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Original Article

The Effects of Low-Dose-Rate γ-irradiation on Forced Swim Test-Induced Immobility and Oxidative Stress in Mice

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The forced swim test (FST) induces immobility in mice. Low-dose (high-dose-rate) X-irradiation inhibits FST-induced immobility in mice due to its antioxidative function. We evaluated the effects of low-dose γ-irradiation at a low-dose-rate on the FST-induced depletion of antioxidants in mouse organs. Mice received whole-body low-dose-rate (0.6 or 3.0 mGy/h) of low-dose γ-irradiation for 1 week, followed by daily FSTs (5 days). The immobility rate on day 2 compared to day 1 was significantly lower in the 3.0 mGy/h irradiated mice than in sham irradiated mice. The FST significantly decreased the catalase (CAT) activity and total glutathione (t-GSH) content in the brain and kidney, respectively. The superoxide dismutase (SOD) activity and t-GSH content in the liver of the 3.0 mGy/h irradiated mice were significantly lower than those of the non-FST-treated mice. The CAT activity in the lungs of mice exposed to 3.0 mGy/h γ-irradiation was higher than that of non-FST treated mice and mice treated with FST. However, no significant differences were observed in the levels of these antioxidant markers between the sham and irradiated groups except for the CAT activity in lungs. These findings suggest that the effects of low-dose-rate and low-dose γ-irradiation on FST are highly organ-dependent.

Key words: low-dose-rate γ-irradiation, forced swim test, antioxidant, oxidative stress

Low-dose irradiations (at low- or high-dose-rates) are known to activate antioxidative [1,2] and immune functions in mice [3,4]. Specifically, low-dose-rate γ-irradiations inhibit the oxidative stress-induced damage in mice. For example, low-dose-rate γ-irradiation ameliorates type II diabetes in mice by maintaining insulin secretion, which is known to gradually decrease during the progression of diabetes due to the degeneration of pancreatic islets [5]. A possible mechanism underlying the inhibition of oxidative stress in the pancreas could involve the antioxidative functions induced by low-dose-rate γ-irradiation [5]. Another report indicated that continuous low-dose-rate γ-irradiation improves diabetic nephropathy and increases the lifespan of db/db mice through the activation of renal antioxidants [6]. Moreover, low-dose-rate irradiation, in contrast to high-dose-rate irradiation, prevents accelerated aging and tissue dysfunction through the attenuation of oxidative damages [7].

Multiple studies have indicated that the forced swim test (FST) induces oxidative stress in animal organs. For example, it was reported that the FST induced immobility in mice and increased the oxidative stress levels in the brains of the mice [8]. Another study revealed that a weight-loaded FST significantly increased the malondialdehyde (MDA) concentration in liver [9]. Rats subjected to a daily 30 min FST for 15

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consecutive days showed high renal contents of nitrite/nitrate and MDA and a depletion of glutathione, suggesting the involvement of oxidative stress in the kidney [10]. The repeated FSTs also increased distal lung constriction, which is associated with increases in actin, interleukin (IL)-4, and 8-iso-prostaglandin 2α levels. It also induced the activation of inducible nitric oxide synthase, cytokines, and oxidative stress pathways [11].

We reported that low-dose (high-dose-rate; 1.2 Gy/min) X-irradiation at 0.5 Gy inhibited FST-induced immobility in mice due to its antioxidative function [12]. However, the health effects of radiation vary depending on the dose rate. For example, an investigation of atomic bomb survivors indicated that the cancer risk increased depending on the dose [13], whereas individuals exposed to low-dose irradiation from the environment at low-dose-rates showed no such increase in risk [14]. After the 2011 nuclear plant accident in Fukushima, Japan, the effects of low-dose (*i.e.*, low-dose-rate) irradiation have been at the forefront of public attention. The residents of Fukushima have also experienced significant levels of stress due to the accident. Since accumulating evidence implicates free radical-mediated pathology, altered antioxidant capacity, neurotoxicity, and inflammation in neuropsychiatric disorders [15], it is important to examine the effects of the combination of radiation exposure, specifically with a low-dose-rate, and psychological stress. Our previous findings demonstrated the inhibitory effects of 0.5 Gy X-irradiation on FST-induced immobility in mice [12], but as the mice were confined to small cages during the irradiation, this stress condition could have affected the immobility results. To exclude this factor, we used γ-rays and no cage restriction in the present study.

The United Nations Scientific Committee on the Effects of Atomic Radiation defined 'low-dose' as 10-100 mGy and 'low-dose-rate' as < 0.1 mGy/m, and a moderate dose as ranging from 100 mGy to 1 Gy [16]. However, in the present study, we defined 'low-dose' as < 0.5 Gy and 'low-dose-rate' as < 0.1 mGy/m, based on our previous observations [1, 2]. We conducted the present to evaluate the effects of low-dose and low-dose-rate γ-irradiation on the FST-induced depletion of antioxidants in mouse organs. We assayed the activities of superoxide dismutase (SOD) and catalase (CAT) and the total glutathione (t-GSH) content in the brain, liver, kidneys, and lungs of mice treated with FST following low-dose-rate γ-irradiation.

Materials and Methods

Animals. Male BALB/c mice (age, 8 weeks; body weight, approx. 21-26 g) were obtained from Charles River (Yokohama, Japan). Ethical approval for all study-related protocols and experiments was obtained from the animal care and use committee of the Central Research Institute of Electric Power Industry and Okayama University.

Experimental design. The mice were divided into four groups: (1) the untreated control mice (non FST); (2) the mice that underwent sham irradiation with FST (sham group); (3) the mice subjected to 0.6 mGy/h irradiation with FST (the 0.6 mGy/h group); and (4) the mice subjected to 3.0 mGy/h with FST (the 3.0 mGy/h group). The mice received their first FST immediately after γ-irradiation.

Low-dose-rate (low-dose) γ-irradiation. The mice in the two radiated groups were subjected to daily whole-body low-dose-rate γ-irradiation at the dose rate of 0.6 or 3.0 mGy/h using ^{137}Cs for 7 consecutive days at the Central Research Institute of Electric Power Industry. The total doses were 0.1 Gy or 0.5 Gy, respectively.

Forced swim test. After low-dose-rate γ-irradiation or sham irradiation, the FST was performed as described by Joram *et al.* [17]. The mouse was placed individually in a transparent polymethylpentene tube (10 cm dia. × 25 cm height) filled with water to a depth of 15 cm (water temperature 25 ± 1°C), and monitored for 10 min by a video camera. The FST was repeated daily for 5 days. The time that the mouse spent in an immobile state was measured by three observers who were not aware of the low-dose-rate γ-irradiation treatment received by the mouse. The immobility ratio was calculated as the ratio of the immobility times on day 2, 3, 4, and 5 to that observed on day 1. After the final FST, the mice were euthanatized using CO_2. The brain, lungs, livers, and kidneys were removed quickly, and their samples were stored at −80°C until analysis.

Biochemical assays. The brain, liver, kidneys, and lungs were homogenized in 10 mM phosphate-buffered saline (PBS; pH 7.4) using a homogenizer (Bio Medical Science, Tokyo). For the SOD and CAT assays, the homogenates were centrifuged at 12,000 g for 45 min and 10,000 g for 15min at 4°C, respectively. For

the t-GSH assays, homogenates were mixed with trichloroacetic acid solution and centrifuged at 3,000 g for 10 min at 4°C. The diluted supernatant was used for each assay.

The SOD activity, t-GSH content, CAT activity, and protein content were assayed by the SOD Assay Kit-WST (Dojindo Molecular Technologies, Kumamoto, Japan), the Bioxytech GSH-420™ assay kit (OXIS Health Products, Portland, OR, USA), the Catalase Assay Kit (Cayman Chemical, MI), and the Protein Quantification Kit-Rapid (Dojindo Molecular Technologies), according to the respective manufacturer's recommendations.

Statistical analysis. The results are presented as the mean ± standard error of the mean (SEM). Each experimental group consisted of samples from seven animals. Statistical significance was determined using a one-way analysis of variance (ANOVA), followed by Tukey's test. Probability (p)-values were considered significant at $p < 0.05$.

Results

Effects of the γ-irradiation on FST-induced immobility. On days 2, 3, and 4, the FST increased the immobility of the sham-irradiated mice, but there were no significant differences in the immobility times. Moreover, no significant differences were observed in the immobility times between the sham-irradiated mice and the 0.6 mGy/h or 3.0 mGy/h irradiated mice (Fig. 1A). The immobility rate on day 2 compared to day 1 was significantly lower in 3.0 mGy/h irradiated mice compared to the sham irradiated mice (Fig. 1B). However, there were no significant differences on days 3, 4, or 5.

Effect of low-dose-rate γ-irradiation on SOD, CAT, and t-GSH in the brain after the FSTs. Although the repeated FSTs resulted in significant decreases in the brain's CAT activity, the γ-irradiation did not decrease the CAT activity. The FSTs slightly decreased the SOD activity and the t-GSH content, but not significantly ($p > 0.05$). There were no significant differences in these antioxidative substances between the sham and irradiated groups following the FSTs (Fig. 2).

Effects of low-dose-rate γ-irradiation on SOD, CAT, and t-GSH in the liver after the FSTs. The SOD activity and the t-GSH content were significantly lower in the livers of the mice irradiated with 3.0 mGy/h compared to the non FST mice, but no significant differences in the amounts of these antioxidative substances were observed between the sham and irradiated groups (Fig. 3).

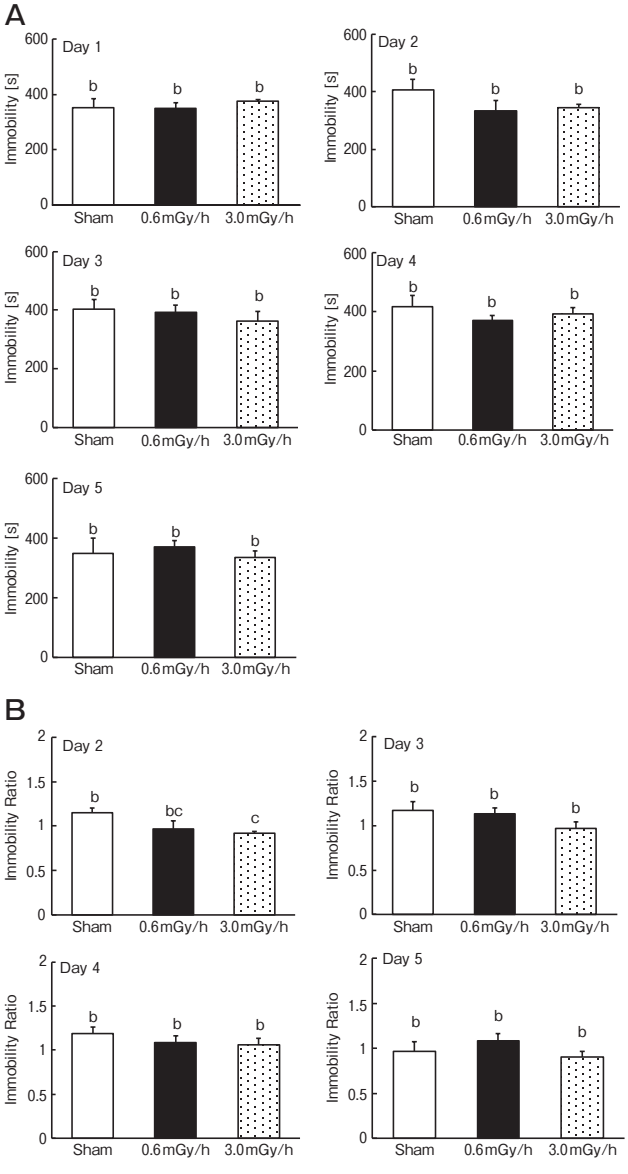

Fig. 1 Effects of low-dose-rate γ-irradiation on FST-induced immobility in mice. A, The average immobility time on each day of the experiment; B, The ratio of immobility. Data are mean ± SEM, n = 7 per group. Bars not sharing the same superscript letters: $p < 0.05$ (B, Day 2; sham vs. 3.0 mGy/h).

Effects of low-dose-rate γ-irradiation on SOD, CAT, and t-GSH in the kidney after the FSTs. Although the repeated FSTs significantly decreased the t-GSH

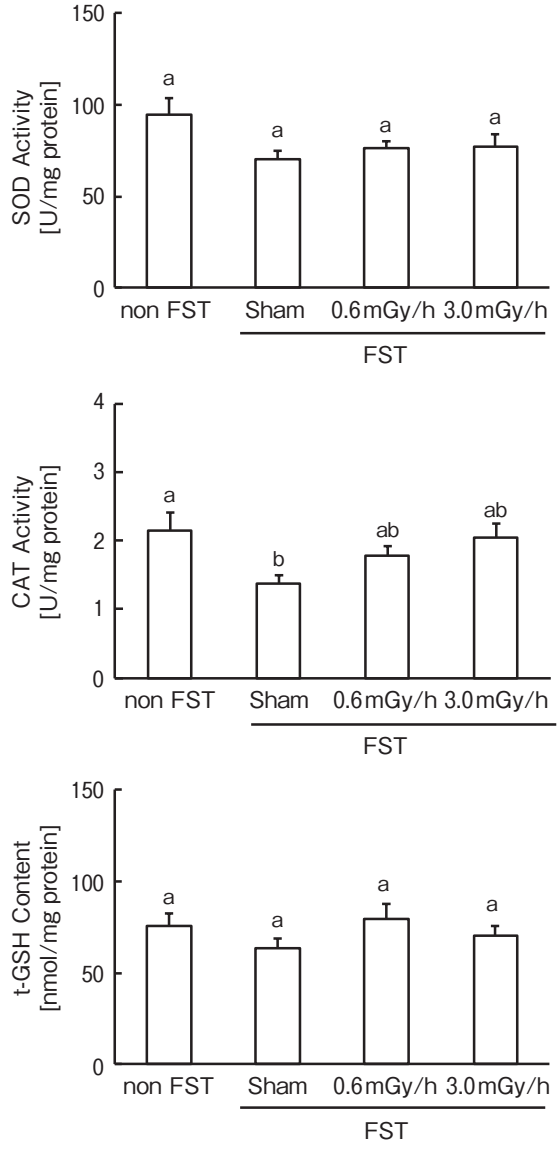

Fig. 2 Effects of low-dose-rate γ-irradiation on antioxidant markers in the mouse brain following FST. Data are mean ± SEM, n = 7 per group. Bars not sharing the same superscript letters: $p < 0.05$ (CAT activity; non FST vs. Sham).

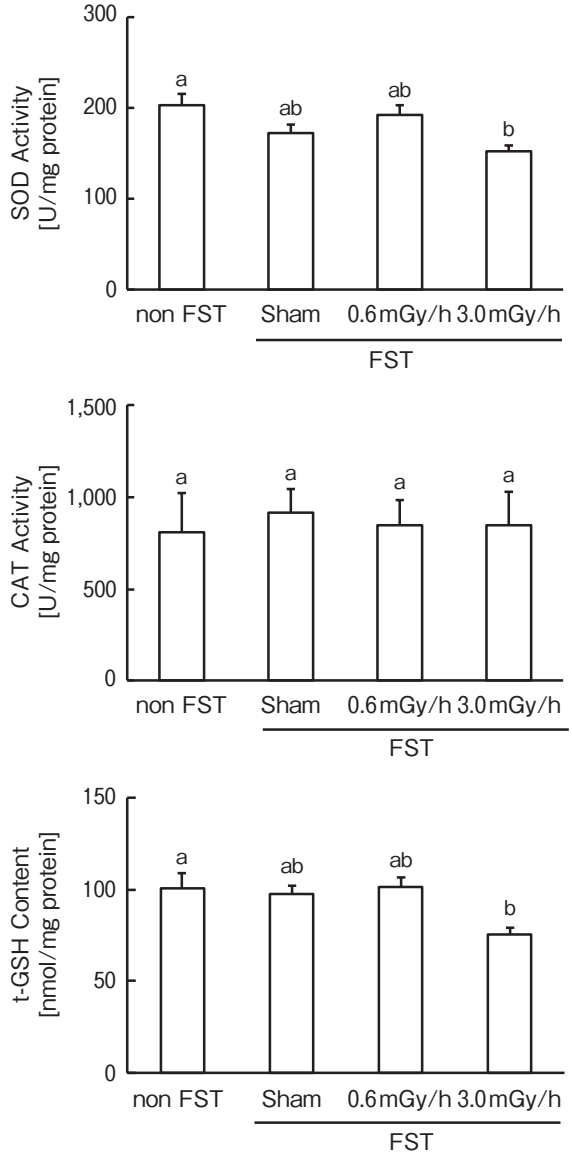

Fig. 3 Effects of low-dose-rate γ-irradiation on antioxidant markers in the mouse liver following FST. Data are mean ± SEM, n = 7 per group. Bars not sharing the same superscript letters: $p < 0.05$ (t-GSH content; non FST vs. 3.0 mGy/h), $p < 0.01$ (SOD activity; non FST vs. 3.0 mGy/h).

content in the kidney, the low-dose-rate γ-irradiation did not cause a decrease in the t-GSH content. The FSTs slightly decreased the SOD activity, but not significantly. The SOD activity was increased by approx. 23% following the 0.6 or 3.0 mGy/h γ-irradiation, but these values were not significantly different. Similarly, there were no significant differences in CAT activities among the sham, 0.6, or 3.0 mGy/h γ-irradiated groups. Although the t-GSH content increased in a dose-dependent manner, these values were also not significantly different among the groups (Fig. 4).

Effects of low-dose-rate γ-irradiation on SOD, CAT, and t-GSH in the lungs after the FSTs. The CAT activity in the lungs of the mice irradiated with

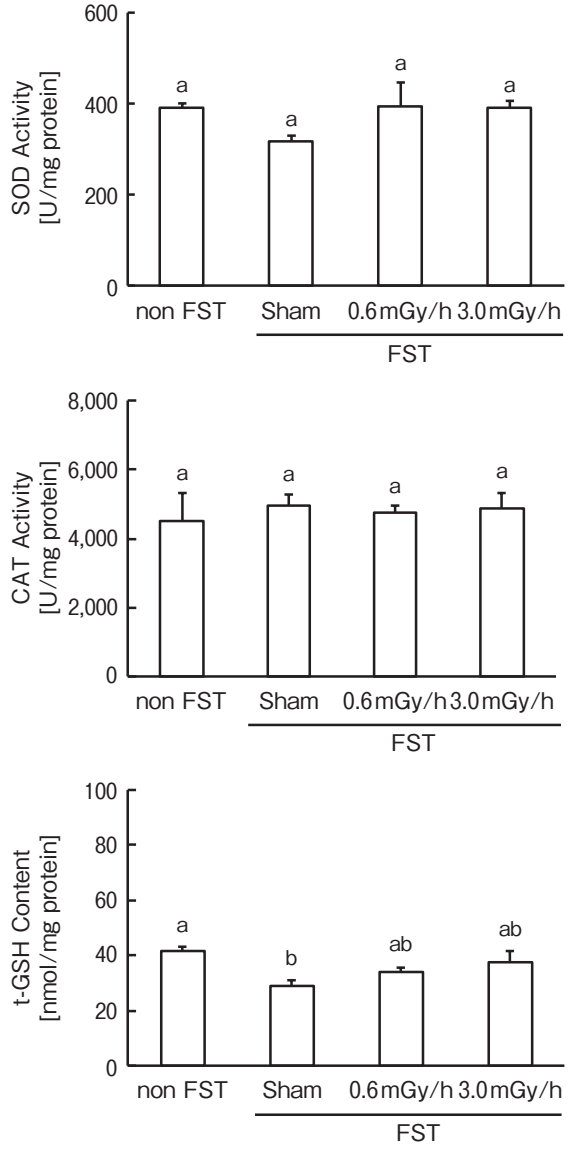

Fig. 4 Effects of low-dose-rate γ-irradiation on antioxidant markers in the mouse kidneys following FST. Data are mean ± SEM, n = 7 per group. Bars not sharing the same superscript letters: $p < 0.05$ (t-GSH content; non FST vs. Sham).

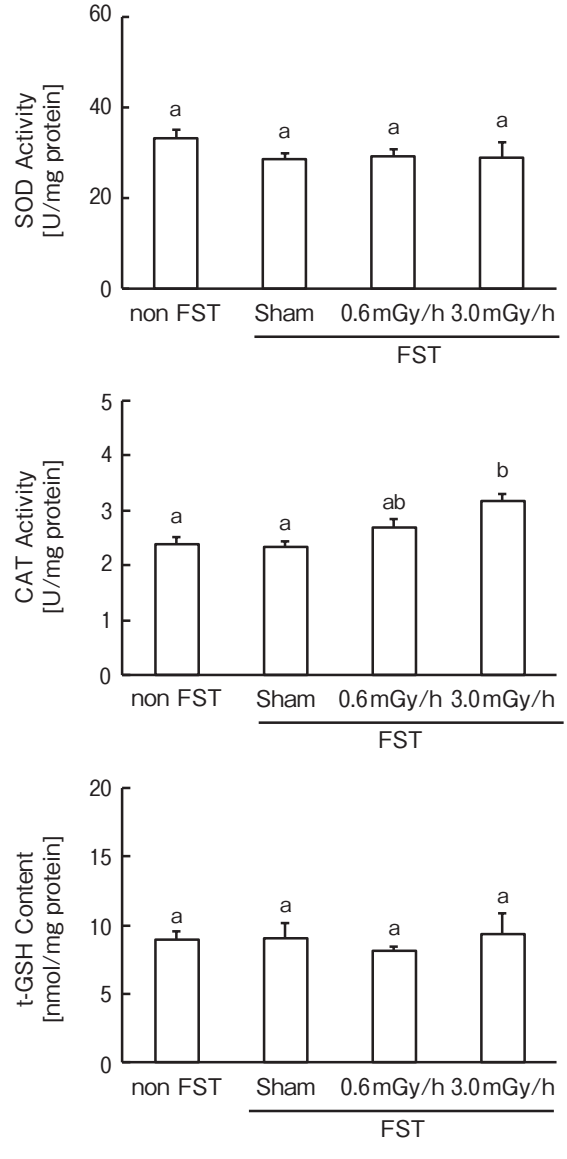

Fig. 5 Effects of low-dose-rate γ-irradiation on antioxidant markers in the mouse lungs following FST. Data are mean ± SEM, n = 7 per group. Bars not sharing the same superscript letters: $p < 0.01$ (CAT activity; non FST vs. 3.0 mGy/h), $p < 0.001$ (CAT activity; Sham vs. 3.0 mGy/h).

3.0 mGy/h γ-irradiation was higher than that in both the non-FST and the sham irradiated mice. However, there were no significant changes in SOD activity or t-GSH content among the sham, 0.6, or 3.0 mGy/h γ-irradiated groups (Fig. 5).

Discussion

Low-dose X- and γ-irradiation both activate antioxidative functions in mouse organs. For example, 0.5 Gy X-irradiation increased the SOD activity and decreased the lipid peroxide (LPO) level in the mouse brain [18]. Although cold injury in the brain causes brain edema

induced by reactive oxygen species (ROS), the activation of antioxidative functions induced by low-dose X-irradiation inhibits brain edema [18]. Another report indicated that the administration of carbon tetrachloride (CCl_4) to mice decreased the activities of SOD and CAT in the liver and resulted in hepatopathy; however, the activities of SOD and CAT of CCl_4-administered mice following 0.5 Gy X-irradiation were much higher than those of non irradiated CCl_4-administered mice, and consequently, CCl_4-induced hepatopathy was inhibited by 0.5 Gy X-irradiation [19].

Another study suggested that the whole-body exposure of mice to γ-irradiation stimulates the antioxidant defense system in the kidneys within 4-24 h after irradiation at doses of 0.25 Gy and 0.5 Gy [20]. In addition, antioxidant enzymes activities for SOD, CAT, glutathione peroxidase (GPx), and glutathione reductase (GR) were increased in the liver at 4 h after exposure to 0.5 Gy, although these antioxidant defense enzymes remained unaltered in the lung [21]. These results indicated that low-dose whole-body γ-irradiation differentially modulates the antioxidant defense system in the liver and lungs of mice [21].

Our present findings demonstrated that 3.0 mGy/h irradiation inhibited the FST-induced immobility in mice on day 2. However, no inhibitory effects were observed from days 3 to 5. Similar results were obtained in our previous study, in which FST-induced immobility was inhibited for 4 h after 0.5 Gy X-irradiation due to an inhibition of oxidative stress [12].

We next evaluated the antioxidative capacity in the mouse brain, liver, kidney, and lung following repeated FSTs. The FSTs decreased the antioxidative capacity in the brain and kidney. These findings suggest that the brain and kidney are vulnerable to oxidative stress after the FST. Irradiation (3.0 mGy/h) along with FST decreased the SOD activity and t-GSH content in the mouse liver, suggesting that the liver is vulnerable to oxidative stress with the combination of low-dose-rate γ-irradiation and the FST. In contrast, we observed that the 3.0 mGy/h irradiation along with the FST increased the CAT activity in the lungs of mice, indicating that the lung is resistant to oxidative stress under this experimental condition.

Our previous study revealed a significant decrease in the level of LPO (a marker of oxidative stress) and a slight increase in CAT activity (~28%) following irradiation with 0.5 Gy X-ray in the mouse brain [12]. The present study also showed a 50% increase in brain CAT activity following 3.0 mGy/h γ-irradiation (total dose, 0.5 Gy). Taken together, these data suggest that the stimulation of CAT activity may play an important role in the inhibition of FST-induced immobility in mice.

In conclusion, the effects of low-dose-rate γ-irradiation on FST vary depending on the organs. Specifically, under the experimental conditions used herein, the mouse liver was vulnerable to oxidative stress, and the lungs were resistant to oxidative stress. Although, the brain and kidneys are vulnerable to oxidative stress following the FST, antioxidative functions come close to the normal levels by low-dose-rate γ-irradiation. The results of this study can provide new insights into the effects of radiation. However, further research is needed to clarify the health effects of low-dose-rate irradiation.

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