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Radiation Protection of Mice by Mixtures of β-Mercaptoethylguanidine (MEG) and Cysteamine (MEA)

Yoshinari Takagi,[1a] Setsuko Shimizu,[1b] Mikio Shikita,[1a] Masato Shinoda,[1b] and Sanya Akaboshi[1a]

National Institute of Radiological Sciences[1a]

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The injection of β-mercaptoethylguanidine bromide hydrobromide (MEG) together with cysteamine hydrochloride (MEA) afforded better protection of mice against X-irradiation than the injection of either compound alone, while the two compounds did not act additively in the display of the acute toxicity. The powerful effectiveness of the MEG–MEA mixture was not attributable to the formation of a mixed disulfide, aminoethyl guanidinoethyl disulfide (AEGE) in the solution or in the animal body. The latter compound was synthesized and proved very toxic but weak in the radioprotective activity.

It has been known that a combination of two kinds of radioprotective compounds affords better protection of the experimental animals than the administration of either compound alone. Huber and Spode listed a number of reports on such "antiradiation cocktails."[2] Most of these cocktails consist of the combination of a type of aminothiol with other radioprotective agents of the different mode of action, such as vasorepressive agents, serotonin and other hormones, antibiotics, narcotics, surface-active compounds, bone marrow cells, and so on. In this short communication, we would like to report that cysteamine (MEA) and β-mercaptoethylguanidine (MEG), which are chemically related to each other and radioprotective seemingly through a same mechanism of action, also offered a better effect when these compounds were used together than when either compound was used alone.

Experimental

Animals——Male mice of the ddY strain, aged 5 weeks at the time of irradiation, were employed. The animals were intraperitoneally injected with 0.2 ml of the neutral solution of the radioprotective compounds, and irradiated with a total dose of 800 R of X-rays 5 to 10 min after the injection.

Radioprotective effectiveness of the compound was expressed in the term of average survival time in 30 days after the irradiation; the survival time of the animals which survived longer than 30 days was regarded as 30 days. Further details of the experimental conditions were described in the previous papers.[3,4]

Chemicals——Cysteamine hydrochloride (MEA) and 2-aminoethylisothiuronium bromide hydrobromide (AET) were commercially obtained. The latter compound was converted to β-mercaptoethylguanidine (MEG) by the treatment with an alkaline solution shortly before use in the animals.

2-Aminoethyl guanidinoethyl disulfide (AEGE) was synthesized by the reaction of β-aminoethylthiosulfric acid with MEG in 2% methanolic NaOH under nitrogen gas, and purified by Amberlite-IR-120 column chromatography. The compound was then crystallized as hydroiodide (mp 166—169°).[5]

In the case of combined use of MEA and MEG, neutral solutions of the respective compounds were mixed together in appropriate proportions prior to injection into the animals. Under this condition, formation of disulfide was practically negligible.

1) *a*) Location: *Anagawa 4-9-1, Chiba-shi*; *b*) Present address: *Hoshi College of Pharmacy, 2-4-41 Ebara, Shinagawa-ku, Tokyo.*
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Result

Toxicity of Compounds

Acute toxicity of the compounds was examined after the intraperitoneal injection of the compounds in mice. By the toxic doses of the compounds, the animals were killed within several hours after the injection. In the animals which survived this acute toxicity of the chemicals, no sign of subacute toxicity was observed within a month thereafter. As it is shown in Fig. 1, MEG was approximately twice as toxic as MEA in comparison on equimolar basis. It is interesting to notice that a combined use of MEA and MEG did not exhibit an additive effect in the acute toxicity. For example, the mortality observed after the injection of 4.9 mmoles of MEA plus 2.4 mmoles of MEG was only slightly larger than the mortality observed after the injection of 2.4 mmoles of MEG alone. It seems that the decreased toxicity of the MEA–MEG cocktail was not due to the formation of AEGE, *i.e.*, the mixed disulfide of MEA and MEG, in the solution or in the animal body, because AEGE itself was rather much strongly toxic (LD_{50} in mice was found 0.5 mmole/kg).

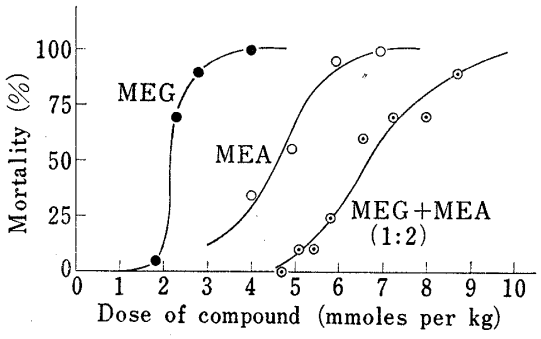

Fig. 1. Acute Toxicity in Mice of β-Mercaptoethylguanidine Br·HBr (MEG), Cysteamine (MEA) and an 1:2 Mixture of MEG and MEA as a Function of Dose of the Compound

The chemicals were intraperitoneally injected. Each point represents mortality observed within 24 hr after the injection in a group of 20 mice.

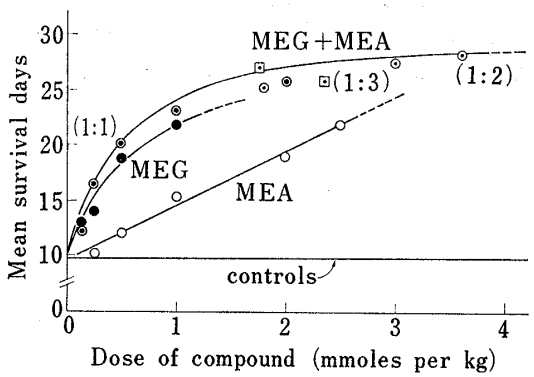

Fig. 2. Radioprotective Effect in Mice of β-Mercaptoethylguanidine Br·HBr (MEG), Cysteamine (MEA) and the Mixtures of MEG and MEA

The proportions of the two compounds in the mixtures are given in the figure. Each point represents an average survival time of 30—40 mice which had been irradiated with 800 R of X-rays. The control animals (40 mice) received the injection of 0.9% NaCl solution.

Rodioprotective Effect in Mice

The relationship between the dose of the compounds and the radioprotective effect was depicted in Fig. 2. It is seen that MEG is much more effective than MEA on the molar basis, but, because of its lower toxicity, MEA can be administered in amounts twice as large as MEG to afford an effectiveness equivalent to that of MEG. However, in both cases of MEA and MEG, it was impossible to increase the dose of the compound in order to obtain better protection without having a portion of the animals dead. On the other hand, it was possible to obtain a better protective effect by the use of the MEA–MEG cocktails.

Table I shows the result of an experiment on the radioprotective effect of AEGE. Although this compound was very much toxic in mice, a weak radioprotective effect was attainable by a dose of 0.25 mmole/kg of this compound. Anyhow, it is evident that the powerful radioprotective effect of the MEA–MEG cocktail is not attributable to the formation of AEGE.

Table I. 2-Radioprotective Effect of Aminoethyl Guanidinoethyl Disulfide (AEGE) in Mice irradiadiated with X-Rays (800 R)

Dose of compound (mmole/kg)	Survival (30 days)	
	Ratio	Days (average)
Zero	zero/20	9.5
0.0625	zero/20	9.7
0.125	zero/20	11.6
0.25	4/20	15.2

Discussion

In a previous paper, we reported that the radioprotective effect of MEA in $HeLaS_3$ cells developed very rapidly, while the effect of MEG developed rather gradually with time, and this difference was interpreted by the term of penetration problem.[6] It is plausible, therefore, that these radioprotective compounds differently distribute in the animal body. The combined effect of MEA and MEG observed in the present experiment might be due to this difference in the mode of action of these compounds. The present observation gives a fundamental basis for the benefit of the combined use of the radioprotective agents, even if the chemical structures of the compounds are closely related to each other.

6) F. Sato, M. Shikita, T. Terasima, and S. Akaboshi, *Radiation Res.*, **44**, 660 (1970).