

Radiation Protection by Oral Administration of L-Cysteine Ethyl Ester Hydrochloride in Mice

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Cysteine ethyl ester hydrochloride was as effective as cysteine in saving the life of X-irradiated (600 R) mice, regardless of whether it was given orally or intraperitoneally. However, the compound did not prolong the life of the animals who received higher dose (800 R) of X-rays. Notwithstanding rather weak effectiveness in radiation protection, cysteine ethyl ester will be useful because of its low toxicity; median lethal oral dose in mice was 35.8 mmoles/kg, while the radioprotective effect was observable in an oral dose of 2.0 mmoles/kg.

A radioprotective drug which can be used by oral route is needed for the practical convenience of use. Acute toxicity of a drug will be smaller, when it is given orally than when it is given parenterally. Oral administration of a radioprotective drug will be advantageous in protecting the alimental canal which is one of the major radiosensitive organs in the body. Besides, if there occurs a rapid gastro-intestinal absorption of the drug, many other organs will be protected as well eventually by the oral administration of the compound.

We found that L-cysteine ethyl ester (cystanin) exhibited a lifeprolonging effect in acutely and lethally X-irradiated mice, when the compound was given to the animals orally by the stomach tube. The toxicity of this compound is considerably low and it is widely used as an expectorant. Thus, there is a sufficient accumulation of knowledge about its toxicity and other properties for its clinical use.

Experimental

Animals—Male mice of the Na-2, ddN and ICR strains, all at the age of 5–6 weeks, were employed in the experiment on the acute toxicity of the compounds. The medium lethal dose (LD_{50}) of the chemicals was determined by the method described by Finney.²⁾

The radioprotective action of the compounds was studied exclusively in the mice of the ICR strain. These mice aged 5 weeks and weighed 25–30 g at the time of X-irradiation. The animals were housed 5 in each cage and observed for 30 days thereafter. The method of X-irradiation was same as reported in a previous paper.³⁾

Chemicals—L-Cysteine ethyl ester hydrochloride (cystanin) was dissolved in redistilled water. The solution was not neutralized, but diluted with water so that 0.2 ml aliquots might contain the compound in amounts as specified in each case. The pH of the solution was found at 1.5–2.5. The solution of cysteine hydrochloride was prepared by a similar procedure. It has been known that these compounds are quite stable against auto-oxidation in such solutions as described above. The solutions of β -mercaptoethyl-guanidine were prepared by dissolving β -aminoethylisothiuronium bromide hydrobromide in an equivalent molarity of NaOH solution. Unless otherwise specified, the chemicals were given to the animals 10–15 min prior to X-irradiation. For oral administration of the chemicals, a stainless steel stomach tube was used. In the case where the chemicals were intraperitoneally injected in mice, the solutions were neutralized to pH 6–7 with concentrated solution of NaOH shortly before injection.

1) Location: a) Anagawa 4-9-1, Chiba-shi; b) 26-1 Nishigawara-1, Kita-ku, Tokyo.

2) D.J. Finney, "Probit Analysis" Cambridge Univ. Press, London (1952).

3) Y. Takagi, F. Sato, M. Shikita, M. Shinoda, T. Terasima, and S. Akaboshi, *Radiation Res.*, **42**, 79 (1970).

Result

Acute Toxicity of the Compounds

Cystanin was compared with typical radioprotectors, cysteamine and β -mercaptoethylguanidine, in regard to their acute toxicity in mice of three different strains. It was observed that the mice of the ICR strain were more resistant against the toxicity of these SH compounds than the animals of other two strains (Table I). Among the three compounds examined above, cystanin was least toxic, when it was administered *via* oral route.

TABLE I. Acute Toxicity in Mice of Cysteine Ethyl Ester Hydrochloride as compared with the Toxicity of β -Mercaptoethylguanidine (MEG) and Cysteamine (MEA)

Compound	Route	LD ₅₀ (mmoles/kg)		
		Na-2	ddN	ICR
β -Mercaptoethylguanidine	<i>i.p.</i>	—	2.3	—
bromide hydrobromide	<i>p.o.</i>	—	4.1	6.0
Cysteamine hydrochloride	<i>i.p.</i>	—	4.5	—
	<i>p.o.</i>	—	11.9	13.9
L-Cysteine ethyl ester	<i>i.p.</i>	4.6	—	—
hydrochloride	<i>p.o.</i>	23.3	25.5	35.8

The animals were observed for 24 hr after administration of the drug

Intraperitoneal Injection of Compounds and Radiation Protection

The radioprotective action of cystanin was compared with that of cysteine in mice in which the chemicals were intraperitoneally injected 10 min before X-irradiation (700 R). As it was shown in Fig. 1, cystanin exhibited a radioprotective effect better than (in a dose of 2-mmoles/kg) or comparable with (in a dose of 1 mmole/kg) the effect of cysteine.

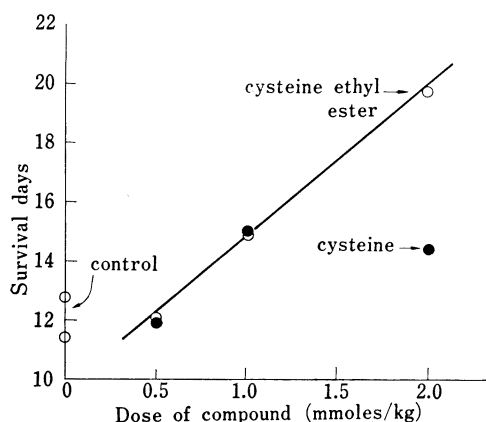


Fig. 1. Radiation Protection by Intraperitoneal Injection of Cysteine Ethyl Ester Hydrochloride and Cysteine Hydrochloride in X-Irradiated (700 R) mice

Each point represents an average of survival days observed on 10 mice. The survival time of the animals which survived longer than 30 days was regarded as 30 days

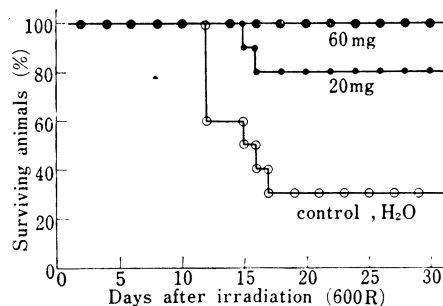


Fig. 2. Radiation Protection by Oral Administration of Cysteine Ethyl Ester Hydrochloride in X-Irradiated (600 R) mice

Each group consisted of 10 mice on day 0.

Oral Administration of Compounds and Radiation Protection

Cystanin was effective in protecting the animals against x-rays, even when it was given to the animals by oral route. In the control non-treated animals, 7 out of 10 had died between 11 and 16 days after they received X-irradiation of 600 R. On the other hand, in the animals who had been given 60 mg/animal of the compound, all survived the irradiation of 600 R (Fig. 2).

Duration of the Effectiveness after Oral Administration

In the experiment shown in Fig. 3., cystanin was given orally to mice, 5, 30 or 60 min prior to irradiation (600 R). The radioprotective effectiveness of 60 mg of the compound was maintained nearly constant for one hr after the compound was administered to the animals. The effectiveness of the compound seemed to decay gradually in 60 min in the animals who received smaller doses of the compound.

In a separate experiment, the compound was given to the animals shortly after the animals had received X-rays. The compound was administered either orally or intraperitoneally, but there observed no significant beneficial effect of the compound in these animals. Necessity of administration of the compound prior to irradiation has been observed for all other radioprotective substances of this category.⁴⁾

Dose of the Compound

Fig. 4 shows the effect of dose of cystanin on the radioprotective action of the compound. It was noted that a small extent of protection could be achieved by the compound in the dose as one-tenth of the median lethal dose. The dose-response curve for the radioprotective effect of the compound is not sigmoidal, unlike the dose-response curve usually observable with the action of many other drugs. Similar non-sigmoidal dose-response curves have been obtained in the cases of the radioprotective action of other chemicals.^{5,6)}

Radiation Dose and the Effectiveness of the Compound

In the same fashion as above but with various doses of X-irradiation, the radioprotective effect of orally administered cystanin was examined in mice. It will be seen in Fig. 5 that the compound was protective in the animals who received 600 R of irradiation, but was nearly powerless in the animals who received 800 R of irradiation.

Discussion

By the experiment reported above, it has been proved that L-cysteine ethyl ester hydrochloride exhibits a radiation protective effect in lethally X-irradiated mice, regardless of

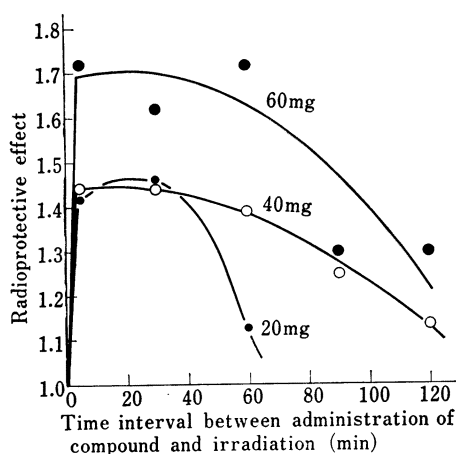


Fig. 3. Duration of the Radioprotective Action of Cystein Ethyl Ester Hydrochloride after Oral Administration

Each point represents an average of the observation on 10 mice. The radioprotective effect of the compound was expressed in terms of the ratio of mean survival days of the protected and control non-protected animals. X-irradiation=600 R

4) Z.M. Bacq, "Chemical Protection against Ionizing Radiation" Charles C. Thomas Publisher, Springfield (1965).

5) A.T. Hasegawa and H.D. Landahl, *Radiation Res.*, **44**, 738 (1970).

6) J.M. Yuhas, *Radiation Res.*, **44**, 621 (1970).

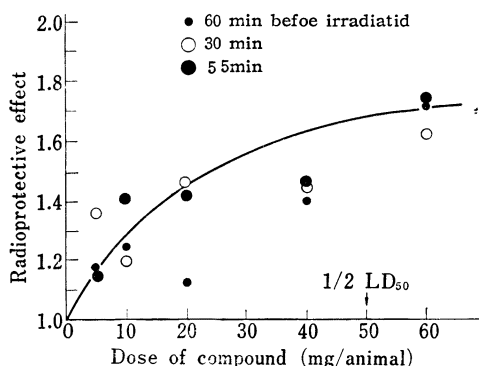


Fig. 4. Effect of Dose of Cystein Ethyl Ester Hydrochloride on the Radioprotective Action of the Compound

The animals (10 mice in each group) received the compound orally. X-irradiation=600 R.

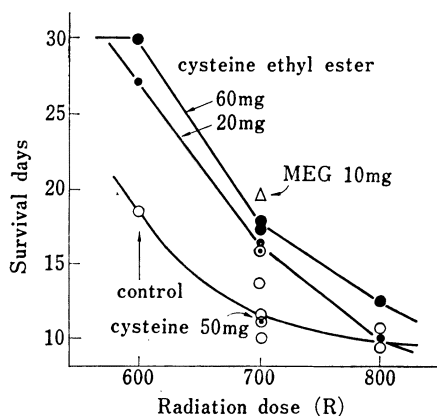


Fig. 5. The Radioprotective Action of Cystein Ethyl Ester Hydrochloride as Influenced by the Dose of X-Irradiation

All chemicals were administered orally to mice. Each point represents average survival days of 10 mice.

whether the compound is given to the animals by oral route or it is injected intraperitoneally. The radioprotective action of this compound is nearly comparable with that of cysteine, but seemingly weaker than that of β -mercaptoethylguanidine which is the most powerful radioprotector hitherto known. However, cystanin is much less toxic than other radioprotective substances, and this low toxicity will be advantageous for its practical use as a radioprotector.

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