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Durable Radioprotection by Oral Administration of 2-Aminoethanethiosulfuric Acid in Mice

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Oral administration of 2-aminoethianethiosulfuric acid offered a powerful protective action in mice against X-radiation of 600—800 R. The effectiveness continued for more than 6 hr after ingestion of the compound. The radiation protective effect of the compound was not observed, however, in the animals which received 900 R radiation. S-Sulfocysteine was also radioprotective orally, but the effectiveness faded away in one hr.

A Bunte salt, 2-aminoethanethiosulfuric acid, exists as zwitterions and is unaffected by air-oxidation unlike its corresponding thiol, 2-mercaptoethylamine (cysteamine), which has been known as one of the most powerful radioprotectors. Furthermore, the thiosulfate ester is odorless and less toxic than cysteamine.[2] It seems that these properties of 2-aminoethanethiosulfuric adid offer a good usefulness of the compound as an anti-radiation drug, although it is not as effective as cysteamine when it is injected intraperitoneally in mice.[2] The present paper reports that the thiosulfate ester offers a better radioprotective action than cysteamine if it is given orally to the animals. The radioprotective effect of the compound was sustained for more than 6 hr after its ingestion.

Experimental

Animals——Male mice of the ddY strain were used in all the experiments described below. The animals were 5 weeks old at the time of irradiation and were observed for 30 days thereafter as reported previously.[3] The survival time of the animal which survived throughout the experiment was regarded as 30 days. In the experiment shown in Fig. 1, the animals were starved for one night prior to the ingestion of the drug, while in the other experiments the animals were given food *ad libitum*.

X-Irradiation——The X-ray generator (Shimazu, Shin-ai 250-II) was operated at 200 kVp and at 20 mA. The rate of the radiation dose was 95—98 R/min as monitored with a Radocon ionization gauge.

Chemicals——2-Aminoethanethiosulfuric acid and related compounds were provided by Research Laboratories, Yoshitomi Pharmaceutical Industries, Tokyo. The compounds were dissolved in double distilled water and neutralized to pH 7.0. The solution was administered to mice orally by a stainless steel stomach tube or injected intraperitoneally in an amount of 0.2 ml/animal. By a separate experiment, it was shown that the organic thiosulfates and the thiophosphate were sufficiently stable in the aqueous solution, and there was no liberation of the sulfhydryl group which could be detected by the Ellman's reagent.[4]

Result and Discussion

Intraperitoneal Injection of 2-Aminoethanethiosulfuric Acid and Related Compounds

The radioprotective action of various organic thiosulfates was examined in mice after the compounds were injected intraperitoneally in the animals. It will be seen in Table I that

1) Location: *Anagawa, 4-9-1, Chiba-shi.*
2) B. Holmberg and B. Sörbo, *Nature*, **183**, 832 (1959).
3) Y. Takagi, F. Sato, M. Shikita, M. Shinoda, T. Terasima, and S. Akaboshi, *Radiation Research*, **42**, 79 (1970).
4) G.L. Ellman, *Arch. Biochem. Biophys.*, **74**, 443 (1958).

Table I. Radiation protective Effect of 2-Aminoethanethiosulfuric Acid and Rrelated Compounds after Intraperitoneal Injection in Mice

Compounds	Dose (mmoles/kg)	Radioprotective effect	
		Survival days	Survival ratio
$NH_2CH_2CH_2SH \cdot HCl$	0.7	14.4	0.10
	1.4	24.5	0.40
$NH_2CH(COOH)CH_2SSO_3Na$	0.5	13.7	0.10
	1.0	17.2	0.20
	2.0	18.7	0.30
$NH_2CH_2CH_2SSO_3H$	2.0	18.6	0.40
	4.0	23.0	0.40
$NH_2CH_2CH(C_6H_5)SSO_3H$	0.25	10.0	zero
	0.50	10.4	zero
$NH_2CH_2CH_2OSO_3H$	4.0	10.0	zero
	8.0	10.5	zero
None (saline)	—	10.5	zero

The chemicals were injected 10 min prior to X-irradiation (700 R). Each group consisted of 10 animals.

2-aminoethanethiosulfuric acid gave a radioprotective effect comparable with that given by cysteamine, if a larger amount of the compound was injected. S-Sulfocysteine was also as effective as 2-aminoethanethiosulfuric acid, while *o*-sulfate of ethanolamine and 1-phenyl derivative of 2-aminoethanethiosulfuric acid were both ineffective.

Oral Administration of 2-Aminoethanethiosulfuric Acid

In the case of oral administration, the median lethal doses of cysteamine hydrochloride and 2-aminoethanethiosulfuric acid were determined as 11.9 and 18.5 mmoles/kg, respectively. Thus, cysteamine is about 1.6 times as toxic as its thiosulfate ester on the molar basis. It has been reported that the median lethal doses of these compound were 4.4 and 5.7 mmoles/kg, when the compounds were injected intraperitoneally in mice.[2)]

Fig. 1 shows the radioprotective effect of these two compounds as influenced by the dose of X-irradiation. The chemicals were given orally to mice in a dose of 0.6—0.7 LD_{50}. It is noted that the increase in the X-ray dose counteracted the radioprotective effectiveness of these compounds. Seemingly, 2-aminoethanethiosulfuric acid was slightly more effective than cysteamine.

Fig. 2 shows the continuance of the radioprotective effect of 2-aminoethanethiosulfuric

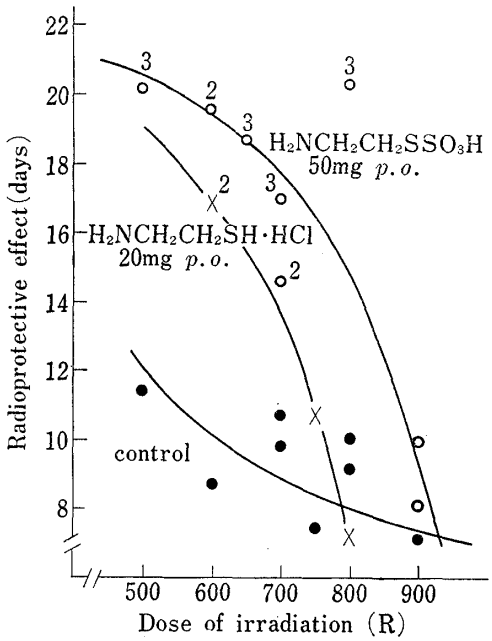

Fig. 1. The Radioprotective Effectiveness of Cysteamine Hydrochloride and 2-Aminoethanethiosulfuric Acid as a Function of Dose of X-radiation. The chemicals were given orally to the mice 15—30 min prior to irradiation. The radioprotective effect of the compound is expressed in the term of average survival days of the animals. Each point represents an average on 10 mice. The number on each point means the survival ratio; *e. g.*, 3=3 out of 10 survived. The point without number on it means that no animal survived.

acid in the case of either oral or intraperitoneal administration. It will be seen that the effectiveness of the compound continued for more than 6 hr when it was given orally, while the effectiveness of intraperitoneally injected compound ended in one hr. Sodium 2-aminoethanethiophosphate also showed a durable radioprotection after oral administration. In this experiment, the development of the radioprotection by 2-aminoethanethiosulfuric acid was not sulfficient in 15—30 min after its ingestion; the result is not consistent with that shown in Fig. 1. Probably, it was due to the fact that the animals were starved for one night in the experiment shown in Fig. 1.

Oral Administration of S-Sulfocysteine

In a same manner as above, S-sulfocysteine was orally or intraperitoneally given to mice prior to irradiation, and the duration of the radioprotective action of the compound was compared with the duration of the effectiveness of cysteine hydrochloride which was injected

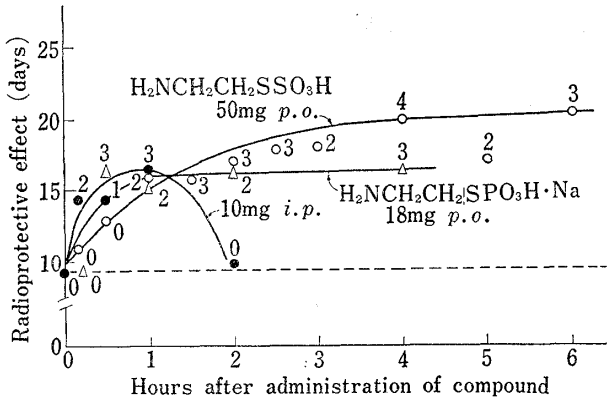

Fig. 2. Duration of the Radioprotective Effectiveness of 2-Aminoethanethiosulfuric Acid (○, ●) and Sodium 2-Aminoethanethiophosphate (△) after Oral or Intraperitoneal Administration in Mice. The amimals were irradiated with a total dose of 700 R of X-rays.

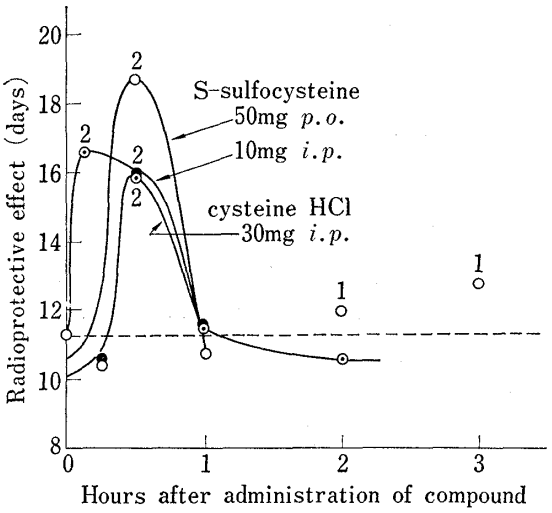

Fig. 3. Duration of the Radioprotective Effectiveness of Sodium S-Sulfocysteine and Cysteine Hydrochloride after Oral or Intraperitoneal Administration in Mice. X-irradiation=700 R.

intraperitoneally. As it was shown in Fig. 3, the radioprotective effect of S-sulfocysteine and cysteine both faded away in one hr after administration. It has been known that S-sulfocysteine is metabolized by liver to pyruvic acid with the liberation of thiosulfate and ammonium ions, while 2-aminoethanethiosfuluric acid is not subjected to this metabolic decomposition.[5] Probably, it is because of this difference of the metabolism that the action of S-sulfocysteine is more rapidly terminated than the action of 2-aminoethanethiosulfuric acid. Conversion of 2-aminoethanethiosulfuric acid to cysteamine ocurrs in the animal body presumably by a nonenzymic process.[6]

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