A comparative study on effect of continuous radon inhalation on several-time acute alcohol-induced oxidative damages of liver and brain in mouse

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We examined the effect of continuous radon inhalation on acute alcohol-induced oxidative damage of mouse liver and brain. Assay of antioxidative functions indicated that lipid peroxide levels in both the liver and brain of the alcohol-treated mice were significantly higher than those of the saline-treated mice. However, the lipid peroxide level in the liver, but not in the brain, of alcohol-treated mice was significantly decreased by radon inhalation whereas that in the brain of saline-treated mice, but not in the liver of saline-treated mice, was significantly increased by radon inhalation. These findings suggest that radon inhalation inhibits alcohol-induced oxidative damage of liver due to activation of antioxidative functions and that radon inhalation exert only a week effect on the brains in comparing with the livers. They further suggest that alcohol administration protects against oxidative damage of the brain that is induced by radon inhalation.

Key words: radon inhalation, antioxidative function, alcohol-induced oxidative damage, liver, brain

1. Introduction

Therapy using radon gas, which is volatilized from radon-enriched water, is performed for various diseases in the Misasa Medical Center of Okayama University Hospital.¹,²) Radon is a gaseous radioactive element that emits mainly α-rays. If radon is inhaled, the lungs will be subjected to the actions of free radicals generated by irradiation and may become inflamed. Although radon inhalation is generally believed to be hazardous to health, radon springs have been reported to have therapeutic effects on osteoarthritis and asthma.¹,²) Other disease for which radon therapy is used include diabetes mellitus, hyperpiesia, emphysema, and articular rheumatism; however, the mechanisms by which radon alleviates the symptoms of these diseases are unknown. The use of radon therapy is limited to a few locations including Misasa in Japan and Badgastein in Austria. To clarify the mechanisms underlying radon therapy, we have co-developed the Radon Mist Generator and have shown that radon inhalation using this generator activates antioxidative functions in some organs of mice.³)

Reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), hydroxyl radical (·OH), and superoxide anion radicals (O₂·⁻), damage DNA, lipids, and enzymes, and are highly toxic. Cells can be injured or killed when the ROS level exceeds the cellular antioxidant capacity.⁴) There is evidence that oxidative stress plays an important role in the development of alcoholic liver disease.⁵) Alcohol administration has been found to cause accumulation of ROS, including O₂·⁻, ·OH, and H₂O₂,⁶) and increases lipid peroxide levels in liver⁷) and brain.⁸)

Low-dose X-irradiation or radon inhalation increases antioxidative functions and decreases lipid peroxide levels in the liver, kidney, lung and brain of mouse.³,⁹–¹³) Low-dose irradiation promotes a small induction of ROS in vivo and induces the production of antioxidant substances, including superoxide dismutase (SOD) and catalase, in various organs.¹²–¹⁵) Activation of
Effect of radon on alcohol-induced damage

antioxidative functions inhibits oxidative damage including fatty liver and ischemia-reperfusion injury.16–19)

To clarify the underlying mechanisms of radon effects, in the present study we examined the effects of radon inhalation on acute alcohol-induced oxidative damage of mouse liver and brain. Assay of biochemical parameters including SOD, catalase, total glutathione (total GSH), and lipid peroxide levels in liver and brain were investigated.

Materials and methods

Conditions of radon inhalation

The radon exposure system is shown in Fig. 1. To generate conditions for inhalation of low or high radon concentrations, approximately 800 or 6,300 g respectively of the “Doll Stone” radon source (Ningyotoge Gensiryoku Sangyo, Co., Ltd. Okayama, Japan) was placed in a radon exposure box (370 mm × 260 mm × 272 mm). Air was blown into the box at a rate of 0.5 L/min and an exhaust vent was opened. Mice were maintained under controlled conditions (temperature: 25°C; humidity: 75%) and had free access to food and water during radon inhalation. The radon concentration in the box was measured using a radon monitor (CRM-510, Measure Work, Chiba, Japan). The results are shown in Fig. 2. The radon concentration decreased at the 12 and 24 hr time points because the box was opened in order to administer ethanol to the mice. The mean radon concentrations were 600 and 3,500 Bq/m³, respectively.

Radioactivity determination

The 226Ra activity of the radon source was determined by gamma-ray spectrometry. The crushed radon source (130 g) was placed in a plastic container that was then sealed with an epoxy resin adhesive. After achieving radioactive equilibrium among 226Ra and its progeny, gamma rays from the radon source were measured for 3 hr using a high-purity germanium detector (GMX-15200, SEIKO EG&G, Japan). The gamma rays from 214Pb (295 and 352 keV) and 214Bi (609 keV) were used for the analysis of 226Ra activity. Based on these measurements 226Ra activity was calculated as 14.9 ± 0.7 Bq/g.

Experimental protocol

Male BALB/c mice (age: eight weeks; body weight: approximately 25 g) were obtained from the Department of Animal Resources Advanced Science Research Center Okayama University. Ethics approval was obtained from the animal experimental committee of Okayama University. The study protocol was in accordance with the animal experimental guidelines of Okayama University. Each experimental group consisted of 4–7 mice.

The alcohol used, 99.5% ethanol, was diluted to 30% (v/v) in saline solution. Alcohol (6 g/kg body weight) or saline solution (control) was administrated to the mice by gavage administration using medication syringe every 12 hr for a total of 3 doses. Mice inhaled 20, 600, or 3,500 Bq/m³ radon for 28 hr immediately after the first alcohol administration. At 12 or 24 hr

![Schematic diagram of the radon exposure system](image.png)

Fig. 1 Schematic diagram of the radon exposure system

![Changes in the radon concentration in the radon exposure box over the period of radon inhalation. Each value indicates the mean radon concentration. The radon exposure box was opened at 12 and 24 hr. The mean radon concentrations were A) 600 and B) 3,500 Bq/m³, respectively.](image.png)

Fig. 2 Changes in the radon concentration in the radon exposure box over the period of radon inhalation. Each value indicates the mean radon concentration. The radon exposure box was opened at 12 and 24 hr. The mean radon concentrations were A) 600 and B) 3,500 Bq/m³, respectively.
after the start of radon inhalation, these mice stopped radon inhalation temporary (about 10 min) in order to administration of alcohol. Livers and brains were quickly excised for analyses of lipid peroxide levels, total GSH, and SOD and catalase activity.

**Biochemical assays**

Lipid peroxide (malondialdehyde (MDA)) levels were assayed using Bioxytech LPO-586™ assay kit (OXIS Health Products, Inc., OR, USA). Briefly, livers and brains were homogenized in 20 mM phosphate buffer (PBS; pH 7.4) on ice. Prior to homogenization, 10 µL of 0.5 M butylated hydroxytoluene in acetonitrile were added per 1 mL of tissue homogenate. After homogenization, the homogenate was centrifuged at 15,000 × g, for 10 min at 4°C and the supernatant was used for assay. The MDA assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylidole, with MDA at 45°C. The optical density of the colored products was read at 586 nm in a spectrophotometer.

Total glutathione content was measured using the Bioxytech GSH-420™ assay kit (OXIS Health Products). Briefly, livers and brains were homogenized in 10 mM phosphate buffer (pH 7.4) on ice, and then mixed with ice-cold 7.5% trichloroacetic acid solution. The homogenates were centrifuged at 3,000 × g for 10 min. The supernatant was used for the assay. Total glutathione content was measured at 420 nm using a spectrophotometer. This assay is based on the formation of a chromophoric thione the absorbance of which, measured at 420 nm, is directly proportional to the total glutathione concentration.

Mouse liver and brain were homogenized in a 1 M Tris-HCl buffer containing 5 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4) on ice. The homogenate was centrifuged at 12,000 × g for 45 min at 4°C and the supernatant was used for assay of the activity of SOD and catalase.

SOD activity was measured by the nitroblue tetrazolium (NBT) reduction method20) using the Wako-SOD test (Wako Pure Chemical Industry, Co., Ltd., Osaka, Japan). Briefly, the extent of inhibition of the reduction in NBT was measured at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as 50% inhibition of NBT reduction.

Catalase activity was measured as the hydrogen peroxide (H₂O₂) reduction rate at 37°C and was assayed at 240 nm using a spectrophotometer.21) The assay mixture consisted of 50 µl of 1 M Tris-HCl buffer containing 5 mM EDTA (pH 7.4), 900 µl of 10 mM H₂O₂, 30 µl deionized water, and 20 µl supernatant. Activity was calculated using a molar extinction coefficient of 7.1 × 10⁻³ M⁻¹cm⁻¹. Catalase activity was measured by the amount of hydrogen peroxide split by catalase at 37°C. The reactions were started by addition of the supernatant.

The protein content was measured by the Bradford method, using Protein Quantification Kit-Rapid (Dojindo Molecular Technologies, Inc., Kumamoto, Japan).22)

**Statistical analyses**

The data values are presented as the mean ± standard error of the mean (SEM). Statistically significant differences between two groups were determined using Student’s t-test.

**Results**

**Changes in antioxidative function in liver of alcohol-treated mouse by radon inhalation**

Lipid peroxide levels in livers of the alcohol-treated mice were significantly higher than those of the saline-treated mice. Radon inhalation of a concentration of 600 or 3,500 Bq/m³ significantly decreased lipid peroxide level of saline or alcohol-treated mice (Fig.3).

![Fig. 3](image-url) Changes in lipid peroxide level in the livers and brains of alcohol-treated mice following radon inhalation. Each value indicates the mean ± SEM. The number of mice per experimental point is 4–7. *P < 0.05, **P < 0.01, ***P < 0.001 vs. sham inhalation, ^P < 0.05 vs. saline.
Alcohol administration significantly decreases the total GSH content of liver following sham or radon inhalation of a concentration of 3,500 Bq/m³. Total GSH contents of saline or alcohol-treated mice were significantly decreased by radon inhalation of a concentration of 600 or 3,500 Bq/m³ (Fig.4).

The SOD activities in livers of the alcohol-treated mice were significantly increased by radon inhalation of a concentration of 3,500 Bq/m³ (Fig.5).

Catalase activities in livers of the saline-treated mice were significantly increased by radon inhalation of a concentration of 3,500 Bq/m³ (Fig.6).

Changes in antioxidative function in brain of alcohol-treated mouse by radon inhalation

Lipid peroxide levels in brains of alcohol-treated mice were significantly higher than those of the saline-treated mice. Radon inhalation of a concentration of 3,500 Bq/m³ significantly increased lipid peroxide level of the saline-treated mice (Fig.3).

Total GSH contents in brains of saline- or alcohol-treated mice were significantly increased by radon inhalation of a concentration of 3,500 Bq/m³ (Fig.4).

Fig. 5 Changes in SOD activity in the livers and brains of alcohol-treated mice following radon inhalation. Each value indicates the mean ± SEM. The number of mice per experimental point is 4–7. *P < 0.05 vs. sham inhalation.

Fig. 6 Changes in catalase activity in the livers and brains of alcohol-treated mice following radon inhalation. Each value indicates the mean ± SEM. The number of mice per experimental point is 4–7. *P < 0.05 vs. sham inhalation.
centration of 600 Bq/m³, and significantly decreased by radon inhalation of a concentration of 3,500 Bq/m³. Total GSH contents in brains of the alcohol-treated mice were significantly lower than those of the saline-treated mice following radon inhalation of a concentration of 3,500 Bq/m³ (Fig.4).

No significant differences were observed in SOD activities in brains (Fig.5).

Catalase activities in brains of saline-treated mice were significantly increased by radon inhalation of a concentration of 600 or 3,500 Bq/m³ and those of alcohol-treated mice were significantly increased by radon inhalation of a concentration of 600 Bq/m³ (Fig.6).

**Discussion**

It is well established that peroxidation of unsaturated fatty acids of cell membrane phospholipids is accompanied by alterations in membrane structural and functional characteristics, and that lipid peroxidation affects the physical properties of membranes, including membrane fluidity. As shown in Fig. 3, alcohol administration significantly increase the level of lipid peroxide in liver and brain following sham inhalation, suggesting that alcohol decreases membrane fluidity and inhibits normal metabolism.

It is generally accepted that lipid peroxides play an important role in the pathogenesis of alcohol-induced cellular injury and that thiol groups, including GSH, are vital in cellular defense against endogenous or exogenous oxidants. Acetaldehyde, which is a metabolic product of alcohol, is one substance that reacts with GSH. Alcohol administration leads to GSH depletion in liver and other tissues, suggesting direct conjugation of GSH with acetaldehyde and reactive intermediates of alcohol oxidation. Other mechanisms by which alcohol induces hepatic GSH depletion include alcohol-induced increased efflux of GSH from the liver and enhanced utilization of GSH for detoxification of ROS produced as a result of alcohol administration. The role of GSH in protection against alcohol-induced oxidative stress is well-established. In agreement with this fact, as shown in Fig. 4, our data show that alcohol administration significantly decreases the total GSH content of liver following sham inhalation.

On entry to the body through the lungs, radon reaches the blood stream and is then distributed throughout the body. Because it is rather lipid soluble, radon tends to accumulate in organs rich in fat, such as nerve fibers, which are surrounded and protected by a lipid-containing layer. In the present study, radon inhalation of a concentration of 3,500 Bq/m³ significantly increased the catalase activity (shown in Fig.6) in liver, and that of 600 Bq/m³ significantly increased the total GSH (shown in Fig.4) content and catalase activity (shown in Fig.6) in brain, suggesting that radon inhalation activates antioxidative function in liver and brain. Our results also show that the peak of enhancement of antioxidative function in brain, but not in the liver, is following radon inhalation of a concentration of 600 Bq/m³. These findings may indicate that brain is more susceptible to oxidation by radon than liver. Low-dose X-irradiation inhibits non-alcoholic liver injury induced by Fe³⁺ and CCl₄-induced hepatopathy. These results suggested that antioxidant enzymes, which are elevated by low-dose X-irradiation, reduce liver injury and that antioxidant molecules play an important role in the alleviation of liver injury. We have also previously demonstrated that low-dose X-irradiation inhibits edema in mouse paw induced by ischemia-reperfusion injury. The combined results indicate that low-dose X-irradiation is clearly effective for alleviation of oxidative stress. Consistent with these previous reports, we show in the present study that radon inhalation, lipid peroxide levels in liver of alcohol- and saline-treated mice were decreased. These findings suggest that radon inhalation is also effective against oxidative stress and that radon alleviates the dysfunctions induced by alcohol. In addition, since radon inhalation of a concentration of 600 Bq/m³ does not increase the activities of SOD (shown in Fig.5) and catalase (shown in Fig.6), lipid peroxide level in liver (shown in Fig.3) may decrease due to activation of another antioxidant such as thioredoxin. Therefore, it is likely that radon effect is not consistent with radon concentration. On the other hand, alcohol-induced oxidative damage causes blood brain barrier dysfunction. In this study, following radon inhalation, no significant changes were observed in lipid peroxide levels in brain of alcohol-treated mice, suggesting that radon inhalation dose not alleviate alcohol-induced oxidative damage in brain. These findings indicate the different effects of radon inhalation on acute alcohol-induced oxidative damage of liver and brain in mouse. The reason may be the difference radiosensitivity between liver and brain.

As shown in Fig. 4, the total GSH content decreases in liver and increases in brain following radon inhalation of a concentration of 600 Bq/m³, regardless of whether these mice were given alcohol or not. It is likely that GSH in liver increases the efflux from the liver because brain is sensitive to radon. Therefore, there may be no marked differences in total GSH content in
liver of radon inhalation of 600 Bq/m$^3$ between saline- or alcohol-treated mice.

The high susceptibility of the brain to damage by lipid peroxidation is related to the high oxygen consumption of brain and brain is susceptible to oxidative damage. However, the activity of antioxidative enzymes such as catalase in brain is very low compared to that in liver. As shown in Fig. 6, the catalase activity of brain is 150 times lower than that of liver. Hence, in addition to the susceptibility of the brain to ROS formed during alcohol metabolism the brain is also vulnerable to alcohol damage that is due to the lipid soluble nature of alcohol. This effect of alcohol can cause local disordering of lipid membrane structures and of proteins embedded therein. The metabolism of alcohol by the brain is comparatively small and is approximately 1/3,700 of that of the liver. This fact indicates that the residence time of alcohol in the brain is considerably longer than in the liver. On the other hand, alcohol also has some positive antioxidative functions and protects DNA strand breaks from radiation.

The findings of the present study suggest that radon inhalation inhibits alcohol-induced oxidative damage of liver due to activation of antioxidative functions. However, they further suggest that alcohol administration protects against oxidative damage of the brain that is induced by inhalation of a high radon concentration.

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References