Original Article

Suppression of Streptozotocin-induced Type-1 Diabetes in Mice by Radon Inhalation

Yuichi Nishiyama[1], Takahiro Kataoka[1], Junichi Teraoka[1], Akihiro Sakoda[2], Hiroshi Tanaka[2], Yuu Ishimori[2], Fumihiro Mitsunobu[3], 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]Misasa Medical Center, Okayama University Hospital, 827 Yamada, Misasa-cho, Tohaku-gun, Tottori 682-0192, Japan

Address for correspondence: Kiyonori Yamaoka, Professor

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:

Short Title: Suppression of Type-1 diabetes by Radon Inhalation

Summary

We examined the protective effect of radon inhalation on streptozotocin (STZ)-induced type-1 diabetes in mice. Mice inhaled radon at concentrations of 1000, 2500, and 5500 Bq/m^3 for 24 hours before STZ administration. STZ administration induced characteristics of type-1 diabetes such as hyperglycemia and hypoinsulinemia; however, radon inhalation at doses of 1000 and 5500 Bq/m^3 significantly suppressed the elevation of blood glucose in diabetic mice. Serum insulin was significantly higher in mice pre-treated with radon at a dose of 1000 Bq/m^3 than in mice treated with a sham. In addition, superoxide dismutase activities and total glutathione contents were significantly higher and lipid peroxide was significantly lower in mice pre-treated with radon at doses of 1000 and 5500 Bq/m^3 than in mice treated with a sham. These results were consistent with the result that radon inhalation at 1000 and 5500 Bq/m^3 suppressed hyperglycemia. These findings suggested that radon inhalation suppressed STZ-induced type-1 diabetes through the enhancement of antioxidative functions in the pancreas.

Keywords: radon inhalation, antioxidative function, streptozotocin, type-1 diabetes

Introduction

Free radicals and other reactive oxygen species (ROS) are constantly formed in the human body. Free-radical mechanisms have been implicated in the pathology of several human diseases, such as cancer, atherosclerosis, and malaria (Aruoma 1998). On the other hand, it has been reported that low-dose irradiation induces various biological effects, such as increased resistance to ROS (Kojima *et al*. 1997) and enhanced immune function (Ishii *et al*. 1995, Kojima *et al*. 2004). We have reported that low-dose X- or γ-irradiation increases or induces antioxidant substances such as superoxide dismutase (SOD) (Yamaoka *et al*. 1991, Yamaoka *et al*. 1999), catalase (CAT) (Kojima *et al*. 1999), and glutathione (GSH) (Kojima *et al*. 2004) in some organs of small animals. These findings indicate that low-dose irradiation may contribute to preventing or alleviating ROS-related injury (Yamaoka 2006). In fact, we previously reported that low-dose X-irradiation inhibited oxidative damage, such as brain edema (Yoshimoto *et al*. 2012), ischemia-reperfusion injury (Kataoka *et al*. 2007), and carbon tetrachloride (CCl_4)-induced hepatopathy in mice (Kataoka *et al*. 2005, Yamaoka *et al*. 2004a).

Therapy using radon (^{222}Rn), which is volatilized from radon-enriched water and mainly emits α-rays, is performed for ROS-related diseases, such as arteriosclerosis, osteoarthritis (Yamaoka *et al*. 2004b), and bronchial asthma (Mitsunobu *et al*. 2003) at Misasa Medical Center, Okayama University Hospital; however, the mechanisms of the therapy have not been fully understood. To clarify the effects of the therapy, we investigated the effects of radon inhalation on mice. We previously reported that radon inhalation increased antioxidant substances and inhibited CCl_4-induced hepatopathy (Kataoka *et al.* accepted by *J Radiat Res*) and renal damage (Kataoka *et al*. 2011a, Nishiyama *et al*. 2012). These findings suggested that radon inhalation has antioxidative effects similar to low-dose X- or γ-irradiation.

Diabetes mellitus is now a worldwide disease and, in particular, the number of young patients is increasing (Harjutsato *et al*. 2008, Mayer-Davis *et al*. 2009). The β cells in the pancreas are susceptible to ROS because they express very low levels of antioxidants (Lenzen *et al*. 1996);

therefore, it is considered that β cells are easily subjected to oxidative stress. It is well known that oxidative stress caused by ROS contributes to β cell death or dysfunction of the pancreas in type-1 diabetes (Cnop *et al*. 2005). On the other hand, we recently reported that radon inhalation activated SOD activity in various organs, including the pancreas of mice (Kataoka *et al*. 2011b), suggesting that radon inhalation may prevent type-1 diabetes; however, there has been no report of a protective effect of radon inhalation on type-1 diabetes.

Streptozotocin (STZ) is widely used in studies of experimental type-1 diabetes because it selectively destroys pancreatic β cells through the generation of ROS and alkylation of deoxyribonucleic acid (DNA) (Lenzen 2008, Szkudelski 2001). To assess the protective effect of radon inhalation on STZ-induced type-1 diabetes, we examined the following antioxidant- and diabetes-associated parameters and histological changes of the pancreas: SOD activity, CAT activity, total glutathione (t-GSH) content, lipid peroxide (LPO), blood glucose, serum insulin, and body weight.

Methods

Animals

Male C57BL/6J mice (9 weeks of age, body weight 25-28 g) were purchased from CLEA Japan Inc. (Tokyo, Japan). The animals were housed in clear plastic cages with wood-chip bedding in a temperature-controlled room (20 ± 1 °C). They were fed Oriental MF diet (Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum*. Each group consisted of 5-8 mice. Ethics approval was obtained from the animal experimental committee of Okayama University.

Radon inhalation

Mice were exposed to radon using a large-scale facility developed at Misasa Medical Center, Okayama University Hospital (Ishimori *et al*. 2010). Briefly, it was designed to examine a number of animals at various radon concentrations at the same time, and the facility has adopted a whole-body exposure system. Air with radon was blown into an exposure box and then blown out of the box. The radon concentration was measured using a radon monitor (Genitron Co., Ltd., Germany).

Experimental procedure

Mice were divided into eight groups: control (Control), radon inhalation only (1000, 2500, and 5500 Bq/m^3), sham inhalation with STZ administration (Sham + STZ), and radon inhalation with STZ administration (1000 Bq/m^3 + STZ, 2500 Bq/m^3 + STZ, and 5500 Bq/m^3 + STZ). Mice were exposed to radon at doses of 1000, 2500, and 5500 Bq/m^3 for 24 hours. A single high-dose of STZ (200 mg/kg weight, 50 g/L in saline solution) was administrated into the peritoneum immediately after radon inhalation. A single dose of STZ causes mild to severe types of diabetes according to the dosage used. A single high dose of STZ (200 mg/kg weight) destroys most of the β cells present in the islets and induces a rapid and permanent insulin-dependent diabetic condition in C57BL strain mice (Shertzer *et al.* 2009). On the other hand, it has been reported that a single low dose of STZ (approximately 100 mg/kg weight) sometimes causes a non-insulin-dependent diabetic condition (such as type 2 diabetes) in some strains of mice because the dose level is not sufficient to destroy most of the β cells (Ito *et al.* 2001); therefore, we injected a single high dose of STZ into C57BL strain mice, which are sensitive to the toxicity of STZ and develop complete insulin-dependent type-1 diabetes. Changes in blood glucose and body weight were monitored during the experiment. Blood glucose was measured by Glucose Pilot (Aventir Biotech, LLC, CA, USA) using tail tip blood. Four days after STZ administration, mice were killed by an overdose of ether anesthesia and blood was collected from the heart for analysis of insulin in serum. Serum was obtained by centrifugation at 3,000 × *g* for 5 min at 4 °C. The pancreas was quickly excised for SOD, CAT, t-GSH, and LPO analyses. Part of each pancreas was fixed in 10 % formalin for histological examination.

Biochemical assays

Mouse pancreas was homogenized in 1 M Tris-HCl buffer containing 5 mM ethylendiaminetetraacetic 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 CAT. SOD activity was measured by the nitroblue tetrazolium (NBT) reduction method (Braehner *et al*. 1975) 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.

CAT activity was measured as the hydrogen peroxide (H_2O_2) reduction rate at 37 °C and was assayed at 240 nm using a spectrophotometer (Aebi *et al*. 1976). 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 H_2O_2, 30 μl deionized water, and 20 μl pancreas supernatant. Activity was calculated using a molar extinction coefficient of 7.1×10^{-3} $M^{-1}cm^{-1}$. CAT activity was measured by the amount of H_2O_2 split by CAT at 37 °C. The reactions were started by addition of the supernatant.

The t-GSH content was measured using the Bioxytech GSH-420 assay kit (OXIS Health Products, Inc., Portland, OR, USA). Briefly, the pancreas was suspended in 10 mM phosphate buffer saline (PBS; pH 7.4), mixed with ice-cold 7.5% trichloroacetic acid solution and then homogenized. The homogenates were centrifuged at 3,000 × *g* for 10 min. The supernatant was used for the assay. The t-GSH 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 t-GSH concentration.

LPO (malondialdehyde (MDA)) was assayed using the Bioxytech LPO-586 assay kit (OXIS Health Products, Inc.). Briefly, the pancreas was homogenized in 20 mM PBS (pH 7.4) on ice. Before homogenization, 10 μL of 0.5 M butylated hydroxytoluene in acetonitrile was added per 1 mL tissue homogenate. 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 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 using a spectrophotometer.

The protein content was measured by the Bradford method (Bradford 1976), using Protein Quantification Kit-Rapid (Dojindo Molecular Technologies, Inc., Kumamoto, Japan).

Serum insulin was measured by the enzyme linked immunosorbent assay (ELISA) using an insulin assay kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan).

Histological examination

Tissue samples fixed in 10 % formalin were embedded in paraffin. Six micrometer-thick tissue sections were prepared and stained with hematoxylin-eosin (HE). The size of pancreatic islets was measured using image-editing software.

Statistical analyses

Data are presented as the mean ± standard error of the mean (SEM). The statistical significance of differences was determined by Dunnett's tests and Tukey's tests for multiple comparisons where appropriate. $P < 0.05$ was considered significant.

Results

Effects of radon inhalation on antioxidant-associated substances in the pancreas

SOD activity and t-GSH content in the pancreas were significantly higher and the LPO level was significantly lower in the 1000 Bq/m^3 group than in the control group. CAT activity was significantly higher in the 5500 Bq/m^3 group than in the control group (Figure 1A-D)

Effects of radon inhalation on body weight following STZ administration

Significant decreases were observed in body weight 4 days after STZ administration in Sham + STZ, 2500 Bq/m^3 + STZ, and 5500 Bq/m^3 + STZ groups compared with the control group; however, there were no significant differences in body weight throughout the experiment between the 1000 Bq/m^3 + STZ group and control group (Figure 2).

Effects of radon inhalation on blood glucose and serum insulin following STZ administration

Four days after STZ administration, blood glucose was significantly higher in all groups pre-treated in the presence or absence of radon inhalation than in the control group; however, blood glucose was significantly lower in 1000 Bq/m^3 + STZ and 5500 Bq/m^3 + STZ groups than in the Sham + STZ group (Figure 3A).

STZ administration significantly decreased serum insulin in all groups pre-treated in the presence or absence of radon inhalation compared with the control group; however, serum insulin was significantly higher in the 1000 Bq/m^3 + STZ group than in the Sham + STZ group (Figure 3B).

Histological observation

A significant decrease in the mean size of pancreatic islets was observed in all groups pre-treated in the presence or absence of radon inhalation compared with the control group (Figure 4A-E); however, the mean sizes of pancreatic islets were significantly larger in 1000 Bq/m^3 + STZ and 5500 Bq/m^3 + STZ groups than in the Sham + STZ group (Figure 4F).

Effects of radon inhalation on antioxidant-associated substances following STZ administration

To assess the protective effect of radon inhalation on STZ-induced type-1 diabetes, antioxidant substances in pancreas were assayed (Figure 5A-D). SOD activity and the t-GSH content were significantly lower and the LPO level was significantly higher in the Sham + STZ group than in the control group. CAT activity was 51% lower in the sham group than in the control group, but these differences were not significant; however, SOD activities were significantly higher in 1000 Bq/m^3 + STZ, 2500 Bq/m^3 + STZ, and 5500 Bq/m^3 + STZ groups than in the Sham + STZ group. The t-GSH contents were significantly higher and the LPO levels were significantly lower in 1000 Bq/m^3 + STZ and 5500 Bq/m^3 + STZ groups than in the Sham + STZ group.

Discussion

We have reported that radon inhalation activates antioxidative functions in various organs of BALB/c strain mice (Kataoka *et al*. 2011b). In this study, we used C57BL/6J strain mice, which have low sensitivity to radiation compared with BALB/c (Kallman and Kohn 1956) because in this strain of mice it is especially easy to induce insulin-dependent type-1 diabetes by STZ administration (Cardinal *et al*. 1998); however, the effects of radon inhalation on antioxidative functions in the pancreas of C57BL/6J strain mice have never been examined and were assessed here for the first time. Our results showed that antioxidative functions in the pancreas were significantly higher in mice that inhaled radon at doses of 1000 and 5500 Bq/m^3 than in control mice. These findings suggested that radon inhalation may contribute to preventing oxidative stress-related disease in the pancreas.

We examined diabetic conditions 4 days after STZ administration, considering that this is an adequate interval to induce type-1 diabetes when STZ is used. STZ administration induces permanent hyperglycemia within about 7 days and complete degranulation of β cells is seen within 12 to 48 hrs (Lenzen 2008). In addition, metabolic alterations are usually found in animals 3 to 5 days after STZ administration (Catanzaro *et al*. 1994).

STZ administration induces certain typical characteristics of type-1 diabetes, such as hyperglycemia, hypoinsulinemia and body weight loss (Tomlinson *et al*. 1992). In the present study, all groups pre-treated in the presence or absence of radon inhalation finally showed hyperglycemia and hypoinsulinemia; however, pretreatment with radon inhalation at doses of

1000 and 5500 Bq/m^3 significantly suppressed blood glucose elevation and body weight decrease compared with the Sham + STZ group. In addition, serum insulin was significantly higher in the 1000 Bq/m^3 + STZ group than in the Sham + STZ group. These results indicated that radon inhalation partially suppressed type-1 diabetes induced by STZ administration.

Histological observation of the pancreatic tissue further substantiated the claim that radon inhalation has protective effects on pancreatic tissue. STZ administration induced severe injury to the pancreas, such as a decrease of the islet size, which was probably due to the reduction in the number of β cells; however, mice that inhaled radon at doses of 1000 and 5500 Bq/m^3 showed slight pancreatic islet damage compared with sham-treated mice.

To clarify the mechanism of radon inhalation suppressing STZ-induced type-1 diabetes, we examined antioxidative functions in the pancreas. STZ is a nitric oxide (NO) donor and NO partially restricts adenosine triphosphate (ATP) generation in mitochondria and increases xanthine oxidase (XOD) (Lenzen 2008, Szkudelski 2001). XOD catalyzes the synthesis of superoxide anion radical ($O_2^{\bullet-}$) and, as a result, H_2O_2 and hydroxyl radical ($OH^{\bullet-}$) are formed (Szkudelski 2001). In the case of SOD deficiency or increased $O_2^{\bullet-}$ production, it reacts with NO to produce peroxynitrite (ONOO), which is a highly toxic agent that can cause direct damage to proteins, lipids and DNA (Szkudelski 2001). The scavenging activity of SOD, which catalyzes the conversion of $O_2^{\bullet-}$ into H_2O_2, and CAT, which transforms H_2O_2 into H_2O as well as GSH, is well known. ROS scavengers such as SOD protect β cells against ROS attack induced by STZ administration (Kubisch *et al*. 1994, Robbins *et al*. 1980). It was reported that low-dose γ-irradiation increased SOD activity in the pancreas and suppressed β cell apoptosis and diabetes incidence in non-obese diabetic mice (Takahashi *et al*. 2000). A similar result was reported in alloxan-induced type-1 diabetic rats, in which single γ-irradiation at 0.5 Gy prevented the elevation of pancreatic lipid peroxidation and blood glucose (Takehara *et al*. 1995). Our results showed that STZ administration caused oxidative damage, represented by decreased SOD activity and t-GSH content and increased LPO in the pancreas; however, SOD activities and t-GSH contents were significantly higher and the LPO was significantly lower in 1000 Bq/m^3 + STZ and 5500 Bq/m^3 + STZ groups than in the Sham + STZ group. These results are consistent with the result that radon inhalation at doses of 1000 and 5500 Bq/m^3 suppressed hyperglycemia. These findings suggested that radon inhalation suppressed STZ-induced type-1 diabetes through the enhancement of antioxidative functions in the pancreas.

It is well known that insulin is the main hormone to lower blood glucose. Although hypoinsulinemia in the 5500 Bq/m^3 + STZ group was not improved, hyperglycemia was significantly suppressed. These findings may suggest that radon inhalation has a blood-glucose-lowering effect which is non-insulin dependent; however, no report has shown that low-dose irradiation, including radon, has such effects. It was reported that low-dose X-irradiation enhanced the ability to regulate energy metabolism and the membrane transport mechanism, as reflected by the increase in adenosine triphosphatase (ATPase) activity (Yamaoka *et al.* 1994). Low-dose irradiation may contribute to the suppression of hyperglycemia through the enhancement of glycolytic metabolism in cells. Further study is required to clarify this point.

Radon inhalation did not prevent type-1 diabetes in a dose-related fashion. We previously reported that SOD activity changes in some organs, such as the pancreas and liver, had a complex response to radon (Kataoka *et al*. 2011b). Briefly, radon inhalation of low (500 Bq/m^3) or high (4000 Bq/m^3) concentrations for 24 hrs increased SOD activity; however, there was little change in SOD activity following the inhalation of intermediate (2000 Bq/m^3) radon concentration for 24 hrs. That is, the distinctive feature was that it had two activation points of SOD activity, although it was not clear whether the total antioxidative function, including CAT and t-GSH, changed in a similar manner with SOD, which may correlate with poor efficacy in suppressing type-1 diabetes by radon inhalation at a dose of 2500 Bq/m^3. Further study is required to understand the effects of radon inhalation on antioxidant systems in the living body.

High-level radon has been found to cause lung cancer (Lubin *et al.* 1995); however, based on the recommendations of the International Commission on Radiological Protection (ICRP), the risks associated with exposure to radon therapy are low. In fact, adverse effects and negative effects of radon therapy have not been reported in the past. The dose of radon absorbed under our experimental conditions was also very low according to previous reports (Franke *et al.* 2000, Sakoda *et al.* 2010), and radon inhalation has only a small risk compared to lifestyle influences, such as smoking (Sethi *et al.* 2012).

In conclusion, radon inhalation activated antioxidative functions in the pancreas and partially suppressed STZ-induced type-1 diabetes. The data presented in this study provide a substantial basis for future studies aimed at assessing new radon-based therapies for the treatment of type-1 diabetes in humans.

Acknowledgments

The authors are grateful to Ms. Keiko Yamato (Graduate School of Health Sciences, Okayama University) for her technical support.

Abbreviations

ATP – adenosine triphosphate; ATPase – adenosine triphosphatase; CAT – catalase; CCl_4 – carbon tetrachloride; DNA – deoxyribonucleic acid; EDTA – ethylendiaminetetraacetic acid; ELISA – enzyme linked immunosorbent assay; GSH – glutathione; HE – hematoxylin-eosin; H_2O_2 – hydrogen peroxide; ICRP – International Commission on Radiological Protection; LPO – lipid peroxide; MDA – malondialdehyde; NBT – nitroblue tetrazolium; NO – nitric oxide; $O_2^{\bullet-}$ – superoxide anion radical; $OH^{\bullet-}$ – hydroxyl radical; ONOO – peroxynitrite; PBS – phosphate buffer saline; ROS – reactive oxygen species; SEM – standard error of mean; SOD – superoxide dismutase; STZ – streptozotocin; t-GSH – total glutathione; XOD – xanthine oxidase.

References

AEBI H, WYSS SR, SCHERZ B, GROSS J: Properties of erythrocyte catalase from homozygotes and heterozygotes for Swiss-type acatalasemia. *Biochem Genet* **14**: 791–807, 1976.

ARUOMA OI: Free radicals, oxidative stress, and antioxidants in human health and disease. *J Am Oil Chem Soc* **75**: 199–212, 1998.

BAEHNER RL, MURRMANN SK, DAVIS J, JOHNSTON RB Jr: The role of superoxide anion and hydrogen peroxide in phagocytosis-associated oxidative metabolic reactions. *J Clin Invest* **56**: 571–576, 1975.

BRADFORD MM: 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, 1976.

CARDINAL JW, ALLAN DJ, CAMERON DP: Differential metabolite accumulation may be the cause of strain differences in sensitivity to streptozotocin-induced β cell death in inbred mice. *Endocrinology* **139**: 2885–2891, 1998.

CATANZARO OL, MARINA-PRENDES MG, HOPE SI, ZUCCOLLO A, DOMINGUEZ A: Streptozotocin-induced hyperglycemia is decreased by nitric oxide inhibition. *Braz J Med Biol Res* **27**: 2043–2047, 1994.

CNOP M, WELSH N, JONAS JC, JÖRNS A, LENZEN S, EIZIRIK DL: Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* **54**: S97–S107, 2005.

FRANKE A, REINER L, PRATZEL HG, FRANKE T, RESCH KL: Long-term efficacy of radon spa therapy in rheumatoid arthritis–a randomized, sham-controlled study and follow-up. *Rheumatology* **39**: 894–902, 2000.

HARJUTSATO V, SJÖBERG L, TUOMILEHTO J: Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet* **371**: 1777–1782, 2008.

SHERTZER HG, SCHNEIDER SN, KENDIG EL, CLEGG DJ, D'ALESSIO DA, JOHANSSON E, GENTER MB: Tetrahydroindenoindole inhibits the progression of diabetes in mice. *Chem Biol Interact* **177**: 71–80, 2009.

ISHII K, YAMAOKA K, HOSOI Y: Enhanced mitogen-induced proliferation of rat splenocytes by low-dose whole-body X-irradiation. *Physiol Chem Phys Med NMR* **27**: 17–23, 1995.

ISHIMORI Y, MITSUNOBU F, YAMAOKA K, TANAKA H, KATAOKA T, SAKODA A: Primary functions of the first Japanese large-scale facility for exposing small animals to radon. *Jpn J Health Phys* **45**: 65–71, 2010.

ITO M, KONDO Y, NAKATANI A, HAYASHI K, NARUSE A: Characterization of low dose streptozotocin-induced progressive diabetes in mice. *Environ Toxicol Pharmacol* **9**: 71–78, 2001.

KALLMAN RF, KOHN HI: The influence of strain on acute X-ray lethality in the mouse I. LD50 and death rate studies. *Radiat Res* **5**: 309–317, 1956.

KATAOKA T, MIZUGUCHI Y, YOSHIMOTO M, TAGUCHI T, YAMAOKA K: Inhibitory effects of prior low-dose X-irradiation on ischemia-reperfusion injury in mouse paw. *J Radiat Res* **48**: 505–513, 2007.

KATAOKA T, NISHIYAMA Y, TOYOTA T, YOSHIMOTO M, SAKODA A, ISHIMORI Y, AOYAMA Y, TAGUCHI T, YAMAOKA K: Radon inhalation protects mice from carbon-tetrachloride-induced hepatic and renal damage. *Inflammation* **34**: 559–567, 2011a.

KATAOKA T, NISHIYAMA Y, YAMATO K, TERAOKA J, MORII Y, SAKODA A, ISHIMORI Y, TAGUCHI T, YAMAOKA K: Comparative study on the inhibitory effects of antioxidant vitamins and radon on carbon tetrachloride-induced hepatopathy. *J Radiat Res* (in press), DOI: 10.1093/jrr/rrs057.

KATAOKA T, NOMURA T, WANG DH, TAGUCHI T, YAMAOKA K: Effects of post low-dose X-ray irradiation on carbon tetrachloride-induced acatalasemic mice liver damage. *Physiol Chem Phys Med NMR* **37**: 109–126, 2005.

KATAOKA T, SAKODA A, ISHIMORI Y, TOYOTA T, NISHIYAMA Y, TANAKA H, MITSUNOBU F, YAMAOKA K: Study of the response of superoxide dismutase in mouse organs to radon using a new large-scale facility for exposing small animals to radon. *J Radiat Res* **52**: 775–781, 2011b.

KOJIMA S, MATSUKI O, KINOSHITA I, GONZALEZ TV, SHIMURA N, KUBODERA A: Dose small-dose γ-ray radiation induce endogenous antioxidant potential *in vivo*? *Biol Pharm Bull* **20**: 601–604, 1997.

KOJIMA S, MATSUKI O, NOMURA T, YAMAOKA K, TAKAHASHI M, NIKI E: Elevation of antioxidant potency in the brain of mice by low-dose γ-ray irradiation and its effect on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced brain damage. *Free Radic Biol Med* **26**: 388–395, 1999.

KOJIMA S, NAKAYAMA K, ISHIDA H: Low dose gamma-rays activate immune functions via induction of glutathione and delay tumor growth. *J Radiat Res* **45**: 33–39, 2004.

KUBISCH HM, WANG J, LUCHE R, CARLSON E, BRAY TM, EPSTEIN CJ, PHILLIPS JP: Transgenic copper/zinc superoxide dismutase modulates susceptibility to type 1 diabetes. *Proc Natl Acad Sci U S A* **91**: 9956–9959, 1994.

LENZEN S, DORINKGERN J, TIEDGE M: Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse organs. *Free Radic Biol Med* **20**: 463–466, 1996.

LENZEN S: The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* **51**: 216–226, 2008.

LUBIN JH, BOICE JD Jr, EDLING C, HORNUNG RW, HOWE GR, KUNZ E, KUSIAL RA, MORRISON HI, RADFORD EP, SAMET JM, TIRMARCHE M, WOODWARD A, YAO SX, PIERCE DA: Lung cancer in radon-exposed miners and estimation of risk from indoor exposure. J Natl Cancer Inst **87**: 817–827, 1995.

MAYER-DAVIS EJ, BEYER J, BELL RA, DABELEA D, D'AGOSTINO R Jr, IMPERATORE G, LAWRENCE JM, LIESE AD, LIU L, MARCOVINA S, RODRIGUEZ B; SEARCH for

Diabetes in Youth Study Group: Diabetes in African American youth: prevalence, incidence, and clinical characteristics: the SEARCH for Diabetes in Youth Study. *Diabetes Care* **32**: 112–122, 2009.

MITSUNOBU F, YAMAOKA K, HANAMOTO K, KOJIMA S, HOSAKI Y, ASHIDA K, SUGITA K, TANIZAKI Y: Elevation of antioxidant enzymes in the clinical effects of radon and thermal therapy for bronchial asthma. *J Radiat Res* **44**: 95–99, 2003.

NISHIYAMA Y, KATAOKA T, TERAOKA J, SAKODA A, ISHIMORI Y, YAMAOKA K: Inhibitory effects of pre and post radon inhalation on carbon tetrachloride-induced oxidative damage in mouse organs. *Radioisotopes* **61**: 231–241, 2012.

ROBBINS MJ, SHARP RA, SLONIM AE, BURR IM: Protection against streptozotocin-induced diabetes by superoxide dismutase. *Diabetologia* **18**: 55–58, 1980.

SAKODA A, ISHIMORI Y, KAWABE A, KATAOKA T, HANAMOTO K, YAMAOKA K: Physiologically based pharmacokinetic modeling of inhaled radon to calculate absorbed doses in mice, rats, and humans. *J Nucl Sci Technol* **47**: 731–738, 2010.

SETHI TK, EL-GHAMRY MN, KLOECKER GH: Radon and lung cancer. *Clin Adv Hematol Oncol* **10**: 157–64, 2012.

SZKUDELSKI T: The mechanisms of alloxan and streptozotocin action in B cells of rat pancreas. *Physiol Res* **50**: 537–546, 2001.

TAKAHASHI M, KOJIMA S, YAMAOKA K, NIKI E: Prevention of type 1 diabetes by low-dose gamma irradiation in NOD mice. *Radiat Res* **154**: 680–685, 2000.

TAKEHARA Y, YAMAOKA K, HIRAKI Y, YOSHIOKA T, UTSUMI K: Protection against alloxan diabetes by low-dose ^{60}Co γ irradiation before alloxan administration. *Physiol Chem Phys Med NMR* **27**: 149–159, 1995.

TOMLINSON KC, GARDINER SM, HEBDEN RA, BENNETT T: Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. *Pharmacol Rev* **44**: 103–150, 1992.

YAMAOKA K, EDAMATSU R, MORI A: Increased SOD activities and decreased lipid peroxide levels induced by low dose X irradiation in rat organs. *Free Radic Biol Med* **11**: 299–306, 1991.

YAMAOKA K, ISHII K, EDAMATSU R, ITO T, MORI A: Changes in specific amino acid residues and Na^+, K^+-ATPase activity in cell membranes of rat cerebral cortex by low dose X-irradiation. *Physiol Chem Phys* **26**: 323–328, 1994.

YAMAOKA K, KATAOKA T, NOMURA T, TAGUCHI T, WANG DH, MORI S, HANAMOTO K, KIRA S: Inhibitory effects of prior low-dose X-ray irradiation on carbon tetrachloride-induced hepatopathy in acatalasemic mice. *J Radiat Res* **45**: 89–95, 2004a.

YAMAOKA K, KOJIMA S, NOMURA T: Change of SOD-like substances in mouse organs after low-dose X-ray irradiation. *Physiol Chem Phys Med NMR* **31**: 23–28, 1999.

YAMAOKA K, MITSUMOBU F, HANAMOTO K, MORI S, TANIZAKI Y, SUGITA K: Study on biologic effects of radon and thermal therapy on osteoarthritis. *J Pain* **5**: 20–25, 2004b.

YAMAOKA K: Activation of antioxidant system by low dose radiation and its applicable possibility for treatment of reactive oxygen species-related disease. *J Clin Biochem Nutr* **39**: 144–133, 2006.

YOSHIMOTO M, KATAOKA T, TOYOTA T, TAGUCHI T, YAMAOKA K: Inhibitory effects of prior low-dose X-irradiation on cold-induced brain injury in mouse. *Inflammation* **35**: 89–97, 2012.

Figure Legends

Figure 1

Effects of radon inhalation on antioxidant-associated substances in the pancreas. **(A)** SOD activity, **(B)** CAT activity, **(C)** t-GSH content, and **(D)** LPO level in the pancreas. Each value indicates the mean ± SEM. The number of mice per experimental point was 5-6. $^{*}P<0.05$, radon inhalation vs. no inhalation.

Figure 2

Effects of radon inhalation on body weight following STZ administration. Each value indicates the mean ± SEM. The number of mice per experimental point was 5-8. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, radon or sham inhalation before STZ administration vs. no inhalation at the same time point. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, radon inhalation before STZ administration vs. sham inhalation before STZ administration at the same time point.

Figure 3

Effects of radon inhalation on blood glucose and serum insulin following STZ administration. **(A)** Blood glucose and **(B)** serum insulin. Each value is the mean ± SEM. The number of mice for each experiment and significance are the same as in Figure 2.

Figure 4

Histological changes in the pancreas after STZ administration. **(A)** Control, **(B)** sham inhalation under STZ administration, **(C)** radon inhalation of 1000 Bq/m^3 under STZ administration, **(D)** radon inhalation of 2500 Bq/m^3 under STZ administration, and **(E)** radon inhalation of 5500 Bq/m^3 under STZ administration. Arrow indicates pancreatic islets. Scale bar = 50 μm. All samples were stained with H.E. **(F)** Larger pancreatic islets of mice pre-treated with radon inhalation than those of mice treated with sham inhalation. Each value is the mean ± SEM. The number of mice for each experiment and significance are the same as in Figure 2.

Figure 5

Effects of radon inhalation on antioxidant-associated substances following STZ administration. **(A)** SOD activity, **(B)** CAT activity, **(C)** t-GSH content, and **(D)** LPO in the pancreas. Each value is mean ± SEM. The number of mice for each experiment and significance are the same as in Figure 2.

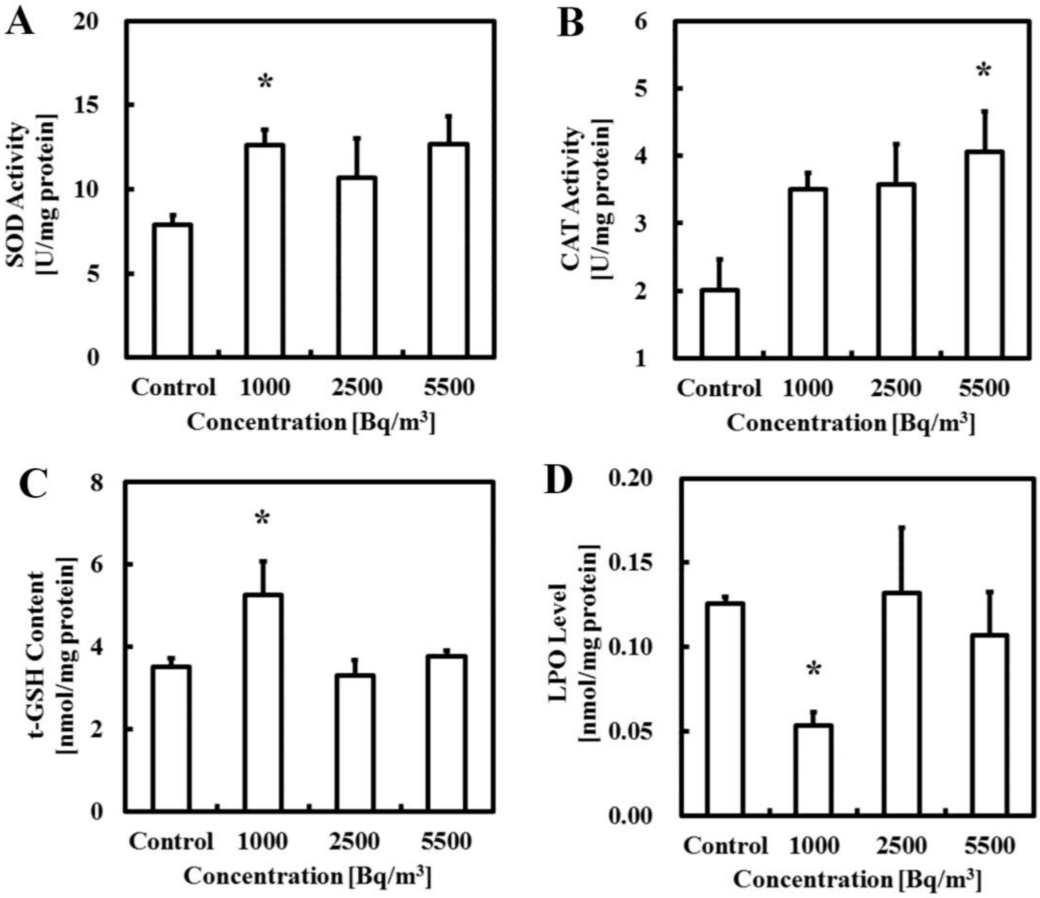
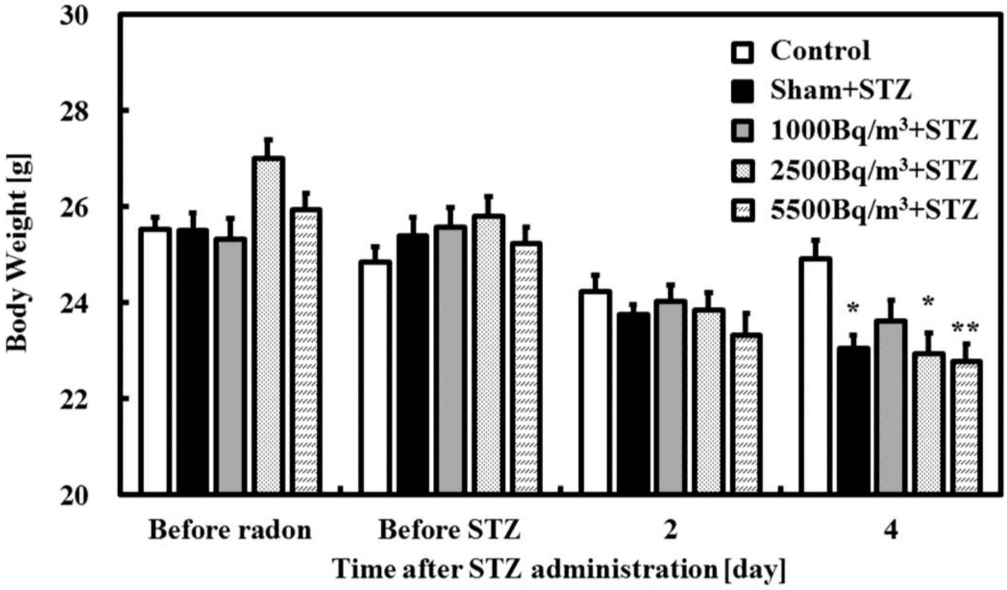

A
B
C
D
SOD Activity [U/mg protein]
CAT Activity [U/mg protein]
t-GSH Content [nmol/mg protein]
LPO Level [nmol/mg protein]
Control
1000
2500
5500
Concentration [Bq/m³]
*

Figure 1



30
28
26
24
22
20
Body Weight [g]
Control
Sham+STZ
1000Bq/m³+STZ
2500Bq/m³+STZ
5500Bq/m³+STZ
*
*
**
Before radon
Before STZ
2
4
Time after STZ administration [day]

Figure 2

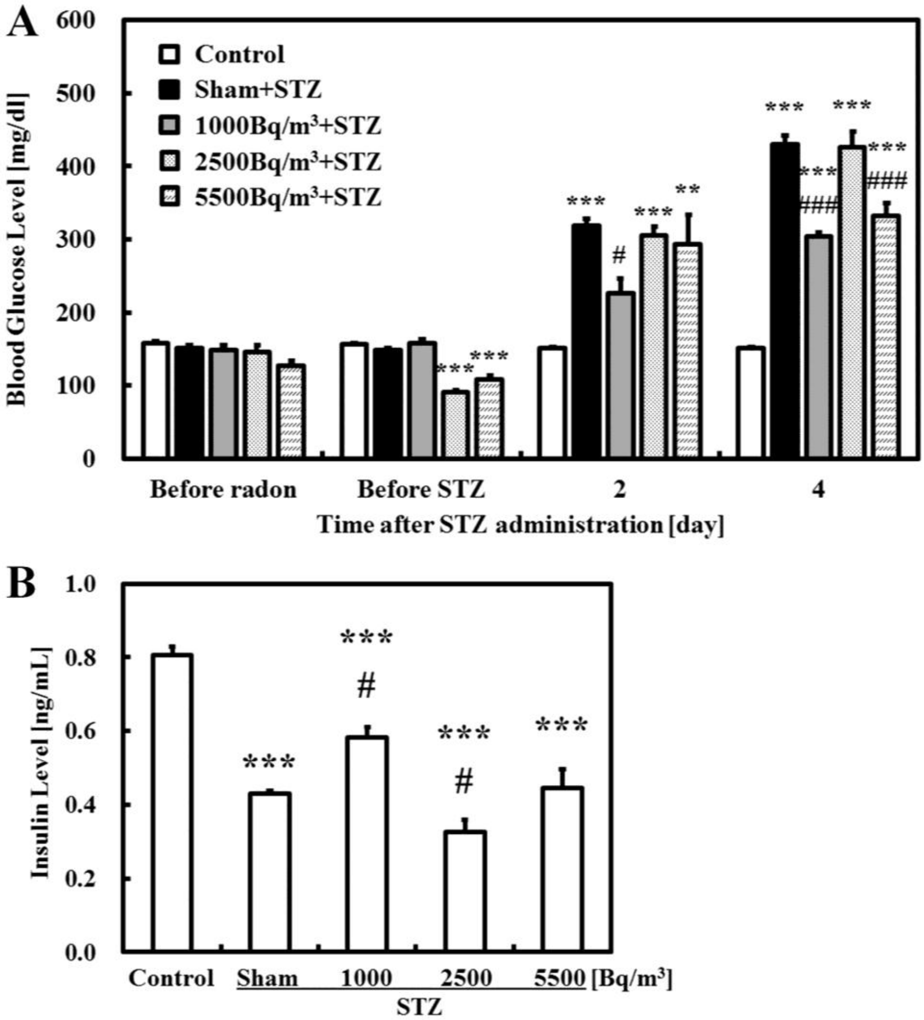

A
Control
Sham+STZ
1000Bq/m³+STZ
2500Bq/m³+STZ
5500Bq/m³+STZ
Blood Glucose Level [mg/dl]
0
100
200
300
400
500
600
Before radon
Before STZ
2
4
Time after STZ administration [day]
B
Insulin Level [ng/mL]
0.0
0.2
0.4
0.6
0.8
1.0
Control
Sham
1000
2500
5500 [Bq/m³]
STZ

Figure 3

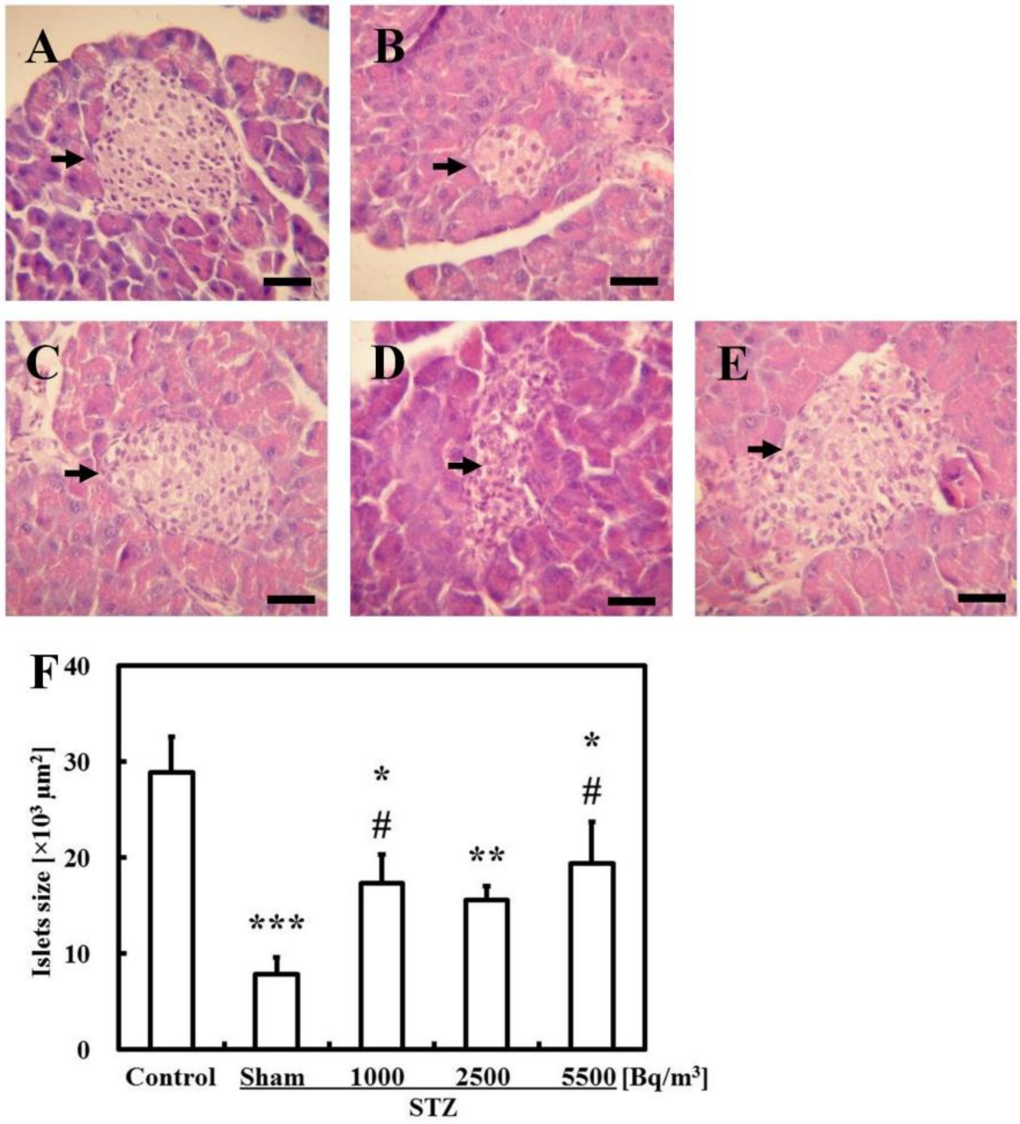

A
B
C
D
E
F
Islets size [×10³ µm²]
0
10
20
30
40
***
*
#
**
*
#
Control
Sham
1000
2500
5500 [Bq/m³]
STZ

Figure 4

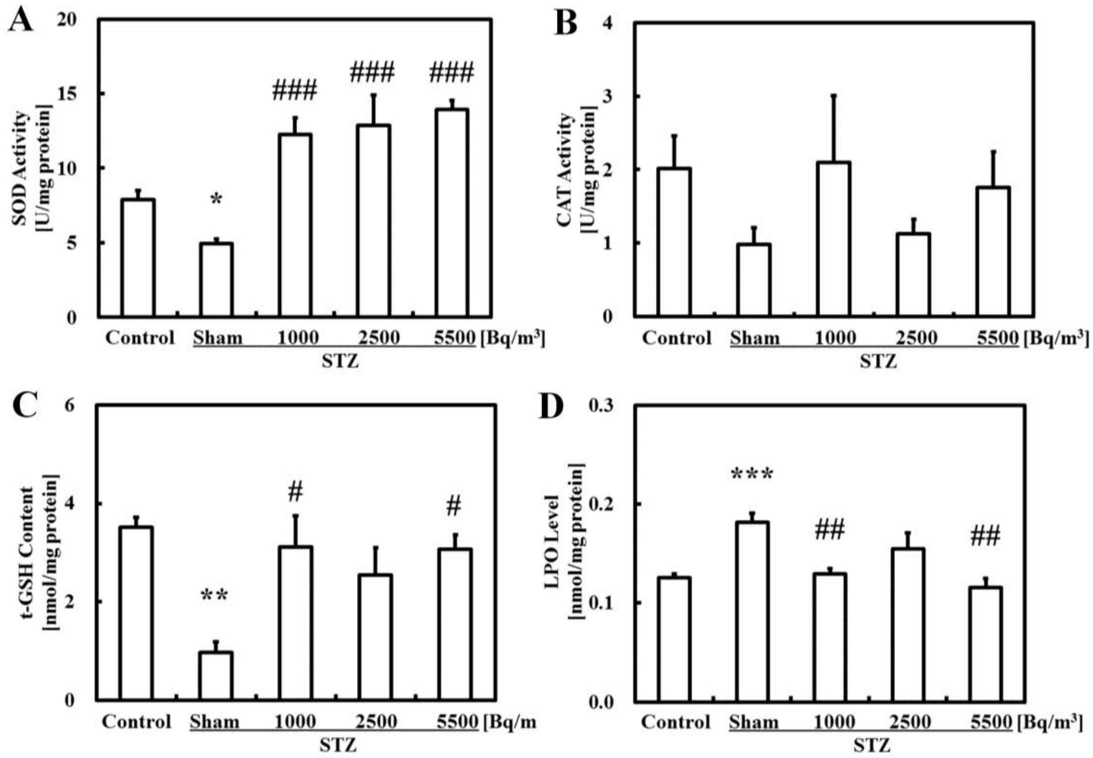

A
SOD Activity [U/mg protein]
20
15
10
5
0
*
###
###
###
Control
Sham
1000
2500
5500
[Bq/m³]
STZ
B
CAT Activity [U/mg protein]
4
3
2
1
0
Control
Sham
1000
2500
5500
[Bq/m³]
STZ
C
t-GSH Content [nmol/mg protein]
6
4
2
0
**
#
#
Control
Sham
1000
2500
5500
[Bq/m
STZ
D
LPO Level [nmol/mg protein]
0.3
0.2
0.1
0.0
***
##
##
Control
Sham
1000
2500
5500
[Bq/m³]
STZ

Figure 5