CHEMICAL PROTECTION OF MEG AGAINST IONIZING RADIATION REPORT IV COMPARATIVE PROTECTIVE EFFECTS OF MEG AGAINST FAST NEUTRON AND X-RAY IRRADIATION IN MICE

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放射線に対する MEG の化学的保護に関する研究 第 IV 報 速中性子線およびX線照射マウスに対するMEGの保護効果

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200rad の中性子線およびX線の照射によるマウスの骨髄有核細胞数減少，末梢血の Fe-59 摂取能の低下，脾および胸腺の減少に対するMEGの保護効果を調べた．

MEGはX線，中性子線照射マウスの上記示標に対する障害に対して保護効果をもつているが中性子線よりもX線の方がより著明な効果を示した．

化学保護剤はX線致死に対するよりも中性子線致死に対して効果が小さいとされているがこれはMEGの腸障害に対する効果が小さいために由来するだけではなく，中性子照射による血液障害に対してもX線照射による血液障害に比較して効果は少ないことが認められた．

Introduction

It is well known that sulfhydryl-containing compounds such as MEG and MEA markedly modified radiation injuries of animals exposed to X-and γ-ray[1)2)3)4)5)]. However, only a few investigations have been carried out on chemical protection against neutron irradiation[6)7)8)].

These studies indicate that protective agents are much less effective against mortality of mice brought about by fast neutron irradiation than against mortality brought about by X-or γ-irradiation.

In the present paper, a review will be made on the study conducted on the protective effects of MEG with regard to responses of the hematopoietic system such as bone marrow count, spleen weight, thymus weight and iron-59 uptake in mice exposed to X-ray and neutrous.

Material and method

X-ray was delivered by Toshiba KXC-18-2 with tube voltage, 180 kVp; tube current, 10 mA; additional filter, 0.5 mmCu+0.5 mmAl; HVL, 1.18 mmCu; target to center-of-mice distance, 65 cm, and dose rate, 13 to 15 rad/min. Dose measurement was made by a Victoreen Radocon 575 (probe 601). The conversion factor of rad per unit roentgen was calculated to be 0.95 from the data of energy spectrum and ferrous sulfate dosimeter[9)10)].

Neutrons were delivered by a T (d,n) reaction neutron generator (Toshiba NSH-4) with accelerating voltage, 150 kV; deuteron beam current, 300 to 400 μA; neutron energy, 14.1 MeV; $^{3}_{1}$T target to center-of-mice distance, 5 cm and dose rate, 13 to 15 rad/min. Neutron flux measurement was made by a Toshiba scintillation spectrometer equipped with a plastic scintillator. The conversion factor of rad per unit neutron was 6.7×10^{-9}[11)12)].

Irradiation dose was 200 rad of single total body irradiation in both radiations.

Female mice (ddN uniform strain), 7 to 8 weeks old and weighing 20 to 23 g, were used.

MEG was prepared by dissolving AET in distilled water and neutralizing with NaOH to pH 7.2. Final concentration was 4 mg of dissolved AET per 0.2 ml of neutralized water. 0.2 to 0.26 ml (200 mg/kg) of freshly prepared MEG was injected intraperitoneally 5 minutes before irradiation.

Mice were divided to two groups. In first group, determinations were made of bone marrow counts, spleen weight and thymus weight on the lst, 3rd, 6th and 10th day after irradiation.

$^{59}FeCl_3$ in diluted hydrochloric acid solution obtained from Japanese Radioisotope Association was diluted to 5 μc/ml with physiological saline solution and administered by intravenous injection at the dose of 1 μc/mouse.

Mice were sacrificed 48 hours after the administration of Fe^{59} (3rd day after irradiation), and radioactivity of their blood, liver and spleen was measured by a scintillation counter without ashing. The technics used in measuring bone marrow counts and Fe-59 uptake were the same reported earlier[5)].

Result

I Protective effect of MEG on bone marrow counts, spleen weight and thymus weight

In both protected and unprotected groups, all responses show the lowest value on the 3rd day after irradiation. They showed a tendency to recover on the 6th day after irradiation and recovered to their normal values on the 10th day. In protected group, the depression of all responses was less severe and recovery was earlier. Protective effect of MEG was more significant in mice exposed to X-ray than those exposed to neutron and differences between protected and unprotected mice were statistically significant with regard to bone marrow counts and spleen weight on the 3rd and the 6th day after irradiation. On the other hand, in mice exposed to neutron, significant difference between two groups was observed only with regard to thymus weight on the 3rd day after irradiation. Although only one significant difference was observed in neutron exposed mice, all responses were

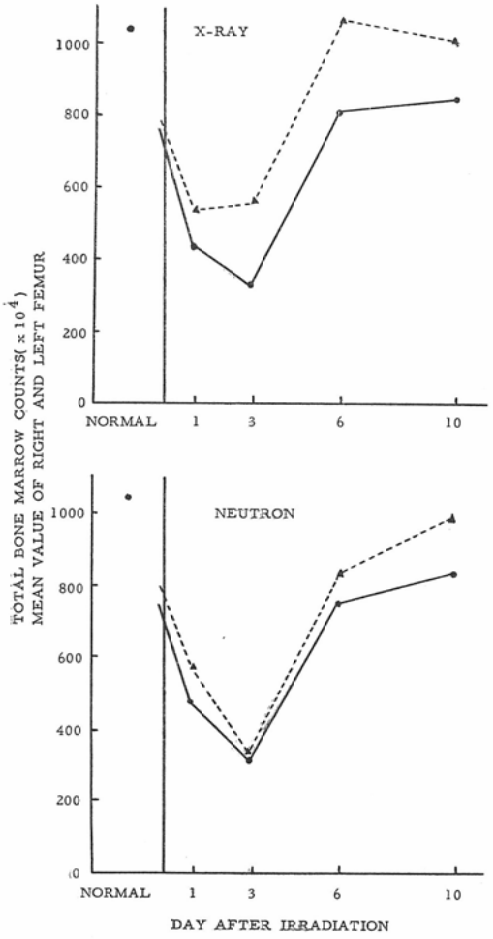

Fig. 1 The protective effect of MEG on bone marrow counts in X-ray and neutron irradiated mice
•—• Unprotected, ▲·····▲ Protected

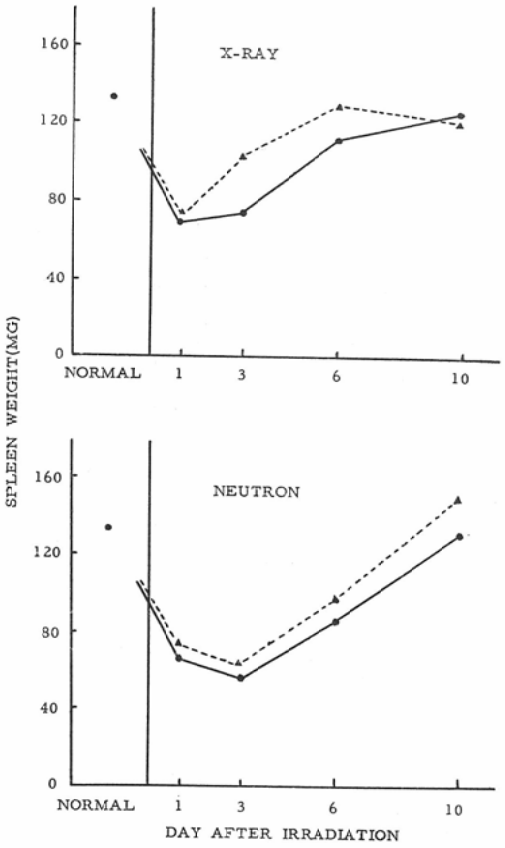

Fig. 2 The protective effect of MEG on spleen weight in X-ray and neutron irradiated mice
•—• Unprotected, ▲·····▲ Protected

of lesser degree and recovery was better in protected mice as compared with unprotected mice. Results are shown in Figs. 1,2 and 3, and Table I.

II Protective effect of MEG on Fe-59 uptake in hematopoietic system

It was found from the above experiment that the protective effect of MEG was the most remarkable on the third day after irradiation. Therefore, the protective effect of MEG on Fe-59 uptake in hematopoietic system was examined on the 3rd day after irradiation, together with bone marrow counts, spleen weight and thymus weight. The results appear in Table II.

In mice exposed to X-ray, statistically significant differences between protected and unprotected were observed with regard to bone marrow counts, spleen weight and Fe-59 uptakes in blood and liver.

In neutron irradiated mice, differences in Fe-59 uptakes in blood and spleen between

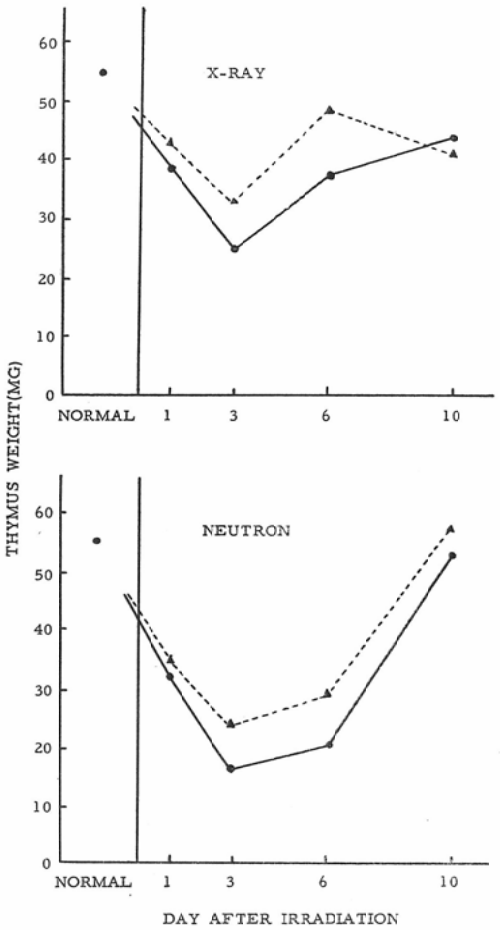

Fig. 3 The protective effect of MEG on thymus weight in X-ray and neutron irradiated mice
•—• Unprotected, ▲·····▲Protected

two groups were significant.

From these results it was concluded that though MEG has protective activity against injuries caused by neutron irradiation, it is less effective against injuries caused by neutron than against those by X-ray.

Discussion

According to Patt et al., cysteine has a dose reduction factor of only 1.1 against fast neutron irradiation, which is significantly lower than the factors reported for X-or γ-irradiations. Vogel Jr. et al have reported that AET and serotonin show effective protection when used before γ-irradiation. However, they are very ineffective against neutrons. Neither agent provided good protection against intestinal syndrome during the first week after neutron irradiation.

Table I The protective effect of MEG on the bone marrow counts and organs weight in X-ray and neutron irradiated mice

Treatment	No. of animals	Bone marrow counts M(σ) x 10^4			Spleen weight M(σ) mg	Thymus weight M(σ) mg
		Left femur	Right femur	Mean		
Control	10	1040 (171)	1044 (177)	1042 (175)	134.0 (15.2)	55.1 (6.1)
1st day after irradiation						
X-ray only	5	411 (71)	466 (102)	439 (60.4)	71.5 (11.2)	38.7 (5.0)
X-ray+MEG	5	543 (84)	526 (105)	535 (86)	73.6 (8.4)	43.1 (6.6)
Neutron only	5	487 (136)	488 (108)	488 (118)	66.2 (10.2)	33.0 (4.1)
Neutron+MEG	5	584 (118)	583 (114)	584 (125)	72.6 (18.1)	34.5 (6.7)
3rd day after irradiation						
X-ray only	5	343 (107)	248 (126)	296 (116) (a)	71.5 (14.6) (b)	32.9 (6.7)
Xray+MEG	5	541 (92)	572 (67)	568 (68)	105.0 (28.5)	33.0 (11.4)
Neutron only	5	316 (104)	307 (66)	313 (78)	55.0 (11.0)	16.6(1.4) (b)
Neutron+MEG	5	354 (34)	318 (37)	338 (33)	61.8 (7.8)	24.2(6.2)
6th day after irradiatio						
X-ray only	5	783 (112)	853 (202)	818 (150) (b)	112.5 (10.0) (b)	37.3 (7.4)
X-ray+MEG	5	1087 (267)	1046 (181)	1064 (198)	130.4 (15.0)	48.5 (10.5)
Neutron only	5	754 (118)	746 (102)	752 (111)	85.6 (23.7)	20.0 (2.2)
Neutron+MEG	5	928 (85)	764 (68)	846 (35)	97.9 (12.3)	29.4 (9.0)
10th day after irradiation						
X-ray only	4	859 (81)	824 (27)	842 (24)	124.0 (12.8)	44.2 (6.2)
X-ray+MEG	4	1031 (318)	971 (143)	1001 (188)	122.2 (19.0)	41.1 (3.1)
Neutron only	4	892 (167)	772 (143)	832 (129)	131.4 (17.2)	53.2 (5.3)
Neutron+MEG	4	933 (228)	1058 (184)	996 (168)	149.1 (13.8)	58.4 (8.9)

a) statistically significant ($P<0.01$) b) statistically significant ($P<0.05$)

Table II The protective effect of MEG on the bone marrow counts, organs weight and Fe-59 uptake in X-ray and neutron irradiated mice

Treatment	No. of animals	Bone marrow counts M (σ) x 10^4		Spleen weight M(σ) mg	Thymus weight M(σ)mg	Fe-59 uptake M(σ) cpm/mg		
		Left femur	Right femur			Blood	Liver	Spleen
Control	5	1300 (142)	1273 (138)	145.2 (39.5)	60.7 (10.1)	12200 (4310)	6660 (2510)	5000 (990)
X-ray only	10	505 (104) (a)	493 (90) (a)	96.1 (24.7) (a)	24.2 (7.1)	3330 (1770) (a)	8690 (2500) (a)	6380 (2270)
X-ray+MEG	10	736 (132)	734 (133)	156.3 (47.5)	26.0 (4.5)	6720 (1890)	6310 (2440)	6830 (1930)
Neutron only	10	521 (144)	514 (130)	69.4 (28.0)	21.1 (4.0)	4590 (1420) (b)	10500 (3060)	4000 (1510) (b)
Neutron +MEG	10	569 (162)	514 (86)	79.0 (20.0)	22.3 (3.1)	6520 (2350)	8980 (1810)	6020 (2000)

a) statistically significant ($P<0.01$) b) statistically significant ($P<0.05$)

In general, gastro-intestinal and hematopoietic injuries are related to acute death of mammals caused by ionizing radiation. Gastro-intestinal injury is remarkable in animals exposed to fast neutrons within the $LD_{50(30)}$ range as compared with X-ray and γ-rays. Most of the protective agents are considerably less effective in protecting the intestinal

epithelium than the hematopoietic system[13][14][15]. Therefore, protective agents must be less effective against radiations which bring more damage to the intestinal system than the hematopoietic system.

If the poor effectiveness of protective agents against neutron irradiation is due to the small dose reduction factor for intestinal injuries, protective agents should be effective for hematopoietic injuries in neutron irradiated mice. However, in this study it was noted that MEG modified hematopoietic injuries, that is bone marrow counts and Fe-59 uptake, in neutron irradiated mice less effectively than in X-irradiated mice.

Therefore, the poor effectiveness of MEG against neutron was not due to the small dose reduction factor for intestinal injuries. This may be taken as evidence of MEG bring about a general reduction in the effective neutron dose rather than making a selective protection of specific physiological system, as reported by Patt et al[6].

Summary

MEG significantly modified the bone marrow counts, Fe-59 uptake in peripheral blood and spleen weight of mice exposed to 200 rad of X-ray. Significant differences were observed in these indices between protected and unprotected mice on the 3rd day after irradiation.

In the neutron irradiated mice, the depression of all these indices were less severe and recovery was earlier, but the protective effect of MEG was not as significant as that in mice exposed to X-ray.

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