CHEMICAL PROTECTION OF MEG AGAINST IONIZING RADIATION

Report II The relationship between irradiation dose and optimum dose of MEG

Shigetoshi ANTOKU

Department of Radiation Biology, (Director: Prof. H. Yoshinaga)
Research Institute for Nuclear
Medicine and Biology, Hiroshima University

放射線に対する MEG の化学的保護に関する研究

第 II 報 照射線量と至適 MEG 投与量の関係

広島大学原爆放射能医学研究所
障害基礎研究部門（主任：吉永春馬教授）
安 徳 重 敏

（昭和38年5月23日受付）

1300r, 1000r, 800r 並びに 600r 照射マウス（ddN 均一系）におけるMEGの最有効投与量（至適投与量）を30日間生存率および体重減少から検討し，次の結果を得た．

MEGは半致死，致死，超致死線量照射マウスの放射線致死に対して著明な保護効果をもつている．

1300r, 1000r並びに 800r 照射に対する至適投与量はそれぞれ 400, 300, 250mg/kg であり，これは線量の増加と共に指数函数的に増加する．

600r 照射における至適投与量は生存率および体重減少に対する効果からは決定出来なかつた．

30日間生存率に対する線量減少率（DRF）は約 1.8であつたが長期間生存率をみれば，この値より少し減少する．

Introduction

In previous papers the protective action of MEG was reported on the survival rate and the hematopoietic injurie in mice exposed to 800 r of total body irradiation[1)2)]. It was shown that the optimum dose of MEG was 250 mg of AET per kg of body weight, optimum administration time was 10 minutes before irradiation and the protective activity was influenced by irradiation time. Although the administration of 250 mg/kg was optimum for 800 r, it is felt that such dose is not always optimum for any irradiation dose and it varies with irradiation dose. It is important to determine the optimum dose for a given irradiation dose in the study of protection action, because the estimation of protective activity of agents must be carried out at the administration of the optimum dose and administration of unnecessary dose must be avoided.

The present paper describes the relationship between irradiation dose and optimum dose

for 30-day mortality.

Material and method

The irradiation source was a Toshiba KXC-18-2, operating at a tube voltage of 180 kvp. Irradiation dose of 600 r, 800 r, 1000 r and 1300 r were administered at a target-to-mice distance of 60 cm for about 40, 53, 67 and 87 r/min, respectivley. In order to obtain a constant irradiation time (15 minutes), 0.3 mmCu+0.5 mmAl filter was used for 1300 r group. For other groups, 0.5 mmCu+0.5 mmAl filter was used and the desired dose rate was obtained by controlling the X-ray filament current. The half value layer was 1.08 mmCu for the former radiation and 1.18 mmCu for the latter.

Female mice (ddN uniform strain), aged 8 weeks and weighing 21 to 25 g, were utilized in the protection study. They were housed in metal cages (4 or 5 animals per cage) and were provided a free supply of CLEA pellet and water. Mice were kept for a minimum of 1 week for careful observation prior to use in the study.

Solution of MEG was prepared by neutralizing 1 to 5 percent AET with dilute NaOH and was intraperitoneally administered in constant volume of 0.35 ml, 10 minutes before irradiation. All doses of MEG are reported in milligrams of AET (not MEG) per kilogram of body weight of mice. This reason has described in a previous paper[1].

The optimum dose of MEG for a given irradiation dose was determined by the survival rate of mice administered graded levels of MEG.

Results and discussion

Mortality data on MEG treated mice and their controls for 30 day period after irradiation are summarized in Table I.

I) Group exposed to 1300 r

All unprotected mice died within six days after irradiation. In protected mice, the 30 day survival was 50 to 10 percent in cases administered 250 mg/kg to 500 mg/kg. The mean survival time of protected mice also increased as compared with unprotected mice. The optimum dose was 400 mg/kg, and for mice given this dose the exposure dose of 1300 r corresponded to $LD_{50(30)}$.

When the dose exceeded 400 mg/kg, some mice died shortly after injection or during irradiation due to MEG toxicity. The figures in parentheses of the survival column in Table I show the number of surviving mice excluding those which died within 24 hours after administration of MEG.

As compared with protected mice exposed to 800 r and 1000 r, the death of protected mice exposed to 1300 r was markedly observed after the 20th day. Furthermore, mice surviving for 30 days did not show recovery of body weight loss and most of them died the following month.

For mice exposed to supralethal dose of 1300 r, it would be more appropriate to say that MEG prolonged the survival time rather to say that it protected the animals from radiation death.

Table 1. The relationship between MEG dose and survival rate for given irradiation doses

Irradiation dose	MEG (mg/kg)	Survival (%)	No. of mice
1300r	0(control)	0	20
	550	30 (50)	10
	450	25 (30)	20
	400	50	20
	350	15	20
	250	10	10
1000r	0(control)	0	20
	450	60 (80)	10
	400	70	20
	350	80	10
	300	90	20
	250	80	10
800r	0(control)	4	50
	350	70	20
	300	70	20
	250	96	50
	200	80	10
	150	40	10
600r	0(control)	80	10
	400	90 (100)	10
	300	100	10
	250	100	10
	200	100	10
	150	100	10
	100	100	10
	50	90	10

It was observed by previous investigators that protective agents are less effective for intestinal injuries than for hematopoietic injuries and that the mean survival time in chemically protected mice is from 5 to 7 days after irradiation[3]. However, in the present study protected mice began to die from the 5th to 7th day after irradiation and continued to die gradually to about the 25th day, A similar tendency was seen in mice irradiated to 1000 r and 800 r.

II Group exposed to 1000 r

MEG had a marked activity in any dose level. As in the 1300 r group, deaths were observed after the 20 th day. However, the recovery of body weight loss was better than that of 1300 r group and about half of the mice recovered body weight loss within 30 days after irradiation. The administration of 300 mg/kg G was optimum for irradiation of 1000 r. The survival rate of mice given this dose was 90%, while the unprotected mice died within about 10 days after irradiation.

III Group exposed ot 800 r

As reported in previous paper[1], the optimum dose was 250 mg/kg. Most mice survived

and recovered body weight loss within 20th to 30th day after irradiation. The survival rate, survival curve, and change of body weight are reported in a previous paper.

IV Group exposed to 600 r

As the 30-day survival rate was 100 percent for mice administered 100, 150, 200 and 300 mg/kg and was 90 percent for mice administered dose of 50 and 400 mg/kg, the determination of optimum dose from the data on the 30-day survival could not be made. Therefore, attempt was made to obtaine the optimum dose from the result regarding recovery of body weight loss. Fig. 1 shows changes in body weight of protected, unprotected and MEG only administered mice. In other protected groups, similar changes in body weight were obtained.

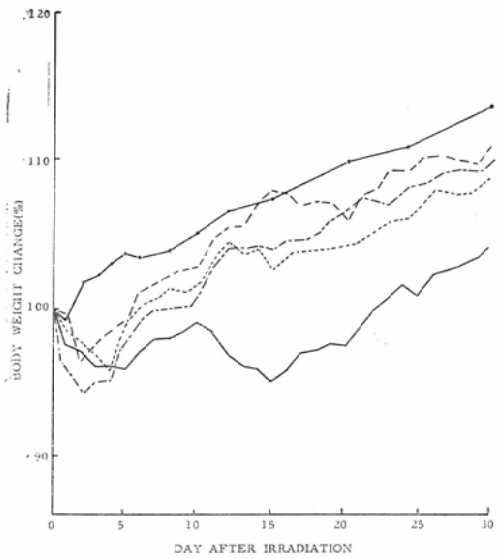

Fig. 1 Body weight change in protected, unprotected and MEG only administered mice
—— X-ray only, —•— MEG 200 mg/kg only, ····· MEG 100 mg/kg + X-ray, ----- MEG 200 mg/kg + X-ray, —·— MEG 300 mg/kg + X-ray

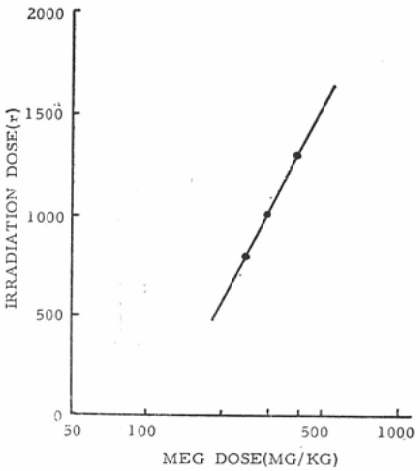

Fig. 2 Relationship between irradiation dose and optimum MEG dose

Body weight curve in unprotected mice has two peaks. The first peak of weight loss appears about the 5th day after irradiation. When there was a temporary recovery, this is followed by a secondary weight loss on about the 15th day, but the secondary body weight loss was not observed in protected mice except for mice administered 50 mg/kg of MEG.

As the difference of body weight loss and recovery was very small among protected groups administered any level of MEG, optimum dose could not obtained from the data on changes of body weight.

From this result it is estimated that the protective activity of MEG was nearly the same at doses from 100 mg/kg to 300 mg/kg. Optimum dose of MEG in irradiation under 600 r will be determined by using other biological indices such as ^{59}Fe uptake, bone marrow counts and atrophy of spleen and thymus.

V Irradiation dose-Optimum dose curve

Fig. 2 shows the optimum dose plotted against irradiation dose. As seen in Fig. 2, the optimum dose increases logarithmically with the increase of irradiation dose. Doherty et al[4] observed that the elevation of radiation $LD_{50(30)}$ was proportional to the dose of MEG administered up to a maximum of 1400 to 1500 r. The result of the present study coincides with that by Doherty et al.

If it can be assumed that this relation can be applied to various biological indices, it follows that the optimum dose for a given irradiation dose and the biological index may be infered from the study of protective effect rather than from survival rate or body weight loss. Therefore, the determination of optimum dose for the biological index in question becomes unnecessary.

VI Dose reduction factor

It has been already reported that MEG provides protection against bone marrow damage and intestinal disorder. According to Doherty et al., dose reduction factor (DRF) is more than 2 for the former, and less than 2 for the latter. Total dose reduction factor for mammalian system is in general about 2 or less 2.[5][6][7]

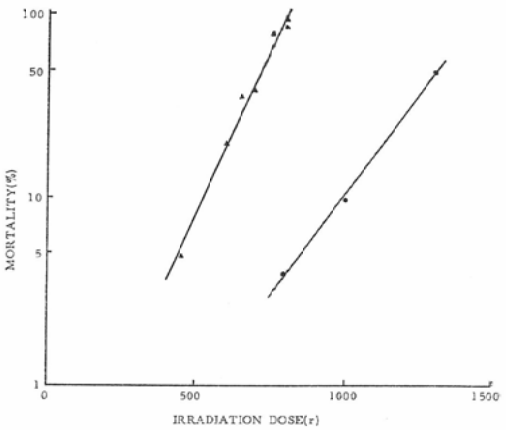

Fig. 3 Mortality curves in protected and unprotected mice
● Protected, ▲ Unprotected

According to author[8] and Sawada,[9] $LD_{50(30)}$ of unprotected ddN mice is 710 r of X-ray, but it is about 1300 r for protected mice as described before (see Fig. 2). Therefore, DRF of MFG for ddN mice is about 1.8, which agrees with that of previous investigations.

Summary

The most effective dose (optimum dose) of MEG for a given irradiation dose was determined by the survival rate and body weight loss of mice. There are summarized below.

MEG has a marked protective activity against the radiation mortality of mice exposed to sublethal, lethal and supralethal dose. The optimum dose was 400, 300 and 250 mg/kg for 1300,

1000 and 800 r irradiation, respectively, and it increased logarithmically with increase of irradiation dose. Optimum dose for 600 r irradiation could not obtained from the data on survival rate and body weight change.

DRF for the 30-day mortality was about 1.8, but it would decrease for long term mortality.

Acknowledgment

The author wishes to express his appreciation to prof. H. Yoshinaga and Prof. A. Tabuchi for their ceaseless kind guidance.

Reference

1) S. Antoku: Nipp. Act. Radiol., in press (1963). — 2) S. Antoku, Y, Kamochi and N. Tanak: J. Kyushu Hemato. Soc., **12**, 71 (1962). —3) J.E. Tomson: Radiation protection in mammals, p. 47 (1962). — 4) D.G. Doherty: Protection and recovery, edited by A. Hollaender, p. 60 (1960). — 5) P. Urso, C.C. Congdon, D.G. Doherty and R. Shapira: Blood, **13**, 665 (1958). — 6) J.R. Maisin, G.D. Novelli, D.G. Doherty and C.C. Congdon: Intern. J. Radiation Biol., **2**, 281 (1961). — 7) W.T. Burnett, Jr and D.G. Doherty: Radation Research, **3**, 217 (1955). — 8) S. Antoku: Unpurblished — 9) S. Sawada: Proc. Hiroshima Univ. RINMB, No. 3,31 (1962).