA and WR-638	5+8	10	4	4	4	4	4	4	
AET and WR-638	8+8	10	10	10	10	10	10	10	

a. ICR mice. 4-8 weeksold weighing 25-35g.
b. Administered intraperitoneally 15 minutes prior to the irradiation.
c. Radiation Dose; 950 rad.[2)8)] of ^{60}Co r-Ray. Dose Rate; 100 rad./min. Total body was irradiated.

Table 5. Radioprotective Effect of AET, WR-638, and Their Combinations.

Radiation Dose[a] (rad.)	Chemicals or Combinations	Drug Dose[b] (mg./25g. of weight)	Number of Animal[c] Irradiated	Numbers of Survived Mice Days after being irradiated							Remarks
				3	5	10	15	20	25	30	
1000	Control	0.2ml. of Saline Sol.	10	10	10	9	5	5	5	5[e]	
	AET	8	10	9	9	9	6	5	5	4[e]	
	WR-638	12[d]	10	9	9	9	9	9	9	9	
	AET and WR-638	8+12	10	9	9	9	9	9	9	9	
1500	AET	8	10	10	10	10	10	9	9	9	
	WR-638	12	10	9	9	9	9	8	5	5	
	AET and WR-638	8+12	10	10	10	10	10	10	9	8	
2000	AET	8	10	10	5	0	0	0	0	0	
	WR-638	12	10	10	0	0	0	0	0	0	
	AET and WR-638	8+12	10	10	9	3	0	0	0	0	

a. ^{60}Co r-Ray Radiation. Dose Rate; 100 rad./min. Total body was irradiated
b. Administered intraperitoneally 15 minutes prior to the irradiation.
c. ICR male mice. 4-8 weeks old weighing 25-35g.
d. Effective drug dose is reported as 13mg./25g. of weight.[10)]
e. These values are showing some deviations from those of data in table 3.

radiation doses were varied in the range of 2000 rad. The results were summarized in the table. 5.

III. RESULTS AND DISCUSSION

As shown in the table. 2, the lethal radiation dose ($LD_{(50)/(30)}$; 850R (γ-ray)) of the ICR mice adopted in the present study was little higher than that of the reported value for a BALB/C$^+$ mice ($LD_{(50)/(30)}$; 600R(X-Ray)).[6)] Probably, this difference in the lethal radiation doses was due to both the strain of mice and the kind of ionizing radiation. Antiradiation screening tests in mice against lethal radiations (825 R(X-Ray) or 950-1050 R(γ-Ray) and 950 rad. (^{60}Co) or 849 rad.(^{137}Cs)[8)]) were reported which resulted no 30 days survival among control mice, but no informations were given on the strain of the mice. It was, therefore, considered to be a reason-

able approach to adopt 950 rad. (^{60}Co) as the lethal radiation in the screening tests shown in tables 3 and 4, which may correspond closely to $LD_{(100)/(30)}$ of the ICR mice as indicated in the table. 2. Both ICR male and female mice had resulted no 30 days survival among control mice as indicated in tables 3 and 4.

Antiradiation screening tests in mice reported in literatures have usually been performed by means of male mice only, but female mice may be considered to be feasible to observe the drug susceptibility of the testing animal. Administering the reported effective drug dose[6] of chemicals and their binary mixtures intraperitoneally, both ICR male and female mice had resulted the significant drug deaths in cases of MEA, AET, and their combinations and the combination of MEA with WR-638. The number of drug deaths was more pronounced in female mice than those of male mice.Especially, the combination of MEA with AET resulted the drug deaths in 60% of the control for the male mice, whereas they reached to 70% of the control for the female mice. Since AET in the neutral aqueous solution was reported to rearrange to MEG[9], which has a sulfurhydryl group at the end of the molecule, the observed high drug deaths for a binary mitxture of MEA and AET would be due to the increased concentration of the sulfurhydryl groups in the mixture, which resulted an additive toxic effect. An additive toxicity was also observed in case of the combination of MEA with WR-638 and it was more pronounced for female mice. The exact cause of this observation is not certain at present. Presumably, the phosporothioate group of WR-638 was dephosphorylated to generate the sulfurhydryl group at the beginning of the mixing by MEA molecules, which may result an additive toxicity as the case of the combination of MEA with AET. On the contrary, the combination of AET with WR-638 resulted no drug deaths for both male and female mice. This may be due to the ineffective dephosphorylation of WR-638 by the rearranged MEG molecules of AET. Existing literatures [5,6] had reported no additive toxic effect of the mixtures of three or more chemical radioprotectants, but there are no definite reports describing the toxicity of the binary mixture of aforementioned chemicals. Present observations covering both male and female mice may be reasonable enough to indicate the tendency of the additive toxic effect of the binary mixtures of chemical radioprotectants of aforementioned structures.

For radiation deaths, existing literatures have been reporting only the survival ratio of the treated mice observed at the end of 30 days treatment. The authors had envisaged to follow the number of radiation deaths in the treated mice every 5 days interval to clarify the general tendency of radiation injury occurred in mice definitely. This attempt of the close checking procedure for radiation deaths seemed to be very promising to grasp the general feature of radiation injury occurred in the treated mice. Thus, around 65% of the reported effective drug dose of WR-638 (Lit. Value 500mg./kg.[10]) when it was administered in mice, had resulted 100% radiation deaths at the end of 15 days treatment of male mice as shown in the table 3, which showed definitely the tendency of the radioprotective effect of this dose of WR-638. Generally, all tested chemicals and their binary mixtures could show 100% survival ratio at the end of 27 days after the initial 3 days of checking drug deaths.

Based on the data obtained in tables 3 and 4, further close study on the additive radioprotective effect for the combination of AET with WR-638, which resulted no drug deaths for both male and female mice, was performed as summarized in the table. 5. Unfortunately, the ICR mice adopted in this study were obtained from the different source, which may result some deviations in the strain itself. As shown in the table.5, the data obtained under the radiation dose of 1000 rad. showed a little deviation from those of the table.3, but the general tendency for radiation deaths was quite reasonable to be evaluated. Even though the combination of AET with WR-638 could not show a definite large additive radiation protection effect under doses of 1000 and 1500 rad., it could show the significant additive radioprotective effect under the dose of 2000 rad. Thus, it resulted 0 % survival at the end

of 10 days treatment in case of administering AET alone, but it was around 33% in case of administering the combination of AET with WR-638. Further definite data on the additive radioprotective effect for this combination of chemicals would, therefore, be expectable under doses of ranging from 1500 to 2000 rad. For the determination of DRF[10] values of prepared chemicals and their combinations, further extensive studies may be required for checking $LD_{(50)/(30)}$ value of the treated mice. However, data in the table.5 would indicate the general tendency of DRF of AET, WR-638, and the combination of AET with WR-638 as follows: AET;~2.5 (Lit. 2.0~[1]), WR-638;~2.0 (Lit. 2.07[10]), and the combination; ~2.5, respectively.

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REFERENCES

1. M. Shikita and M. Okazaki, J. Atom. Energ. Soc. Jap. **22**, 303 (1980).
2. J.R. Pipper et al., J. Med. Chem. **12**, 237 (1969).
3. Am. Chem. Soc., Chem. Eng. News. P.28 (July 20, 1981).
4. J.W. Harris and T.L. Phillips, Radiat. Res. **46**, 362 (1971).
5. P.C. Kalistralov et al., Chem. Abstr. **85**, 171721 y (1976).
6. J.R. Maisin, G. Mattelin, and M. Lambiet-Collier, Radiat. Res. **71**, 119(1977).
7. You Sun Kim et al., The annual research report in 1981 (In Press. KAERI, Seoul)
8. J.R. Pipper et al., J. Med. Chem. **22**, 631(1979).
9. D.G. Doherty et al., J. Am. Chem. Soc. **79**, 5667 (1957).
10. J.M. Yuas, J.O. Procter, and L.H. Smith, Radiat. Res. **54**, 222 (1978).

抄　　錄

Cysteamine(Mercaptoethylamine, MEA), S-2-Aminoethyl isothionronium Bromide HBr(AET) 및 S-(2-Aminoethyl) Dihydrogen phosphorothioate(WR-638)들을 合成하고 이들 各各 또는 그들의 混合物의 ^{60}Co γ-線 放射線에 對한 障害防護效果를 ICR생쥐를 使用하여 檢討하였다. 照射前 15分에 腹腔內에 藥劑를 投與하고 그 效果를 檢討한바 MEA, AET 및 그들 混合物에 있어서는 藥毒作用이 觀察되었으나 WR-638 및 AET와의 混合物에서는 藥毒作用이 輕微하거나 또는 거의 없었다. 試驗한 各各의 化合物 및 그 混合物들은 ^{60}Co γ-線 放射線에 對한 防護效果를 나타냈으며 AET와 WR-638의 混合物에 있어서는 附加된 放射線 防護效果가 觀察되었다.