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Radon inhalation decreases DNA damage induced by oxidative stress in mouse organs via the activation of antioxidative functions

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ABSTRACT

Radon inhalation decreases the level of lipid peroxide (LPO); this is attributed to the activation of antioxidative functions. This activation contributes to the beneficial effects of radon therapy, but there are no studies on the risks of radon therapy, such as DNA damage. We evaluated the effect of radon inhalation on DNA damage caused by oxidative stress and explored the underlying mechanisms. Mice were exposed to radon inhalation at concentrations of 2 or 20 kBq/m^3 (for one, three, or 10 days). The 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels decreased in the brains of mice that inhaled 20 kBq/m^3 radon for three days and in the kidneys of mice that inhaled 2 or 20 kBq/m^3 radon for one, three or 10 days. The 8-OHdG levels in the small intestine decreased by approximately 20–40% (2 kBq/m^3 for three days or 20 kBq/m^3 for one, three or 10 days), but there were no significant differences in the 8-OHdG levels between mice that inhaled a sham treatment and those that inhaled radon. There was no significant change in the levels of 8-oxoguanine DNA glycosylase, which plays an important role in DNA repair. However, the level of Mn-superoxide dismutase (SOD) increased by 15–60% and 15–45% in the small intestine and kidney, respectively, following radon inhalation. These results suggest that Mn-SOD probably plays an important role in the inhibition of oxidative DNA damage.

Keywords: radon; oxidative DNA damage; Mn-superoxide dismutase (SOD); 8-oxoguanine DNA glycosylase

INTRODUCTION

Radon therapy is applied for the treatment of osteoarthritis [1, 2], rheumatoid arthritis [3, 4] and ankylosing spondylitis [5, 6]. The mechanism of action involves the ability of radon to induce anti-inflammatory cytokines and the immune system, especially transforming growth factor-β1, thereby reducing inflammation and subsequently alleviating pain [7, 8]. Reactive oxygen species (ROS) are closely related to inflammation [9–11]. Carrageenan administration to the paws of mice induces edema, accompanied by an increase in tumor necrosis factor-alpha levels in the serum, which is an indicator of inflammation. However, radon inhalation inhibits the development of carrageenan-induced inflammatory paw edema [12]. Radon inhalation inhibits inflammatory pain induced by formalin administration [13] and suppresses colitis induced by dextran sulfate sodium [14]. Therefore, radon-induced activation of antioxidative functions plays a significant role in protection from inflammation. Radon inhalation activates antioxidative functions in several organs in mice [15], which has expanded the possibilities for new applications of radon therapy.

There are several targets affected by oxidative stress, including DNA and proteins. Widely used, 8-Hydroxy-2-deoxyguanosine (8-OHdG) is a sensitive marker of nuclear and mitochondrial oxidative DNA damage. Inhaling a high concentration of radon for several months increases oxidative DNA damage [16]; however, long-term bathing in a radon

hot spring reduces 8-OHdG levels [17]. The radon concentration in the bathroom is usually more than 200 Bq/m^3 during a shower.

However, the aforementioned conditions are different from those used for radon therapy, and there are only a few reports of oxidative DNA damage under radon therapy conditions. The radon concentration in the radon therapy rooms of Misasa in Japan [18] and Montana in the USA [19] are approximately 2 kBq/m^3 and 40 kBq/m^3, respectively. Therefore, further assessment of the oxidative stress induced by radon is needed to clarify the mechanisms of radon therapy. An examination of the risks of radon therapy is also critical to evaluate the risks and benefits of radon therapy. The aim of this study was to evaluate oxidative DNA damage associated with radon inhalation in a mouse model and to examine the mechanisms underlying such effects, based on changes in DNA damage and oxidative stress markers.

MATERIALS AND METHODS

Animals

Eight-week-old male BALB/c mice were obtained from CLEA Japan Inc. (Tokyo, Japan). The animals were housed under standard environmental conditions and a preset light–dark cycle of 12:12 h. Ethics approval was obtained from the Animal Care and Use Committee of Okayama University.

Experimental procedures

Radon inhalation was performed using a radon inhalation system as previously reported [20]. The radon group received radon inhalation for one, three or 10 days. The concentration of radon in the mouse cage was approximately 2 kBq/m^3 (1 day: 2017 ± 138 Bq/m^3, 3 days: 2008 ± 134 Bq/m^3, 10 days: 2020 ± 131 Bq/m^3) or 20 kBq/m^3 (1 day: 20103 ± 516 Bq/m^3, 3 days: 20118 ± 503 Bq/m^3, 10 days: 20340 ± 552 Bq/m^3). The control group received sham inhalation only. The brains, kidneys and small intestines were removed quickly after euthanasia using CO_2 to evaluate the levels of 8-OHdG, 8-oxoguanine DNA glycosylase (OGG1) and SOD.

DNA damage assay

The level of 8-OHdG was used to evaluate the level of oxidative DNA damage. Total DNA was extracted and purified from the tissue samples using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's recommendations. The DNA concentration was measured based on the absorbance at 260 nm. The samples were treated with the 8-OHdG Assay Preparation Reagent Set (FUJIFILM WAKO Pure Chemical Corporation, Osaka, Japan). The level of 8-OHdG was determined using a highly sensitive enzyme-linked immunosorbent assay kit (Nikken Seil Co. Ltd., Shizuoka, Japan).

Western blotting for OGG1 and Mn-SOD

Western blotting with antibodies against OGG1, Mn-SOD and β-actin, including the determination of protein concentrations and band intensities, was performed as previously described [21].

Statistical analyses

The statistical significance of the biochemical assay results among the groups was determined using one-way analysis of variance followed by Tukey's test for multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Dose coefficients of α-rays emitted by radon in mouse organs have already been reported [22]. According to the report, the estimated absorbed doses in the brain, kidney and small intestine are approximately the same. For example, when mice inhaled radon at a concentration of 2 kBq/m^3 for one day or 20 kBq/m^3 for 10 days, the estimated absorbed dose range in these organs was 74–148 nGy and 7.4–14.8 μGy, respectively [22]. However, energy deposition by alpha-emitting radionuclides such as radon was reported [23]. Microdosimetric approaches can be applied when we discuss the activation of antioxidative function induced by radon. This is because it is considered that antioxidant enzymes such as Mn-SOD are produced in response to ROS, which are induced by α-ray emission [21].

Radon inhalation significantly decreased the 8-OHdG levels in the brain (20 kBq/m^3 for three days; Fig. 1) and kidney (2 or 20 kBq/m^3 for one, three or 10 days; Fig. 2). In addition, the 8-OHdG levels in the small intestine decreased by 18% with 2 kBq/m^3 radon for three days, 37% with 20 kBq/m^3 radon for one day and 30% with 20 kBq/m^3 radon for three or 10 days; however, the differences were not significant (Fig. 3). There are two possible mechanisms to explain this decrease; specifically, radon either induces pathways that protect DNA from oxidative damage or induces DNA repair mechanisms. The antioxidative functions are inversely correlated with the 8-OHdG level [24, 25]. Therefore, we examined the effect of radon inhalation on the levels of Mn-SOD, a marker of antioxidative activity and OGG1, which plays a role in DNA repair, to elucidate the mechanisms.

In our previous study, the redox balance, which comprises the dynamics of antioxidants, oxidative stress and ROS, was evaluated. A certain pattern was observed for these organs. The first group included the liver and kidney, which have a high antioxidant capacity. The second group included the brain, stomach and pancreas, which have a low antioxidant capacity and lipid peroxide (LPO) levels. The third group includes the lung, heart, small intestine and large intestine, which have high LPO levels but a low antioxidant capacity. Radon inhalation altered the redox states in each organ; however, the response to radon varied depending on the amount of antioxidants in the specific organ [26]. The characteristics of oxidative DNA damage induced by radon might vary depending on the redox state. Therefore, in this study, oxidative DNA damage in the brain, kidney and small intestine was examined.

There are three SOD isoforms, extracellular SOD, Cu/Zn-SOD and Mn-SOD, which are located in the mammalian extracellular space, cytoplasm and mitochondria, respectively. Radon inhalation at 500 Bq/m^3 increases the level of Mn-SOD (by approximately 30%, but not significant) in the brains of mice via nuclear factor (NF)-κB activation; however, there is no increase in the level of Cu/Zn-SOD. In addition, radon inhalation at 2000 Bq/m^3 does not increase the brain Mn-SOD level [21]. NF-κB regulates the induction of Mn-SOD [27]. NF-κB signaling can be both activated and repressed by ROS, and therefore, this pathway can have both anti- and pro-oxidant roles pertaining to oxidative stress [28]. Radon inhalation (2 kBq/m^3 for one, three or 10 days) increased the Mn-SOD level

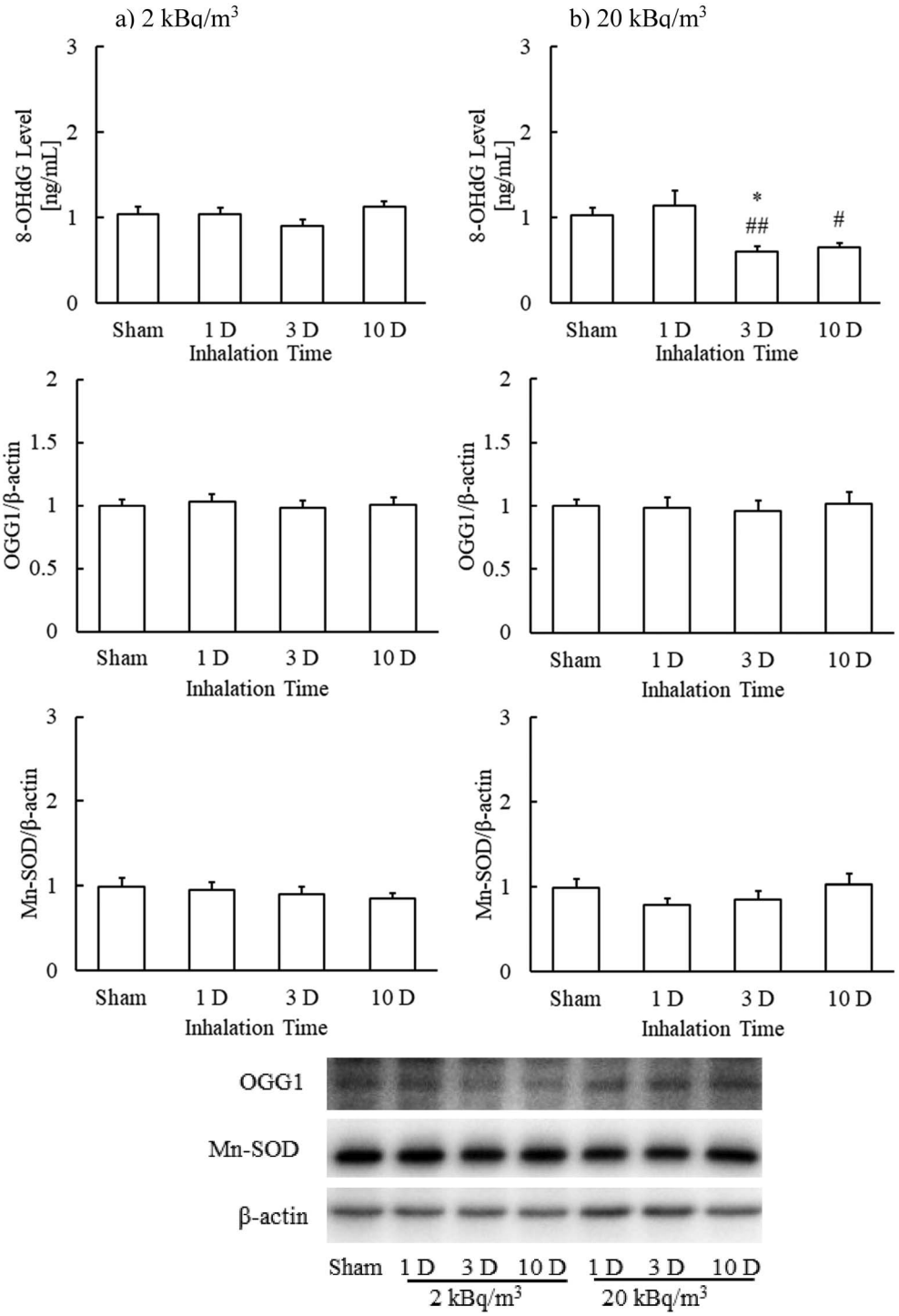

Fig. 1. Changes in 8-OHdG, 8-oxoguanine DNA glycosylase (OGG1), and SOD levels in the brain (n = 6 mice/group). Data are presented as the mean ± standard error of the mean. $^{*}P < 0.05$ vs Sham, $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ vs 1 day (1 d).

in the kidney by 15–45%; however, the difference was not significant (Fig. 2). Radon inhalation at 2 kBq/m^3 for three days increased the small intestine Mn-SOD level by 22%; however, the difference was not significant (Fig. 3).

An evaluation of 8-OHdG and Mn-SOD levels indicated that the activation of antioxidative functions contributes to the suppression of oxidative DNA damage. The increase in Mn-SOD levels in this study corroborates the results from our previous study [21] and provides

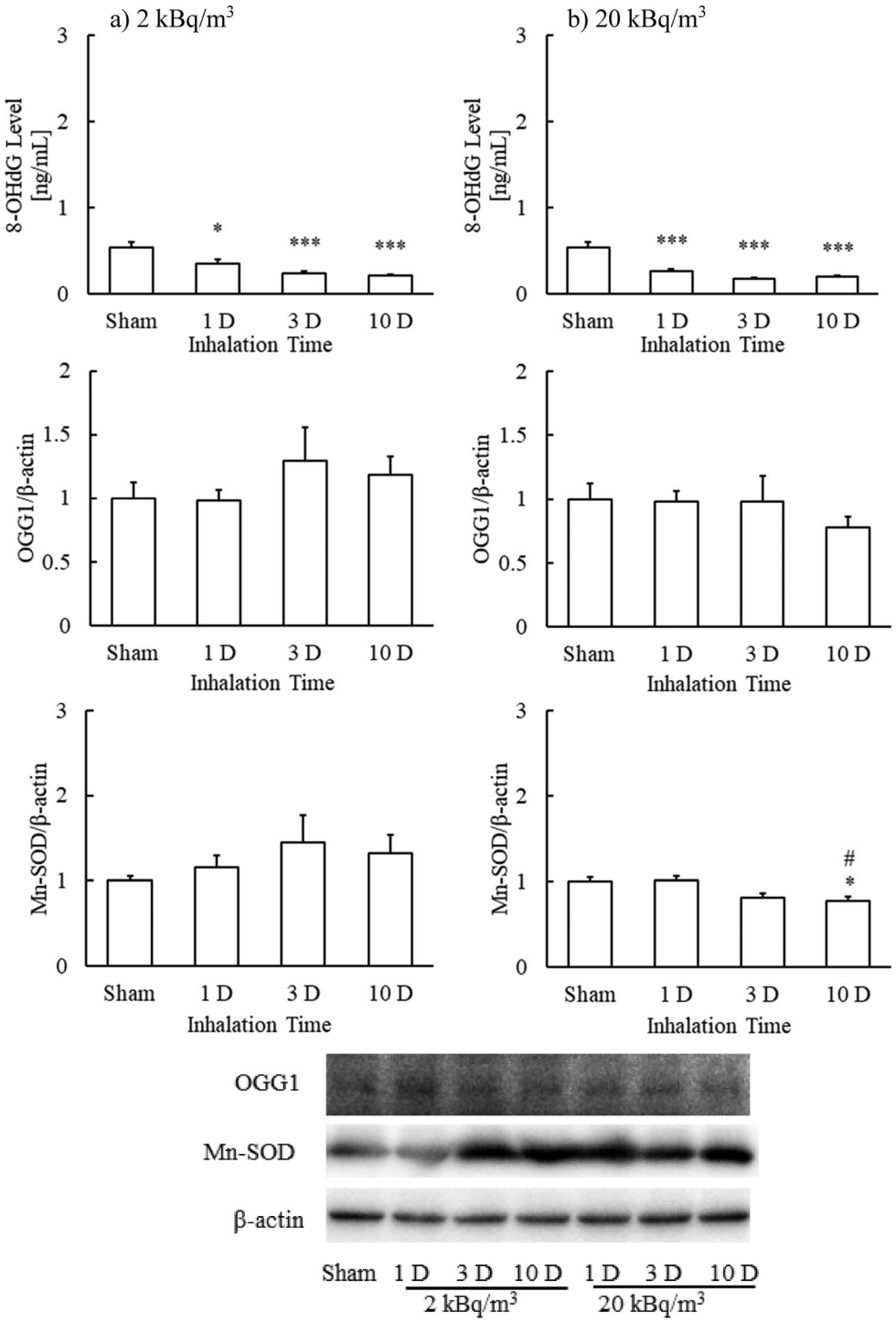

Fig. 2. Changes in 8-OHdG, 8-oxoguanine DNA glycosylase (OGG1), and SOD levels in the kidney (n = 6 mice/group). Data are presented as the mean ± standard error of the mean. $^{*}P < 0.05$, $^{***}P < 0.001$ vs Sham; $^{\#}P < 0.05$ vs 1 day (1 d).

further insights into the time-dependent changes and characteristics in different organs. Radon inhalation alters the redox state in organs. However, the effect is dependent on the total antioxidant capacity of each organ; specifically, the kidney has a high antioxidant capacity, the brain has a low antioxidant capacity and LPO levels, and the small intestine has high LPO levels but a low antioxidant capacity [26]. The 8-OHdG level in the brain and kidney decreased significantly following 20 kBq/m³ radon inhalation; however, the Mn-SOD level

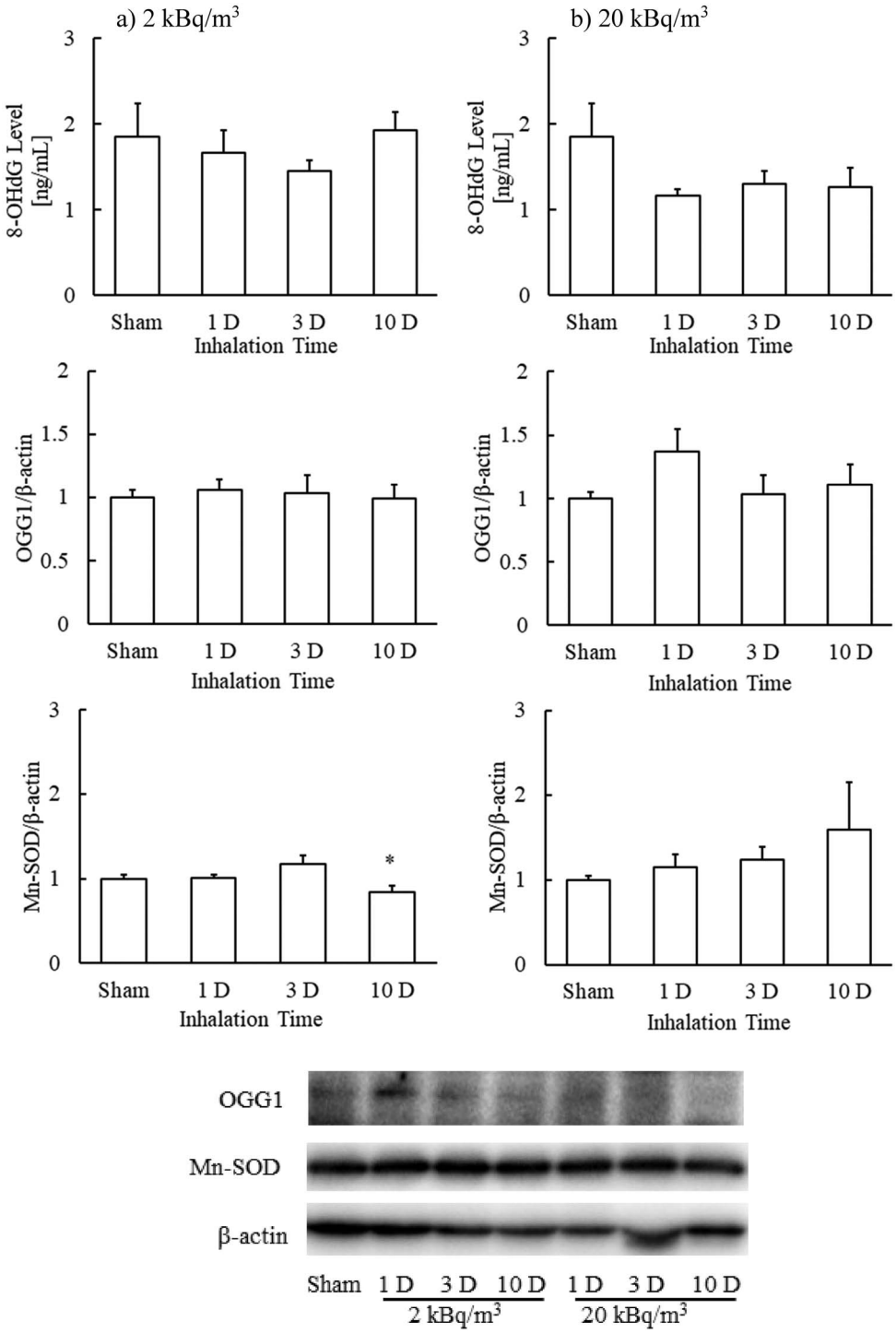

Fig. 3. Changes 8-OHdG, 8-oxoguanine DNA glycosylase (OGG1), and SOD levels in the small intestine (n = 5–6 mice/group). Data are presented as the mean ± standard error of the mean. *P < 0.05 vs Sham.

did not increase under the same condition. The inhalation of a high concentration of radon (20 kBq/m³, 10 days) decreased the Mn-SOD level in the kidney (Fig. 2) and small intestine (Fig. 3). This decrease in Mn-SOD levels following radon inhalation for 10 days might cause excessive oxidative stress. The 8-OHdG levels were decreased in the kidney under the same condition. The kidney has a high antioxidant capacity, and therefore, other antioxidants such as catalase and glutathione might compensate for the shortage of Mn-SOD in response

to oxidative stress. The 8-OHdG level decreased in the brain and small intestine after 20 kBq/m^3 radon inhalation and decreased in the kidney following inhalation of both low (2 kBq/m^3) and high (20 kBq/m^3) doses of radon. Therefore, the redox state in each organ might influence the changes in 8-OHdG levels following radon inhalation, as previously observed for LPO [26].

To evaluate the potential effect of radon on DNA repair as an alternate mechanism, we examined the changes in the levels of OGG1 protein, which removes the 8-oxoguanine (8-oxoG) base from DNA [29]. Urinary excretion of 8-oxoG and the genotype and expression of OGG1 are associated with the risk of cancer [30]. Inhaling a high concentration of radon (64, 121 and 236 working level month) significantly increases the 8-OHdG level and decreases the OGG1 level in rats [16]. However, this inhalation condition is drastically different from the radon therapy condition. In this study, radon inhalation increased OGG1 levels by 19–29% in the kidney (2 kBq/m^3 for three or 10 days; Fig. 2) and by 37% in the small intestine (20 kBq/m^3 for one day; Fig. 3); however, the differences were not significant. The role of OGG1 in suppressing oxidative DNA damage might be limited when compared to the antioxidative functions of Mn-SOD.

In conclusion, Mn-SOD probably played an important role in the inhibition of oxidative DNA damage; however, other antioxidants likely contribute to this response. We examined only Mn-SOD, and therefore, we could not fully elucidate the mechanisms underlying the observed protective effect against oxidative stress with radon inhalation. The protective effects of antioxidative activities against oxidative DNA damage are more important than the DNA repair response under the evaluated radon inhalation conditions. Further studies are needed to clarify the precise mechanisms of these protective effects. In addition, it is critically important to evaluate the risk of lung cancer in patients or single-strand/double-strand breaks when undergoing radon therapy.

CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

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