Comparative Effects of Radon Inhalation According to Mouse Strain and Cisplatin Dose in a Cisplatin-induced Renal Damage Model

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ABSTRACT

Cisplatin (CDDP) is widely used for treating solid cancers; however, it induces nephrotoxicity caused by oxidative stress. Here, we investigated whether radon inhalation has different effects against CDDP-induced renal injury in two mouse strains differing in radiosensitivity, and determined the appropriate dose of CDDP combined with radon inhalation for highly radiosensitive mice. CDDP was administered at 20 mg/kg weight to C57BL/6J and BALB/c mice after radon inhalation at 1000 Bq/m³ and 2000 Bq/m³ for 24 h. Radon inhalation had a slight positive effect against CDDP toxicity in C57BL/6J mice with respect to improved hair condition, whereas radon inhalation exacerbated CDDP-induced toxicity in BALB/c mice, such as a decrease in hair condition, higher creatinine levels, and decreased antioxidant contents (catalase and glutathione). When BALB/c mice were treated with a lower dose of CDDP (15 mg/kg) after 1000 Bq/m³ radon inhalation, the creatinine level was reduced and the superoxide dismutase content was increased, suggesting that this combination might have a protective effect against the CDDP-induced renal damage. The supportive effect of radon inhalation shows its good potential as a candidate treatment to alleviate CDDP-induced renal damage in veterinary medicine.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II, CDDP) is a widely used chemotherapeutic agent for various solid organ tumors such as tumors of the head, neck, testis, ovaries, and bladder (Cetin *et al*., 2006; Rana *et al*., 2016; Santos *et al*., 2007). CDDP is well known to be taken up by the proximal tubule cells and accumulates in the mitochondria of these cells (Hanigan and Devarajan, 2003; Noori and Mahboob, 2010; Prabhu *et al*., 2013; Uehara *et al*., 2011; Yao *et al*., 2007). However, CDDP has a toxic effect by covalently binding to DNA (Hato *et al*., 2014). Although CDDP also has ototoxicity, gastrotoxicity, and myelosuppression, the main dose-limiting toxicity of the drug rests in this nephrotoxicity. Oxidative stress has been proposed as a key contributing factor to CDDP-induced nephrotoxicity (Ozkok and Edelstein, 2014).

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and their removal by antioxidant substances (Galle, 2001). ROS such as superoxide (O_2^-) are caused by continuous oxygen consumption, and are produced within the mitochondria; antioxidant substances such as superoxide dismutase (SOD), catalase (Cat), and glutathione (GSH) eliminate these ROS (Hanigan and Devarajan, 2003; Kruidering *et al*., 1997). However, the uptake of CDDP in cells and their subsequent accumulation in the mitochondria induces a decrease in the activities of SOD, Cat, and GSH; moreover, intracellular GSH levels are further decreased or depleted owing to the rapid reaction with CDDP to promote its transformation to highly reactive forms (Ozkok and Edelstein, 2014; Uehara *et al*., 2011). This phenomenon contributes to the accumulation of ROS, leading to a state of oxidative stress within the cell (Pabla and Dong, 2008). Furthermore, CDDP has been suggested to induce mitochondrial dysfunction, and thus ROS are generated from the respiratory chains of these malfunctioning mitochondria (Kawai *et al*., 2006; Oh *et al*., 2016; Ozkok and Edelstein, 2014; Pabla and Dong, 2008).

Radon (^{222}Rn) is a natural radioactive gas that emits

α-rays and is absorbed in the body through the lungs via respiration as well as through the skin (Kataoka *et al.*, 2016). Radon is delivered to many tissues through the bloodstream and throughout the body (Nishiyama *et al.*, 2013), and is lipo-soluble with a high amount of energy release (Yamato *et al.*, 2013). At the Misasa Medical Center of Okayama University Hospital, radon-based therapy has been performed using a concentration of 2000 Bq/m³ and 42°C at room temperature (Sasaoka *et al.*, 2016). Indications of this therapy are respiratory diseases such as bronchial asthma, pain-related diseases such as osteoarthritis, and digestive diseases such as chronic pancreatitis and gastroenteritis.

We previously reported that radon inhalation could enhance antioxidant function in various organs in mice, such as the brain, lung, pancreas, liver, and kidney (Kataoka *et al.*, 2011). Moreover, radon inhalation was found to mitigate some ROS-related diseases, including the effective prevention and treatment of the renal injury related to streptozotocin-induced type 1 diabetes and of hepatic and renal injuries induced by carbon tetrachloride (Kataoka *et al.*, 2011; Nishiyama *et al.*, 2015). Therefore, radon inhalation might help to suppress the renal damage induced by CDDP owing to its antioxidant functions.

Chronic kidney diseases are observed with a high prevalence in geriatric dogs and cats at 0.4–1.5% and 10%, respectively (Finch *et al.*, 2016; Fiocchi *et al.*, 2017). Thus, the widespread use of CDDP in the treatment of various solid tumors in these animals, as well as in humans, is associated with a problematic issue of nephrotoxicity (Knapp *et al.*, 1987, 1988). In addition, there are several types of dog breeds (approximately 400) and cat breeds (over 40) (Parker, 2012; Vapalahti, 2016), which differ with respect to their risk of developing certain diseases such as cardiovascular, hepatic, kidney, and bone and joint diseases. Therefore, these strain-specific risks and effects of drug administration should be taken into consideration in veterinary practice.

In addition, various mouse strains have been used for animal experiments, such as C57BL/6J, BALB/c, ICR, and Swiss albino mice (Maliakel *et al.*, 2008; Sung *et al.*, 2008), and strain-specific differences with respect to radio-sensitivity have been documented: C57BL/6J mice show relatively low sensitivity, whereas BALB/c mice show relatively high sensitivity (Onoue, 1960). However, no study has yet directly assessed the difference in this sensitivity between strains with respect to the effects of radon inhalation on the CDDP-induced renal injury. Furthermore, it is important to determine the suitable dosage of CDDP administration combined with radon inhalation in a highly radiosensitive mouse strain.

Accordingly, the aims of the present study were to evaluate whether radon inhalation has different effects on CDDP-induced renal injury caused by oxidative stress in two different mouse strains differing in radiosensitivity, and to determine the effective dosage of CDDP when radon inhalation is used. Clarification of these points should be beneficial for the appropriate use of CDDP in small animals in research and veterinary practice.

MATERIALS AND METHODS

Animals

Eight-week-old male C57BL/6J mice, weighing 22.5–26.0 g, and 8-week-old male BALB/c mice, weighing 23.3–27.0 g, were purchased from Japan Charles River Laboratories Inc. (Yokohama, Japan) and housed in clear plastic cages with paper-chipping in a temperature (23.2 ± 2.4°C) and light cycle (12 h of light and 12 h of dark)-controlled room, and maintained on a standard laboratory diet with free access to water. Ethical approval for all protocols and experiments was obtained from the Animal Care and Use Committee of Okayama University.

Experimental procedure

The evaluation of clinical signs, and measurement of urine specific gravity and body weight in each mouse was conducted before radon inhalation, at the time of CDDP administration, and at 72 h after CDDP administration.

In Experiment 1, C57BL/6J mice and BALB/c mice were divided into the following groups with seven mice per cage: control group, 1000 Bq/m³ radon inhalation only group, 2000 Bq/m³ radon inhalation only group, CDDP (20 mg/kg) only group (CDDP 20, sham), CDDP (20 mg/kg) administration after 1000 Bq/m³ radon inhalation group (1000 Bq/m³+CDDP 20), and CDDP (20 mg/kg) administration after 2000 Bq/m³ radon inhalation group (2000 Bq/m³+CDDP 20).

In Experiment 2, BALB/c mice were divided into the following groups with seven mice per cage: control group, CDDP (15 mg/kg) only group (CDDP 15, sham), CDDP (15 mg/kg) administration after 1000 Bq/m³ radon inhalation group (1000 Bq/m³+CDDP 15), and CDDP (15 mg/kg) administration after 2000 Bq/m³ radon inhalation group (2000 Bq/m³+CDDP 15).

After two days of acclimatization, the clinical signs were evaluated by calculating the body condition scores (BCS) for nutritional assessment according to the criteria of the World Small Animal Veterinary Association (Freeman *et al.*, 2011): 1, easily palpable ribs and lumbar vertebrae, pelvic bones and all bones without detectable body fat and muscles; 2, easily visible ribs, lumbar vertebrae, pelvic bones, and all bones without palpable body fat and with slight loss of muscle; 3, easily palpable ribs without palpable fat, visible top of the vertebrae, prominent pelvic bones with an obvious waist; 4, easily palpable

ribs with slight body fat covering, and easily noticeable waist that can be viewed from above; 5, palpable ribs without excessive body fat, and an observable waist; 6, slight excessive body fat covering the ribs, discernible but prominent waist; 7, non-palpable ribs with heavy fat cover, obvious fat deposition over the lumbar area, and absent or barely visible waist; 8, no palpable ribs due to heavy fat cover, fat deposition around the lumbar area, and an absent waist; 9, massive fat deposition over the thorax and spine, absent waist, and abdominal trunk with heavy fat deposition on the neck and limbs. Scale and hair condition scores were based on the system described by Miyagawa et al. (2013): 0, no scales, 1, a few substantial or systemic scales, 2, easily recognized scales systemically, and 3, massive scales systemically; and 0, smooth and glossy hairs, 1, partially dried hair, 2, systemically dried hair, and 3, densely tangled and rough hairs, respectively (Fig. 1). Urine samples were collected either by the urination at the time of the retention of mice or by the bladder pressure of the mice for measuring urine specific gravity. Body weights in all mice were also recorded at the time of evaluation, before radon inhalation and CDDP administration for the CDDP groups, and at 72 h after CDDP administration.

Radon inhalation

Mice were exposed to air only (sham) or radon at the specified concentration for 24 h using a radon exposure system as previously described (Kataoka et al., 2012). The concentration of radon in the mouse cage was supervised using a radon monitor (CMR-510, femo-TECH, Inc., OH, USA) and adjusted to 1000 Bq/m^3 or 2000 Bq/m^3.

CDDP administration

Immediately after radon inhalation, the CDDP groups were administered a single dose of CDDP (Wako Pure Chemical Industry Co. Ltd., Osaka, Japan) at either 15 mg/kg body weight or 20 mg/kg body weight intraperitoneally (i.p.). The dose was determined so as to be sufficiently high to induce severe renal damage in mice (Ueki et al., 2013). After treatment, the mice were allowed to acclimate in the cages under a similar condition as described above for 72 h. Since the greatest severity of renal damage was detected at 72 h following CDDP i.p. injection, all mice were sacrificed by deep diethyl ether anesthesia or inhalation of carbon dioxide gas at 72 h after CDDP administration (Ueki et al., 2013).

Biochemical assay

Blood samples were drawn by cardiac puncture at 72 h after CDDP injection, and then centrifuged at 3,000 ×g for 5 min at 4°C. Sera were kept at −80°C until measurement

of the serum creatinine (Cre) level (as an indicator of renal dysfunction) using the Quantichrom Creatinine Assay Kit (Funakoshi Co., Ltd., Tokyo, Japan) according to the manufacturer's recommendation.
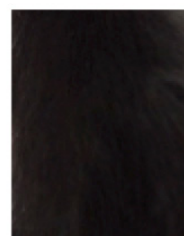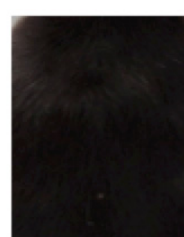

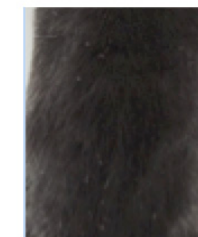A) Hair condition and scale score of control mice
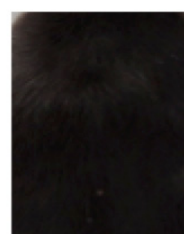
B) Score 1 of Hair condition   Score 2 of hair condition

C) Score 1 of Scale score   Score 2 of Scale score

Fig. 1. Representative photos of hair condition and scale score in mice. A, indicates the normal hair condition and scale score of control mice; B, represents each score of hair condition; C, each score of scale score.

The left and right kidneys were quickly and surgically excised, and the specimens were measured and rinsed in 10 mM phosphate buffer solution (PBS, pH 7.4) and stored at −80°C until analysis of the SOD and Cat activities and GSH protein levels. The left kidneys were homogenized in 10 mM PBS (pH 7.4) on ice, and the homogenates were centrifuged at 12,000 ×g for 45 min at 4°C. The supernatants were used for the assay of SOD activity using the nitroblue tetrazolium (NBT) reduction method with the Wako SOD test (Wako Pure Chemical Industry Co. Ltd., Osaka, Japan) according to the manufacturer instructions (Baehner et al., 1975).
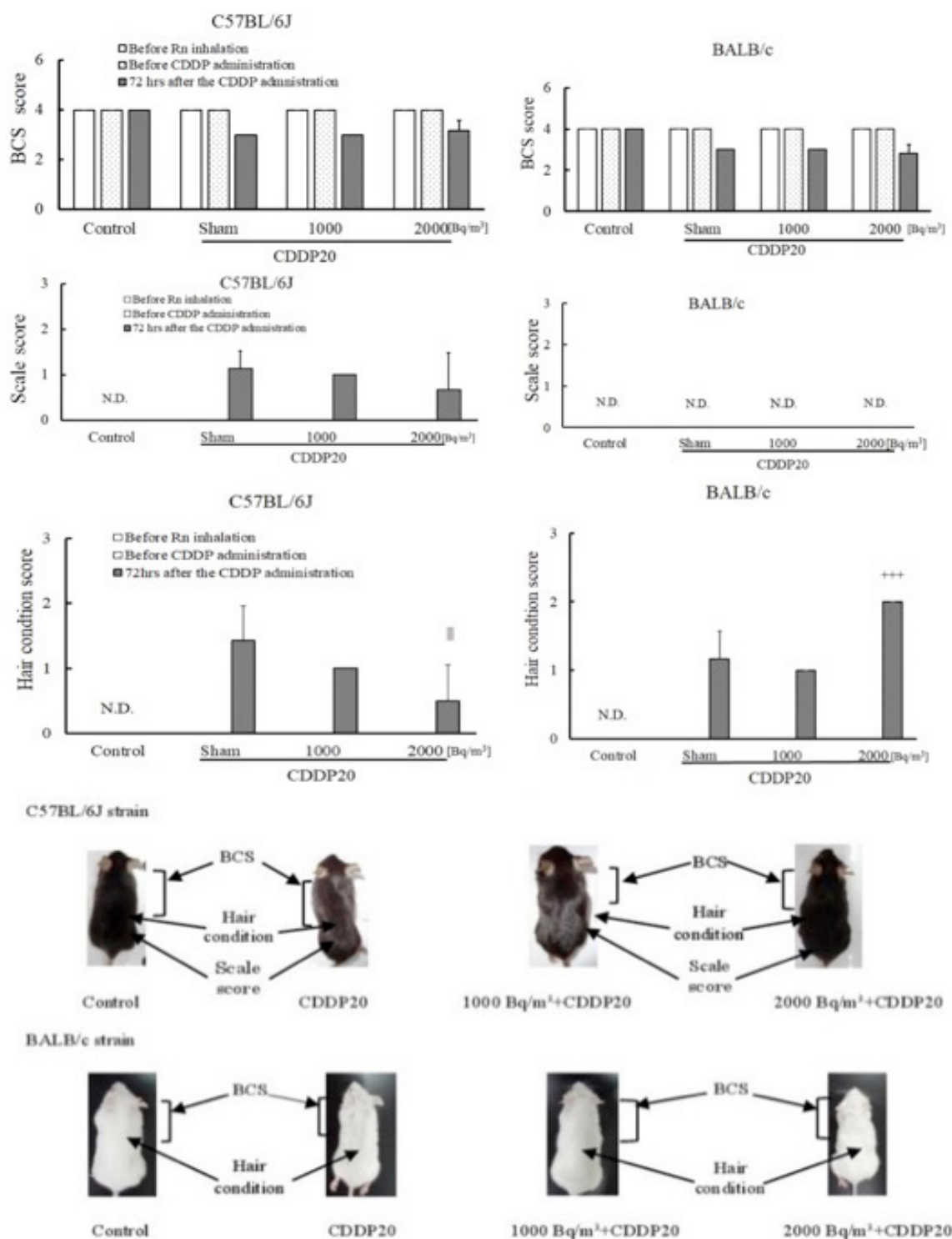
Fig. 2. Effects of radon inhalation on the body condition score (BCS), scale score, and hair condition score in C57BL/6J and BALB/c mice with CDDP-induced renal injury. ||| P < 0.001 compares the effects of the radon inhalation group at 2000 Bq/m³ vs. the sham group in the C57BL/6J strain. +++P < 0.001 indicates the comparison of the radon inhalation group at 2000 Bq/m³ vs. the sham in the BALB/c strain. N.D. means "not detected". Photographs of mice show the methods for measuring the BCS, scale score, and hair condition score.

In brief, inhibition of the reduction of 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 rate of hydrogen peroxide (H_2O_2) reduction at 37°C detected 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 ethylenediaminetetraacetic acid (pH 7.4), 900 μL of 10 mM H_2O_2, 30 μL of deionized water, and 20 μL of the supernatants of the kidneys. Cat activity was calculated using a molar extinction coefficient of 7.1 × 10^{-3} $M^{-1}cm^{-1}$. The GSH content was measured using the GSSG/GSH Quantification Kit (Dojindo Molecular Technologies Inc., Kumamoto, Japan). In brief, the left kidneys were homogenized with 5% 5-sulfosalicylic acid and centrifuged at 8,000 ×*g* for 10 min. The supernatants were used for the assay based on the colorimetric reaction of GSH by detecting the absorption at 415 nm. Protein concentrations were measured using the Bradford method with the protein Quantification Kit-Rapid (Dojindo Molecular Technologies Inc., Kumamoto, Japan) (Bradford, 1976).

Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). The statistical significance of differences was determined by t-tests, Mann-Whitney's U test and Kruskal-Wallis test or post-hoc Tukey's tests or Dunnet test for multiple comparisons where appropriate. P < 0.05 was considered statistically significant.

RESULTS

Clinical signs

In Experiment 1, the BCS tended to be reduced at 72 h after CDDP administration in all CDDP 20 groups compared with the control group for both strains (Fig. 2). Scales were recognized in the C57BL/6J strain and the scale score tended to be reduced in the 2000 Bq/m³+CDDP 20 group compared with that of the CDDP 20 group (Fig. 2). No scales were detected in the BALB/c mice (Fig. 2). Similarly, in Experiment 2 using BALB/c mice only with a lower CDDP dose (15 kg/mg), the BCS tended to be reduced at 72 h after CDDP administration in all groups compared with that of the control group (Fig. 3). However, there was no difference in the overall BSC between the CDDP 15 and CDDP 20 administration groups at 72 h after CDDP administration; no scales were observed (Fig. 3).

In the C57BL/6J strain, the hair condition score of the 2000 Bq/m³+CDDP 20 group was significantly reduced

compared with that of the CDDP 20 group (P < 0.001; Fig. 2). By contrast, in the BALB/c strain, the hair condition score in the 2000 Bq/m³+CDDP 20 group was significantly increased compared with that of the CDDP 20 group (P < 0.001; Fig. 2). In addition, the hair condition score was significantly higher in the 2000 Bq/m³+CDDP 15 group than that of the CDDP 15 group (P < 0.001; Fig. 3).
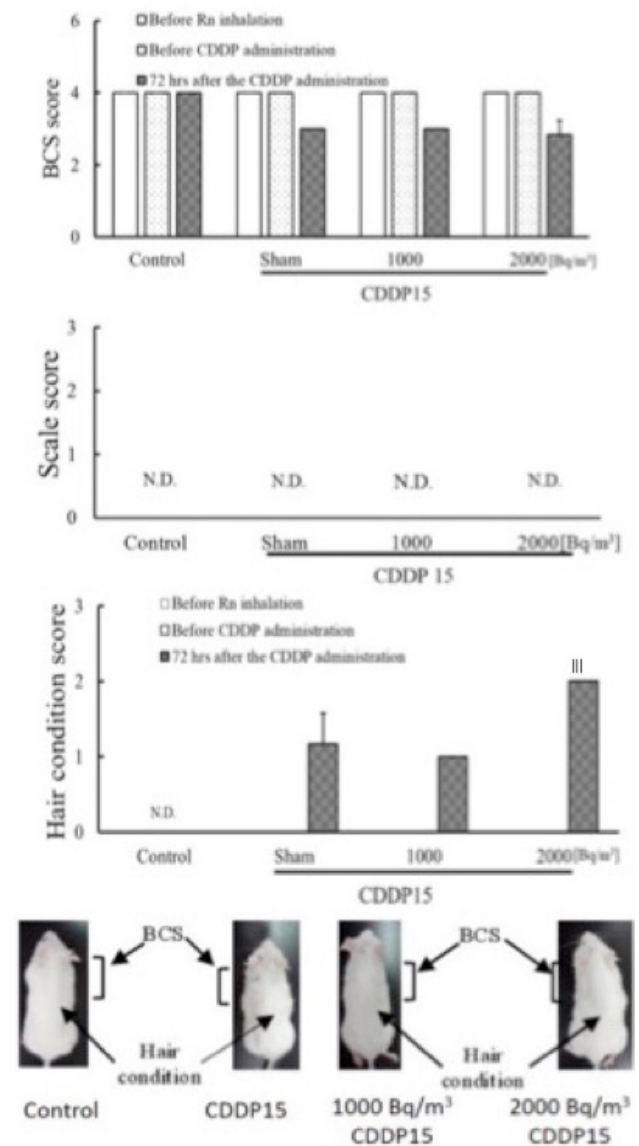

Fig. 3. Effects of radon inhalation on the body condition score (BCS) and hair condition score in BALB/c mice with 15 mg/kg CDDP-induced renal injury. [III]P < 0.001 indicates the comparison of the group treated with 15 mg/kg CDDP after radon inhalation at 2000 Bq/m³ vs. the sham. N.D. means "not detected." Pictures of mice indicate the method of measuring the BCS and hair condition score.
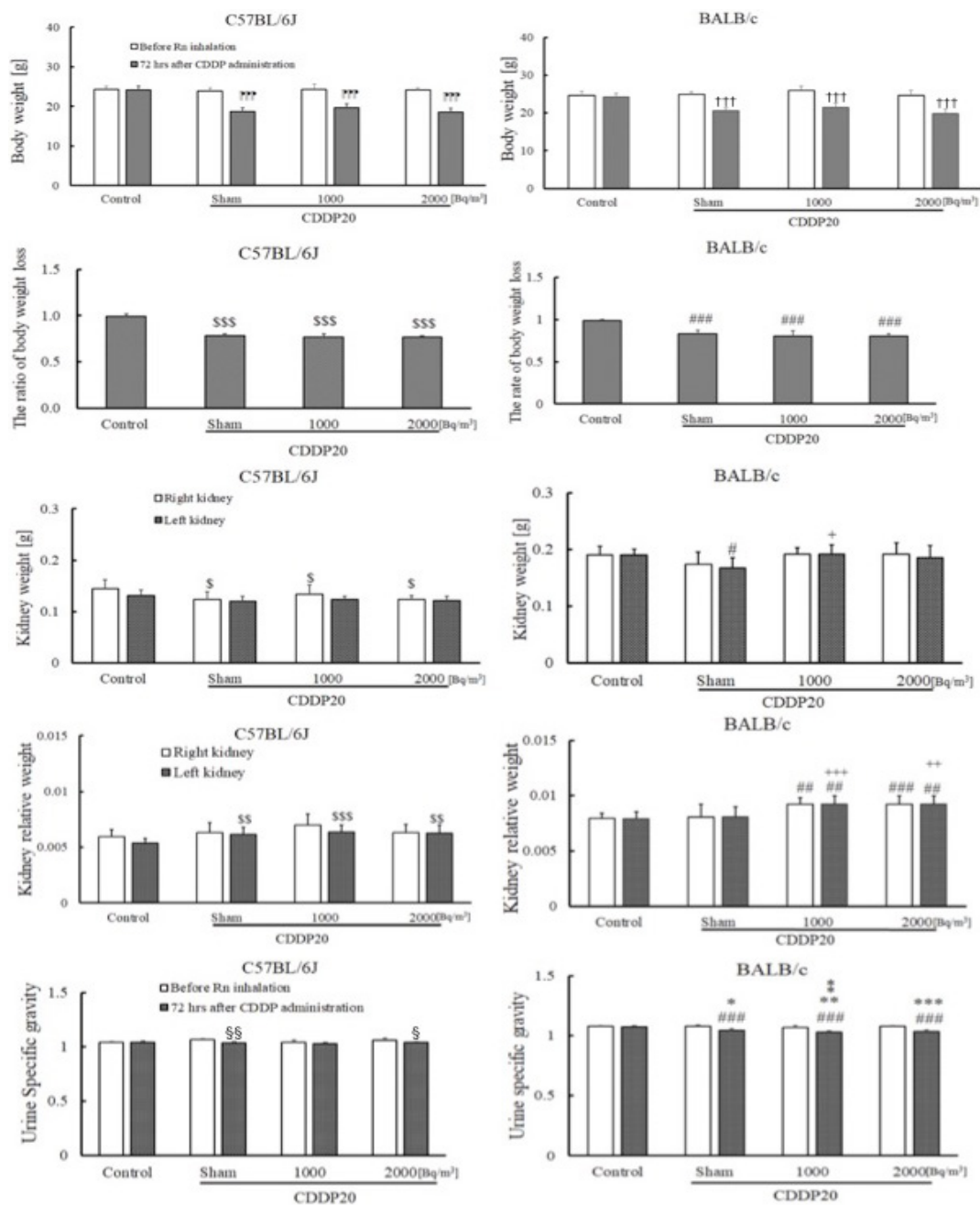
Fig. 4. Effects of radon inhalation on the influence of 20 mg/kg CDDP administration in body weight, the rate of body weight loss, absolute and relative kidney weight, and urine specific gravity in C57BL/6J and BALB/c mice with CDDP-induced renal injury. ¶¶¶P < 0.001 compares the data of the treatment groups vs. the control measured at 72 h after CDDP administration in the C57BL/6J strain; the sham group received 20 mg/kg CDDP only with air inhalation. †††P < 0.001 indicates the comparison of the treatment groups vs. the control in the BALB/c strain at 72 h after CDDP administration. $P < 0.05, $$P < 0.01, $$$P < 0.001 indicate comparisons of the treatment group vs. the control in the C57BL/6J strain.# P < 0.05, ## P < 0.01, ### P < 0.001 indicate the treatment groups vs. the control in the BALB/c strain. §P < 0.05 and §§ P < 0.01 compare data at 72 h after CDDP administration vs. before radon inhalation in the C57BL/6J strain. * P < 0.05, ** P < 0.01, and *** P < 0.001 compare the data at 72 h after CDDP administration in the treatment groups vs. before radon inhalation in the BALB/c strain. +P < 0.05, ++P < 0.01, and +++P < 0.001 indicate the CDDP and radon inhalation groups vs. the sham in the BALB/c strain. ‡P < 0.05 indicates the CDDP and 1000 Bq/m³ inhalation group vs. the sham in the BALB/c strain.
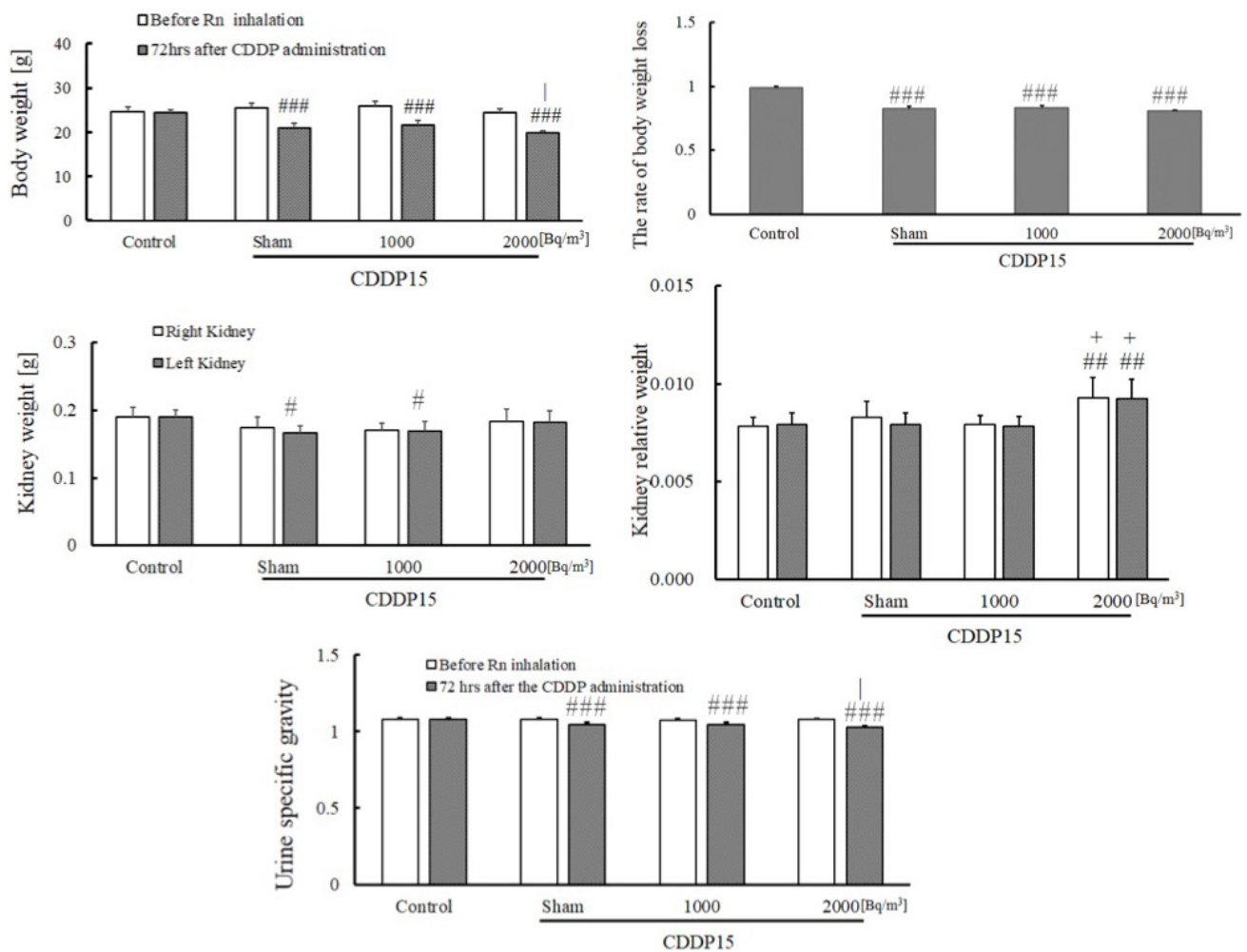
Fig. 5. Effects of radon inhalation on the 15 mg/kg CDDP-induced renal injury clinical signs, body weight, the rate of body weight loss, absolute and relative kidney weight, and urine specific gravity in BALB/c mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ indicate the radon inhalation groups vs. the control. $^{|}P < 0.05$ indicates the data at 72 h after CDDP administration with 2000 Bq/m³ radon inhalation vs. the sham (15 mg/kg CDDP only with air inhalation). $^{+}P < 0.05$ indicates CDDP administration after 2000 Bq/m³ radon inhalation vs. the sham.

Body weight, body weight loss, kidney weight, and urine specific gravity

Body weight was decreased at 72 h after the administration of 20 mg/kg CDDP in all groups compared to the control in both strains (P < 0.001; Fig. 4). The rate of body weight loss of all groups administered CDDP was lower than that of the corresponding control group, with no difference from that of the respective sham group in both strains (P < 0.001; Fig. 4). Body weight significantly decreased in all BALB/c mice treated with 15 mg/kg CDDP at 72 h after CDDP administration compared with that of the control group (P < 0.001; Fig. 5). In addition,

the body weight of the 2000 Bq/m³+CDDP 15 group was significantly decreased compared with that of the CDDP 15 group (P < 0.05; Fig. 5). The rate of body weight loss of all CDDP 15-administered groups was lower than that of the control group (P < 0.001; Fig. 5).

In C57BL/6J mice, the right kidney weight was significantly reduced for all CDDP 20-administered groups compared with the control group (P < 0.05; Fig. 4). In BALB/c mice, the left kidney weight of the CDDP 20 group was significantly decreased compared with that of the control group (P < 0.05; Fig. 4). The left kidney weight of the 1000 Bq/m³+CDDP 20 group was significantly

increased compared that of the CDDP 20 group (P < 0.05; Fig. 4). In C57BL/6J mice, the relative weight of the left kidney was significantly increased in all groups administered CDDP 20 compared with that of the control group (CDDP 20, P < 0.01; 1000 Bq/m³+CDDP 20, P < 0.001; and 2000 Bq/m³+CDDP20, P < 0.01; Fig. 4). In BALB/c mice, the relative weights of both kidneys in the 1000 Bq/m³+CDDP 20 and 2000 Bq/m³+CDDP 20 groups were significantly increased compared with those of the control group (left and right kidneys 1000 Bq/m³+CDDP 20, P < 0.01; right kidney 2000 Bq/m³+CDDP 20, P < 0.001; and left kidney 2000 Bq/m³+CDDP 20, P < 0.01; Fig. 4). The relative weights of the left kidneys of the 1000 Bq/m³+CDDP 20 and 2000 Bq/m³+CDDP 20 groups were also significantly higher than that of the CDDP 20 group (1000 Bq/m³+CDDP 20, P < 0.001; 2000 Bq/m³+CDDP 20, P < 0.01; Fig. 4). As shown in Figure 5, kidney weight was also significantly reduced in the left kidneys of the CDDP 15 and 1000 Bq/m³+CDDP 15 groups compared with that of the control group (both P < 0.05). Moreover, the absolute and relative weights of both kidneys in the 2000 Bq/m³+CDDP 15 group were increased compared with those of the CDDP 15 group (P < 0.05; Fig. 5) and control group (P < 0.01; Fig. 5).

The urine specific gravities of the CDDP 20 and 2000 Bq/m³+CDDP 20 groups were significantly lower than that measured before radon inhalation in C57BL/6J mice (P < 0.01 and P < 0.05, respectively; Fig. 4). In BALB/c mice, the urine specific gravities of all groups administered CDDP at 20 mg/kg were significantly decreased compared with that of the control group (P < 0.001; Fig. 4), and were significantly lower at 72 h after CDDP administration than that measured before radon inhalation (CDDP 20, P < 0.05; 1000 Bq/m³+CDDP 20, P < 0.01; and 2000 Bq/m³, P < 0.001; Fig. 4). The urine specific gravity of the 1000 Bq/m³+CDDP 20 group was significantly decreased compared to that of the CDDP 20 group at 72 h after CDDP administration (P < 0.05; Fig. 4). Urine specific gravities of all groups treated with CDDP 15 were lower than that of the control group (P < 0.001; Fig. 5), and the urine specific gravity of the 2000 Bq/m³+CDDP 15 group was also significantly lower than that of the CDDP 15 group (P < 0.05; Fig. 5).

Index of renal injury

To estimate the ability of radon inhalation to mitigate the renal injury induced by CDDP, Cre levels were determined as an index of renal injury. In C57BL/6J mice, there were no significant differences in Cre levels among any of the CDDP-treated mice. In BALB/c mice, the Cre levels of all CDDP-administered groups were significantly higher than that of control mice (CDDP 20 P < 0.05; 1000 Bq/m³+CDDP 20, P < 0.05, and 2000 Bq/m³, P < 0.01; Fig.

6A), with no difference among the three CDDP-treated groups (Fig. 6A). The Cre levels of the CDDP 15 and 2000 Bq/m³+CDDP 15 groups tended to be increased compared with those of the controls (Fig. 7A). However, there was a decrease in Cre of approximately 70% in the 1000 Bq/m³+CDDP 15 group compared with that of the CDDP 15 group (Fig. 7A).

Effect of radon inhalation on renal antioxidant-related substances

To assess the antioxidative effect of radon inhalation against CDDP-induced renal injury, the antioxidant-related substances SOD, Cat, and GSH were analyzed.

The SOD activity of the CDDP 20 group was significantly decreased compared with that of the control group in C57BL/6J mice (P < 0.05; Fig. 6B). In BALB/c mice, the SOD activity of the 2000 Bq/m³+CDDP 20 group was significantly lower than that of the control group (P < 0.01; Fig. 6B), whereas the SOD activity of the 1000 Bq/m³+CDDP 20 group was significantly increased compared with that of the CDDP 20 group (P < 0.001; Fig. 6B). Similarly, the SOD activity of the 2000 Bq/m³ +CDDP 15 group was significantly decreased compared with that of the control mice (P < 0.01; Fig. 7B), whereas the activities of both the 1000 Bq/m³+CDDP 15 and 2000 Bq/m³+CDDP 15 groups were significantly increased compared with that of the CDDP 15 group (both P < 0.05; Fig. 7B).

The Cat activities of all CDDP administration groups were significantly lower than that of control mice (P < 0.005; Fig. 6B), and there were no significant differences among the CDDP administration groups in C57BL/6J mice. However, in BALB/c mice, Cat activities were significantly decreased in the 1000 Bq/m³+CDDP 20 and 2000 Bq/m³+CDDP 20 groups compared with that of the control group (P < 0.05 and P < 0.01, respectively) (Fig. 6B) and CDDP 20 group (P < 0.05 and P < 0.001, respectively) (Fig. 6B). Similarly, the Cat activity of the 2000 Bq/m³ +CDDP 15 group was significantly lower than that of the control group (P < 0.01; Fig. 7B) and CDDP 15 group (P < 0.001) (Fig. 7B).

In C57BL/6J mice, the GSH activity of the 2000 Bq/m³+CDDP 20 group was 30% higher than that of the CDDP 20 group. However, in BALB/c mice, the GSH activities were significantly decreased in all CDDP-administered groups compared with that of the control group (P < 0.01; Fig. 6B), and the 2000 Bq/m³+CDDP 20 group showed a 26% decrease in GSH activity compared with that of the CDDP 20 group (Fig. 6B). In addition, the GSH activity of the 2000 Bq/m³ +CDDP 15 group showed a 52% decrease compared with that of CDDP15 group (Fig. 7B).
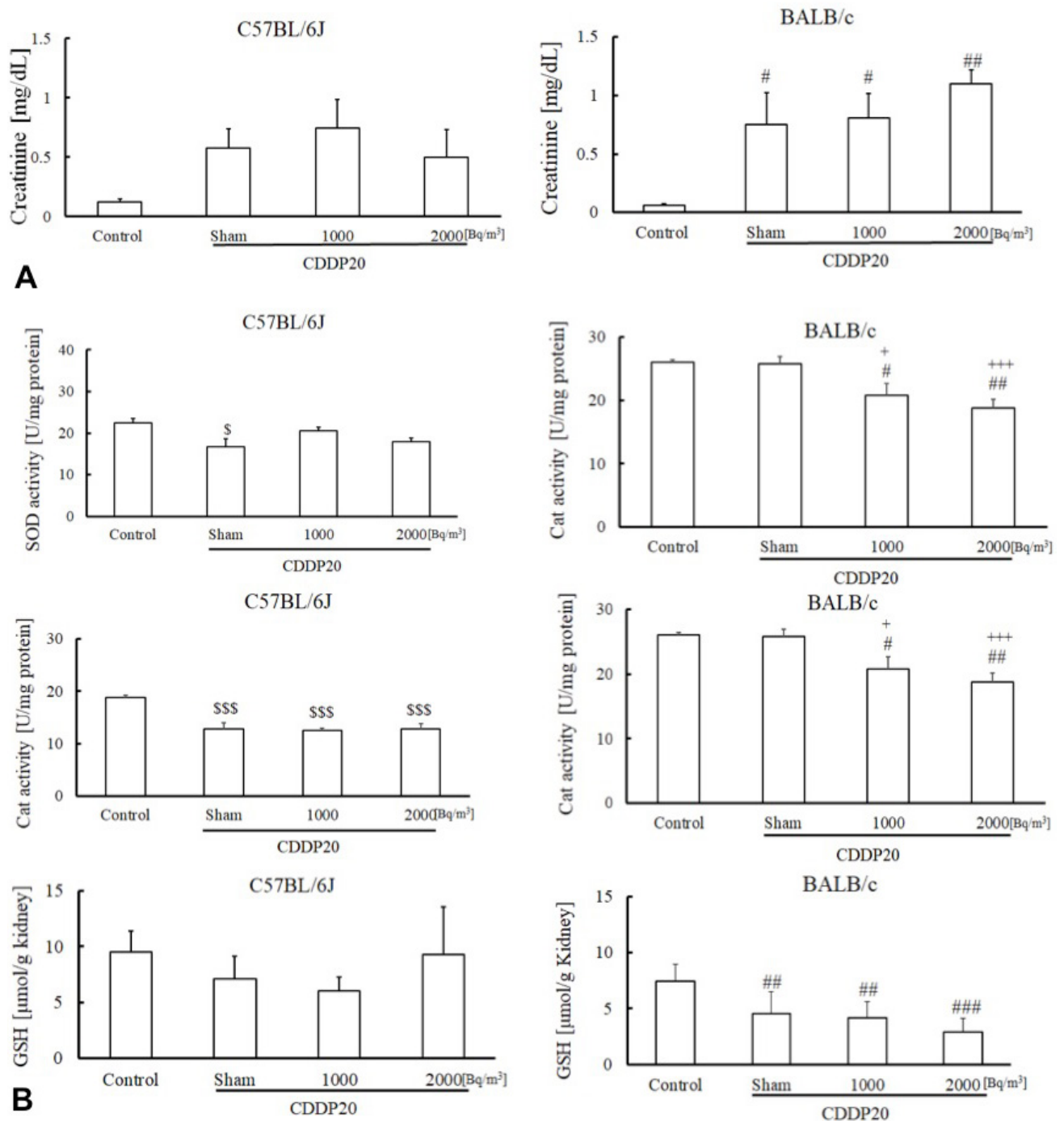
Fig. 6. Effects of radon inhalation on the influence of 20 mg/kg CDDP administration on A) the serum creatinine level, and B) antioxidant-related substances in C57BL/6J and BALB/c mice with CDDP-induced renal injury. $^{\$}P < 0.05$ and $^{\$\$\$}P < 0.001$ indicate the treatment groups vs. the control in the C57BL/6J strain. The sham group represents the group administered with 20 mg/kg CDDP only with air inhalation. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ indicate the treatment groups vs. the control in the BALB/c strain. $^{+}P < 0.05$ and $^{+++}P < 0.001$ indicate the radon inhalation groups vs. the sham in the BALB/c strain.
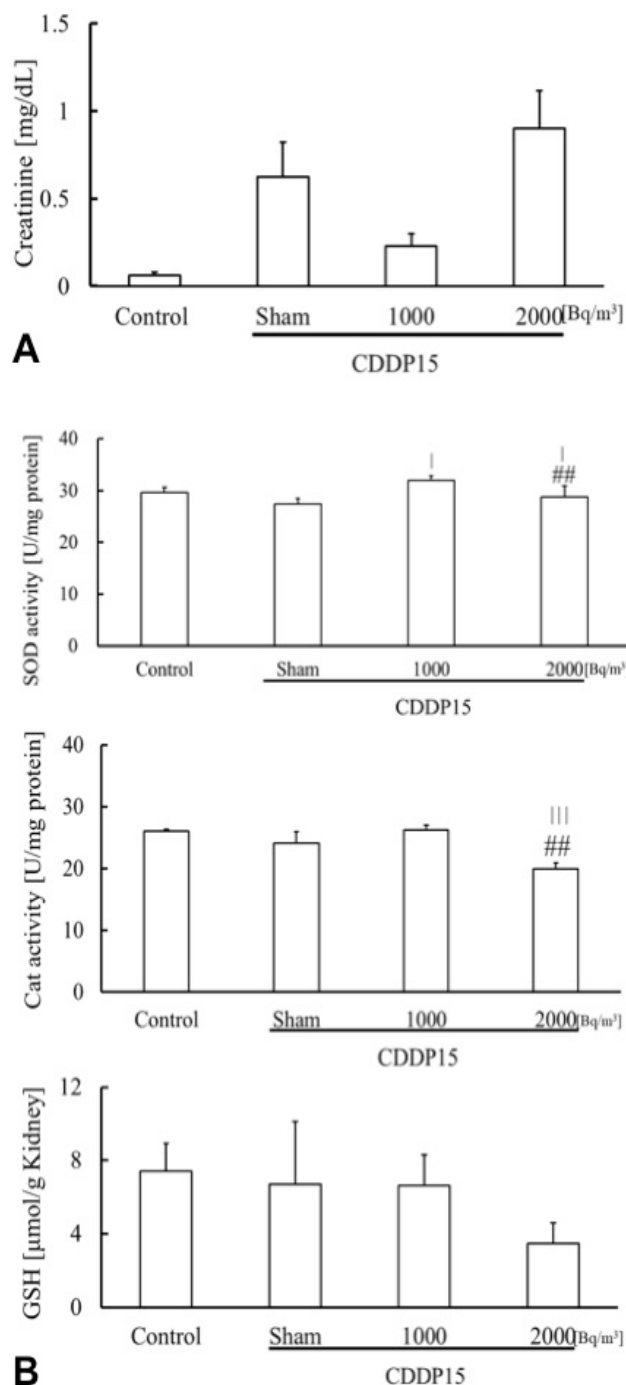
Fig. 7. Effects of radon inhalation on the changes in A) serum creatinine level and B) antioxidant-related substances caused by 15 mg/kg CDDP in BALB/c mice with CDDP-induced renal injury. ##$P < 0.01$ indicates the treatment groups vs. the control. The sham group was treated with 15 mg/kg CDDP only with air inhalation. I$P < 0.05$ and III$P < 0.001$ indicate the radon inhalation groups vs. the sham.

DISCUSSION

Radon inhalation has been suggested to enhance the antioxidant functions of various organs such as the brain, lung, heart, liver, and kidney (Kataoka *et al.*, 2011), and ROS-related diseases of these organs have been shown to be inhibited via the suppression of ROS activity. Furthermore, radon inhalation was found to effectively improve clinical signs in a study with a renal kidney failure animal model (Kataoka *et al.*, 2012). Although CDDP is an effective chemotherapeutic agent, various side effects have been confirmed, including nephrotoxicity as a major adverse effect. The overarching aim of the present study was to evaluate the different effects of radon inhalation on mediating this CDDP-induced renal damage in two different radiosensitivity mouse strains and to determine an appropriate CDDP dose in combination with radon inhalation in the highly radiosensitive mouse strain.

Our results revealed no notable differences between strains with respect to BCS, body weight, and the rate of body weight loss upon 20 mg/kg CDDP administration with or without radon inhalation at two concentrations. Body weight loss is a common outcome of cancer therapy, and therefore nutrition management is an important consideration (Nakahara *et al.*, 2012). Accordingly, both body weight loss and the rate of this loss are important factors for accurately assessing the effect of CDDP. Body weight loss arises from the gastrointestinal toxicity in response to CDDP treatment, and the consequent reduction of food intake leads to a poor nutritional status (Noori and Mahboob, 2010; Rana *et al.*, 2016). The BCS reflects the nutritious condition of animals (Freeman *et al.*, 2011). Therefore, it is considered that the poor nutrition condition observed in C57BL/6J and BALB/c mice was caused by the gastrointestinal toxicity from 20 mg/kg CDDP administration. However, radon inhalation could not protect against this gastrointestinal toxicity. With respect to dose, gastrointestinal toxicity was detected with the administration of both 15 mg/kg and 20 mg/kg CDDP in combination with radon inhalation at 2000 Bq/m³ given the reductions in the BCS, body weight, and rate of body weight loss, indicating that even a relatively high radon concentration (2000 Bq/m³) cannot suppress the reduction of food intake due to the gastrointestinal toxicity induced by CDDP administration at both doses evaluated in this study.

Poor hair quality with an increase of scales is commonly observed in animals suffering from renal injury (Canon, 2016). Therefore, we also evaluated the hair condition as well as scale scores as clinical signs of renal injury induced by CDDP to evaluate the effects of radon inhalation. The results showed that both the hair condition and scale scores were improved in mice administered with 20 mg/kg CDDP after 2000 Bq/m³ radon inhalation in the

C57BL/6J strain, suggesting that this radon concentration may mitigate the CDDP-induced renal injury in this strain. By contrast, the hair condition further deteriorated in BALB/c mice that received 20 mg/kg CDDP after 2000 Bq/m^3 radon inhalation, suggesting that a relatively high radon concentration may exacerbate the CDDP-induced renal damage, which is a mark of the high radiosensitivity of this strain. This same effect was noted with 15 mg/kg CDDP administration, indicating that the extent of renal damage induced by CDDP administration may be aggravated by the combination with a high radon concentration regardless of the CDDP dose.

CDDP-treated animals also show polyuria, indicating a reduction in the urine specific gravity because of a deficiency of urine concentration (Ecelbarger *et al.*, 2001; Nematbakhsh *et al.*, 2013). Polyuria was confirmed following 20 mg/kg CDDP administration after inhaling 2000 Bq/m^3 radon in C57BL/6J mice, and all BALB/c mice administered either 20 mg/kg or 15 mg/kg CDDP after radon inhalation showed significant reductions of urine specific gravity, indicating that radon inhalation does not mitigate the renal damage induced by CDDP. Specifically, there was a significant reduction of urine specific gravity with 15 mg/kg CDDP administration after 2000 Bq/m^3 radon inhalation and with 20 mg/kg CDDP after both 1000 Bq/m^3 and 2000 Bq/m^3 radon inhalation. These findings suggest that the combination of 15 mg/kg CDDP administration and 2000 Bq/m^3 radon inhalation and the combination of 20 mg/kg CDDP administration and 1000 Bq/m^3 and 2000 Bq/m^3 radon inhalation does not provoke the polyuria in CDDP-induced renal damage.

The severity of renal damage can be indicated by a larger kidney weight (Nematbakhsh *et al.*, 2013; Tokunaga *et al.*, 1996). This enlargement of the kidney is not due to a relative effect linked to overall weight loss but rather results from edema caused by necrosis in the tubular cells (Rana *et al.*, 2016). Therefore, both the absolute and relative kidney weights should be considered for assessments of the ability of radon inhalation to improve the CDDP-induced renal injury. The present study showed that absolute and relative kidney weights increased in the C57BL/6J mice administered 20 mg/kg CDDP after radon inhalation, indicating that the edema of the tubular cells in these kidneys was indeed induced by CDDP and could not be mitigated by radon inhalation. Similarly, the BALB/c mice showed significant increases in both the absolute and relative kidney weights with 20 mg/kg CDDP administration after radon inhalation at both concentrations, suggesting that radon inhalation might actually worsen the necrosis of tubular cells in the kidneys in this strain. The kidney is considered to be a relatively weakly radiosensitive organ based on the

different radiosensitivities in various tissues and organs. Furthermore, radon is a highly lipo-soluble substance (Yamato *et al.*, 2013); therefore, it would be expected that radon inhalation would have minimal effects on renal injury in these strains since the kidney has less fat than the other organs. The kidney weights of BALB/c mice also increased with the combination of 15 mg/kg CDDP and 2000 Bq/m^3 radon, as well as 20 mg/kg CDDP and both 1000 Bq/m^3 and 2000 Bq/m^3 radon inhalation, suggesting that these combinations could further exacerbate the CDDP-induced renal injury.

Cre is filtered out of the body by the kidneys, and the level of Cre increases when the filtration function of the kidney is damaged (Prabhu *et al.*, 2013). Likewise, the serum Cre level is elevated with increasing histological damage to the tubular cells (Palipoch and Punsawasd 2013). We did not detect an effect of radon inhalation at either concentration on the Cre level in C57BL/6J mice, indicating that radon inhalation cannot inhibit the effects of CDDP on renal damage in this strain due to its low radiosensitivity. However, in the BALB/c strain, the Cre levels actually increased with 20 mg/kg CDDP after radon inhalation at both concentrations, suggesting that the high radiosensitivity of this strain resulted in exacerbation of the CDDP-induced renal damage by radon inhalation in radon inhalation. Evaluation of the influence of CDDP dose showed that 1000 Bq/m^3 radon inhalation reduced the Cre level with administration of 15 mg/kg CDDP, but not significantly, indicating that radon inhalation may increase the glomerular filtration rate so that the renal injury is mitigated by a relatively low radon concentration (1000 Bq/m^3).

Given this decrease in the Cre level, we further examined antioxidant activity in the C57BL/6J and BALB/c strains and at different CDDP administration doses in the BALB/c strain. SOD converts O_2^- to hydrogen peroxidase (H_2O_2), whereas Cat and GSH transform H_2O_2 to water (H_2O) and then to oxygen (O_2) (Sasaoka *et al.*, 2016). In addition, O_2^- is generated by consuming oxygen in the mitochondria (Kruidering *et al.*, 1997); indeed, O_2^- is mainly generated from the respiratory chain in the mitochondria (Kruidering *et al.*, 1997; Pabla and Dong, 2008). It has also been suggested that CDDP decreases the activity of antioxidants after accumulating in the mitochondria (Noori and Mahboob, 2010). In the present study, the GSH content tended to increase in the C57BL/6J strain after 2000 Bq/m^3 radon inhalation. This suggests that GSH plays an important role for the mitigation of the CDDP-induced renal injury in C57BL/6J mice. However, since this difference was not statistically significant, the effect of radon inhalation in this strain may be small. By contrast, in the BALB/c strain, although the SOD activity

showed an increase in the GSH level following 1000 Bq/m^3 radon inhalation, it was reduced following 2000 Bq/m^3 radon inhalation with 20 mg/kg CDDP administration. The radon concentration employed in this study was determined according to the reference of the radon therapy used at Misasa Medical Center, Okayama University, and based on our previous study examining the change in SOD activity in the kidneys of BALB/c mice (Kataoka *et al.*, 2011). In this previous study, the SOD activity in the kidney sharply increased at 24 h, decreased at 72 h, and then gradually increased with 1000 Bq/m^3 radon inhalation. However, the SOD activity did not increase at 24 h and remained constant with 2000 Bq/m^3 radon inhalation. Therefore, the low SOD activity observed with 2000 Bq/m^3 radon inhalation in the present study likely reflects these dynamics. Consequently, the inhalation of 2000 Bq/m^3 radon may not contribute to the mitigation of CDDP-induced renal injury in this strain. In addition, Cat activity and the GSH contents were significantly reduced following 1000 Bq/m^3 and 2000 Bq/m^3 radon inhalation. As mentioned above, antioxidant activity was enhanced at about 24 h and the effect was reduced by radon inhalation at 72 h (Kataoka *et al.*, 2011). Therefore, these reductions of Cat activity and GSH content with 20 mg/kg CDDP administration might be due to this sequential change in these antioxidants. Moreover, our results showed that SOD activity was significantly high with the combination of 15 mg/kg CDDP and 1000 Bq/m^3 radon inhalation, suggesting that the function of the antioxidant SOD was enhanced by the radon inhalation. This finding is in line with the results of our previous study demonstrating the change of SOD activity in the kidneys of BALB/c mice (Kataoka *et al.*, 2011). Therefore, SOD activity may contribute to the mitigation of 15 mg/kg CDDP-induced renal injury conferred by 1000 Bq/m^3 radon inhalation. Furthermore, according to the analysis of antioxidant activity in paratuberculosis positive cattle, there were no variation of SOD and GSH-PX activities in their sera. Since radon inhalation enhances the antioxidative function, the elevation of antioxidant activity for the biological defense system against the infection might be observed in these cattle after radon exposure (Cenesiz *et al.*, 2016).

Although we used healthy mice for evaluating the effects of radon inhalation against the CDDP induced renal damage model in this study, the further study are needed to assess the effects of radon inhalation with the combination of various CDDP dose and radon concentration in the cancer mouse model.

CONCLUSION

In conclusion, evaluation of the differences in clinical signs, indexes of renal injury, and antioxidant activities

suggests strain-specific effects of radon inhalation on CDDP-induced renal damage, with clear differences detected between C57BL/6J and BALB/c mice. These differences are assumed to result from intrinsic variations in the radiosensitivities of these strains. C57BL/6J mice have relatively low radiosensitivity characteristics, indicating that the effects of radon inhalation on CDDP-induced renal damage would be minimal, whereas BALB/c mice have relatively high radiosensitivity, and exhibited high sensitivity to radon inhalation, which may be associated with radiation-induced DNA damage to further exacerbate the CDDP-induced renal damage. Moreover, comparison of the CDDP administration dose in BALB/c mice suggested that the combination of 15 mg/kg CDDP and 1000 Bq/m^3 radon inhalation is likely to protect against CDDP-induced renal damage to some extent via increasing the SOD content. Based on these results, radon inhalation appears to be a suitable candidate for further studies of mitigating CDDP-induced renal injury in veterinary practice.

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