Original Article

Protective Effects of Radon Inhalation on Carrageenan-induced Inflammatory Paw Edema in Mice

Takahiro Kataoka[1], Junichi Teraoka[1], Akihiro Sakoda[2],Yuichi Nishiyama[1], Keiko Yamato[1], Mayuko Monden[3], Yuu Ishimori[2], Takaharu Nomura[4], Takehito Taguchi[1], and Kiyonori Yamaoka[1]

[1]Graduate School of Health Sciences, Okayama University, 5-1 Shikata-cho 2-chome, Kita-ku, Okayama-shi, Okayama 700-8558, Japan

[2]Ningyo-toge Environmental Engineering Center, Japan Atomic Energy Agency, 1550 Kagamino-cho, Tomata-gun, Okayama 708-0698, Japan

[3]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, 1-Gakuen-cho, Fukuyama-shi, Hiroshima 729-0292, Japan

[4]Radiation Safety Research Center, Central Research Institute of Electric Power Industry, 2-11-1 Iwado-kita, Komae-shi, Tokyo 201-8511, Japan

Address for correspondence: Professor Kiyonori Yamaoka

Graduate School of Health Sciences, Okayama University

5-1 Shikata-cho 2-chome, Kita-ku, Okayama-shi, Okayama 700-8558, Japan,

Phone/Fax: +81-86-235-6852

E-mail: yamaoka @md.okayama-u.ac.jp

Abstract

We assessed whether radon inhalation inhibited carrageenan-induced inflammation in mice. Carrageenan (1% v/v) was injected subcutaneously into paws of mice that had or had not inhaled approximately 2000 Bq/m^3 of radon for 24 hr. Radon inhalation significantly increased superoxide dismutase (SOD) and catalase activity and significantly decreased lipid peroxide levels in mouse paws, indicating that radon inhalation activate antioxidative functions. Carrageenan administration induced paw edema and significantly increased tumor necrosis factor-alpha (TNF-α) and nitric oxide in serum. However, radon inhalation significantly reduced carrageenan-induced paw edema. Serum TNF-α levels were lower the radon-treated mice than in sham-treated mice. In addition, SOD and catalase activity in paws were significantly higher in the radon-treated mice than the sham-treated mice. These findings indicated that radon inhalation had anti-inflammatory effects and inhibited carrageenan-induced inflammatory paw edema.

Keywords: radon inhalation; inflammation; carrageenan; edema; antioxidative function

INTRODUCTION

Low-dose irradiation induces various positive effects, especially activation of antioxidative [1-5] and immune functions [6,7]. Low-dose X- or γ-irradiation activates antioxidative functions in some organs and, consequently, inhibits oxidative injury [8-13]. In mice for example, pretreatment with low-dose X-irradiation inhibits carbon tetrachloride (CCl_4)-induced hepatopathy [8] and treatment with low-dose (0.5 Gy) X-irradiation following exposure to CCl_4 reduced the oxidative damage associated with CCl_4-induced hepatopathy. [9]. In addition, low-dose X-irradiation inhibits brain edema induced by cold injury [14] and paw edema induced by ischemia-reperfusion injury [11]. It is highly possible that low-dose X-irradiation activates the defensive systems in the living body and, therefore, contributes to preventing or reducing reactive oxygen species (ROS)-related injuries, which are thought to involve peroxidation.

Therapy involving radon gas volatilized from radon-enriched water is performed for various diseases at Misasa Medical Center, Okayama University Hospital. Most conditions treated with radon therapy are lifestyle-related diseases, such as arteriosclerosis, osteoarthritis [15], and bronchial asthma [16]. To assess the effects of radon, we have co-developed a radon-exposure system for small animals (OZ PLAN Co., Ltd. Okayama, Japan); using this system we demonstrated that radon inhalation activated antioxidative functions in the liver, kidney, lung, and brain of mice [17]. These findings indicate that radon inhalation may be used as a treatment for liver, kidney, lung, and brain damage. Recently, we also demonstrated that radon inhalation inhibits the oxidative damage associated with CCl_4-induced hepatopathy in mice, indicating that radon inhalation has antioxidative effects [18].

Reportedly, low-dose X- and γ-irradiation each have anti-inflammatory effects. For example, low-dose γ-irradiation attenuates collagen-induced arthritis through suppression of pro-inflammatory cytokines and autoantibody production and through induction of regulatory

T cells [19]. Moreover, radiation, even at low-doses, functionally modulates inflammatory cells, and the mechanism by which low-dose radiation exerts anti-inflammatory effects may involve heat shock proteins [20]. However, there have been no reports on anti-inflammatory effects of radon inhalation in mice.

The purpose of this study was to determine whether radon inhalation has anti-inflammatory effects in mice. We examined the following biochemical and histological parameters to assess the effects of radon treatment on antioxidative and anti-inflammatory responses: superoxide dismutase (SOD) activity, catalase activity, total glutathione content (t-GSH), lipid peroxide levels, tumor necrosis factor-alpha (TNF-α), nitric monoxide (NO), and paw histology.

MATERIALS AND METHODS

Radon inhalation system

The radon inhalation system is shown in Fig. 1 A. To generate conditions for inhalation of a specific concentration, approximately 100 kg of the “Doll Stone” radon source (Ningyotoge Gensiryoku Sangyo, Co., Ltd. Okayama, Japan) was placed in a radon tank. Air with radon was blown into the mouse cages from the tank at a rate of 2.5 L/min/cage. Odor was removed with a high efficiency particulate air (HEPA) filter, and we ensured sufficient oxygen levels in the mouse cages by fresh air intake. As shown in Fig.1 A, the air with radon in the mouse cages was returned to the radon tank, and some air was then released through the exhaust air duct. The volume was controlled by an air displacement pump.

The radon concentrations in the mouse cage are shown in Fig.1 B and C. Radon concentration in mouse cage was controlled by changing the number of Doll stone and the volume of the outlet flow through exhaust air duct. The radon concentration in the mouse cage was measured using a radon monitor (CMR-510, femto-TECH INC., Ohio, USA). The mean concentrations of background radon and treatment radon were approximately 15 Bq/m^3 and

2000 Bq/m^3, respectively.

Animals

Female ICR mice (age, 8 weeks; body weight, approximately 28 g) were obtained from the Charles River Laboratories Japan Inc. (Yokohama, Japan). Ethical approval for all protocols and experiments was obtained from the animal experimental committee of Okayama University. Mice inhaled radon at a concentration of 2000 Bq/m^3 for 24 hr. Mice had free access to food and water during radon inhalation and the sham treatment. Carrageenan was dissolved physiological saline solution (50 μl of 1% v/v) and was injected in to the right hindpaw of the mice immediately after radon inhalation. Paw volume was assessed by comparing paws before and after injection with carrageenan. Paw volumes were measured at 0, 1, 2, 3, 4, or 5 hr after carrageenan administration by measuring the changes in water levels and total volume associated with bathing mouse paws in water-filled container. To assess the effects of radon inhalation, mice were separated into two groups that were treated with air-only (sham) or radon inhalation, and then all mice in both groups were injected with carrageenan solution. Two hours after injection, blood was drawn from the heart for serum analysis, and paws were quickly excised. Serum was separated from the blood samples by centrifugation at 3,000 × *g* for 5 min for the SOD activity, t-GSH content, NO, and TNF-α assays. These samples were preserved at -80 °C until use. Paw tissue samples were fixed in 10% neutral-buffered formalin and decalcified with Plank-Rychlo solution for histological examinations.

Biochemical assays

NO levels were measured using the NO_2/NO_3 assay Kit C II (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the manufacturer's recommendations.

This assay is based on the azo coupling reaction between diazonium salt compound and naphthy ethylenediamine. The optical density of the colored products was read at 540 nm in a spectrophotometer and was directly proportional to the NO level.

Serum TNF-α was measured by a enzyme-linked immunosorbent assay (ELISA) using the Mouse TNF-α ELISA KIT (Shibayagi Co., Ltd Gunma, Japan) according to the manufacturer's recommendations.

Mouse paws were homogenized in a 10 mM phosphate buffer (PBS; pH 7.4), on ice. The homogenates were centrifuged at 12,000 × g for 45 min at 4 °C and the supernatants were used for assay of the activity of SOD and catalase.

SOD activity was measured by the nitroblue tetrazolium (NBT) reduction method [21] using the Wako-SOD test (Wako Pure Chemical Industry, Co., Ltd., Osaka, Japan) according to the manufacturer's recommendations. 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 (H_2O_2) reduction rate at 37 °C and was assayed at 240 nm H_2O_2 using a spectrophotometer [22]. The assay mixture consisted of 50 µl of 1 M Tris-HCl buffer containing 5 mM ethylenediaminetetraacetic acid (pH 7.4), 900 µl of 10 mM H_2O_2, 30 µl deionized water, and 20 µl paw supernatant. Activity was calculated using a molar extinction coefficient of 7.1×10^{-3} $M^{-1}cm^{-1}$. 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.

Total glutathione content was measured using the Bioxytech GSH-420^{TM} assay kit (OXIS Health Products, Inc., Portland, OR, USA) according to the manufacturer's recommendations. Briefly, tissue samples from paw were suspended in 10 mM PBS (pH 7.4), mixed with ice-cold 7.5% trichloroacetic acid solution, and homogenized. The homogenates were

centrifuged at 3,000 × *g* for 10 min. The supernatants or serum were used for the assay. This assay is based on the formation of a chromophoric thione the absorbance of which can be measured at 420 nm and is directly proportional to the total glutathione concentration.

Lipid peroxide levels were assayed using the Bioxytech LPO-586^{TM} assay kit (OXIS Health Products, Inc.) according to the manufacturer's recommendations. Briefly, paw samples were homogenized in 10 mM 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 the buffer-tissue mixture. After homogenization, the homogenate was centrifuged at 15,000 × *g*, for 10 min at 4 °C, and the supernatant was used for the assay. The lipid peroxide level assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylidole, with malondialdehyde and 4-hydroxyalkenals at 45 °C. The optical density of the colored products was read at 586 nm in a spectrophotometer.

The protein content in each sample was measured by the Bradford method, using Protein Quantification Kit-Rapid (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) [23].

Histological examination

Paw samples were fixed in 10% formalin, and decalcified with Plank-Rychlo solution. Fixed, decalcified specimens were dehydrated in a graded ethanol and xylene series and then embedded in paraffin. Tissue sections (6 microns) were prepared and stained with hematoxylin-eosin (HE).

Statistical analyses

The data values are presented as the mean ± standard error of the mean (SEM). Each experimental group consisted of samples from 6-7 animals. The statistical significance of differences was determined by Student's *t*-test for comparisons between two groups and

Tukey's tests for multiple comparisons where appropriate. P-values were considered significant at $P<0.05$.

RESULTS

Effect of radon inhalation on paw edema induced by carrageenan

Mean paw volume increased of approximately 0.07 ml after injection with carrageenan. However, carrageenan-induced paw edema was significantly reduced by treatment with radon at every time point following carrageenan injection (Fig. 2).

Effects of radon inhalation on TNF-α, NO, or antioxidative function in serum

To assess the effects of radon inhalation on the anti-inflammatory response following carrageenan injection, serum levels of NO and TNF-α were measured after mice were exposed to a sham or radon inhalation treatment; similarly, the levels of antioxidative enzymes, e.g., SOD, were measured in serum and paw-tissue samples to assess the effects of radon inhalation on antioxidative functions. There were no significant differences in mean serum TNF-α and NO concentrations between the sham and radon-treated groups (Fig. 3 A and B). Serum SOD activity was significantly higher in the mice exposed to radon than in sham-treated mice (Fig. 3 C). Similarly, SOD and catalase activity was significantly higher in paws from radon-treated mice than in paws from sham-treated mice; in contrast, the lipid peroxide levels were significantly lower in paws from radon–treated mice than in paws from sham-treated mice (Fig. 3 D).

Effects of radon inhalation on NO in mice serum following carrageenan administration

In mice that had not be pretreated with radon, serum NO levels were significantly higher in carrageenan-injected mice than in control mice (sham versus control; Fig. 4). However, in

mice that had been injected with carrageenan, serum NO levels were 33% lower in radon-treated mice than in sham-treated mice, but this difference was not statistically significant (2000 Bq/m^3 versus sham; Fig. 4).

Histological observation in paws following carrageenan administration

Carrageenan administration increased inflammatory leukocytes in paw in the presence or absence of radon inhalation (Fig.5 A, B, C, D). No significant differences were observed in inflammatory leukocytes between control group and radon inhalation group (Fig. 5 A, B, E). However, there were significantly fewer inflammatory leukocytes per unit area in paws from the radon-treated group than in paws from the sham-treated group (Fig. 5E).

Effects of radon inhalation on the TNF-α in mouse serum following carrageenan administration

To assess the anti-inflammatory effect of radon inhalation, serum TNF-α levels were measured. In mice that had not been pretreated with radon, serum TNF-α levels were significantly higher in animals injected with carrageenan than in control animals (sham versus control; Fig. 6). In animals injected with carrageenan, serum TNF-α levels were significantly higher in sham-treated animals than in radon-treated animals (2000 Bq/m^3 versus sham; Fig. 6).

Effects of radon inhalation on oxidative damage levels in mouse serum and paws following carrageenan administration

To assess the protective effects of radon inhalation on carrageenan-induced inflammation, various parameters of oxidative damage were assayed in serum and paw-tissue samples following sham or radon treatments.

SOD activity in serum from the group pretreated with radon was significantly higher than that from the carrageenan-administrated group (2000 Bq/m^3 versus sham; Fig. 7 A). The mean lipid peroxide level in paws was 20% higher in mice injected with carrageenan, but not treated with radon, than in control animals, but this difference was not significant (sham versus control; Fig. 7 B). However, lipid peroxide levels in paws were 16% lower in radon-treated animals than in sham-treated animals following carrageenan injection, but this difference was not significant (2000 Bq/m^3 versus sham; Fig. 7 B).

In mice that had not be pretreated with radon, SOD and catalase activity and t-GHS contents were significantly lower in paws from carrageenan-injected mice than in paws from control mice (sham versus control; Fig. 7A). In mice had been injected with carrageenan, SOD and catalase activity were significantly higher in paws from radon-treated mice than in paws from sham-treated mice (2000 Bq/m^3 versus sham; Fig. 7A).

DISCUSSION

Radon therapy is performed for various diseases, such as ankylosing spondylitis, chronic polyarthritis, spondylosis deformans, osteoarthritis [15], and bronchial asthma [16], at Misasa Medical Center, Okayama University Hospital. Hepatic and renal damage are not the primary indications for radon therapy. However, we have shown previously that radon inhalation activates antioxidative functions in liver, kidney, lung, and brain of mice [17], and our results demonstrate that radon inhalation clearly inhibits oxidative damage in the liver and kidney of mice [18]. It is highly possible that radon inhalation activates defensive systems and, therefore, contributes to preventing or reducing ROS-related injuries, which are thought to involve peroxidation. The present study showed that antioxidative functions were significantly higher in mice that had inhaled radon at a concentration of 2000 Bq/m^3 for 24 hr than in control mice.

Most conditions treated with radon therapy are pain-related diseases such as osteoarthritis [15] and rheumatoid arthritis [24]. Another research group reported that radon inhalation significantly increased β-endorphin and M-enkephalin which both have morphine-like analgesic effects [25]. In addition, it has been reported that low-dose γ-irradiation attenuates collagen-induced arthritis through suppression of pro-inflammatory cytokines and autoantibody production and through induction of regulatory T cells [19]. To assess the anti-inflammatory effects of radon inhalation, we used a carrageenan-induced inflammation model. Our results showed that carrageenan-induced paw edema was significantly reduced by treatment with radon at every time point measured, indicating that radon inhalation had anti-inflammatory effects similar to those of X- or γ-irradiation.

Cuzzocrea suggests that some of the delayed inflammatory pathways involving nitric oxide ($NO^{\bullet}$), superoxide ($O_2^{\bullet}$), hydroxyl radical ($^{\bullet}OH$), and peroxynitrite ($ONOO^{\bullet}$) are induced in carrageenan-induced inflammation [26]. Carrageenan triggers the expression of the inducible NO synthase (iNOS), a process that occurs, at least in part, via activation of nuclear factor kB (NF-kB). NO, in turn, combines with $O_2^{\bullet}$ to yield $ONOO^{\bullet}$. The $^{\bullet}OH$ and $ONOO^{\bullet}$ radicals induce cellular injury. ROSs, such as $NO^{\bullet}$, $O_2^{\bullet}$, $^{\bullet}OH$, and $ONOO^{\bullet}$, were caused by oxidative damage and induced paw edema. To assess the mechanisms mediating the anti-inflammatory effects associated with radon inhalation, we first examined the NO level in serum. Our results indicated that the inhibition of NO synthesis contributed to the reduction in carrageenan-induced inflammation.

Cuzzocrea also suggests that the polymorphonuclear leukocyte infiltration and activation induced $O_2^{\bullet}$ and H_2O_2 production. In this study, our results showed that carrageenan administration increased infiltration of inflammatory leukocyte in paw in the presence or absence of radon inhalation. However, the number of inflammatory leukocytes in paw was significantly lower in the radon-treated group than in the carrageenan-administrated,

sham-treated mice. These findings may indicate that radon inhalation reduced ROS production induced by carrageenan administration.

Next, we examined serum TNF-α levels. TNF-α is major mediator in the inflammatory response and a mediator of carrageenan-induced inflammatory incapacitation. Moreover, TNF-α is able to induce the further release of kinins and leukotrienes, which may have an important role in the maintenance of long-lasting nociceptive responses [27]. Serum TNF-α levels were significantly lower in the radon-treated group than in the carrageenan-administrated, sham-treated group. These findings indicated that radon inhalation inhibited the inflammation induced by carrageenan.

We previously reported that low-dose X-irradiation in mice inhibited paw edema following ischemia-reperfusion [11] and brain edema following cold injury [14]. These reports may indicate that low-dose irradiation activates antioxidative function, especially SOD activity, which catalyzes the conversion of $O_2^{\bullet}$ into H_2O_2. In this study, our data showed that radon inhalation inhibited paw edema induced by carrageenan. To further clarify the mechanisms that inhibited paw edema, antioxidative functions (i.e., lipid peroxide levels, total glutathione content, and SOD and catalase activity) were investigated. The SOD and catalase activity in paws was significantly higher in the radon-treated group in the carrageenan-administrated sham-treated group; these findings indicated that radon inhalation activated antioxidative functions and inhibited carrageenan-induced inflammatory paw edema. These findings also indicated that radon had the same effects as low-dose X-irradiation.

Radon therapy is performed for pain-related diseases at Misasa Medical Center, Okayama University Hospital and Badgastein in Austria. Our present study demonstrated that radon inhalation has anti-inflammation effects. However, our study did not assess whether radon inhalation reduced the pain associated with inflammation. The data presented in this study provide a substantial basis for future studies aimed at assessing relief from pain induced by

inflammation.

REFERENCES

1. Kataoka, T., Yoshimoto, M., Nakagawa, S., Mizuguchi, Y., Taguchi, T., and Yamaoka, K. 2009. Basic study on active changes in biological function of mouse liver graft in cold storage after low-dose X-irradiation. *J. Clin. Biochem. Nutr.* 45: 219-226.
2. Kojima, S., Matsuki, O., Kinoshita, I., Gonzalez, T. V., Shimura, N., and Kubodera, A. 1997. Dose small-dose γ-ray radiation induce endogenous antioxidant potential in vivo? *Biol. Pharm. Bull.* 20: 601-604.
3. Yamaoka, K., Kojima, S., Takahashi, M., Nomura, T., and Iriyama, K. 1998. Change of glutathione peroxidase synthesis along with that of superoxide dismutase synthesis in mice spleen after low-dose X-ray irradiation. *Biochem. Biophys. Acta.* 1381: 265-270.
4. Yamaoka, K., Kojima, S., and Nomura, T. 1999. Changes of SOD-like substances in mouse organs after low-dose X-ray irradiation. *Physiol. Chem. Phys. Med. NMR* 31: 23-28.
5. Yamaoka, K., Edamatsu, R., and Mori, A. 1991. Increased SOD activities and decreased lipid peroxide levels induced by low dose X irradiation in rat organs. *Free Radic. Biol. Med.* 11: 299-306.
6. Kojima, S., Nakayama, K., and Ishida, H. 2004. Low dose gamma-rays activate immune functions via induction of glutathione and delay tumor growth. *J. Radiat. Res.* 45: 33-39.
7. Ishii, K., Yamaoka, K., Hosoi, Y., Ono, T., and Sakamoto, K. 1995. Enhanced mitogen-induced proliferation of rat splenocytes by low-dose whole-body X-irradiation. *Physiol. Chem. Phys. Med. NMR* 27: 17-23.
8. Yamaoka, K., Kataoka, T., Nomura, T., Taguchi, T., Wang, D.H., Mori, S., Hanamoto, K., and Kira, S. 2004. Inhibitory effects of prior low-dose irradiation on carbon tetrachloride-induced hepatopathy in acatalasemic mice. *J. Rad. Res.* 45: 89–95.

9. Kataoka, T., Nomura, T., Wang, D. H., Taguchi, T., and Yamaoka, K. 2005. Effects of post low-dose X-ray irradiation on carbon tetrachloride-induced acatalasemic mice liver damage. *Physiol. Chem. Phys. Med. NMR* 37: 109-126.

10. Yamaoka, K. 2006. Activation of antioxidant system by low dose radiation and its applicable possibility for treatment of reactive oxygen species-related diseases. *J. Clin. Biochem. Nurt.* 39: 114-133.

11. Kataoka, T., Mizuguchi, Y., Yoshimoto, M., Taguchi, T., and Yamaoka, K. 2007. Inhibitory effects of prior low-dose X-irradiation on ischemia-reperfusion injury in mouse paw. *J. Radiat. Res.* 48: 505-513.

12. Tsuruga, M., Taki, K., Ishii, G., Sasaki, Y., Furukawa, C., Sugihara, T., Nomura, T., Ochiai, A., and Magae, J. 2007. Amelioration of type II diabetes in db/db mice by continuous low-dose-rate gamma irradiation. *Radiat Res.* 167: 592-599.

13. Nomura, T. and Yamaoka, K. 1999. Low-dose γ-ray irradiation reduces oxidative damage induced by CCl_4 in mouse liver. *Free Radic. Biol. Med.* 27: 1324-1333.

14. Yoshimoto, M., Kataoka, T., Toyota, T., Taguchi, T., and Yamaoka, K. in press. Inhibitory effects of prior low-dose X-irradiation on cold-induced brain injury in mouse. *Inflammation* DOI: 10.1007/s10753-011-9293-9

15. Yamaoka, K., Mitsunobu, F., Hanamoto, K., Mori, S., Tanizaki, Y., and Sugita, K. 2004. Study on biologic effects of radon and thermal therapy on osteoarthritis. *J. Pain.* 5: 20-25.

16. Mitsunobu, F., Yamaoka, K., Hanamoto, K., Kojima, S., Hosaki, Y., Ashida, K., Sugita, K., and Tanizaki, Y. 2003. Elevation of antioxidant enzymes in the clinical effects of radon and thermal therapy for bronchial asthma. *J. Radiat. Res.* 44: 95-99.

17. Nakagawa, S., Kataoka, T., Sakoda A., Ishimori, Y., Hanamoto, K., and Yamaoka, K. 2008. Basic study on activation of antioxidation function in some organs of mice by radon inhalation using new radon exposure device. *Radioisotopes* 57: 241-251. (in Japanese)

18. Kataoka, T., Nishiyama, Y., Toyota, T., Yoshimoto, M., Sakoda, A., Ishimori, Y., Aoyama, Y., Taguchi, T., and Yamaoka, K. in press. Radon inhalation protects mice from carbon-tetrachloride-induced hepatic and renal damage. Inflammation DOI: 10.1007/s10753-010-9263-7

19. Nakatsukasa, H., Tsukimoto, M., Ohshima, Y., Tago, F., Masada, A., and Kojima, S. 2008. Suppressing effect of low-dose gamma-ray irradiation on collagen-induced arthritis *J. Radiat. Res.* 49: 381-389.

20. Schaue, D., Jahns, J., Hildebrandt, G., and Trott. K.R. 2005. Radiation treatment of acute inflammation in mice. *Int. J. Radiat. Biol.* 81: 657-667.

21. Baehner, R.L., Murrmann, S.K., Davis, J., and Johnston, R.B. 1975. The role of superoxide anion and hydrogen peroxide in phagocytosis-associated oxidative metabolic reactions. *J. Clin. Invest.* 56: 571-576.

22. Aebi, H., Wyss, S.R., Scherz, B., and Gross, J. 1976. Properties of erythrocyte catalase from homozygotes and heterozygotes for Swiss-type acatalasemia. *Biochem. Genet.* 14: 791-807.

23. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.

24. Franke, A., Reiner, L., Pratzel, H.G. Franke, T., and Resch, K.L. 2000. Long-term efficacy of radon spa therapy in rheumatoid arthritis--a randomized, sham-controlled study and follow-up. *Rheumatology* 39: 894-902.

25. Yamaoka, K.,and Komoto, Y. 1996. Experimental study of alleviation of hypertension, diabetes and pain by radon inhalation. *Physiol. Chem. Phys. Med. NMR* 28: 1-5.

26. Cuzzocrea, S., Zingarelli, B., Hake, P., Salzman, A.L., and Szabó, C. 1998. Antiinflammatory effects of mercaptoethylguanidine, A combined inhibitor of nitric

oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. *Free Radic. Biol. Med.* 24: 450-459.

27. Tonussi, C.R., and Ferreira, S.H. 1999. Tumour necrosis factor-alpha mediates carrageen in-induced knee-joint incapacitation and also triggers overt nociception in previously inflamed rat knee-joints. *Pain* 82: 81-87.

Figure Legends

Fig. 1 Schematic diagram of the radon exposure system (A). Changes in radon concentration in mouse cages due to outlet flow (B) or the number of Doll Stones (C). (D) Changes in the radon concentration in the mouse cage over the period of radon inhalation.

Fig. 2 Effect of radon inhalation on carrageenan-induced paw edema. Each value indicates the mean paw volume ± SEM. The number of mice per experimental point was 6-7. *P < 0.05, **P < 0.01, ***P < 0.001 vs Sham.

Fig. 3 Changes in serum levels of TNF-α (A), NO (B), and antioxidant-associated parameters (C), and antioxidant-associated parameters (D) in paw. Each value indicates the mean ± SEM. Each experimental point represents data from 6-7 mice. *P < 0.05, **P < 0.01, ***P < 0.001 vs Control.

Fig. 4 Effects of radon inhalation on serum NO levels of carrageenan-administrated mice. Each value indicates the mean ± SEM. Each experimental point represents data from 7 mice. *P < 0.05 vs Control.

Fig. 5 Histological changes in mouse paw after carrageenan administration. Mouse paws were examined histologically. (A) Control, (B) radon inhalation, (C) carrageenan administration, (D) radon inhalation before carrageenan administration. The length of the scale bar is 50 μm. All samples were stained with HE. (E) Fewer inflammatory leukocytes infiltrated tissues of the mice pretreated with radon than of those treated only with carrageenan administration. Each value indicates the mean ± SEM. Each experimental point represents data from 6 mice. ***P<0.001 vs control, $^{\#}$P<0.05 vs radon inhalation before carrageenan administration.

Fig. 6 Effects of radon inhalation on serum TNF-α levels in carrageenan-administrated mice. Each value indicates the mean ± SEM. Each experimental point represents data from 6-7 mice. **P < 0.01 vs Control, $^{\#\#}$P < 0.01 vs Sham.

Fig. 7 Effects of radon inhalation on antioxidant-associated parameters in serum (A) and in paws (B) of carrageenan-administrated mice. Each value indicates the mean ± SEM. Each experimental point represents data from 6-7 mice. **P < 0.01, ***P < 0.001 vs Control, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 vs Sham.

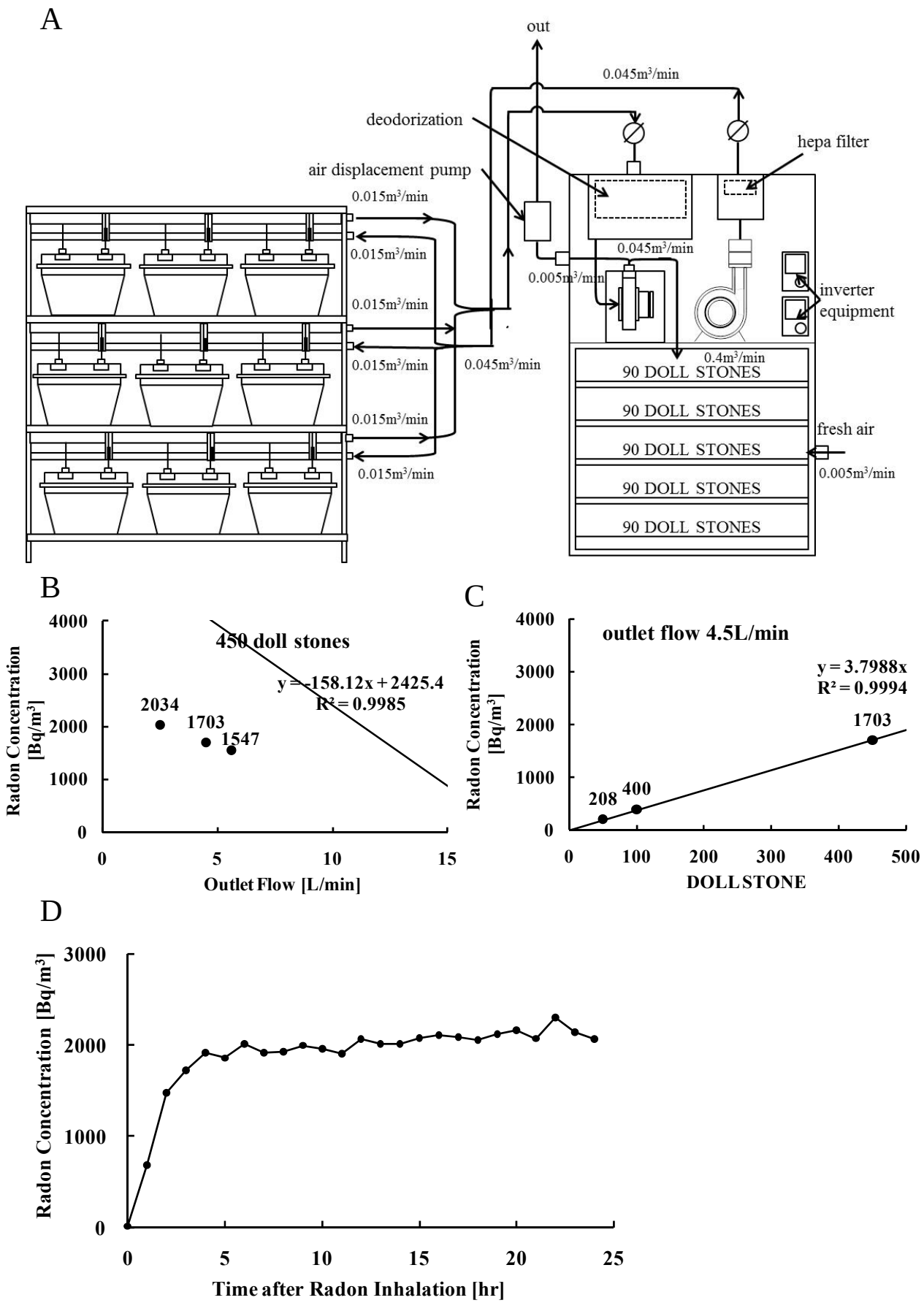

A
out
0.045m³/min
deodorization
hepa filter
air displacement pump
0.015m³/min
0.015m³/min
0.015m³/min
0.015m³/min
0.015m³/min
0.015m³/min
0.045m³/min
0.005m³/min
0.045m³/min
inverter
equipment
0.4m³/min
90 DOLL STONES
90 DOLL STONES
90 DOLL STONES
90 DOLL STONES
90 DOLL STONES
fresh air
0.005m³/min
B
450 doll stones
y = -158.12x + 2425.4
R² = 0.9985
2034
1703
1547
Radon Concentration [Bq/m³]
Outlet Flow [L/min]
C
outlet flow 4.5L/min
y = 3.7988x
R² = 0.9994
1703
400
208
Radon Concentration [Bq/m³]
DOLL STONE
D
Radon Concentration [Bq/m³]
Time after Radon Inhalation [hr]

Fig.1

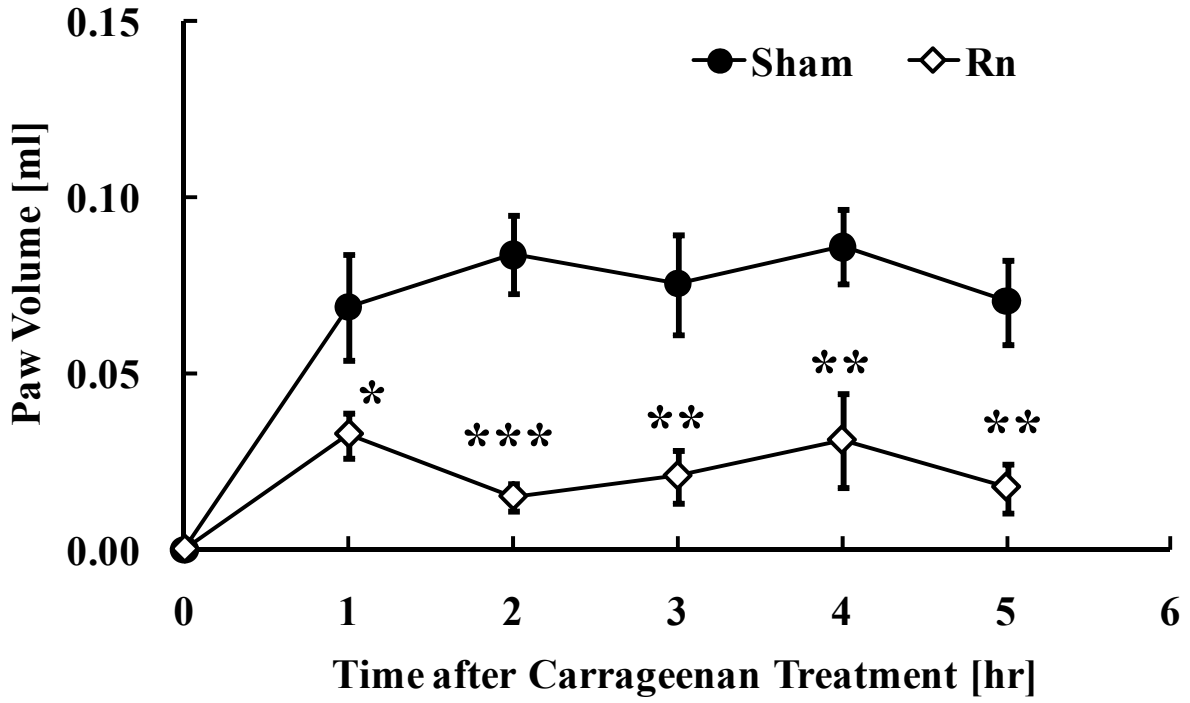

0.15
0.10
0.05
0.00
Paw Volume [ml]
Sham
Rn
*
***
**
**
**
0
1
2
3
4
5
6
Time after Carrageenan Treatment [hr]

Fig.2

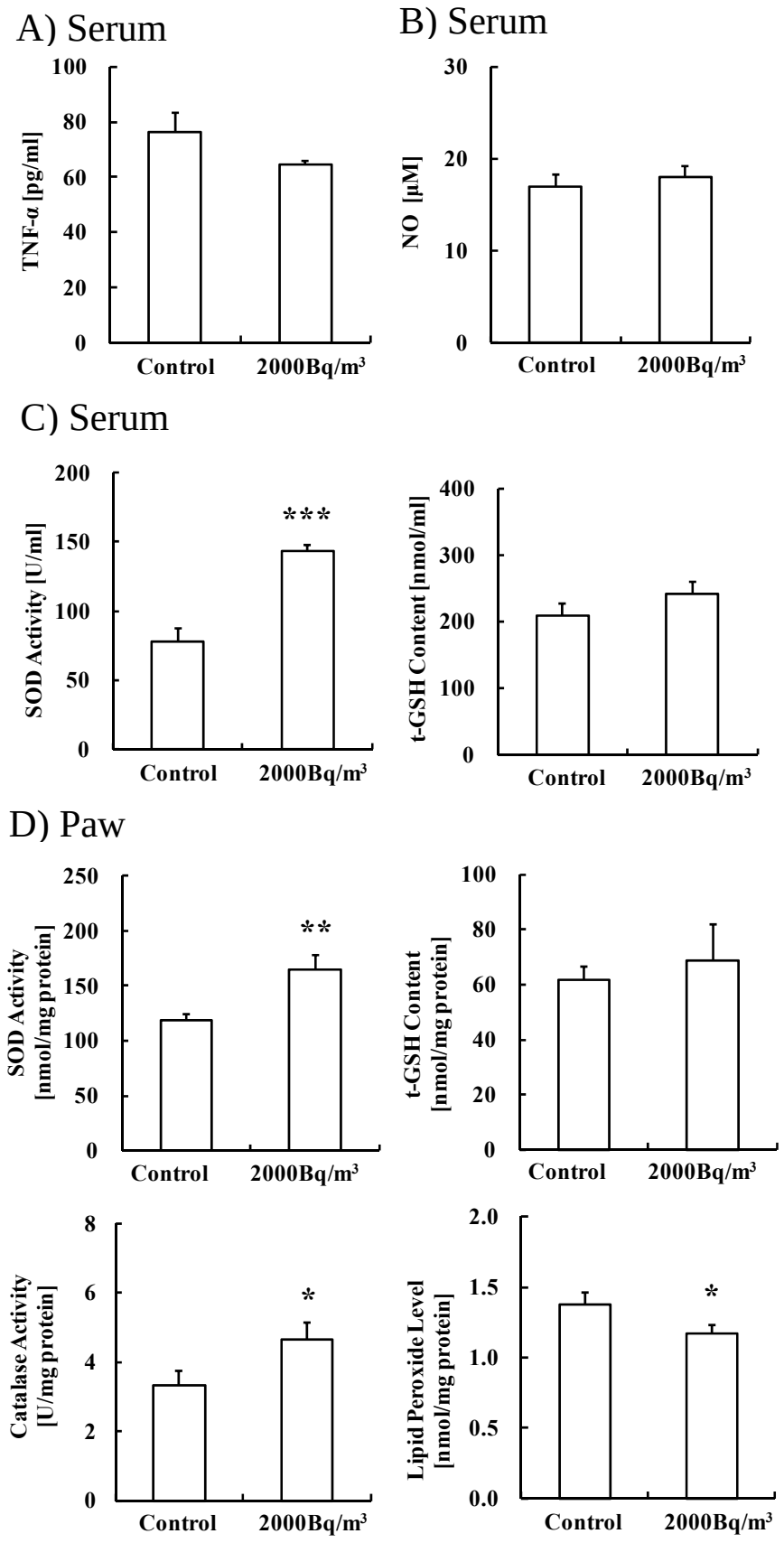

A) Serum
TNF-α [pg/ml]
100
80
60
40
20
0
Control
2000Bq/m3
B) Serum
NO [μM]
30
20
10
0
Control
2000Bq/m3
C) Serum
SOD Activity [U/ml]
200
150
100
50
0
***
Control
2000Bq/m3
t-GSH Content [nmol/ml]
400
300
200
100
0
Control
2000Bq/m3
D) Paw
SOD Activity [nmol/mg protein]
250
200
150
100
50
0
**
Control
2000Bq/m3
t-GSH Content [nmol/mg protein]
100
80
60
40
20
0
Control
2000Bq/m3
Catalase Activity [U/mg protein]
8
6
4
2
0
*
Control
2000Bq/m3
Lipid Peroxide Level [nmol/mg protein]
2.0
1.5
1.0
0.5
0.0
*
Control
2000Bq/m3

Fig.3

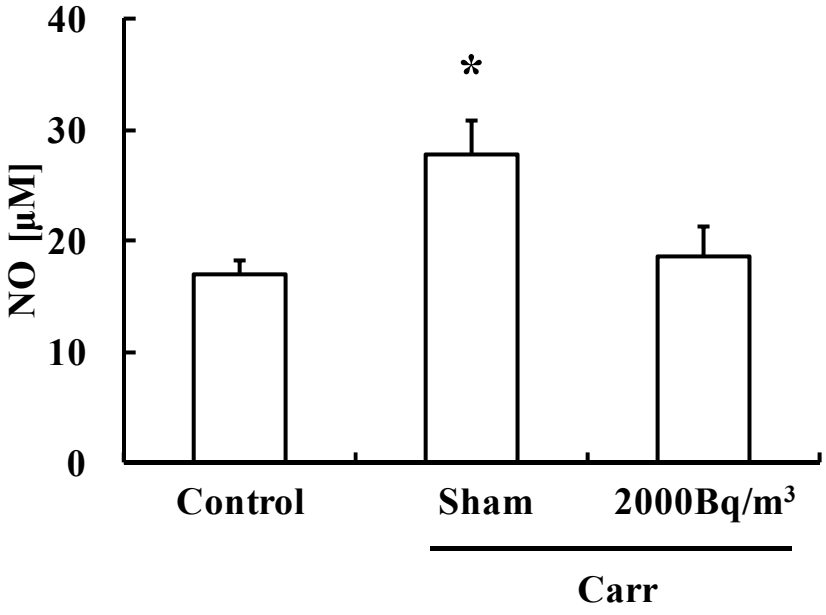
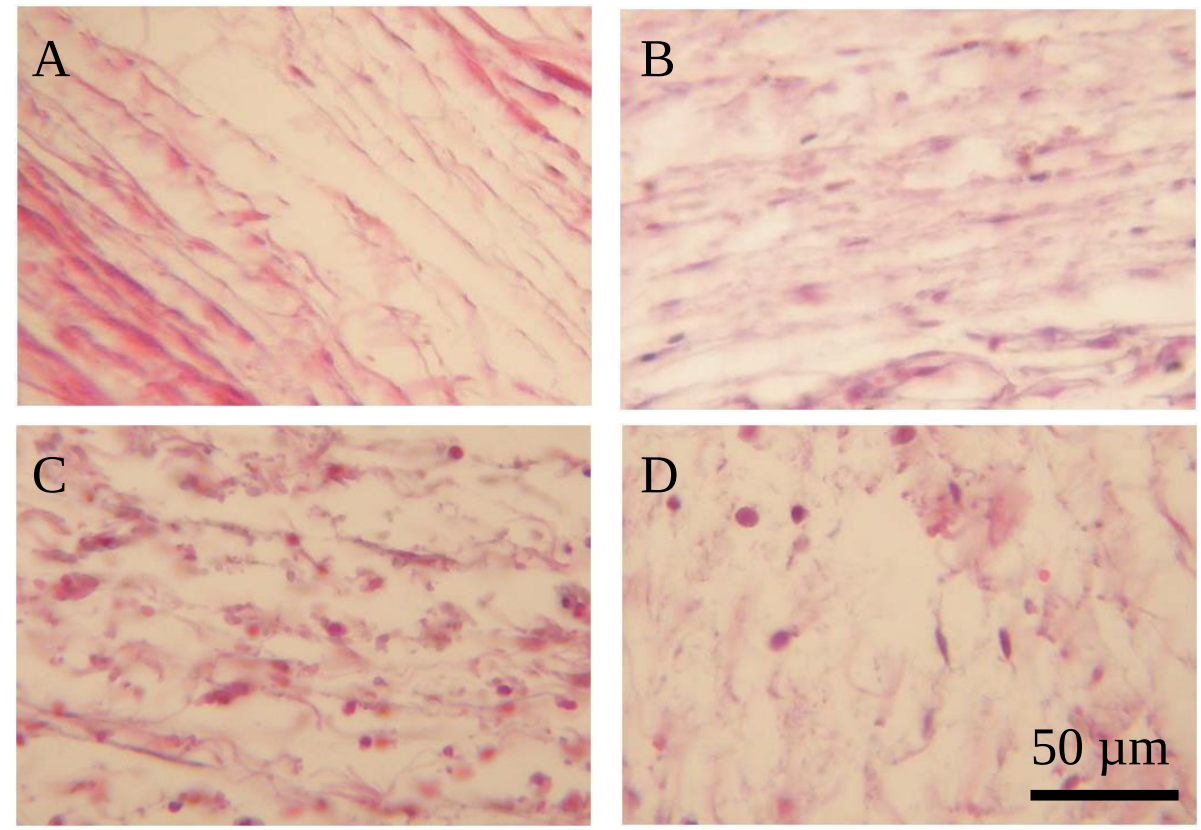


Fig.4


A
B
C
D
50 µm

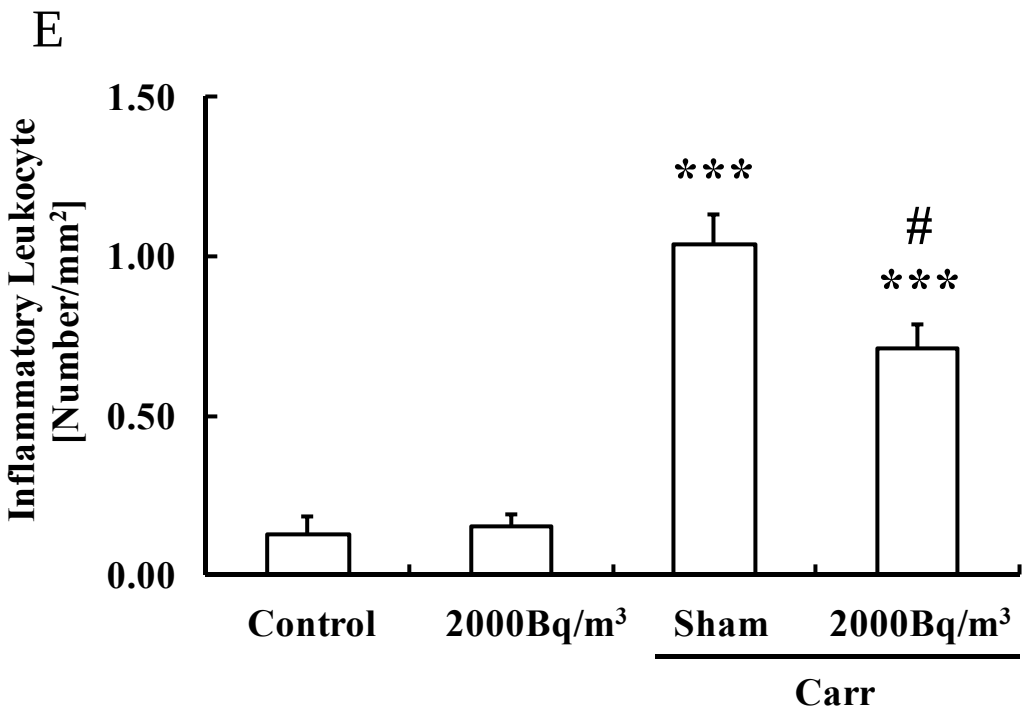

E
Inflammatory Leukocyte
[Number/mm²]
1.50
1.00
0.50
0.00
***
#
***
Control
2000Bq/m³
Sham
2000Bq/m³
Carr

Fig.5

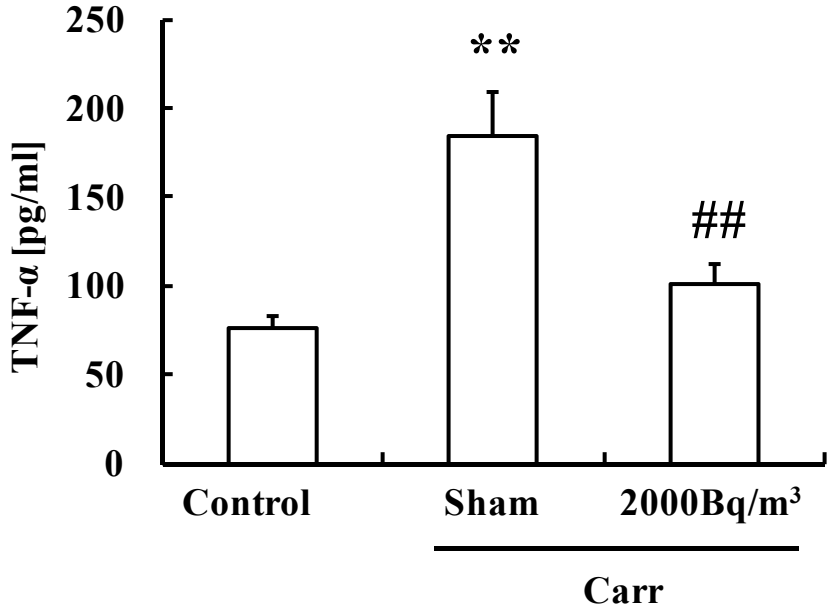
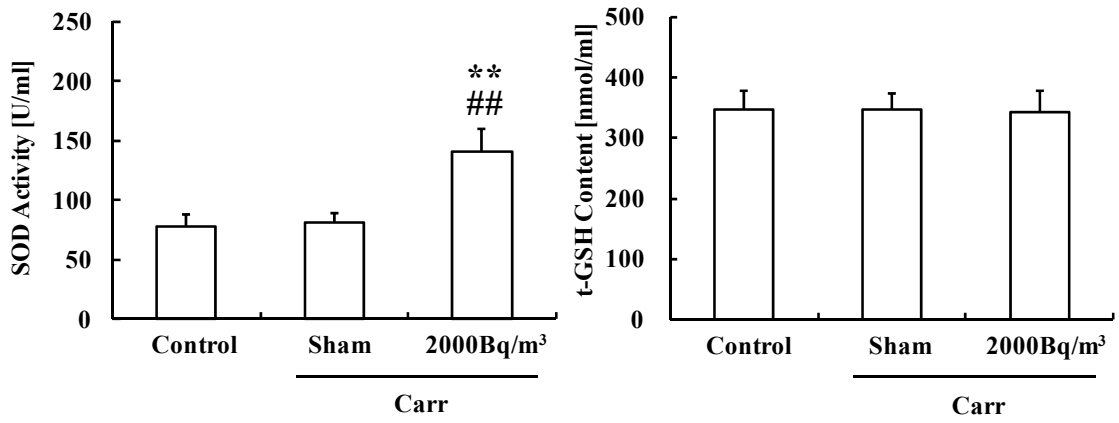


Fig.6

A) Serum



B) Paw

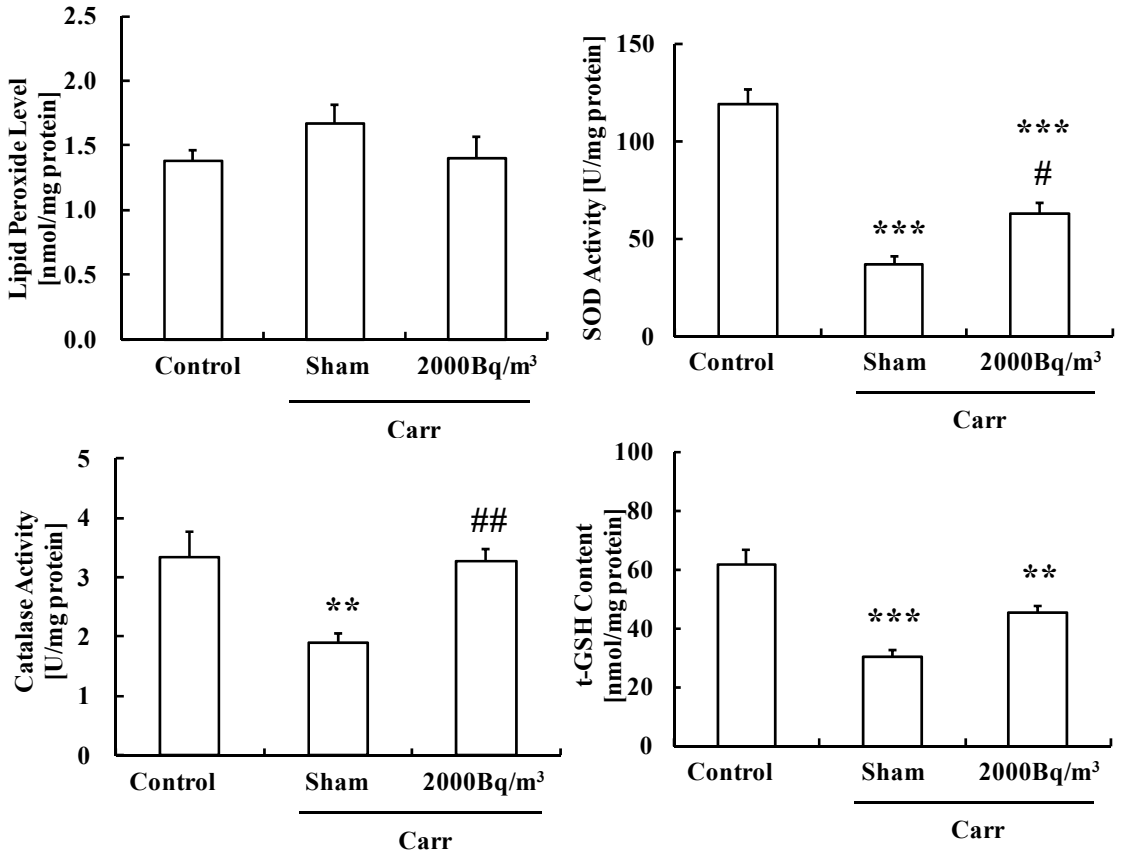


Fig. 7