PROTECTIVE EFFECT OF MEG ON RADIATION INJURIES OF HEMATOPOIETIC SYSTEM IN MICE AND RATS

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マウスおよびラットの放射線による造血障害に対する MEG の保護効果

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MEGの放射線に対する保護効果を主に血液学的に検討し，次の結果を得た．

MEGはX線照射マウス (ddN 系) の骨髄，白血球，体重，脾重量，胸腺重量ならびに血液の Fe-59 摂取に及ぼす放射線の影響をいちじるしく減少させる．

MEGはラット (Wister および Donryu 系) に対して毒性が強く，且つ末梢血の Fe-59 摂取，血液像ならびに臓器重量からみてX線に対する保護は認められない．

Introduction

The study of chemical protection against radiation injuries is of interest because some protective agents significantly modify radiation injuries and also because mechanism of chemical protection are correlated to that of radiation action on animals. The effectiveness of AET_{Br} (MEG_{Br}) on the survival of X-ray irradiated mice first reported by Doherty et al,[1)2)] was recently confirmed[3)4)5)]. The object of this investigation was to study the protective effect of MEG on hematopoietic system through the changes in Fe-59 uptake, bone marrow counts, peripheral blood leukocytes, erythrocytes, spleen weight, thymus weight and body weight in mice and rats exposed to sublethal and lethal X-ray irradiation. Parts of the paper have been already reported in another journal[6)].

I) Protective effect of MEG on Fe-59 uptake

1) Material and method

Irradiation was performed with a Toshiba KXC-18-2 operated at 180 kVp, 25 mA with filter, 0.5 mmCu+0.5 mmAl; HVL, 1.18 mmCu; target to skin distance, 65 cm; and dose rate, 50 r/min for mice and with filter, 0.3 mmCu+0.5 mmAl; HVL, 1.09 mmCu; distance, 70 cm; and dose rate, 45 r/min for rats. Dose measurement was made by a Victoreen Radocon 575 (probe 601) placed in the center of one of acrylite irradiation boxes. All animals were exposed to 800 r of total body irradiation.

Female mice (ddN uniform strain) and female rats (Wister and Donryu uniform strain) were used as experimental animals.

MEG_{SO_4} (80 mg/kg and 160 mg/kg) was mainly used as the protective agent. In another case, neutralized AET_{Br} (250 mg/kg), namely MEG_{Br}, was used. MEG_{SO_4} at 160 mg/kg is almost equivalent to AET_{Br} at 250 mg/kg from the molar base. MEG_{SO_4} was first synthesized by Taguchi[7] in order to reduce the toxicity attributable bromine by substituting bromine ion to sulfate ion and increase the protective activity. The protective activity on the survival rate of X-ray irradiated mice and toxicity were similar to AET_{Br} on the basis of molar concentration. However, MEG_{SO_4} has a remarkable activity without the adjustment of pH and its most effective time after administration is slightly slower than that of AET_{Br}[3)8)]. Differences in effectiveness between MEG and AET are now being studied in detail.

Chemicals were administered by intraperitoneal injection 10 minutes before irradiation.

$^{59}FeCl_3$ in dilute hydrochloric acid solution obtained from Japanese Isotope Association was diluted to 4 μc/ml with physiological saline solution and administered by intracadial injection at the dose of 2.0 μc/rat under light ether anesthesia and by intraperitoneal injection at the dose of 0.75 μc/mouse.

In the examination of Fe-59 uptake, radioactivity of blood is generally measured on about the second day after administration of Fe-59. In this study, it was performed 24 hours after administration in order to examine the uptake of bone marrow, this was decreased on the second day after administration. The animals were sacrificed 24 hours after administration of Fe-59, and the radioactivity of their blood, liver, bone marrow (left and right femurs) and spleen was measured by a scintillation counter (Toshiba medical spectrometer UCH 23103) without ashing. Therefore, the relative value of protected and unprotected groups was compared. The examination was done on the first, seventh and fourteenth day after irradiation for rats and the third and seventh day for mice.

2) Result and discussion

Fig. 1 shows the radioactivity of blood, femurs, spleen and liver of Donryu rats plotted against days after irradiation. In both protected and unprotected rats, the uptake of Fe-59 in peripheral blood was decreased on the first day and more so on the 7 th day, but significantly increased on the 14 th day after irradiation. In protected group, the depression of Fe-59 uptake on the 1 st and the 7 th day is greater than in the unprotected rats but on the 14 th day it was smaller than in unprotected rats. However, the difference between the two groups was not statistically significant. Although the behaviour in changes of Fe-59 uptake in

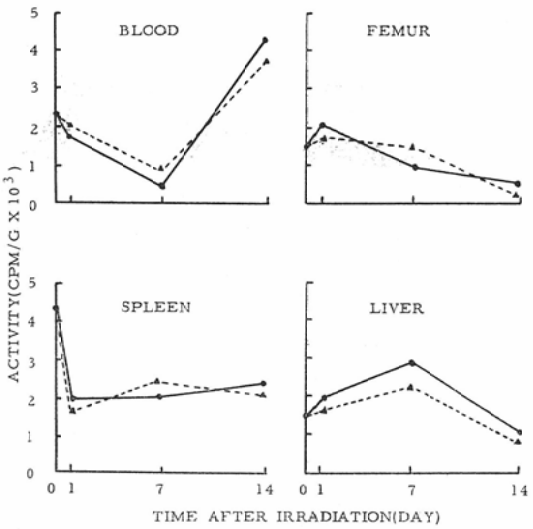


Fig. 1 Protective effect of MEG on Fe-59 uptake in Donryu rats
●—● 600 r only, ▲···▲ MEG+600 r
(MEG_{SO_4}: 80 mg/kg, Fe-59; 2 μc/rat)

femurs, spleen and liver differed from those in blood, no significant differences were observed. The weight changes in liver, spleen, thymus, kidney, adrenal and lung were also examined, but they were in general identical in both groups.

Donryu rats were more sensitive to the toxicity of MEG than Wister rats and ddN mice. As three out of five rats died when administered 160 mg/kg, dose at which there were no deaths in ddN mice, half dose (80 mg/kg) was administered in the foregoing study.

As reported in a previous paper[6], the lack of protective effect was assumed to be the administration of an insufficient dose of MEG. Other reasons for the lack of effect of MEG in rats are as follows: 1) MEG cannot modify radiation injuries in rats, 2) 600 r exceeds the critical dose where MEG cannot demonstrate any protective effect on highly radiosensitive responses such as Fe uptake and bone marrow and blood disorders, 3) There are differences in protective activity between AET_{Br} and MEG_{SO_4}, 4) The distribution of MEG in critical organs of rats is small.

Therefore, Wister rats which have a considerably high resistance against the toxicity of MEG used as test animals. Using MEG_{SO_4} and AET_{Br} as protective agents, changes in Fe-59 uptake were examined on the 7th day after irradiation when protective effect is the most significant for various responses.

Wister rats are more resistant against MEG than Donryu rats. When 160 mg/kg of MEG_{SO_4} and 250 mg/kg of AET_{Br} were administered, only two out of ten rats died.

The results are listed in Table I. In general, Fe-59 uptakes in blood, femurs and spleen are depressed by X-ray irradiation, but that in liver is increased. Particularly, Fe-59 uptake in blood and liver changes quantitatively with radiation dose[9]. If MEG has protective activity against Fe-59 uptake, the amount of Fe-59 in these organs should approach that of un-

Table I Protective effect of MEG on Fe-59 uptake, organ weight and body weight in Wister rats (MEG $_{SO_4}$: 160mg/kg, AET $_{Br}$: 250mg/kg, Fe—59 : 2.0 μc/rat)

Treatment	No. of animals	Body weight M (σ) g		Spleen weight M (σ) mg	Thymus weight M (σ) mg	Fe-59 uptake M (σ) cpm/g of wet tissue			
		Before irradiation	After irradiation			Blood	Femur	Spleen	Liver
Control (Not irr.).	10	184 (10.4)	—	483.2 (120.1)	358.1 (78.5)	2224 (454)	1061 (309)	2099 (338)	1310 (305)
AET only	5	181 (34.9)	181 (39.6)	379.3 (113.2)	278.2 (89.4)	1524 (357)	1365 (277)	3600 (1985)	1691 (201)
600 r only	10	163 (15.3)	159 (15.0)	175.9 (29.8)	84.6 (27.5)	249.3 (84.4)	1061 (305)	2026 (452)	2461 (409)
AET + 600 r	8	171 (14.5)	161 (12.4)	194.8 (29.9)	106.3 (24.2)	357.6 (353.1)	956.8 (535.0)	2001 (537)	2468 (576)
MEG + 600 r	8	169 (10.8)	156 (6.5)	170.9 (29.0)	85.4 (34.3)	295.4 (507.4)	665.9 (644.8)	1337 (455)	2766 (624)

irradiated animals. As seen in Table I, the differences in radioactivity between protected and unprotected groups were not significant, but those in blood and liver between the above two groups and the non-irradiated group were statistically significant ($p<0.01$). These facts indicate that the effect of radiation on depression of Fe-59 uptake is remarkable, but the protective effect of MEG is not remarkable.

From the foregoing results, it may be concluded that none or little effectiveness of MEG for rats is not due to the administration dose of MEG, difference in strain of rats, and difference in protective activity of AET_{Br} and MEG_{SO_4}.

In the third experiment, mice were irradiated under the similar conditions to rats with and without chemical protection. The radioactivity in organs described before was examined on the 3 rd and 7 th day after irradiation. The results are shown in Table II. On the 3 rd day after irradiation the amount of Fe-59 was depressed in the peripheral blood, femurs and spleen and was increased in liver in protected and unprotected groups, while the difference in radioactivity in four organs between two groups was not significant. On the 7 th day after irradiation, radiation injuries for Fe-59 uptake began to recover in both groups.

Table II Protective effect of MEG on Fe-59 uptake and organ weight in ddN mice (MEG $_{SO_4}$: 160mg/kg, Fe-59 : 0.7 μc/mouse)

Treatment	No. of animals	Spleen weight M (σ) mg	Thymus weight M (σ) mg	Fe-59 uptake M (σ) cpm/g of wet tissue			
				Blood	Femur	Spleen	Liver
Control	10	148.3 (18.3)	49.5 (12.5)	3900 (1124)	1573 (412.0)	9438 (1447)	2683 (1396)
3 rd day after irradiation 600 r only	10	47.7 (7.9)	12.4 (4.9)	141.7 (57.9)	474.5 (166.7)	3913 (549.9)	6829 (1197)
MEG+ 600r	10	48.1 (3.8)	15.9 (3.3)	107.7 (71.5)	483.9 (100.0)	4417 (1014)	4719 (703.3)
7 th day after irradiation 600 r only	10	51.7 (9.4)	25.7 (8.1)	657.9 (546.9)	935.3 (261.2)	7906 (3655)	4952 (951.3)
MEG+ 600r	10	101.7 (45.0)	23.0 (13.4)	2461 (1512)	1196 (336.7)	9385 (4010)	3896 (1730)

Radioactivity in blood was 3900 cpm/g in the un-irradiated group, 2500 cpm/g in the protected group and 660 cpm/g in unprotected group. The protected group recovered considerably but the unprotected group showed a slow recovery rate. The difference in radioactivity between the protected and unprotected groups was significant ($p<0.01$).

From these results, it was shown in mice that the protective effect of MEG could be observed with statistical significance for sensitive responses which change markedly in the low irradiation dose range, such as Fe-59 uptake.

This fact indicates that MEG has no or little effect on rats. In other organs excluding blood, Fe-59 uptake changes with a different pattern between mice and rats or between different strains. In Donryu rats and ddN mice, the amount of Fe-59 uptake was depressed by irradiation in spleen and femurs and increased in liver as compared with the control group. In Wister rats, it was depressed slightly in spleen and femur and increased in liver.

II Protective effect on the bone marrow and peripheral blood picture in rats and mice

1) Material and method

Under the same irradiation conditions and administration dose and time of MEG_{SO_4} as the foregoing experiment, mice were exposed to total body irradiation of 800 r and rats were exposed to 600 r.

Mice were divided to two groups. In the first group, mice were sacrificed on the 0.5 th, 9 th, and 24 th hour (1 st day), 48 th hour (2 nd day) and 72 nd hour (3 rd day) following irradiation. Studies were made of the bone marrow counts, spleen weight and thymus weight. In the second group, mice were sacrificed on the 3 rd, 7 th and 12 th day following irradiation. The determinations were made of bone marrow counts, periphearl blood leukocyte counts of tail vein, spleen weight, thymus weight and body weight.

Bone marrow cells in the right femur 8 mm in length were forced out from the marrow cavity with 1.0 ml of physiological saline solution. Bone marrow counts in this solution were determined in a method similar to that for peripheral leukocyte counts.

In rats, determinations were made of the hemoglobin value, total leukocyte counts, differential leukocyte counts, reticulocyte counts and erythrocyte counts of the peripheral blood from the tail vein on the 1 st, 3 rd, 7 th and 14 th day following irradiation.

2) Result and discussion

Protective effect of MEG in rats

Erythrocyte counts began to decrease on the 7 th day after irradiation and greatly decreased on the 14 th day in both prot ected and unprotected groups.

Hemoglobin value showed no change from normal values until the 7 th day after irradiation and greatly decreased on the 14 th day. Difference between protected and unprotected groups was not statistically significant. Peripheral blood leukocytes were markedly depressed on the 1 st and 3 rd day, but recovered gradually with lapse of days in both groups. Although

leukocyte counts in protected group were slightly higher than in unprotected group on the 3rd, 7th and 14th day, no significant difference could be observed. Granulocyte and angranulocyte counts responded to the effect of irradiation in a manner similar to that of leukocyte counts and the difference between the protected and unprotected groups was not significant. The results are shown in Table III.

Table III Protective effect of MEG on white blood cell counts, red blood cell counts, hemoglobin value and reticulocyte counts in Donryu rats (MEG SO_4 :80mg/kg)

Treatment	No. of animals	WBC M (σ)			RBC M (σ)	Hb M (σ)	Ret M (σ)
		Total	Granulo.	Angranulo.			
Control	25	11576 (2560)	8692 (2226)	2774 (857)	801.8 (91.8)	92.4 (4.3)	42.1 (8.7)
lst day afier irradiation 600 r	10	2575 (829)	145.7 (55.3)	2429 (882)	763.7 (86.3)	85.1 (5.9)	26.0 (13.0)
MEG+ 600 r	10	2260 (601)	292.2 (155.0)	1968 (445)	772.6 (62.6)	86.2 (2.0)	29.4 (10.6)
3 rd day after irradiation 600 r	3	400 (87)	197.6 (28.9)	202.3 (105.0)	763.0 (130.0)	89.3 (3.7)	0 0
MEG+ 600 r	5	625 (184)	253.0 (101.0)	373.2 (96.5)	881.0 (70.3)	87.5 (4.8)	0 0
7th day after irradiation 600 r	10	1340 (878)	622.7 (465.4)	717.4 (442.3)	628.1 (57.0)	89.0 (7.9)	0.7 1.2
MEG+ 600 r	10	1810 (1165)	729.8 (427.1)	1084 (823)	702.0 (75.7)	92.3 (6.9)	1.0 1.7
14th day after irradiation 600 r	10	2215 (1263)	1614 (1143)	601.0 (507.0)	416.2 (62.0)	57.3 (10.6)	40.2 (24.6)
MEG+ 600 r	9	2822 (1686)	2048 (1212)	774.2 (556)	424.0 (104.0)	58.1 (11.7)	47.2 (26.4)

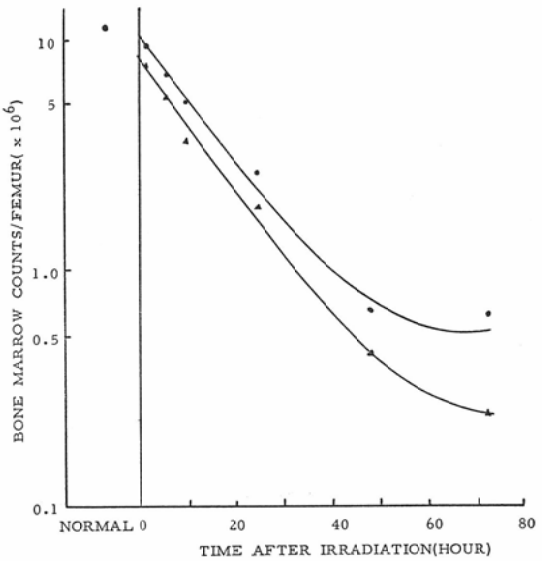
Protective effect of MEG in mice

Table IV shows changes in bone marrow counts, spleen weight and thymus weight from 30 minutes to 72 hours after irradiation. Within this period, spleen and thymus weight decreased gradually with lapse of time in both groups. MEG was not effective in modifying the atrophy of these organs.

Bone marrow counts reduced logarithmically with time after irradiation excluding the count at the 72 nd hour. Although statistically significant differences were not always observed between protected and unprotected groups, the depression of bone marrow counts was less severe in the protected group as seen in Fig. 2. Marked protective activity of MEG against the response of the bone marrow counts cannot be detected during the first three days, but it became marked on the 7th to 12th day when recovery was noted as shown in Fig. 3 and Table V. Thus in protected group the depression was less severe and recovery was early. Statistical differences in the bone marrow counts on the 7th and 12th day were observed between protected and unprotected groups. In protected group, leukocyte counts showed a low value on the third day but gradually recovered.

Table Ⅳ Protective effect of MEG on bone marrow cell counts and organ weight in ddN mice -early effect (MFG SO_4 : 160mg/kg)

Time after irradiation (hr)	800 r only			MEG + 800 r		
	Spleen weight M (σ) mg	Thymus weight M (σ) mg	Bone marrow counts of right femur M (σ) ×10⁴	Spleen weight M (σ) mg	Thymuf weight M (σ) mg	Bone marrow counts of right femur M (σ) ×10⁴
0.5	108.4 (18.6)	41.7 (10.8)	763 (168)	111.8 (12.5)	37.3 (5.8)	953 (117)
5	104.4 (15.8)	39.7 (9.4)	566 (37.0)	81.4 (12.1)	45.6 (19.8)	671 (44.2)
9	71.2 (15.4)	29.9 (7.6)	362 (92.7)	72.3 (6.7)	31.4 (19.9)	508 (200)
24	46.9 (10.0)	19.3 (3.1)	191 (137)	53.0 (6.5)	17.5 (5.8)	273 (70.2)
48	35.0 (7.1)	11.9 (1.4)	43.0 (14.0)	39.7 (6.0)	13.3 (1.9)	69.0 (14.6)
72	34.1 (3.7)	10.0 (5.1)	24.5 (10.9)	37.3 (4.9)	9.7 (2.0)	66.0 (25.3)



Eig. 2 Protective effect of MEG on bone marrow counts in ddN mice
··· early effect ···
▲—▲ X-ray only, ●—● MEG+X-ray
(MEG: 160 mg/kg, Irradiation dose: 800 r)

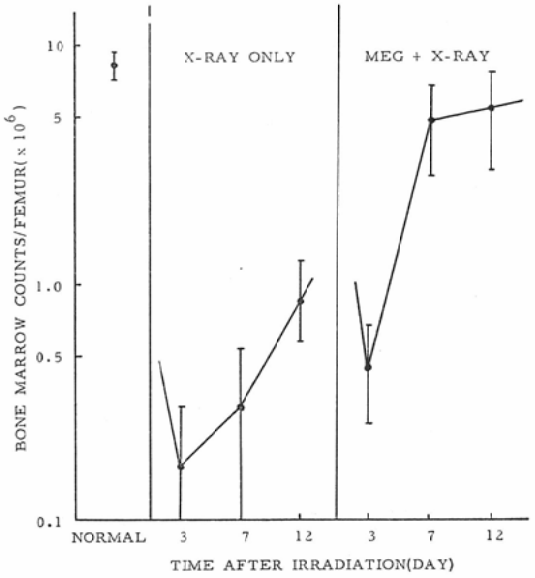


Fig. 3 Protective effect of MEG on bone marrow counts in ddN mice
(MEG SO_4: 160 mg/kg, Irradiation dose: 800 r)

In unprotected group, they were depressed severely on the 3rd day and the depression continued through the 7th day when recovery was noted in the protected group. Recovery only began on the 12th day.

In general, recovery from leukopenia occured at a slower rate as comapred with bone marrow counts.

Granulocytic elements (heterophils and eosinophils) in protected group responded in a

Table V Protective effect of MEG on bone marrow cell counts, leukocyte counts, organ weight and body weight in ddN mice (MEG_{SO_4} : 160 mg/kg)

Treatment	No. of animals	Body weight $\left(\frac{M}{\sigma}\right)$ g		Spleen weight $\left(\frac{M}{\sigma}\right)$ mg	Thymus weight $\left(\frac{M}{\sigma}\right)$ mg	Total bone marrow counts of right femur $\left(\frac{M}{\sigma}\right) \times 10^6$	Leukocyte counts/mm³ $\left(\frac{M}{\sigma}\right) \times 10^2$		
		Before irradiation	After irradiation				Total	Granulo.	Angranulo.
Control	10	23.4 (1.1)	—	117 (22)	48 (9)	8.31 (1.36)	119 (39.6)	15.3 (6.9)	104.0 (35.2)
3 rd day after irradiation 800 r only	10	23.0 (1.2)	22.0 (1.7)	43 (9)	15 (4)	0.17 (0.13)	3.5 (2.8)	1.2 (1.5)	2.3 (1.3)
MEG+ 800 r	10	23.0 (0.8)	23.0 (1.0)	47 (7)	15 (3)	0.46 (0.21)	4.9 (2.2)	1.3 (0.8)	3.6 (1.7)
7th day after irradiation 800 r only	10	23.7 (1.7)	22.0 (2.5)	34 (4)	21 (8)	0.30 (0.22)	3.1 (1.7)	0.7 (0.3)	2.4 (1.5)
MEG+ 800 r	10	23.4 (0.7)	24.0 (1.2)	82 (25)	33 (3)	4.86 (1.99)	13.8 (5.5)	7.6 (3.8)	6.2 (3.7)
12th day after irradiation 800 r only	7	22.7 (0.7)	18.7 (1.9)	79 (27)	22 (12)	0.89 (0.49)	5.9 (2.6)	1.0 (0.3)	4.8 (0.9)
MEG+ 800 r	9	21.8 (1.1)	21.8 (1.6)	124 (37)	45 (11)	5.40 (2.60)	25.5 (6.2)	9.9 (1.8)	15.6 (1.4)

similar manner to that of bone marrow counts to effects of irradiation and recovered to their normal values on the 12 th day, but in unprotected group the count was about one tenth of the protected group.

Angranulocyte counts (lymphocytes and monocytes) were slightly modified by MEG, and recovery was slower than granulocytes. The results were nearly identical to those obtained by Urso et al[10].

There is the question of whether protective agents, such as MEG and MEA, act mainly by minimizing the early lesions to a certain degree or by stimulating or making possible important regenerative processes. De Schryver et al[11] have reported that in the early stage after X-ray irradiation, the early protective effect of MEG on Fe-59 uptake in bone marrow and the red blood cells of the rats could not be demonstrated. In this study, similar results were obtained with regard to response of bone marow counts. However, even in the early stage, MEG showed its effects on the bone marrow counts, Fe-59 uptake and organs weight in mice irradiated to X-ray and neutrons of 200 rad[12].

Although many papers[13]~[17] have been reported on this problem, no satisfactory solution has been presented. For the investigation of this problem, the biological response, dose reduction factor of protective agent for such response, and irradiation dose must be taken into consideration. If they were not, such protective effect would fail to be detected even though the protective agent is effective, and thus it would be almost impossible to elucidate the mechanism of radiation action. As Fe-59 uptake and bone marrow counts are very sensitive, it is felt that 600 r and 800 r used in this study exceeded the critical dose where no early protective effect can be demonstrated.

Summary

MEG markedly modified the response of Fe-59 uptake by hematopoietic systems, bone marrow, peripheral blood leukocytes and spleen, thymus and body weight to lethal X-irradiation in ddN mice.

On the other hand, MEG failed to modified the response of the Fe-59 uptake, peripheral blood leukocytes, erythrocytes, hematocrit, reticulocytes, organ weight and body weight to sublethal X-irradiation in Wister and Donryu rats.

Donryu rats and Wister rats were more sensitive to toxicity of AET_{Br} and MEG_{SO_4} as compared with ddN mice.

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