Studies on Chemical Protectors against Radiation. XXXV.[1] Effects of Radioprotective Chinese Traditional Medicines on Radiation-Induced Lipid Peroxidation *in Vivo* and *in Vitro*[2]

Cheng-Ming WANG, Setsuko OHTA and Masato SHINODA*

Faculty of Pharmaceutical Sciences, Hoshi University, 2–4–41, Ebara, Shinagawa-ku, Tokyo 142, Japan. *Received July 9, 1991*

The fluctuation of lipid peroxidation (LP) in 9 tissues was investigated in mice for 7 d after whole-body X-irradiation with a lethal dose of bone marrow death. LP increased significantly in bone marrow, thymus, spleen and liver following irradiation, and slightly in brain and testis, but not in blood plasma, submaxillary gland or kidney.

The effects of 7 radioprotective Chinese traditional medicines (CTMs) and cysteamine (MEA) on the radiation-induced LP in 4 tissues were studied by i.p. injection before or after irradiation and their LP content in tissues was measured 2 d after irradiation. Most CTMs showed significant inhibition of radiation-induced LP in bone marrow and liver, especially when injected prior to irradiation. Some CTMs also showed such inhibition in spleen. MEA only inhibited the increase of LP in liver when injected before irradiation, but enhanced the increase of LP in spleen. None of these radioprotectors including MEA was recognized to inhibit radiation-induced LP in thymus.

The *in vitro* experiments were carried out using mouse liver microsomal suspensions (MS). The MS were prepared from normal (non-irradiated) mice. Each of the 8 radioprotectors was added to MS before or after irradiation and then post-irradiation-incubated at 37 °C. All markedly inhibited radiation-induced LP if added before irradiation, but were slightly less effective if added after.

Keywords radiation protection; radioprotector; X-irradiation; lipid peroxidation; malondialdehyde; Chinese traditional medicine; cysteamine; radiation injury; survival effect; microsome

It is well documented that irradiation *in vitro* causes increased lipid peroxidation (LP) in subcellular components such as microsomes, lysosomes, mitochondria, nuclei[3] and endoplasmic reticulum,[4] and such an increase was also observed in some animal tissues after whole-body irradiation.[5–8] LP induced by radiation is known to be due to the attack of free radicals on the fatty acid chains of membrane lipids and that this consequently causes radiation-induced damage to functional biological membranes.[3] Indirect evidence that the damage of biological membranes may be an important factor in cell and animal death was suggested by researchers.[3–8] Several radioprotectors are known that can inhibit radiation-induced LP in irradiated microsomal suspensions (*in vitro*) or in some tissue homogenates after whole-body irradiation, for example, glutathione (GSH),[4] vitamin E,[4,5] 2-mercaptopropionyl glycine (MPG)[9] and *S*-2-(3-aminopropylamino)-ethyl-phosphorothioic acid (WR-2721).[8] These reports have indicated that one protective mechanism of these radioprotectors can be explained on the basis of a reduction of LP.

In a previous paper,[1] we reported the survival effects of 60 Chinese traditional medicines (CTMs) against radiation-induced bone marrow death in mice; 15 of the CTMs are recognized to have a significant survival effect. The radioprotective mechanisms of these CTMs, however, have not yet been clarified, and it was for this reason that the present investigation was carried out. We report here on the results obtained of the effects of several radioprotective CTMs on radiation-induced LP *in vivo* and *in vitro*.

The same irradiation dose and administration dose were used in the *in vivo* experiment as in the survival test,[1] and we were able to judge whether the exhibition of survival effect was *via* the inhibition of radiation-induced LP. A microsomal electron transport system was used for the *in vitro* experiment. This method avoided the indirect effects of the various physiological activities of CTM and the complicated defense system of a living body on radiation-induced LP, and enabled us to assess the direct effect of CTM itself on radiation-induced LP in a relatively simple environment.

TABLE I. Survival Effects of Aqueous Extracts of Various Chinese Traditional Medicines and Cysteamine on 2100 R (5.418×10^{-1} Ckg^{-1}) Soft X-Rays (70 kVp) Irradiated Mice

No.	Chinese traditional medicine[a] and Cysteamine	Yield of extract[b] (%)	Administration dose (mg/kg)	Survival effect[c]	
				Pre-irradiation[d]	Post-irradiation[d]
1	Jeou-Wei-Bin-Lang-Tang (Kumi-binro-to)	25.7	1000	2.04[f]	1.02
2	Da-Chai-Hu-Tang (Dai-saiko-to)	35.0	750	2.00[f]	0.77
3	Gui-Zhi-Shao-Yao-Zhi-Mu-Tang (Keisi-syakuyaku-chimo-to)	32.6	1000	1.50[f]	1.52[e]
4	Jing-Jie-Lian-Qiao-Tang (Keigai-rengyo-to)	36.7	1000	1.83[f]	1.94[f]
5	Si-Wu-Tang (Simotu-to)	45.0	2000	1.68[e]	1.67[f]
6	Shao-Yao-Gan-Cao-Tang (Syakuyaku-kanzo-to)	37.8	1000	1.48[e]	1.70[e]
7	Ban-Xia-Xie-Xin-Tang (Hange-syasin-to)	38.2	1000	1.95[f]	1.98[f]
Cysteamine (MEA)		—	227	2.82[f]	1.25

a) The Japanese reading of Chinese traditional medicines is indicated in parentheses. *b*) Percentage of the weight of extract to the weight of total material of a Chinese traditional medicine. *c*) Survival effect is the ratio of mean survival time in a treated group to that of the control 30 d after irradiation. *d*) Intraperitoneal injection 5 min before or after irradiation. *e*) $p<0.05$, *f*) $p<0.01$ *vs.* control group ($n=10$).

Of the 15 radioprotective CTMs,[1] only the 7 were investigated in the present paper, because of their strong survival effect. Cysteamine (MEA),[10] a powerful and typical sulfhydryl radioprotector, was also tested. For convenience of description, the data of the 7 CTMs and MEA are shown in Table I. As seen, Nos. 1 and 2 showed no survival effect in post-irradiation injection, so post-irradiation experiments *in vivo* and *in vitro* were not carried out.

Materials and Methods

Radioprotectors CTMs prepared by their traditional recipe,[11] were received as a gift from Uchida Wakanyaku Co., Ltd. The 7 kinds of CTMs used are listed in Table I with both their Chinese and Japanese readings, according to the designation of Wakan Iyaku Gakkai.[12] MEA was purchased from Tokyo Kasei Kogyo Co., Ltd. Japan.

Preparation of CTM Extracts Thirty grams of dried and smashed CTM was added with 750 ml of purified water, and boiled until the volume of aqueous extract was reduced to 250 ml. The procedure was repeated four times, then all extracts were pooled and filtered through absorbent cotton. The filtrate was then concentrated under reduced pressure and freeze-dried to give a powder.

Animals Male mice of ICR strain, 5 weeks old, purchased from Charles River Japan, Inc., were given nutritional chow (NMF of Oriental Yeast Co., Ltd.) and water *ad libitum*. Acidified water, adjusted to pH 3 by adding HCl to purified water, was given to the mice to prevent contamination by *Pseudomonas* bacteria. Mice weighing 30 ± 2 g (about 6 weeks old) were used throughout the *in vivo* and *in vitro* studies.

Administration MEA and freeze-dried powders of CTMs were dissolved in purified water, adjusted to pH 7—8 with 4 N NaOH, and the administration volume regulated with purified water to 0.3 or 0.4 ml/30 g of mouse. The administration dose and manner of these radioprotectors were carried out under the same conditions described for their survival test[1,10] (Table I).

Irradiation Irradiation of the *in vivo* and *in vitro* experiments was carried out by a soft X-rays irradiator of Softex-CMBW, purchased from Softex Co., Ltd., Tokyo, Japan. 1) *In vivo*: Mice were placed in a circular polystyrene cage (diameter: 15 cm, height: 2.3 cm), and covered with a net (mesh: 1 cm) made with fine stainless steel threads (diameter: 0.3 mm), then put on a revolving stage at a distance 40 cm below the source. The animals were whole-body irradiated from dorsum with 2100 R (5.418×10^{-1} Ckg^{-1}) under the conditions of 70 kVp, 10 mA, a 10 cm acrylic filter and 70 R (1.806×10^{-2} Ckg^{-1})/min. The lethal effect of 2100 R (5.418×10^{-1} Ckg^{-1}) at 70 kVp on mice was equivalent to that of 7—7.5 Gy of hard X-rays at 200 kVp.[13] 2) *In vitro*: Microsomal suspensions in plastic tubes (dimensions: 23×12 mm, volume: 1 ml), with or without addition of radioprotectors, were set on a revolving stage at a distance 30 cm below the source. These suspensions were irradiated in air at room temperature with soft X-rays in the range of 18—72 kR (4.644—18.576 Ckg^{-1}) under the condition of 70 kVp, 10 mA, 1200 R (3.096×10 Ckg^{-1})/min without filter. Dose rate of the *in vivo* and *in vitro* experiments was determined with a Victoreen model 500 rate meter using a model 550-6A probe.

Preparation of Tissue Homogenates and Microsomes Mice were killed by drawing blood from the carotid under pentobarbital sodium anesthesia. All of the following manipulations were carried out at around 4 °C. 1) *In vivo*: Plasma was prepared by the method of Yagi,[14] using 0.05 ml of blood as sample. Organs were immediately removed and all, except, femurs, were washed with ice-cold 0.9% NaCl and weighed, then homogenized in ice-cold 1.15% KCl using a glass-Teflon homogenizer. For thymus samples, each was obtained from thymus pooled from four or five mice. The w/v% of tissues to homogenizing media used was as follows: 10% for brain, submaxillary gland, liver and kidney; and 5% for thymus, spleen and testis. Bone marrow was flushed out from the two femurs of each mouse as indicated by Waynforth,[15] except that 5 ml of ice-cold 1.15% KCl was used. The suspension of dissociated bone marrow cells was centrifuged at $400 \times g$ for 4 min. The supernatant was discarded, and the remaining bone marrow was resuspended in 1 ml of ice-cold 1.15% KCl. 2) *In vitro*: Isolated livers from normal (non-irradiated) mice were homogenized with 4 volumes of ice-cold 0.15 M KCl and 10 mM Tris–HCl buffer (THB), pH 7.5. Homogenate was centrifuged at $9000 \times g$ for 30 min and the pellet was discarded. The resultant supernatant was centrifuged at $10500 \times g$ for 60 min to obtain the microsomal pellet. The microsomes were resuspended and diluted using THB, so that four final concentrations of microsomal protein were constantly maintained at 0.5, 1.0, 1.5 and 2.0 mg protein/ml as determined by the method of Lowry *et al.*[16] Microsomal suspensions were not added with any preservative, and were not refrigerated or frozen overnight; that is, fresh microsomes were used immediately after preparation.

Addition of Radioprotectors in Microsomal Suspensions Each radioprotector was dissolved in THB, and adjusted to 10 μl of solution containing 100, 10 or 1 μg of CTMs or 22.7, 2.27 or 0.227 μg of MEA. Each solution (10 μl) was then added into microsomal suspension (0.5 mg protein/990 μl) with a microsyringe before or after irradiation.

Determination of LP The LP in plasma was determined by the thiobarbituric acid (TBA) method of Yagi.[14] The LP in other tissue homogenates and in microsomal suspensions was determined by the TBA method of Ohkawa *et al.*[17] The amount of LP in both methods was expressed in terms of malondialdehyde (MDA). As described in Results, most microsomal suspensions were post-irradiation-incubated at 37 °C for 30 min before measuring LP, but plasma, tissue homogenates and a few microsomal suspensions were not incubated.

Expression of Results and Statistical Analysis 1) *In vivo*: Irradiation caused hematosteon in the marrow cavity, and erythochromia was observed while collecting the bone marrow cells. The absorbance of LP (a red-colored product from the reaction of MDA with TBA) was determined at 532 nm,[17] and the color of erythochromia resources from red protoheme was also absorbed at 532 nm. To obtain the actual amount of LP from bone marrow, each sample was divided into two parts. To the 1st part (P_1), all reagents were added as indicated in the TBA method. To the 2nd part (P_2), an equal amount of purified water was added instead of TBA. For correction, one more part (W) was also prepared, and an equal amount of purified water was added instead of sample. The three parts were determined at the same time by the TBA method, then their MDA value was substituted into the following equation, so that the actual amount of LP could be easily calculated.

$$\text{LP of bone marrow} = P_1 - P_2 - W$$

where P_1, P_2 and W represent respective MDA values measured from the absorbable color of their own solution, and the absorbable colors were as follows: MDA (appeared after TBA reaction), TBA (reagent itself) and bone marrow suspension for P_1; bone marrow suspension for P_2; TBA for W. All of the bone marrow samples, in both irradiated and non-irradiated groups regardless of administration, were treated in the same manner as above. For statistical analysis, Student's t test was used. The probability values (p) are also presented in figure captions. 2) *In vitro*: Inhibitory effects of radioprotectors on radiation-induced LP were expressed as an inhibition percentage by the following equation.

$$\text{inhibition } \% = \frac{C_x - A_x}{C_x - N} \times 100$$

where C_x is the MDA value (nmol/mg of microsomal protein) of irradiated sample, A_x is the MDA value of added and irradiated sample, and N is the MDA value of non-irradiated sample.

Results

In Vivo **1) Effect of Whole-Body X-Irradiation on LP in Mouse Tissues** As indicated in our previous papers,[1,18] all the mice irradiated with 2100 R (5.418×10^{-1} Ckg^{-1}) of soft X-rays died within 1—2 weeks. To determine the temporal changes of LP (MDA level) in 9 mouse tissues after whole-body irradiation with 2100 R (5.418×10^{-1} Ckg^{-1}), therefore, studies were made within 7 d (Fig. 1). The irradiation had no significant effect on the temporal changes of LP in plasma, submaxillary gland and kidney, as compared to normal (non-irradiated) mice. In brain, irradiation not only caused an increase in the LP rate on the 1st and 4th days, but also caused a decrease on the 5—7th days. In testis, LP levels on the 1st and 2nd days were enhanced after irradiation, but on the 7th day the level was lowered. LP rates in liver and spleen showed almost the same change, although they differed slightly.

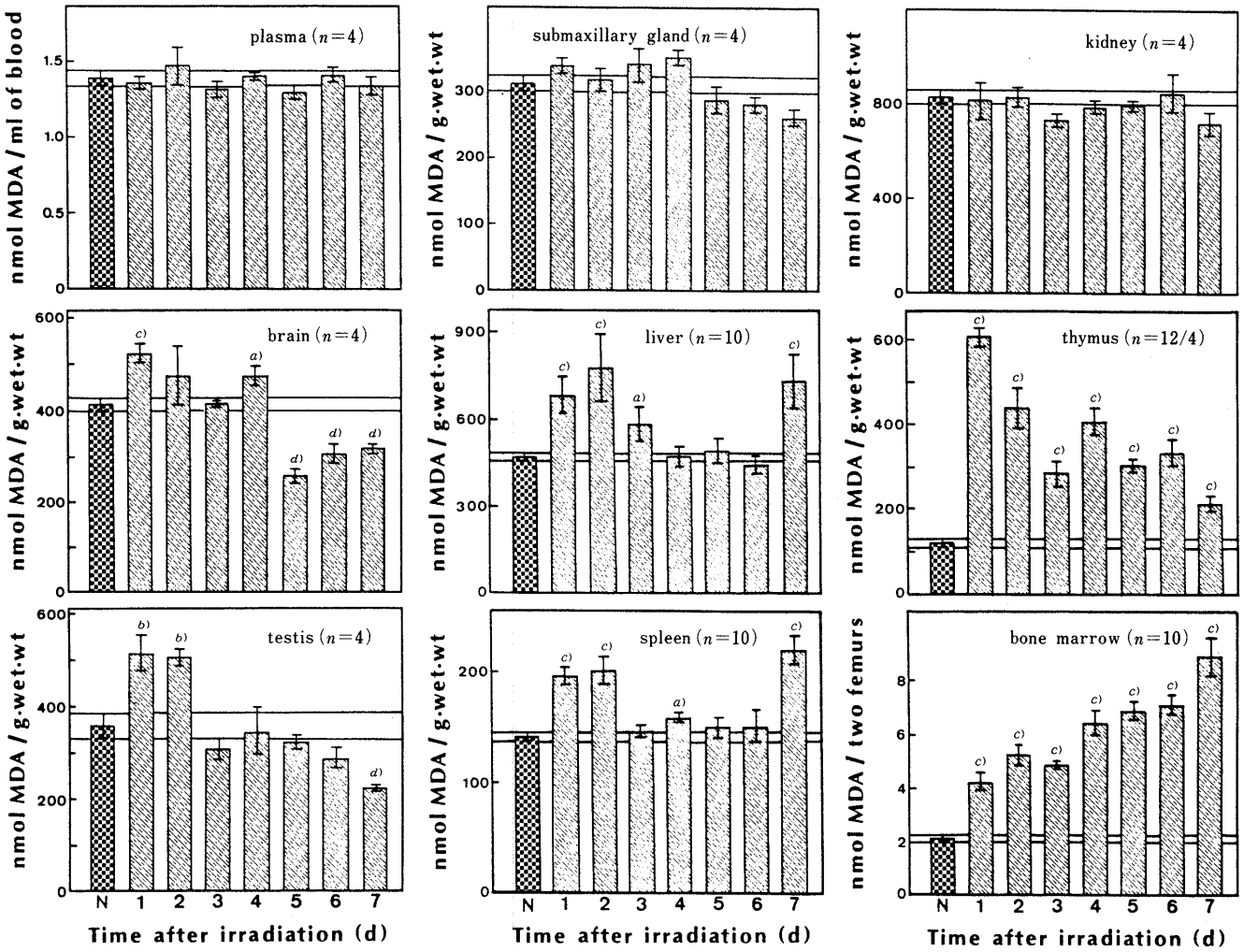

Fig. 1. Effect of Whole-Body Soft X-Irradiation with 2100 R (5.418×10^{-1} Ckg^{-1}) on Lipid Peroxidation (MDA Level) in Mouse Tissues

N, normal (non-irradiated) group. The number of experimental animals is expressed by the sign *n*. In thymus, numerator figure indicates the total number of animals used in a group, and denominator figure indicates the pooled number of animals used in a sample. The MDA values are expressed as the mean ± S.E. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ increased *vs.* normal group; *d*) $p<0.001$ decreased *vs.* normal group.

Two ascending peaks appeared simultaneously in both on the 2nd and 7th days. The thymus and bone marrow showed a considerable increase in LP on all of the 7 d observed following irradiation. A striking increase in the thymus was seen on the 1st day when the LP increased to about 5 times the normal level; this increasing tendency then abated with time. LP in the bone marrow, in contrast, increased slowly as time passed.

2) Effects of Radioprotectors on Radiation-Induced LP in Mouse Tissues Based on the results of 1), the effects of 7 CTMs and MEA on radiation-induced LP were evaluated in mouse liver, spleen, thymus and bone marrow on the 2nd day after whole-body irradiation with 2100 R (5.418×10^{-1} Ckg^{-1}) of soft X-rays. Each radioprotector was i.p. injected to three groups; the pre-irradiation-injected group (PRE), the post-irradiation-injected group (POST) and the non-irradiation-injected group (IJ). PRE, POST and IJ were studied at different times and a normal (non-irradiated) group or a control (irradiated) group was established in each of them. Because no significant difference was found in the MDA level between the normal and the control groups, however, these two groups were pooled and are shown in the same figure (Fig. 2).

In liver, all of the investigated radioprotectors showed significant inhibition against radiation-induced LP in PRE (Fig. 2). Such inhibition was also observed in POST in Nos. 3, 4 and 6. In IJ, Nos. 3 and 4 decreased the intrinsic LP level significantly. In spleen, only Nos. 1 and 3 showed the inhibition in PRE and POST, respectively. MEA, on the contrary, caused an increase in LP exceeding that of the control group in both PRE and POST (Fig. 2). None of these radioprotectors were recognized to have inhibition on radiation-induced LP in thymus (Fig. 2). In bone marrow, considerable inhibition was shown to radiation-induced LP, with the exception of MEA and the POST of Nos. 4, 5 and 7. Furthermore, the PRE of Nos. 2—7 and the POST of No. 6 reduced the radiation-induced LP to the normal level significantly (Fig. 2).

In Vitro **1) Effects of Radiation Doses and Incubation Times on LP in Mouse Liver Microsomal Suspensions** To determine a suitable condition for study of the effects of radioprotectors on radiation-induced LP in microsomes, the following preparatory examinations were performed. As shown in Fig. 3, radiation-induced LP was increased with increasing dose (18—72 kR or 4.644—18.576 Ckg^{-1}) in the four microsomal concentrations (0.5—2.0 mg protein/ml). But extensive formation occurred in the most diluted suspension (0.5 mg protein/ml), and irradiation in

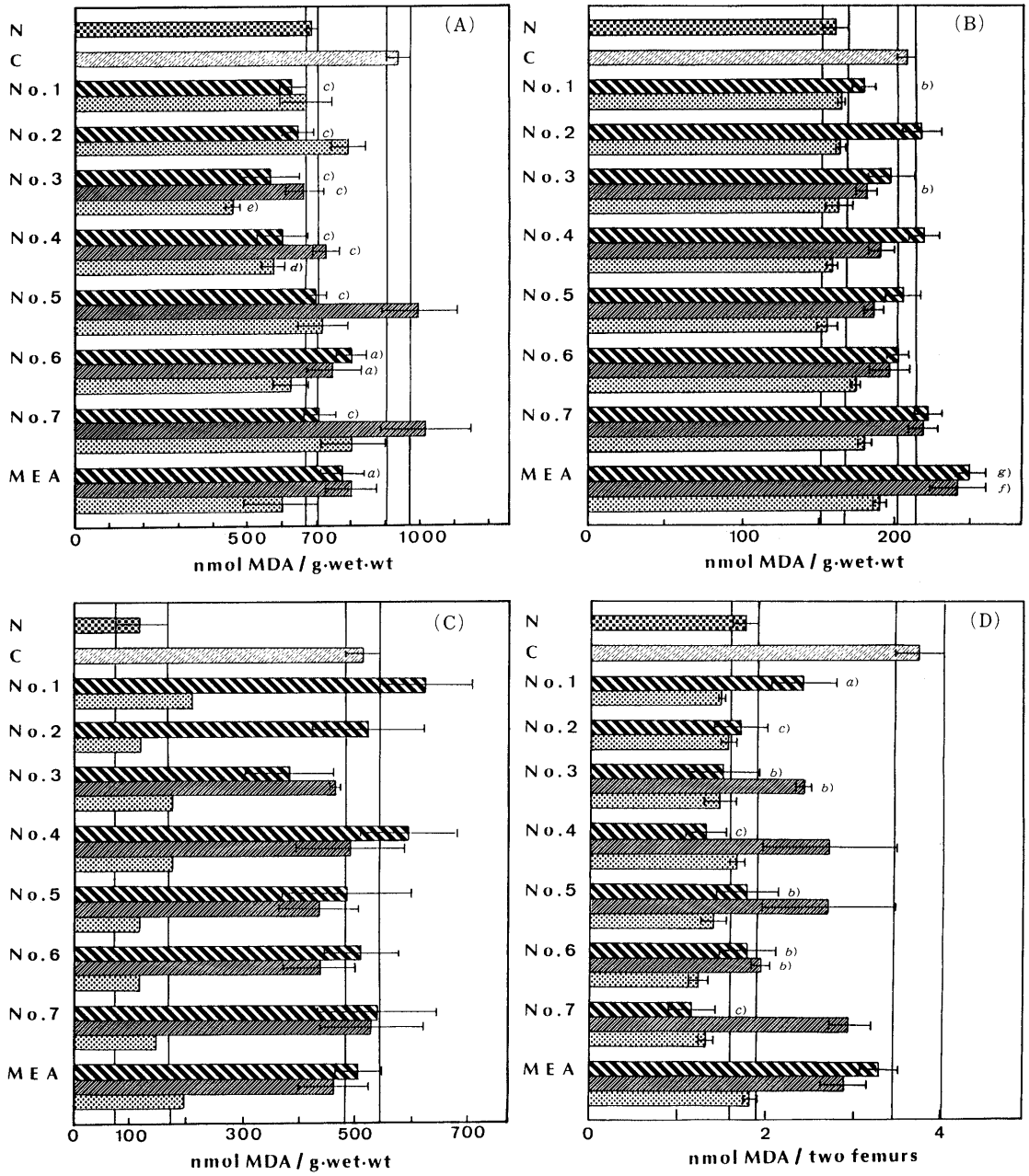

Fig. 2. Effects of Radioprotectors on Lipid Peroxidation (MDA Level) in Mouse Liver (A), Spleen (B), Thymus (C) and Bone Marrow (D) 2 d after Whole-Body Irradiation (2100 R or 5.418×10^{-1} Ckg^{-1}) or Injection Only

▩, N = normal group ($n=25$ for liver and spleen, $n=35/5$ for thymus and $n=13$ for bone marrow); ▨, C = control group ($n=20$ for liver and spleen, $n=30/5$ for thymus and $n=10$ for bone marrow); ▩, pre-irradiation-injected group ($n=10$ for liver and spleen, $n=15/5$ for thymus and $n=5$ for bone marrow); ▨, post-irradiation-injected group ($n=10$ for liver and spleen, $n=15/5$ for thymus and $n=5$ for bone marrow); ▨, non-irradiation-injected group ($n=5$ for liver and spleen, $n=5/5$ for thymus and $n=3$ for bone marrow). The MDA values are expressed as the mean ± S.E. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ decreased *vs.* control group; *d*) $p<0.05$, *e*) $p<0.01$ decreased *vs.* normal group; *f*) $p<0.05$, *g*) $p<0.01$ increased *vs.* control group.

the range of 54—72 kR (13.932—18.576 Ckg^{-1}) therefore caused a maximum formation of LP when the concentration of microsomes was low. To investigate the effect of incubation time after irradiation, two concentrations (0.5 and 1.0 mg protein/ml) of microsomes were used, and they were incubated at 37 °C for 10—40 min after 54 kR (13.932 Ckg^{-1}) irradiation. The formation of LP was increased with incubation time after irradiation in both microsomal concentrations, but the lower the concentration, the greater was the LP (Fig. 4). Thus, 0.5 mg protein/ml of microsomal concentration, 54 kR (13.932 Ckg^{-1}) of radiation dose and 30 min of incubation time were used in the following studies.

2) Effects of Radioprotectors on Radiation-Induced LP in Mouse Liver Microsomal Suspensions Each concentration of radioprotector was added to a microsomal suspension (approximately 0.5 mg protein/ml) before or after 54 kR (13.932 Ckg^{-1}) irradiation, and then incubated at 37 °C for 30 min. LP was determined immediately after incubation, and the inhibitory effect on radiation-induced LP was expressed in terms of percentage inhibition. As is evident from Table II, all radioprotectors markedly inhibited the radiation-induced LP if added before irradiation, but were slightly less effective if added afterward. The inhibitions of all radioprotectors were dependent on added concentration, namely the higher the concentration, the stronger the

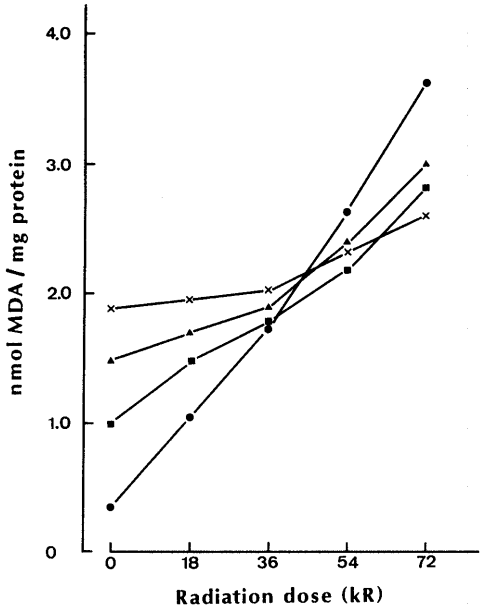

Fig. 3. Effects of Radiation Doses on Lipid Peroxidation in Microsomal Suspensions

Various concentrations of microsomal suspensions were irradiated with 18—72 kR (4.644—18.576 Ckg^{-1}) of soft X-rays and lipid peroxidation was determined thereafter. Values are means of two experiments. ●, 0.5 mg protein/ml; ■, 1.0 mg protein/ml; ▲, 1.5 mg protein/ml; ×, 2.0 mg protein/ml.

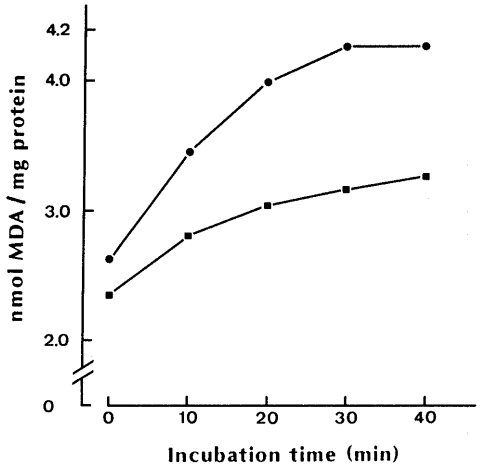

Fig. 4. Effects of Incubation Times on Lipid Peroxidation in Microsomal Suspensions after Irradiation

Two concentrations of microsomal suspensions were incubated in air at 37 °C for various times after 54 kR (13.932 Ckg^{-1}) soft X-irradiation, and lipid peroxidation was determined thereafter. Values are means of two experiments. ●, 0.5 mg protein/ml; ■, 1.0 mg protein/ml.

inhibition.

Discussion

Kergonou *et al.*[6] studied the influence of whole-body gamma irradiation of rats on LP content in plasma, erythrocyte, brain, heart, lung, kidney, spleen, liver, thymus and bone marrow over a period of 10 d after 800 rads (8 Gy) of irradiation. LP levels were increased in all studied tissues except lung; the highest increases were observed in the most radiosensitive bone marrow, thymus and spleen. They reported that irradiated rats began dying on the 8th day. It is known from the survival days that the radiation dose is lethal to the bone marrow, causing it to die. As our method showed, such a high dose will

TABLE II. Inhibitory Effects of Radioprotectors on Radiation-Induced Lipid Peroxidation in Mouse Liver Microsomal Suspensions

Addition	μg/ml	Percentage inhibition of lipid peroxidation[a]	
		Pre-irradiation[b]	Post-irradiation[b]
No. 1	100	90.7	—
	10	60.4	—
	1	23.0	—
No. 2	100	76.7	—
	10	27.7	—
	1	−7.3	—
No.3	100	80.5	34.1
	10	9.7	19.5
	1	−19.5	9.7
No.4	100	90.7	30.6
	10	39.4	14.3
	1	13.7	8.2
No. 5	100	51.0	20.4
	10	−11.9	8.2
	1	−7.3	6.1
No. 6	100	76.7	40.8
	10	9.1	10.2
	1	−7.3	4.1
No. 7	100	76.7	34.1
	10	20.7	17.1
	1	2.6	7.3
MEA	22.7	51.0	19.7
	2.27	8.2	16.3
	0.227	−9.1	8.2

a) Inhibition % = $[(C_x - A_x)/(C_x - N)] \times 100$; C_x, irradiation only; A_x, added and irradiated; N, non-irradiated. *b*) Added immediately before or after 54 kR soft X-irradiation, then incubated in air at 37 °C for 30 min.

cause hematosteon[19]; this is a problem which should not be ignored in measuring the LP in bone marrow, but Kergonou and his colleagues did not mention it. Spectrofluorometric measurement (excitation: 515 nm; emisson: 553 nm) was used by Kergonou *et al.* to determine LP,[6] and we used spectrophotometric measurement (visible wavelength: 532 nm). The red protoheme was measured by both means in our preliminary examination, but the data are not shown here. If we consider the effect of hematosteon on LP in bone marrow, the increase in LP is not as marked as reported by Kergonou *et al.*[6] However, our experimental results approximately agree with theirs for the temporal changes of LP in the bone marrow, thymus and spleen.

The cause of bone marrow death is known to be damage by radiation (up to about 10 Gy or 2.58×10^{-1} Ckg^{-1}) primarily of the blood-forming tissues, and impairment of the renewal of stem cells in the bone marrow. Accompanied by the hematopoietic syndrome, widespread cell death occurs in lymphoid tissues. This injury to the cellular and immunological defense mechanisms of the organism may cause death from anemia, bleeding or infection from one to several weeks after exposure.[20a] It seems that the increased LP in the three radio-sensitive organs is closely related to damage to blood-forming tissues and lymphoid tissues, and radiation-induced LP may be one of the aggravating factors causing bone marrow death.

Diluted microsomal suspensions from much more LP than concentrated suspensions (Fig. 3). This is in agreement with the results of Wills[4,21] who reported that ascorbate-induced, reduced nicotinamide adenine dinucleotide phos-

phate (NADPH)-dependent or radiation-induced microsomal LP increased with decrease of the concentration of microsomal protein. Post-irradiation incubation in air or oxygen stimulates lipid peroxide formation in the subcellular fractions, and oxygen is an essential factor in enduring maximum peroxide formation, as reported by Wills and Wilkinson.[3] The effect of incubation in air was also reconfirmed by our experiments (Fig. 4).

Although all the PRE of CTMs showed similar inhibition of radiation-induced LP in liver and bone marrow, some different radioprotective mechanism could be recognized between them from the following results: (1) in POST, some CTMs showed inhibition in liver and bone marrow, but some did not; (2) in spleen, only Nos. 1 and 3 showed inhibition in PRE and POST, respectively; (3) in IJ, Nos. 3 and 4 decreased the intrinsic LP level in liver. In some cases, the radioprotective mechanism of pre-irradiation and post-irradiation injection in the same CTM differed. For instance, Nos. 5 and 7 showed an inhibition in PRE of liver and bone marrow, but this was not observed in POST. Most CTMs, however, showed significant inhibition against radiation-induced LP in liver and bone marrow. These results suggest that the inhibition of those CTMs may be an important factor in their survival effect. But, PRE and POST of MEA and POST of Nos. 4, 5 and 7 showed no such inhibition in any radiosensitive organs; it thus appears that their survival effect is due to another protective mechanism (Fig. 2). The experimental results of CTMs and MEA in spleen and bone marrow were different, indicating that the radioprotective mechanisms of the two are somewhat different.

As shown in Table II, all radioprotectors themselves have an inhibitory effect on radiation-induced LP, and the inhibition of pre-irradiation addition is higher than that of post-irradiation, suggesting that the radioprotectors added to microsomal suspensions before irradiation began to inhibit the radical chain reaction of lipid peroxide formation during the irradiation. The CTMs and MEA which showed inhibiting effects on radiation-induced LP *in vitro* were not always effective *in vivo*, but one demonstration of inhibition *in vivo* is thought to be due to the direct inhibition of the radioprotector itself *in vitro*.

The inhibitory effect on radiation-induced LP and the protective effect against radiation-induced injuries of sulfhydryl compounds such as MEA, GSH and MPG are considered to be due to their peculiar chemical structure, as part of the sulfhydryl group they scavenge the radiation-induced radicals.[22] It is well known that CTMs are compound medicines which are combined with various crude drugs, and the exhibition of their pharmacologic action is the result of the pharmacodynamic interaction of the combined drugs and their components. The structure–activity relationship of these CTMs is thus still unknown and requires further research.

Extensive studies have revealed that various injuries may result in a living body after whole-body irradiation. A primary inductive factor of such injuries is believed to be radiation-induced radicals which react to biomolecules, and cause functional and structural damage to biological membranes, deoxyribonucleic acid and enzymes.[20b] Clarification of the various radioprotective mechanisms of CTMs or MEA will necessitate much more study.

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