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Radioprotective Effect of Pyridoxamine-5-thiophosphate and Related Compounds

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Among 6 derivatives of 5-mercaptopyridoxine, pyridoxamine-5-thiophosphate was most powerful in protecting mice from X-radiation. Its efficacy was nearly comparable with that of the typical radioprotector, cysteamine. It was less toxic than any other derivatives tested, the ratio of the median lethal dose to the minimum radioprotective dose was smaller than 1/4. It was effective when administered orally as well as when injected intraperitoneally. Quicker recovery of leucocyte number was observed in the circulating blood of the protected animals than in the controls. The compound protected an enzyme protein (β-glucuronidase) from radiation-inactivation in aqueous solution as powerfully as cysteamine did. However, it was much less effective than cysteamine in protecting HeLa S_3 cells from the loss of reproductive integrity caused by radiation.

It has been reported that various pyridoxine analogues have a prophylactic effect of protecting animals from radiation injury: the list of radioprotective compounds includes pyridoxine,[2] N-pyridoxylidene-L-cysteine,[3] 5-mercaptopyridoxine,[4] pyridoxal-5-phosphate,[5] pyridoxine-4-isothiuronium bromide[6] and so on. Among these compounds, 5-mercaptopyridoxine is most effective and exerts the radioprotective action even when it is given orally to the animals.[4] The efficacy is specific for 5-mercaptopyridoxine; 4-mercaptopyridoxine is hardly radioprotective.[4] The present workers synthesized several new derivatives of 5-mercaptopyridoxine and found that pyridoxamine-5-thiophosphate is less toxic and more potent for radioprotection than the mother compound, 5-mercaptopyridoxine.

Experimental

Animals——Male mice of the ICR strain were employed for the radiation protection study. The animals were 5 weeks old when they received whole-body X-radiation. Details of the experimental conditions were described previously.[7] Toxicity of the compounds was examined by determination of the median lethal dose (LD_{50}) in male mice of the ddY strain (5 weeks old) by the method of Finney.[8]

Chemicals——Sodium pyridoxine-5-thiophosphate (mp 70—73°) and pyridoxamine-5-thiophosphate (mp 216—218°) were synthesized by the reaction of trilithium phosphorothioate with 5-bromopyridoxine or with 5-bromopyridoxamine. Sodium thiosulfate was used in the place of trilitium phosphorothioate to prepare pyridoxine-5-thiosulfate (mp 191°). The isothiuronium derivative (mp 150—151°) was the product of the reaction of thiourea with 5-bromopyridoxine. Other compounds were obtained commercially. These chemicals were dissolved in sterilized water and given to the animals orally (0.5 ml/animal, 30 min prior to irradiation) or intraperitoneally (0.2 ml/animal, 15 min prior to irradiation).

Analytical Methods——Leucocyte number in the circulating blood was counted with an autocytometer.[9] The effect of the chemicals to protect an enzyme protein from radiation-inactivation was studied with use

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8) D.J. Finney, "Probit Analysis," Cambridge Univ. Press, London (1952).
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of β-glucuronidase and according to the method reported in our previous paper.[10] The enzyme activity was assayed by the rate of hydrolysis of *p*-nitrophenyl β-D-glucopyranosiduronic acid in sodium acetate buffer (0.1 M, pH 4.5). HeLa S_3 cells were cultured in plastic petri dishes and the effect of X-radiation (250 to 1000 R) was examined by counting the number of cell-colonies developed in 2 weeks after irradiation. Details of the methods have been described in the previous paper.[7]

Result and Discussion

Radioprophylactic Action of 5-Mercaptopyridoxine and Its Derivatives in Mice

All the derivatives of 5-mercaptopyridoxine, except pyridoxine-5-isothiuronium bromide, were effective in protecting animals from the radiation injury. The number of the animals which survived exposure to 600 R of X-radiation was increased by the intraperitoneal injection of the compounds, whereas all the control animals were killed by the radiation (Table I).

Table I. Radiation Protective Effect of 5-Mercaptopyridoxine and Its Derivatives in Mice

Compounds	Dose (mg/kg body weight)	Radioprotective effect	
		survival days	survival ratio
Control (saline)	—	10.2±0.9	0/20
Cysteamine hydrochloride	266	27.7±0.8	14/20
5-Mercaptopyridoxine hydrochloride	100	14.6±2.6	2/10
	200	17.1±2.6	5/20
	300	22.9±3.2	4/10
Sodium pyridoxine-5-thiosulfate	300	10.8±0.5	0/10
	500	12.8±2.5	1/10
	600	22.2±3.1	5/10
Pyridoxine-5-isothiuronium bromide	50	10.8±1.4	0/10
	100	10.4±0.3	0/10
Monosodium pyridoxine-5-thiophosphate	500	19.2±3.1	8/20
	750	14.1±1.8	1/10
	1000	20.6±4.0	2/10
Pyridoxamine-5-thiophosphate	125	10.4±0.5	0/10
	250	20.6±2.4	8/20
	500	27.0±2.1	7/10
Pyridoxine-5-thioacetate	100	21.4±3.0	5/10
	200	14.0±4.0	3/10

Among 6 pyridoxine derivatives tested, pyridoxamine-5-thiophosphate was most effective; its effectiveness is nearly comparable with that of cysteamine which is known as a typical radioprotective substance.

Table II. Acute Toxicity of 5-Mercaptopyridoxine and Its Derivatives in Mice

Compounds	LD_{50} (mg/kg body weight)		
	i.p.	*i.v.*	*p.o.*
5-Mercaptopyridoxine hydrochloride	350	278	1769
Sodium pyridoxine-5-thiosulfate	—	804	—
Pyridoxine-5-isothiuronium bromide	—	160	—
Sodium pyridoxine-5-thiophosphate	—	1415	4121
Pyridoxamine-5-thiophosphate	>1550	—	2646
Pyridoxine-5-thioacetate	—	222	1950

10) Y. Takagi, F. Sato, M. Shikita, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2514 (1970).

Acute Toxicity of the Pyridoxine Derivatives in Mice

As it is shown in Table II, pyridoxamine-5-thiophosphate was least toxic; it was difficult to determine an accurate value for its LD_{50} because of its small solubility in the aqueous solution. Anyhow, it will be seen that the radioprotective dose of this compound is less than 1/4 of its LD_{50}, while there is no such large difference between the minimum effective dose and LD_{50} in the case of other compounds.

Radioprotective Action of Pyridoxamine-5-thiophosphate

Because pyridoxamine-5-thiophosphate was most effective and least toxic in the screening test described above, its radioprotective action was studied further in detail. Fig. 1 shows the radioprotective action of the compound as a function of the dose of radiation. It will be seen in the figure furthermore that, even when it was given orally to the animals, the compound was as effective as when it was injected intraperitoneally.

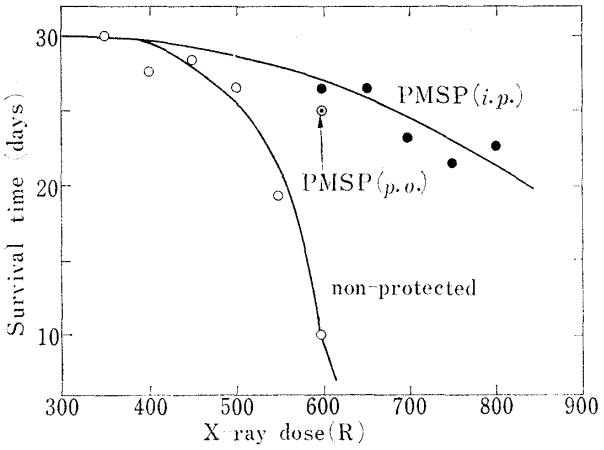

Fig. 1. Radioprotective Effect of Pyridoxamine-5-thiophosphate (PMSP) in Mice: Effect of the Dose of X-radiation.

i.p.=intraperitoneal injection (500 mg/kg), *p.o.*= oral administration (1000 mg/kg)

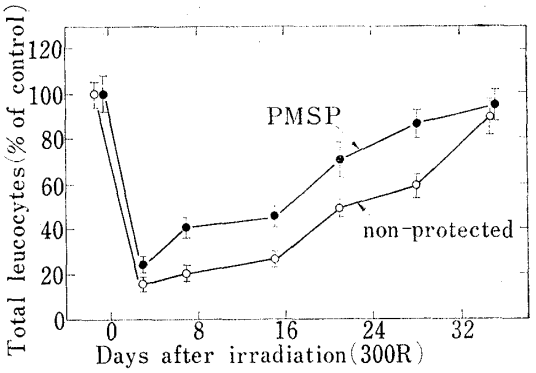

Fig. 2. Increase in the rate of post-irradiation (300 R) recovery of the leucocyte number in the animals protected by pyridoxamine-5-thiophosphate (PMSP) (*i.p.*, 500 mg/kg). Each point represents the average of the measurements in 10 animals. The standard error of the determination is shown by the vertical bars

However, the compound should be administered shortly before the animal receives radiation. Post-irradiation treatment with the compound had no life-prolonging effect; when the compound was injected intraperitoneally in a daily dose of 500 mg/kg body weight and for 6 days after irradiation (600 R), average survival time of these animals was 10.9 ± 0.9 days which was not significantly different from that of the control animals. Similarly, pyridoxine-5-thiophosphate had no effect on the survival time of the X-irradiated mice, when it was given after irradiation.

In a separate experiment, the effect of pyridoxamine-5-thiophosphate on the recovery of the number of leucocytes in the circulating blood was studied in the sublethally (300 R) X-irradiated mice. It will be seen in Fig. 2

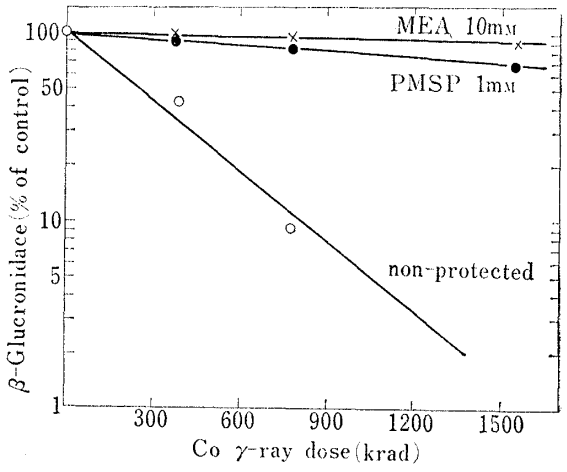

Fig. 3. Inactivation by Radiation of β-Glucuronidase in Aqueous Solution (2 mg protein/ml acetate buffer, pH 4.5): Protection of the Enzyme Activity by Cysteamine (MEA) and Pyridoxamine-5-thiophosphate (PMSP)

that there was no difference between the control and the treated animals in the initial rapid decrease of leucocytes, while there occurred significantly quicker recovery of the leucocyte number in the treated animals.

Fig. 3 shows that this compound is a powerful radioprotector at the molecular level; inactivation by gamma-radiation of an enzyme in the aqueous solution was prevented by the presence of the compound in the solution. The result suggests that it may exhibit the radioprotective effect in the animal body before it is hydrolyzed to 5-mercaptopyridoxamine.

The damage of the reproductive integrity of HeLa S_3 cells caused by X-radiation was prevented by the addition of the compound to the culture medium prior to irradiation. Dose reduction factor (DRF), which is the ratio of the X-ray exposure giving 10% survival of the protected cells to that giving the same survival of the control cells, was 1.2 when the compound was added in a concentration of 30 mM. Under the same conditions of experiment, 30 mM cysteamine gave a DRF of 3.4. Thus, it can be said that the effectiveness of pyridoxamine-5-thiophosphate is much smaller than that of cysteamine in this HeLa S_3 cell assay system. The result suggests a possibility that pyridoxamine-5-thiophosphate does not protect all kinds of cells in the animal body to a same extent.