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Radioprotective Effect of Sodium Hydrogen S-(2-L-Pyrrolidylmethyl)phosphorothioate in Mice

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Several aminoethyl and pyrrolidylmethyl thiosulfate and thiophosphate derivatives were synthesized and tested for the radioprotective effect in X-irradiated mice. Sodium hydrogen S-(L-2-pyrrolidylmethyl)phosphorothioate was most protective among ten compounds tested. It was effective to a same extent as cysteamine which is a typical radioprotector. N-Methylpyrrolidyl derivative of the compound was completely inactive. Furthermore, no radioprotection was observed in case of the corresponding thiosulfate.

It has been long known that 2-aminoethanethiol (cysteamine) protects animals from deleterious effect of ionizing radiation, and that many cysteamine derivatives with substitute(s) on its nitrogen atom are less effective than cysteamine.[2] On the other hand, thiophosphate and thiosulfate derivatives of cysteamine are more effective than cysteamine, presumably because of greater cellular uptake and subsequent rapid hydrolysis to the thiol form.[3,4] This paper reports similar radioprotective action of various cysteamine analogues of the thiophosphate and thiosulfate form. It was noted that sodium hydrogen S-(L-2-pyrrolidylmethyl)phosphorothioate (compound I) which possesses 2-aminoethanethiol structure in its molecule was as powerful as cysteamine under a certain condition of irradiation.

Experimental

Chemicals——Compound I and its N-methyl derivative were synthesized by the reaction of L-2-(bromomethyl)pyrrolidine (or-N-methylpyrrolidine) hydrobromide with trisodium monothiophosphate. Other thiophosphates were synthesized also by similar Na_3SPO_3 reactions according to Åkerfeldt.[5] L-2-Pyrrolidylmethyl thiosulfuric acid was prepared by refluxing an aqueous solution containing L-2-(bromomethyl)pyrrolidine hydrobromide and sodium thiosulfate. Other thiosulfuric acid derivatives were synthesized by the method of Schimmelschmit, *et al.*[6] with use of corresponding aminoethyl chloride derivatives. S-(2-Pyrrolidylmethyl)isothiouronium bromide hydrobromide was prepared by refluxing isopropyl alcohol solution of 2-pyrrolidylmethyl bromide and thiourea. S-(N-Methyl-2-pyrrolidyl)isothiouronium bromide hydrobromide was synthesized by the same method as above but with use of *n*-butanol as the solvent. Details of the method of synthesis and physical properties of these new compounds will be reported separately.[7]

Animals——Male mice (ICR strain, 5 weeks old, 25—30 g body weight) were employed throughout the experiments. Methods of X-irradiation and other experimental conditions were same as those reported previously.[8] Chemicals were dissolved in water and the solutions were neutralized and injected intraperitoneally in the animals in a single dose of 0.2 ml/animal. The amount of the chemicals injected is shown in the table and figures.

Determination of Sulfhydryl Concentration——Tissues were homogenized with 0.1M sodium phosphate buffer (pH 7.4) and extracted with perchloric acid (final concentration, 5%). The extract was neutralized

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2) H. Langendorff and R. Koch, *Strahlen Therapie*, **99**, 567 (1956).
3) S. Åkerfeldt, *Acta Radiol. Ter. Phys. Biol.*, **1**, 465 (1963).
4) J.J. Kelley, N.F. Hamilton and O.M. Friedman, *Cancer Res.*, **27**, 143 (1967).
5) S. Åkerfeldt, *Acta Chem. Scand.*, **13**, 1479 (1959); *idem, ibid.*, **16**, 1897 (1962).
6) K. Schimmelschmit, H. Hoffman and E. Mundlor, *Chem. Ber.*, **96**, 38 (1963).
7) K. Uoji, S. Ikegami and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), in preparation.
8) Y. Takagi, F. Sato, M. Shikita, M. Shinoda, T. Terasima and S. Akaboshi, *Radiation Res.*, **42**, 79 (1970).

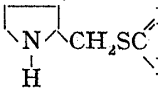
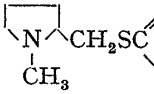
with 5N KOH and resulting precipitate of potassium perchlorate was removed by centrifugation. An aliquot of this supernatant was used for the determination of sulfhydryl concentration by the 5,5′-dithiobis(2-nitro)-benzoic acid method.[9]

Result and Discussion

It was found in a screening test that compound I was most effective in protecting animals against the radiation injury (Table I). It is interesting that corresponding thiosulfuric acid and isothiuronium compounds had no such powerful radioprotective action. Furthermore, N-methylpyrrolidyl derivative of compound I was nonprotective. This difference of the effectiveness was not due to the difference of the rate of hydrolysis of these thiosulfates and thiophosphates in the animal body, because the increase of free sulfhydryl content of tissues (spleen and liver) was similarly observed after the injection of any of these compounds in mice. At the present moment, there is no reasonable explanation on the large difference of effectiveness of these compounds.

The radioprotective action of compound I was confirmed under various conditions of injection and irradiation. The minimum radioprotective dose of this compound was 3—5

Table I. Radioprotective Action of Thiosulfate and Thiophosphate Derivatives

Compound	Dose (mg/animal)	30-Day survival ratio (%)	Survival time (relative to control)
$H_2NCH_2CH_2SSO_3H$	5	10	1.25
	10	20	1.24
$CH_3NHCH_2CH_2SSO_3H$	10	65	2.24
$(CH_3)_2NCH_2CH_2SSO_3H$	5	20	1.46
	7.7	30	1.40
	10	70	2.18
	5	5	1.09
$CH_3NHCH_2CH_2SPO_3HNa$	5	10	1.17
	10	50	1.82
	15	70	2.13
$(CH_3)_2NCH_2CH_2SPO_3HNa$	5	zero	1.29
	10	20	1.34
	15	zero	0.95
 (compound I)	5	30	1.63
	7.5	70	2.26
	10	80	2.31
	5	zero	1.05
	10	zero	0.94
	15	12	0.88
	0.62	zero	0.81
	1.25	zero	1.11
	1.25	zero	0.57
	2.5	zero	0.95
Saline	0.2 (ml)	zero	0.90
$NH_2CH_2CH_2SH \cdot HCl$ (cysteamine)	7	20	1.31
	10	68	2.32

The chemicals were injected in mice (10—40 animals for each group), 10 min prior to X-irradiation (700 R). Average of the survival time of the control animals (non-injected) was 11.4±1.6 days.

9) G.L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

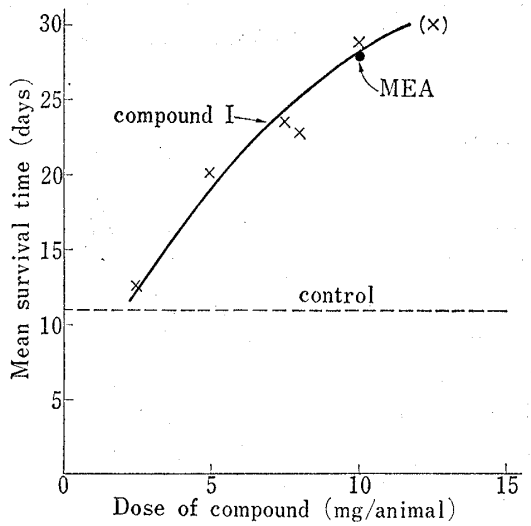


Fig. 1. Radioprotection of Mice by Sodium Hydrogen S-(2-L-Pyrrolidylmethyl)phosphorothioate (Compound I): Effect of the Dose of the Compound

The chemical was injected 15 min prior to X-irradiation (700 R). The ordinate represents the average survival time of the irradiated animals (10 mice in each group); survival time of the animal which was not killed by the radiation was regarded as 30 days. MEA; cysteamine

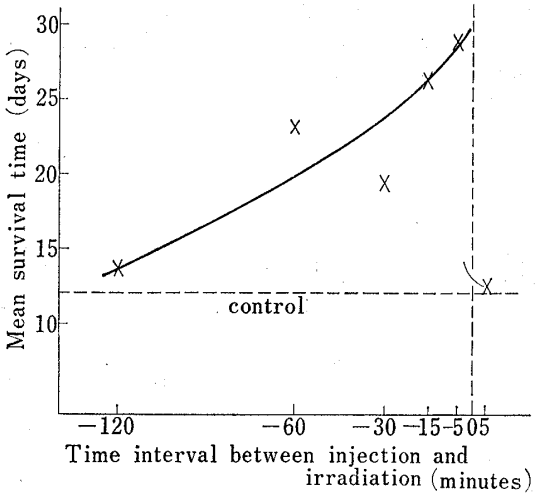


Fig. 2. Radioprotective Action of Sodium Hydrogen S-(2-L-Pyrrolidylmethyl)phosphorothioate (Compound I): Effect of Interval between Injection of the Compound and Irradiation

The dose of the compound was 7.5 mg/animal. See the legend of Fig. 1 for other details of the experiment.

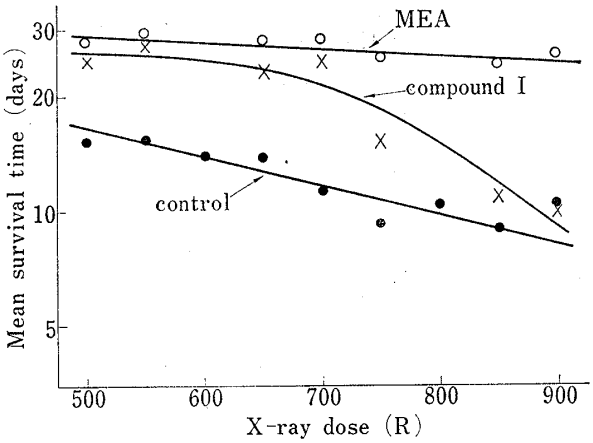


Fig. 3. Radioprotective Action of Sodium Hydrogen S-(2-L-Pyrrolidylmethyl) phosphorothioate (Compound I): Effect of Dose of X-Ray on the Effectiveness of the Compound

Tne radioprotective compounds were injected 5—10 min prior to X-irradiation. Compound I, 8 mg/animal; MEA (cysteamine) 10 mg/animal

mg/animal (Fig. 1), while its LD_{50} in non-irradiated mice was 13 mg/animal. It must be given to the animal shortly before irradiation. When injected 2 hours prior to or 5 min after irradiation, it showed no protection (Fig. 2). This is analogus to the mode of action of cysteamine. However, compound I was not effective when the animals were heavily irradiated, while the effectiveness of compound I was comparable with that of cysteamine in the range of radiation dose of 500—700 R (Fig. 3). This result suggests that the mode of action of compound I is somewhat different from that of cysteamine.

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