J. Radiat. Res., 52, 775–781 (2011)

Study of the Response of Superoxide Dismutase in Mouse Organs to Radon Using a New Large-scale Facility for Exposing Small Animals to Radon

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Radon/Superoxide dismutase/Dose/Dose rate/Large-scale facility.

We examined dose–dependent or dose rate–dependent changes of superoxide dismutase (SOD) activity using a new large-scale facility for exposing small animals to radon. Mice were exposed to radon at a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. When mice were exposed to radon at 2000 day•Bq/m^3, activation of SOD activities in plasma, liver, pancreas, heart, thymus, and kidney showed dose–rate effects. Our results also suggested that continuous exposure to radon increased SOD activity, but SOD activity transiently returned to normal levels at around 2 days. Moreover, we classified the organs into four groups (1. plasma, brain, lung; 2. heart, liver, pancreas, small intestine; 3. kidney, thymus; 4. stomach) based on changes in SOD activity. Thymus had the highest responsiveness and stomach had lowest. These data provide useful baseline measurements for future studies on radon effects.

INTRODUCTION

Low-dose X- or γ-irradiation induces various effects, especially activation of biological defense system, including antioxidative[1–5] and immune functions.[6,7] Low-dose irradiation increases endogenous antioxidants, such as superoxide dismutase (SOD),[3,8] glutathione peroxidase (GPx),[8] glutathione reductase (GR),[2] glutathione,[2,8] catalase,[8] and thioredoxin,[8] in animal tissue. We previously reported that low-dose irradiation activated antioxidative functions and inhibited oxidative damage, such as hepatopathy[9–11] and ischemia-reperfusion injury.[12] It is highly possible that adequate activation of antioxidative functions induced by low-dose irradiation can contribute to preventing or reducing oxidative damage, which are related to lifestyle-related diseases.

Therapy using radon gas, which is volatilized from radon-enriched water, is performed for various diseases in Misasa Medical Center of Okayama University Hospital. The indications for radon and thermal therapy are lifestyle-related diseases such as arteriosclerosis, osteoarthritis,[13] and bronchial asthma.[14] Moreover, radon inhalation activated SOD activity in rats.[15] We also reported that radon inhalation activated the activity of SOD and catalase and decreased lipid peroxide levels in mouse organs.[16] To clarify the effects of radon, we have developed a radon exposure system at Misasa Medical Center of Okayama University Hospital.[17] The system allows different groups of mice to be exposed to radon at six different concentrations simultaneously. In order to lower thoron concentration, a decay chamber (i.e., an empty 0.2 m^3 drum) was positioned after the radon source. The equilibrium equivalent radon concentration, the radon progeny concentration, was quite low compared with the concentration of the coexisting radon even if the air balance in the mouse cage was considered because air supply through a HEPA filter. These results suggest that the effect of thoron and radon progeny is negligible in our radon exposure system.

Exposure to radon activates antioxidative functions in mouse and rat.[15,16] However, dose–dependent or dose rate–dependent changes in antioxidative functions in organs exposed to radon are still unknown. The purpose of this study was to investigate the response of plasma, brain, lung, thymus, heart, liver, stomach, pancreas, kidney, and small intestine to radon. We examined changes in SOD activity in

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doi:10.1269/jrr.10072

the plasma and organs as an indicator of the effects of radon.

MATERIALS AND METHODS

Animals

Male BALB/c mice (age: seven weeks, body weight of approximately 25 g) were obtained thorough the Department of Animal Resources at the Advanced Science Research Center of Okayama University. Ethics approval was obtained from the animal experiment committee of Okayama University. Each experimental group consisted of 5 mice.

Mice were exposed to radon at of a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. These mice were in an air-conditioned room (temperature: 21°C) during radon exposure. Blood containing ethylendiaminetetraacetic acid was collected from the heart under ether anesthesia and plasma was obtained by centrifugation of the blood samples at 3,000 × *g* for 5 min at 4°C. Brain, lung, thymus, heart, liver, stomach, pancreas, kidney, and small intestine were quickly excised for analyses of SOD activity. These organs were preserved at –80°C until used in the biochemical assay.

Biochemical assays

SOD activity was measured by the nitroblue tetrazolium (NBT) reduction method[18] using the Wako-SOD test (Wako Pure Chemical Industry, Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. Briefly, brain, lung, thymus, heart, liver, stomach, pancreas, kidney, and small intestine samples were homogenized in a 10 mM phosphate buffer (pH 7.4) on ice. Homogenate was centrifuged at 12,000 × *g* for 45 min at 4°C, and the supernatant was used for assay of SOD activity. The extent of inhibition of the reduction in NBT was measured by absorbance at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as 50% inhibition of NBT reduction. The protein content was measured by the Bradford method, using Protein Quantification Kit-Rapid (Dojindo Molecular Technologies, Inc., Kumamoto, Japan)[19] according to the manufacturer's instructions.

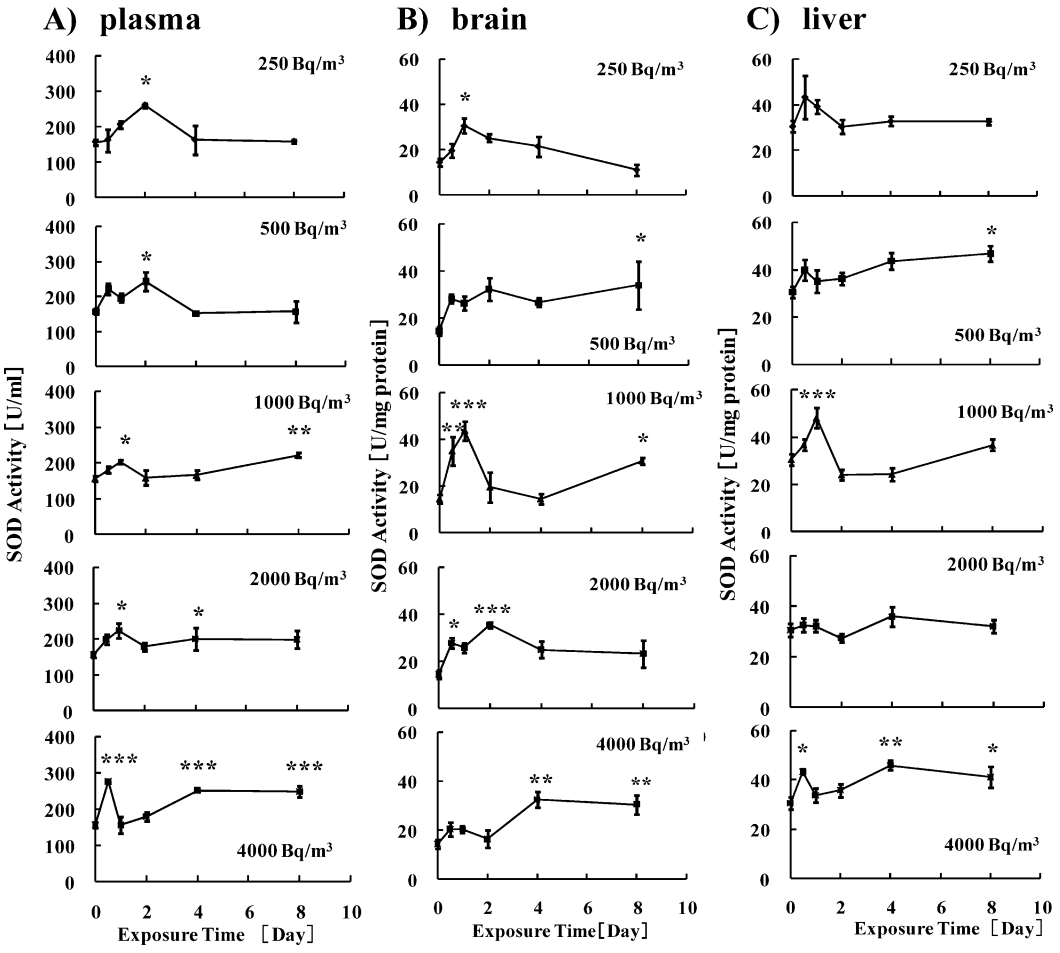

Fig. 1-1. Changes in SOD activity in A) plasma, B) brain, and C) liver following exposure to radon. Mice were exposed to radon at a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. The number of mice per experimental point was 3-5. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

Statistical analyses

The data values are presented as the mean ± standard error of mean (SEM). The statistical significance of differences was determined by Dunnett's tests for multiple comparisons.

RESULTS

Changes in SOD activities in organs

The peak in SOD activity in plasma occurred after exposure to radon at a concentration of 250 or 500 Bq/m^3 for 2 days, after exposure to radon at a concentration of 1000 or 2000 Bq/m^3 for 1 day, and after exposure to radon at a concentration of 4000 Bq/m^3 for 0.5 days. In addition, SOD activity in plasma increased at radon concentrations of 1000 (8 days), 2000 (4 days), or 4000 Bq/m^3 (4 or 8 days) (Fig. 1-1 A). The SOD activity in brain, liver, pancreas, small intestine, and heart also show a tendency to transient activation of SOD within 2 days exposure (Fig. 1-1 B, C; Fig. 1-2 D, E, F). In contrast, there were no significant changes in SOD activity in liver, pancreas, and small intestine of mouse exposed to radon at a concentration of 250 Bq/m^3 (Fig. 1-1 C; Fig. 1-2 D, E). The peak in SOD activity in kidney occurred after exposure to radon at a concentration of 250, 500, or 1000 Bq/m^3 for 1 day (Fig. 1-3 G). The changes in SOD activity in thymus in response to radon were similar to those in the kidney (Fig. 1-3 H). There were no significant changes in SOD activity in stomach after exposure to radon (Fig. 1-4 I). The changes in SOD activity in lung in response to radon were different from those in other organs. When mice were exposed to radon at a concentration of 250, 500, or 2000 Bq/m^3, SOD activity in lung was significantly activated after exposure for 1, 2, 4, or 8 days (Fig. 1-4 J).

Radon concentration dependent changes in SOD activities in organs

When mice were exposed to 2000 day•Bq/m^3 of radon for 0.5 or 1 day, SOD activity in plasma significantly increased, and SOD activity in liver and pancreas significantly

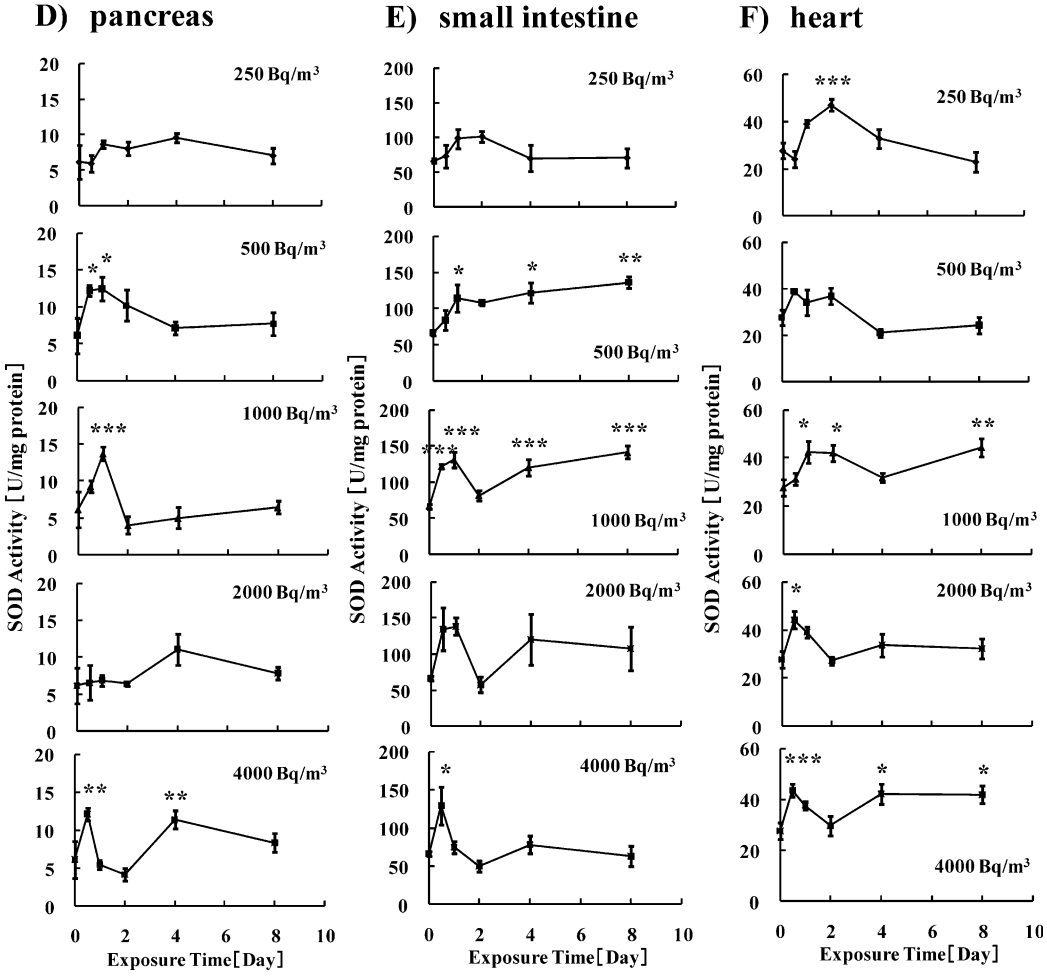

Fig. 1-2. Changes in SOD activity in D) pancreas, E) small intestine, and F) heart following exposure to radon. Mice were exposed to radon at a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. The number of mice per experimental point was 3-5. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

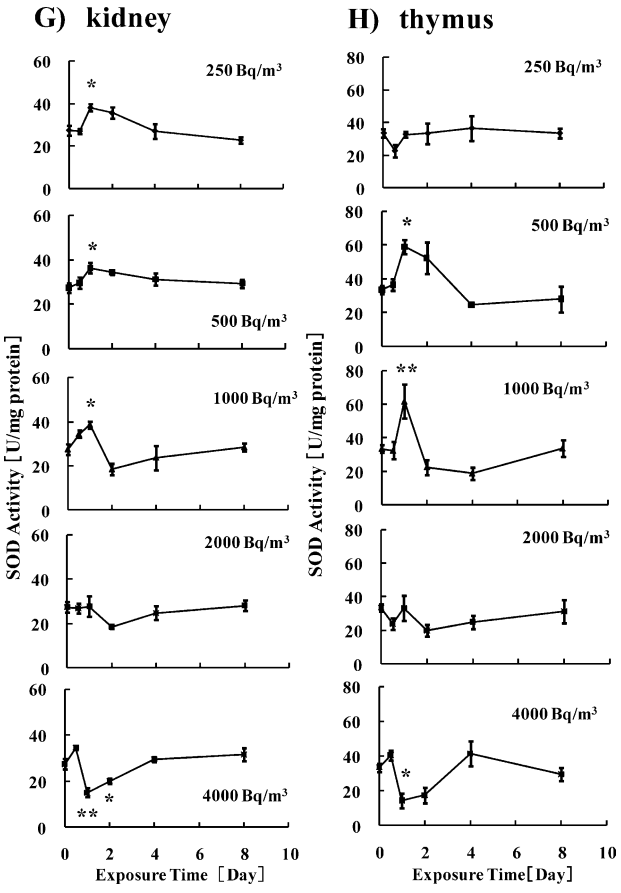

Fig. 1-3. Changes in SOD activity in G) kidney and H) thymus following exposure to radon. Mice were exposed to radon at a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. The number of mice per experimental point was 4-5. *P < 0.05, **P < 0.01 vs. control.

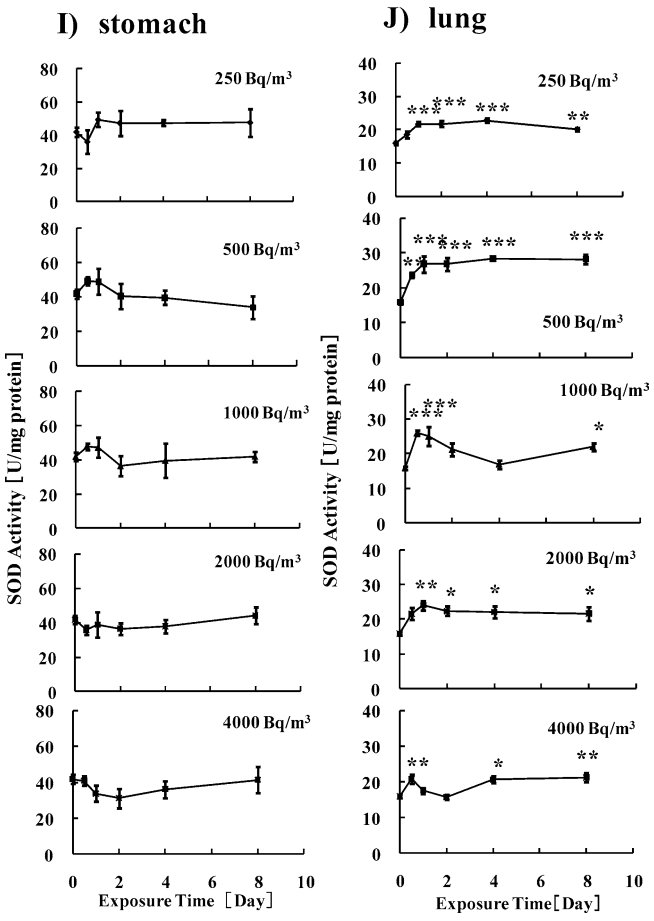

Fig. 1-4. Changes in SOD activity in I) stomach and J) lung following exposure to radon. Mice were exposed radon at a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. The number of mice per experimental point was 4-5. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

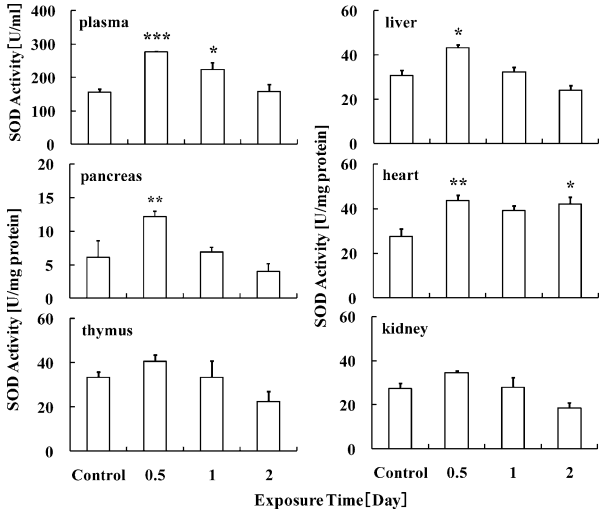

Fig. 2-1. Changes in SOD activity in plasma, liver, pancreas, heart, thymus, or kidney following exposure to 2000 day•Bq/m^3 of radon. The number of mice per experimental point was 4-5. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

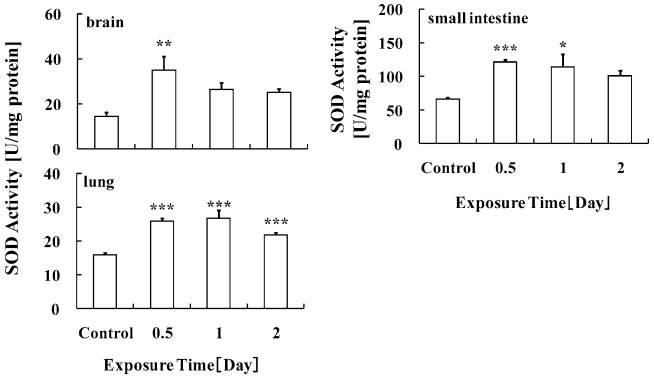

Fig. 2-2. Changes in SOD activity in brain, small intestine, or lung following exposure to 500 day•Bq/m^3 of radon. The number of mice per experimental point was 4-5. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

increased following exposure to this exposure rate for 0.5 days. Moreover, SOD activity in heart significantly increased following exposure for 0.5 or 2 days (Fig. 2-1). When mice were exposed to 500 day•Bq/m^3 of radon for 0.5 days, SOD activity in the brain significantly increased, and that in lung significantly increased following exposure for 0.5, 1, or 2 days. Moreover, SOD activity in the small intestine significantly increased following exposure to 500 day•Bq/m^3 of radon for 0.5 or 1 day (Fig. 2-2). The peaks of enhancement of SOD activities in these organs are at 0.5 days of exposure to radon.

DISCUSSION

It is reported that a single low-dose of X-irradiation activated SOD activity in some organs.[20–23] Low-dose X- or γ-irradiation promotes a small induction of reactive oxygen species (ROS) *in vivo* and induces the production of antioxidant substances, including SOD and catalase, in various organs.[20–23] This activation of antioxidant functions inhibits some oxidative injury, such as hepatopathy[10] and ischemia-reperfusion injury.[12]

The effects of radon that are induced by α-particles may be mediated by the generation of ROS. The relatively large transfer of energy that is associated with the absorption of α-particles causes a series of complicated reactions within animal organs. When mice inhaled radon, radon concentration in mouse organs increased with increasing exposure time, and then reached a constant value within 1 hr.[24] In this study, exposure times were much longer than 1 hr. So, exposure dose to the organs was nearly proportional to exposure time in our experimental system. In addition, the report suggests that the radon concentration in organs (brain, lung, thymus, heart, liver, stomach, pancreas, kidney, and small intestine) after continuous inhalation of radon with a concentration of 1 Bq m^{-3} is about 3 mBq kg^{-3}.[24] Since the report shows the concentration of radon taken into blood, it may underestimate the absorbed dose of lung. Therefore, the changes of SOD activity in organs may show the response to radon except for lung.

Reports suggest that exposure to radon alleviates osteoarthritis[13] and bronchial asthma.[14] To understand the effects of radon, we have co-developed another radon exposure system that is a small-scale facility for exposing small animals to radon.[16] Exposure to radon for up to two days using this system activated the activities of SOD and catalase in the liver, kidney, lung, and brain of mice. In this study, mice were exposed to radon for up to 8 days. Our results also reveal activation of SOD. For example, SOD activity in plasma of mice exposed to radon at a concentration of 4000 Bq/m^3 was significantly increased at 0.5 days. However, SOD activity transiently returned to normal level at 1 day, and then increased again. These findings suggest that radon inhalation for a long period results in two distinct types of SOD activation. This result is similar to the reported effects of low-dose X-irradiation in rats.[20] These findings may be due to homeostasis. In addition, inhalation of a radon of concentration of 4000 Bq/m^3 resulted in transient inactivation of SOD in kidney. This finding may also be due to homeostasis. Though radon inhalation resulted in transient inactivation of SOD in kidney, radon inhalation under our experimental condition has only a small risk based on finding in a recent study because the exposure dose is very low.[24]

Another report suggests that suppression of the induction of SOD following inhalation of a high radon concentration (approximately 1000 kBq/m^3) predominates after long exposures (about 16 hr) and overcomes the stimulating effects of radon.[15] However, our data showed that SOD activity in many organs increased after 0.5–8 days of exposure because of the low radon concentration.

The small-scale facility for exposing small animals to radon enabled only five mice to inhale radon at a time. In addition, it was difficult to change radon concentration at will in the exposure box. Therefore, we developed a large-scale facility that has potential to test approximately 150 mice simultaneously.[17] To clarify the effects of dose rate–dependent changes in antioxidative functions in mouse organs exposed to radon, we examined SOD activity in plasma, liver, pancreas, heart, thymus, kidney, brain, small intestine, or lung of mice that were exposure to a total amount of radon of 500 or 2000 day·Bq/m^3. The results showed that the peak in SOD activity occurred around 0.5 days after radon inhalation (Fig. 2-1, Fig. 2-2). These findings strongly indicate that activation of SOD activities induced by radon inhalation showed dose-rate effect under these exposure conditions.

Our results indicated that the response to radon varies in different organs. We classified these organs into four groups according to the changes in SOD activity in response to radon exposure (Fig. 3). The first group comprised plasma, brain, and lung and had a strong rapid response to radon.

Our results showed that these organs activated SOD at all radon concentrations. The reasons may be as follow: brain is highly fatty organs and has more fats than any other organ, and radon dissolves easily in fat. Therefore, radon may accumulate in the brain. However, our previous report suggested that the absorbed–dose rate was not much difference for brain than for other organs.[24] Another report suggests that SOD in brain readily responds to radiation, since the brain contains abundant phospholipids that are sensitive to active oxygen.[20] These findings may indicate that brain readily responds to radon.

The lung was the most susceptible to radon. Our results demonstrated that SOD activity in the lung was more readily activated than that in any other organ. However, another report shows that almost no changes in the SOD activities in the lungs occurred even in the cases of high dose X-irradiation. Since the reason for no changes in SOD activity after X-

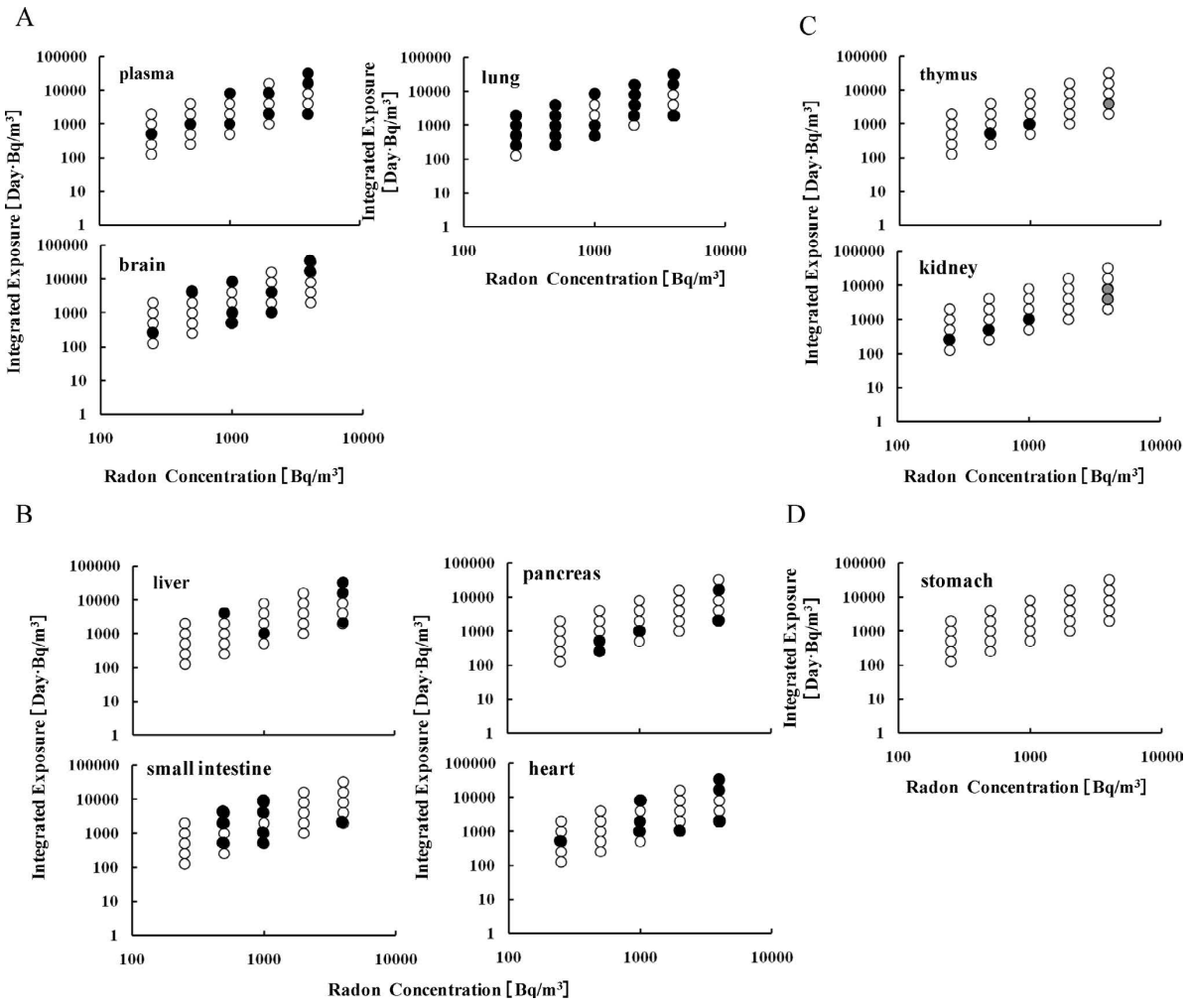

Fig. 3. Changes in SOD activity in A) plasma, lung, and brain, B) liver, pancreas, small intestine, and heart, C) kidney and thymus and D) stomach. Mice were exposed to radon at a concentration of 250, 500, 1000, 2000, or 4000 Bq/m^3 for 0.5, 1, 2, 4, or 8 days. The black circle (●) shows the activation of SOD activity compared with control group. The white circle (○) show the no change of SOD activity compared with control group. The gray circle (◍) show the inactivation of SOD activity compared with control group. Integrated exposure is expressed as a product of radon concentration and exposure time. The number of mice per experimental point was 3-5.

irradiation is attributed to the fact that lungs are exposed to oxygen more than other organs,[20] and lung may be insusceptible to oxidation induced by oxygen and radiation. These findings may indicate that radon exposure, but not X-irradiation, is particularly suited for treatment of lung disease. Moreover, radon therapy may also suit for treatment of brain disease because brain is in the same group as lung.

The second group comprised the liver, heart, pancreas, and small intestine and had a "more complex response to radon". The distinctive feature of this group was that it had two activation points. Radon inhalation of low or high concentrations increased SOD activity. However, there was little change in SOD activity following inhalation of middle radon concentrations. However, our experiments were not designed to investigate the mechanism of SOD activation. Report suggests that the SOD activity increases in liver for a slightly higher dose of X-ray in the range compared to other organs except for lung,[20] which pointed out to have relatively low radiosensitivity. Our results also suggest that response to radon of liver is slightly higher dose compared to SOD activity in plasma. There is no report of changes of SOD activity in heart, pancreas, and small intestine after low-dose irradiation.

The third group comprised thymus and kidney and was "the most susceptible to radon". It is well known that immune organs are susceptible to ionizing radiation. Report suggests that SOD activities in the thymus showed a sharp increase at a low dose than the brain and liver. This indicates that the immunity-related cells, which have been pointed out to have relatively high radiosensitivity, exhibit highly radiosensitive SOD activities than other cells.[20] Our results also show that low concentrations of radon significantly increased

in SOD activities in thymus. In contrast, high concentration of radon significantly decreased in SOD activities in thymus. The findings suggest that thymus was as susceptible to α particles as it is to X-ray.[20] We previously reported that the response of SOD in kidney is similar to that in liver.[16] However, our present results were not consistent with these previous results. One of the differences between this study and previous study is radon exposure conditions, such as the concentrations of thoron and radon progeny. The radon exposure system in our previous study is uncontrolled their concentrations. Therefore, our results in present study indicate that SOD in kidney is relatively susceptible to radon.

The fourth group included only the stomach and it had no response. There was little or no change in SOD activities in the stomach tissue after radon inhalation. This result may indicate the lowest responsiveness to radon. Alternatively, gastroenteritis is indication for radon and thermal therapy. These findings may indicate that the thermal effect is greater than the radon effect. In fact and consistent with this hypothesis, the stomach cancer mortality rates of residents at Misasa hotspring area in Japan are lower than that of a control area.[25]

The radon therapy is used to meet the indications of lifestyle diseases, such as arteriosclerosis, osteoarthritis,[13] bronchial asthma,[14] and emphysema. Our data suggested some new indications for radon treatment. Specifically, it is highly possible that radon inhalation inhibits brain disorder induced by ROS. The data presented in this study provide an essential basis for future studies aimed at the assessment radon therapy for the treatment of brain disorder induced by ROS.

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Received on May 28, 2010
Revision received on January 7, 2011
Accepted on August 8, 2011