EFFECT OF DIMETHYL SULFOXIDE PRETREATMENT ON ACTIVITIES OF LIPID PEROXIDE FORMATION, SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE IN THE MOUSE LIVER AFTER WHOLE-BODY IRRADIATION

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ABSTRACT — We investigated the effects of dimethyl sulfoxide (DMSO) on radiation damage in the mouse. DMSO (i.p. 0.11 g/mouse) administered 30 min before exposure protected the mice from the gamma-whole body irradiation: the 30 days lethality was significantly decreased from 44% to 16%(P<0.05). The contents of thiobarbituric acid reactive substances(TBA-RS) in the mouse liver increased linearly between days 2 and 10 after 9 Gy gamma ray irradiation. The TBA-RS contents in the liver on days 2 to 10 after irradiation were reduced by DMSO pretreatment. The irradiation decreased superoxide dismutase (SOD) activity in the liver on day 10. Decrease in SOD activity was prevented by DMSO pretreatment. In the electron microscopic study, the mitochondria in the irradiated mouse liver were swollen, but we could observe no change after DMSO pretreatment. The results suggest that DMSO has radioprotective effects, probably due to inhibition of lipid peroxidation.

KEY WORDS: Dimethyl sulfoxide, Radiation, Lipid peroxide, Superoxide dismutase, Glutathione peroxidase.

INTRODUCTION

Radiation produces the active oxygens, such as O_2^-, HO^-, ^1O_2, H_2O_2, etc. in living organisms. These active oxygens attack the membrane lipid resulting in lipid peroxidation. Petkau and Che-lack (1976) reported that lipid peroxidation of model phospholipid membranes was increased by irradiation. Therefore lipid peroxidation should be closely monitored to determine its possible role in radiation injury in vivo. Dimethyl sulfoxide (DMSO) has a radioprotective effect which was reported by Ashwood-Smith in 1961. Several workers have confirmed radioprotective properties of DMSO in their systems: Dod and Shewell reported that topical application of DMSO to the skin of 16-day-old nesting rats was very successful (1968), Hagemann et al. reported that the topical use of DMSO protected the
mouse cataract completely (1970). Therefore the mechanism of radioprotection is still not understood. In the present study, we investigated the protective effects of DMSO in mouse liver lipid peroxidation after irradiation as an index of radiation injury. We determined thio-barbituric acid reactive substances (TBA-RS) as lipid peroxide. The changes of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities in the mouse liver after irradiation were also investigated.

MATERIALS AND METHODS

1. Animals:
   Male ddY mice, weighing 25 g, were fed a standard diet and maintained under light-controlled conditions (6:00 a.m.–6:00 p.m.).

2. 30-day lethality of irradiated mice:
   Whole mice were irradiated by a gamma-ray from 60Co (Toshiba RCR-120-CI) (field size, 35 cm square; distance, 80 cm; exposure rate, 0.49 Gy/min). The 30-day lethality after 9 Gy gamma-ray irradiation was investigated in the two groups of mice: 1) untreated group, without DMSO pretreatment; 2) DMSO treated group, the DMSO pretreatment [0.11 g i.p. injection, according to the report of Ashwood-Smith(1961)] 30 min before irradiation. There were 32 mice in each group. Statistical analysis was by $X^2$ test.

3. Lipid peroxidation, SOD and GSH-PX activity:
   We investigated that the effects of radiation and DMSO pretreatment on the lipid peroxidation, the SOD and the GSH-PX activities in the mouse liver: 1) the control group (without irradiation), 2) the irradiated group, 3) the DMSO pretreated group (0.11 g i.p. injection 30 min before 9 Gy whole-body irradiation). There were 5–6 mice in each group. On days 2, 4, 7 and 10 after irradiation, the mice were killed by dislocation of the cervical vertebrae; the liver was perfused in situ with 0.9% NaCl solution and homogenized with 9 vol. of a 0.1% phosphate buffer (pH 7.4). The lipid peroxide was determined as thiobarbituric acid reactive substance (TBA-RS) according to the method of Uchiyama and Mihrara (1978). The reaction mixture contained 10% liver homogenate 0.5 ml, 1% phosphate 3 ml and 0.6% TBA 1 ml, and was incubated for 45 min at 100°C. n-Butanol was added to the above mixture, mixed for 3 min and centrifuged at 3,000 rpm for 10 min. The absorbance differences between 535 nm and 520 nm in n-butanol layer were recorded. The TBA-RS content in the liver was calculated from the slope of the 1,1,3,3-tetraethoxypropane standard line as nmol malonaldehyde (MDA)/mg protein. The SOD activity was assayed using xantine-xanthine oxidase (XOD) and 2-methyl-6-(p-methoxypyphenyl)-3,7-dihydroimidazo[1,2-a] pyrazin-3-one hydrochloride (MCLA) (Nakano, 1990). The reaction mixture contained 1× 10⁻⁷M MCLA, 5×10⁻⁵M hypoxanthine, 6.5 U XOD, enzyme source (0.1% homogenate 10 μl) or none, and the 50 mM tris-HCl buffer contained 0.1 mM EDTA at pH 7.8, in a total volume of 3.0 ml. The chemiluminescence measurement was initiated by addition of MCLA to the standard incubation mixture excluding XOD and preincubated for 2 min; XOD was added and incubated for 4 min. The chemiluminescence was measured with a luminescence reader (Aloka, BLR102) at 25°C. The SOD activity was calculated from the slope of the SOD standard line. The standard reaction mixture was contain SOD (1 ng·50 ng) instead of the enzyme source. The GSH-PX activity was assayed with a modification of the coupled assay procedure of Paglia and Valentine (1967). The reaction mixture consisted of a 50 mM phosphate buffer (pH 7.0), 2 mM EDTA, 1 mM NaN₃, 0.15 mM NADPH, 1 mM glutathione, 0.5% glutathione reductase solution in a total volume of 180 μl. The enzyme source (20 μl) was added to the above mixture and incubated for 5 min at 25°C before initiation of the reaction by the addition of 10 μl t-butyl hydroperoxide. Absorbance at 340 nm was recorded for 3 min 30 seconds and the activity was calculated from the slope of these lines as μmoles NADPH-oxidized per min. (1 unit is equivalent to and absorbance change of 0.1 per min.) The enzyme activity was shown as units per mg protein. The protein was measured by the method of Markwell et al. (1978). Statistical analysis was by Student’s t-test.

4. Ultrastructural study:
   The mice were anaesthetized. The liver was removed and perfused at 4°C, with 2% parafor-
maldehyde, 2% glutaldehyde overnight. The liver, cut in 1 mm³ pieces, was rinsed with a 0.1 M sodium phosphate buffer. The specimens were postfixed at 4°C for 1 hr in 2% OsO₄, buffered with a 0.1 M sodium phosphate buffer (pH 7.4) washed in a 0.1 M sodium phosphate buffer (pH 7.4), dehydrated in an alcohol series and embedded in Epon-Araldite resin. The ultrathin sections (silver-gold) were cut with a diamond knife. These thin sections were stained with uranyl acetate and lead acetate and were examined in an electron microscope (Hitachi H 800).

RESULTS

1. Percent lethality of ddY mice 30 days after 9 Gy irradiation:

The effects of DMSO pretreatment on the 30-days lethality of ⁶⁷Co-gamma ray irradiated mice is shown in the Table 1. The death of mice in the untreated group were recognised on the 7th day (two mice); the 12th day (three mice); the 13th day (one mouse); the 14th day (six mice); the 17th day (one mouse); and the 19th day (one mouse) after 9 Gy irradiation. The death of mice in the DMSO pretreated group were recognised on the 4th day (one mouse); the 7th day (one mouse); the 15th day (two mice); and the 17th day (one mouse) after 9 Gy irradiation. Therefore, the 30-days lethality of the control group was 44% after 9 Gy irradiation and 16% in the 0.11 g DMSO i.p. pretreated group. These result were significant (P<0.05) by X²-test.

Table 1. Percent lethality of ddY mice in 30 days after 9 Gy irradiation.

<table>
<thead>
<tr>
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<th>Percent lethality</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>44% (14/32)</td>
</tr>
<tr>
<td>DMSO treated</td>
<td>16% (5/32)</td>
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Mice were pretreated with DMSO (0.11 g i.p.) 30 min before irradiation. There were 32 mice in each group.

2. Lipid peroxidation in the mouse liver after irradiation:

Fig. 1 shows the irradiation dose effects for the contents of TBA-RS in the mouse liver the 2nd day after irradiation. The TBA-RS contents in the mouse liver after 8, 9 and 10 Gy irradiation increased significantly, 0.671±0.074 (P<0.01), 0.703±0.214 (P<0.05) and 0.775±0.174 nmol/mg protein (P<0.01), respectively. After 9 Gy irradiation, mice were sacrificed on days 2, 4, 7 and 10, and TBA-RS content was determined in the liver. From 2 to 10 days after irradiation, the contents of TBA-RS in the mouse liver increased significantly (Fig. 2). On the 4th, 7th and 10th days after 9 Gy irradiation, the TBA-RS contents were 0.874±0.058, 0.886±0.167 and 1.070±0.130 nmol/mg protein (P<0.05, vs. control).

![Fig. 1 Radiation dose effects on TBA-RS contents in mouse liver 2 days after whole-body irradiation. The ordinate shows TBA-RS content (nmol/mg protein). The abscissa shows the irradiated doses. Each value is derived from 5 experiments. Error bars indicate S.E. from the mean of 5 experiments to each group. *: P<0.05, **: P<0.01 (vs. control), mean±S.E.](image1)

![Fig. 2 Change of TBA-RS contents in mouse liver as a function of time after 9 Gy whole-body irradiation. The TBA-RS content in the control group : without irradiation (■); in the irradiated group (□); and the DMSO pretreated group (△) (i.p. injection 30 min before 9 Gy whole-body irradiation) are shown. Ordinate as in Fig. 1. The abscissa shows, days after irradiation. Error bars indicate S.E. from the mean of 5 mice. *: P<0.05 (vs. control group), #: P<0.05, ##: P<0.01 (vs. irradiated group), mean±S.E., n=5.](image2)
control), respectively. The radiation-induced lipid peroxidation in the mouse liver was inhibited by DMSO pretreatment (0.11 g DMSO i.p., 30 min before irradiation). The inhibition effects of DMSO in the lipid peroxidation was recognized by the 10th day after irradiation. During these experiment, there were no differences of the TBA-RS contents in the mouse liver between the intact group and DMSO treated group (without irradiation).

3. The SOD and GSH-PX activities:

On the 10th day after 9 Gy irradiation, the SOD activity in the mouse liver decreased significantly to 22.93±1.42 μg/mg protein (P<0.05, vs. the control group, 30.65±2.2 μg/mg protein); the decrease in SOD activity was prevented by DMSO pretreatment (Fig. 3). The GSH-PX activity in the mouse liver did not change for at least 10 days after 9 Gy irradiation, and no effect of DMSO pretreatment was observed (Fig. 4).

4. Ultrastructural study:

Ultrastructural study of the mouse liver after 9 Gy irradiation is shown in Photo. 1. On the 2nd and 7th days after irradiation, the luminal spaces were gradually expanded in cisternas of endoplasmic reticulum. On the 10th day, the

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**Photo. 1** Electron micrograph of a thin section of mouse liver (×9000). N: nucleus, M: mitochondria, ER: endoplasmic reticulum.
mitochondria showed swelling after 9 Gy irradiation, however, no swelling was observed in the mitochondria that received pretreatment of 0.11 g DMSO.

DISCUSSION

The TBA-RS in the mouse liver markedly increased after whole-body irradiation; the amount of increase was related to the irradiation dose (from 6 to 10 Gy) and the time lapse (after the irradiation from 2 to 10 days). These results were similar to those of the in vitro study of Petkau and Chelack (1976). Nakazawa and Nagatsuka (1980) reported that the lipid peroxidation in glucose-retaining liposomes after gamma-irradiation was correlated with the increase in glucose efflux, and Narabayashi et al. (1982) reported that the electron transport in mitochondria was damaged by lipid peroxidation. Accordingly, the TBA-RS level in the mouse liver after whole-body irradiation could be used as an index of radiation injury. The increase of TBA-RS in the mouse liver after irradiation was not observed in groups of mice who received DMSO pretreatment. The SOD activity in the mouse liver decreased markedly 10 days after whole-body 9 Gy irradiation, but we did not observe this decrease in mice pretreated with DMSO. Accordingly, it is supposed that the decrease of SOD activity on day 10 indicates a functional disorder of the cell that is caused by an increase of lipid peroxidation. In the same way, Akita et al. (1984) reported that the decrease of SOD activity in the mouse salivary glands after irradiation was caused from the leakage through membrane damage induced by lipid peroxidation. The activity of GSH-PX, the metabolic enzyme of lipid peroxide, in the mouse liver did not change after irradiation. It is not clear whether the GSH-PX is not influenced by irradiation or whether the enzyme activity preserves despite of cell injury. In the ultramicroscopical study, the mitochondria and the endoplasmic reticulum in the irradiated mouse liver cell were swollen, but we did not observe these changes in DMSO pretreated mice. We suppose that the swelling of mitochondria was caused by the increase of lipid peroxidation in the mitochondrial membrane. These results suggest that DMSO has a radical scavenging effect and prevents the radical generation immediately after irradiation and inhibits the chain reaction of membrane lipid peroxidation which follows thereafter.

DMSO has a radioprotective effect. This is shown clearly by the result from the 30-days lethality after 9 Gy irradiation. We suppose that DMSO inhibits the chain reaction of lipid peroxidation in the cell membrane and inhibits radiation injury caused by lipid peroxidation.

REFERENCES


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