

Study of antioxidative effects and anti-inflammatory effects in mice due to low-dose X-irradiation or radon inhalation

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Low-dose irradiation induces various stimulating effects, especially activation of the biological defense system including antioxidative and immune functions. Oxidative stress induced by reactive oxygen species (ROS) can cause cell damage and death and can induce many types of diseases. This paper reviews new insights into inhibition of ROS-related diseases with low-dose irradiation or radon inhalation. X-irradiation (0.5 Gy) before or after carbon tetrachloride (CCl₄) treatment inhibits hepatopathy in mice. X-irradiation (0.5 Gy) before ischemia-reperfusion injury or cold-induced brain injury also inhibits edema. These findings suggest that low-dose X-irradiation has antioxidative effects due to blocking the damage induced by free radicals or ROS. Moreover, radon inhalation increases superoxide dismutase activity in many organs and inhibits CCl₄-induced hepatic and renal damage and streptozotocin-induced type I diabetes. These findings suggest that radon inhalation also has antioxidative effects. This antioxidative effect against CCl₄-induced hepatopathy is comparable to treatment with ascorbic acid (vitamin C) at a dose of 500 mg/kg weight, or α -tocopherol (vitamin E) treatment at a dose of 300 mg/kg weight, and is due to activation of antioxidative functions. In addition, radon inhalation inhibits carrageenan-induced inflammatory paw edema, suggesting that radon inhalation has anti-inflammatory effects. Furthermore, radon inhalation inhibits formalin-induced inflammatory pain and chronic constriction injury-induced neuropathic pain, suggesting that radon inhalation relieves pain. Thus, low-dose irradiation very likely activates the defense systems in the body, and therefore, contributes to preventing or reducing ROS-related injuries, which are thought to involve peroxidation.

Keywords: low-dose irradiation; radon inhalation; antioxidative effect; anti-inflammatory effect; pain relief

INTRODUCTION

Low-dose irradiation induces various effects, especially activation of the biological defense system including antioxidative [1–6] and immune functions [6–8]. For example, X-irradiation at a dose of 0.2 Gy increases superoxide dismutase (SOD), glutathione peroxidase (GPx), and the amount of GPx mRNA in spleens of BALB/c or C57BL/6NJcl mice, in contrast to irradiation at 4 Gy [1]. Another report suggested that the levels of reduced glutathione (GSH), glutathione reductase (GR), γ -glutamylcysteine synthetase (γ -GCS), and thioredoxin (TRX) in liver increase soon after irradiation with 0.5 Gy of γ -rays and that GSH, GR, γ -GCS, and TRX mRNAs are slightly increased after γ -ray irradiation at a dose of 0.5 Gy [2]. In addition, the levels of GSH, GR, γ -GCS, and TRX in the brain

increase soon after irradiation with 0.5 Gy of γ -rays [3]. These findings suggest that low-dose irradiation increases antioxidative functions in several organs due to the induction of mRNA for antioxidant-related molecules. The activation of antioxidative functions is mediated by transcriptional regulation of the γ -GCS gene, predominantly through the activator protein-1 binding site in its promoter [9]. In addition, intercellular Ca²⁺ is involved in induction of γ -GCS mRNA by low-dose irradiation [10]. Thus, the mechanisms of activation of antioxidative functions have been clarified at the molecular level.

There are many sources of oxidative stress in daily life, such as smoking [11], exercise [12] and radiation [13]. Reactive oxygen species (ROS) may induce many types of diseases, such as Parkinson's disease [14], cataracts [15], atherosclerosis [16], nephrotic syndromes [17], cancer [18],

rheumatoid arthritis [19], ailments associated with ageing [20], stroke [21], and many others [22]. SOD detoxifies superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2), and then catalase converts H_2O_2 into H_2O and O_2 . GSH reacts directly with ROS, and this reaction generates oxidized glutathione (GSSG). GPx catalyzes the destruction of H_2O_2 and hydroxyl radical ($^{\cdot}OH$). However, GR catalyzes the regeneration of GSH from GSSG. Thus, GR and GPx are enzymes in the glutathione regeneration pathway. Oxidative stress induced by ROS can damage cells and lead to cell death. Melatonin, which is a natural antioxidant and free radical scavenger, protects against neurobehavioral and mitochondrial deficits in a chronic mouse model of Parkinson's disease [23]. Another report suggests that the antioxidant resveratrol protects against inflammatory arthritis in rabbits [24]. In addition, CuZn SOD has been reported to protect against focal cerebral ischemic injury [25]. Thus, antioxidants play an important role in protection from ROS-related diseases.

As described above, low-dose irradiation increases various kinds of antioxidants. Therefore, activation of antioxidative functions induced by low-dose irradiation likely inhibits several types of oxidative damage. In fact, 0.5 Gy irradiation inhibits ferric-nitritotriacetate (Fe^{3+} -NTA)-induced hepatopathy [26–27], and carbon tetrachloride (CCl_4)-induced hepatopathy in mice [28–29]. Irradiation with 0.5 Gy of γ -rays has been reported to induce endogenous antioxidative potency in the brain of mice and to inhibit 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced brain damage [30]. In addition, treatment with 0.5 Gy of γ -rays suppresses progression of type I diabetes in non-obese diabetic (NOD) mice [31]. These findings suggest that activation of antioxidative functions induced by low-dose irradiation protects animals from oxidative damage in several organs.

To further clarify these effects, my colleagues and I have examined whether low-dose irradiation or radon inhalation inhibits oxidative damage in mice. This paper reviews new insight into inhibition of ROS-related diseases with low-dose irradiation or radon inhalation.

Activation of antioxidative functions by low-dose X-irradiation and inhibition of free radical- or ROS-induced damage

Effects of low-dose X-irradiation before or after CCl_4 -induced hepatopathy in acatalasemic mice

Low-dose γ -irradiation before or after Fe^{3+} -NTA [26–27] or CCl_4 treatment activates antioxidative functions in mouse liver and inhibits hepatopathy [28–29]. These reports indicated that the activation of antioxidative functions that are induced following low-dose irradiation plays an important role in the inhibition or alleviation of hepatopathy induced by free radicals. To further clarify the role of antioxidant enzymes against CCl_4 -induced hepatopathy, we examined the inhibitory effects of pretreatment with low-dose

X-irradiation on CCl_4 -induced hepatopathy in acatalasemic mice (C3H/AnLCs^bCs^b), which have lower catalase activity than normal mice (C3H/AnLCs^aCs^a) [32]. For example, the catalase activities in organs of acatalasemic mice are one-tenth to half the levels found in normal mice [33]. Therefore, acatalasemic mice are likely to be sensitive to oxidative stress. Our results showed that although the catalase activity in the liver of non-treated acatalasemic mice is 50% lower than that in non-treated normal mice, the GPx activity in the liver of non-treated acatalasemic mice is 50% higher than that in non-treated normal mice. Consequently, no significant changes were observed in the lipid peroxide level, which shows the level of oxidative damage, in the liver between acatalasemic and normal mice. These findings suggest that the free radical reaction induced by the lower catalase levels is properly neutralized by high GPx activity. Catalase activity in the liver of both strains significantly increased after 0.5 Gy irradiation, suggesting the activation of antioxidative functions. CCl_4 administration significantly increases glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in the blood and lipid peroxide levels in the liver in both strains of mice, suggesting CCl_4 -induced hepatopathy. However, pathological examination and analysis of blood GOT and GPT activity and lipid peroxide levels in the livers of both strains showed that CCl_4 -induced hepatopathy is inhibited by low-dose irradiation. These findings suggest that the effects of CCl_4 are properly neutralized by high GPx activity and low-dose irradiation in the acatalasemic mouse liver.

Furthermore, to clarify the alleviation of CCl_4 -induced hepatopathy in acatalasemic mice, we examined the effects of low-dose X-irradiation on liver damage after CCl_4 treatment of acatalasemic mice [34]. Although low-dose irradiation accelerated the rate of recovery from CCl_4 -induced hepatopathy in acatalasemic mice, the recovery of the acatalasemic mice was not as good as that of normal mice. These findings suggest that catalase plays an important role in recovery from CCl_4 -induced hepatopathy. In this study, we also examined high-dose X-irradiation after CCl_4 -induced acatalasemic mouse liver damage. However, no recovery of liver damage was observed with 15 Gy irradiation. These findings strongly suggest that low-dose irradiation has positive effects against oxidative damage that are different from high-dose irradiation.

Inhibitory effects of 0.5 Gy X-irradiation prior to ischemia-reperfusion injury or cold-induced brain injury in mice

ROS, such as O_2^- and $^{\cdot}OH$, contribute significantly to the development of edema induced by ischemia-reperfusion or cold injury [35–38]. In fact, several reports suggested that the treatment of SOD inhibits ischemia-reperfusion injury [39] and cold injury [40]. To determine whether low-dose irradiation inhibits oxidative damage in other tissues, we

examined the inhibitory effects of low-dose X-irradiation prior to ischemia-reperfusion injury in mouse paw [41] or cold injury in mouse brain [42]. Our results showed that ischemia-reperfusion increases paw thickness, and cold injury increases the water content in the brain, suggesting paw edema and brain edema. However, low-dose irradiation prior to ischemia-reperfusion or cold injury inhibited paw edema and brain edema. The possible mechanism of the inhibitory effect is that low-dose irradiation activates antioxidative functions and that the ROS generated by ischemia-reperfusion injury or cold injury was reduced by these activated antioxidative functions. In particular, prevention and treatment of brain edema is of clinical importance because severe brain edema increases intracranial pressure, resulting in brain herniation and a decrease in cerebral blood perfusion, which leads to secondary ischemia. Vasogenic edema such as ischemia-reperfusion injury or cold injury is associated with disruption of endothelial cells and a resultant increase in vascular permeability. These findings suggest that low-dose irradiation may protect vascular endothelial cells from ROS-induced damage.

Basic study of active changes in biological function of mouse liver graft in cold storage after low-dose X-irradiation

Ischemia-reperfusion injury is an inevitable result of transplantation and is closely related to the development of non-functional and dysfunctional primary grafts [43]. Our previous report suggested that low-dose irradiation inhibits ischemia-reperfusion injury. Therefore, activation of antioxidative functions in grafts very likely inhibits ischemia-reperfusion injury following transplantation. However, no reports of changes in the antioxidative function of grafts following low-dose irradiation after exenteration have been published. Therefore, we examined changes in antioxidative functions induced in extirpated mouse liver by low-dose X-irradiation [44]. Immediately after irradiation, the graft was stored in preservation solution (Via Span; Bristol-Myers Squibb Co.) at 4°C or in physiological saline solution. The catalase activity of mouse liver stored in preservation solution after 1.0 Gy irradiation, or in saline solution after 0.25 or 0.5 Gy irradiation, was significantly higher than that after sham irradiation. At 24 h after cold storage, catalase activity of mouse livers stored in preservation solution after 1.0 Gy irradiation was significantly higher than in liver stored in saline solution. Moreover, catalase activity in livers stored in preservation solution after sham, 0.25 or 0.5 Gy irradiation was significantly lower than in liver stored in saline solution. The preservation solution contains glutathione, allopurinol, and adenosine. Glutathione and allopurinol prevent cytotoxic injuries from ROS. Therefore, it is likely that ROS induced by X-irradiation are neutralized by glutathione in the solution. The dose at which enhancement of antioxidative function occurs in livers stored in preservation solution

is significantly higher than that required when livers are stored in saline solution. The data presented in this study provide an essential basis for future studies aimed at determining prevention of ischemia-reperfusion injury in livers after transplantation.

Use of radon as medicine

A large number of patients are treated in various countries with traditional spa therapy (e.g. Japan, [45–48] and central Europe [49–50]). 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 and thermal therapy are ROS-related diseases such as arteriosclerosis, osteoarthritis [45], and bronchial asthma [46]. Participants do not bathe in but only stay in a bathroom with a high radon concentration. The room temperature is 42°C, and the room radon concentration is 2080 Bq/m³ (about 100-fold higher than background level). Every 2 days, nasal inhalation of vapor from the hot spring in the room is performed for 40 min in high humidity (90%) conditions. The estimated effective dose of the radon therapy at the Misasa Medical Center is 50–67 μSv. In addition, the radon effect is larger than the thermal effect [47].

Radon therapy is widely performed in central Europe and Russia. Although most papers are written in Russian, German or Dutch, Falkenbach *et al.* reviewed these reports to elucidate whether radon therapy in rheumatic diseases is associated with a significant alleviation of pain compared to another or no intervention in patients suffering from rheumatic disease [49]. For treatment purposes, radon is commonly applied by bathing for about 20 min in water with a radon concentration of 0.3–3 kBq/l or staying in caves or galleries with natural radon concentrations of about 30–160 kBq/m³ for about 1 h [49]. A meta-analysis of controlled clinical trials of radon therapy demonstrated a positive effect of radon therapy on pain in rheumatic diseases [49]. In addition, a report estimated the radon progeny activity in Heilstollen as a Working Level. For the total 10-hour stay, patients were exposed to a cumulative dose of 0.536 Working Level Months [50]. Thus, many clinical studies have been reported, but no data are available explaining why radon inhalation results in positive effects. Therefore, my colleagues and I have been studying the mechanisms of the effects of radon using small animals. Below, I review studies of the effects of radon that have already been demonstrated.

Activation of antioxidative functions by radon inhalation and inhibition of free radical- or ROS-induced damage

Basic study of activation of antioxidation functions in brain, lung, liver and kidney of mice by radon inhalation using a new radon exposure device

Radon inhalation at a concentration of 1000–4000 kBq/m³ for 4 h has been reported to activate antioxidative functions

in rat organs [51]. However, this radon concentration is considerably higher than the concentration in the treatment area of the Misasa Medical Center. To examine whether radon inhalation activates antioxidative functions in brain, lung, liver and kidney in mice, we first developed a trial manufacture of a radon exposure system for small animals [52]. Mice inhaled 400 Bq/m³ or 4000 Bq/m³ radon for up to 2 days with this device. Results showed that in brain, lungs, liver and kidney, both the activities of SOD and catalase increased, and lipid peroxide levels decreased. This suggests that radon inhalation enhances antioxidative functions.

Studying the response of SOD in mouse organs to radon using a new large-scale facility for exposing small animals to radon

As described above, radon inhalation enhances antioxidative functions in brain, lung, liver and kidney of mice [52]. Investigation of the response of antioxidative functions in other organs to radon may contribute to finding new indications for radon therapy. Moreover, dose-dependent or dose rate-dependent changes in antioxidative functions in organs exposed to radon are still unknown. To clarify the effects of dose rate-dependent changes in antioxidative functions in mouse organs exposed to radon, we examined SOD activity in plasma, liver, pancreas, heart, thymus, kidney, brain, small intestine, lung and stomach of mice [53]. Mice were exposed to radon at a concentration of 250, 500, 1000, 2000 or 4000 Bq/m³ for 0.5, 1, 2, 4 or 8 days. Our results suggested that continuous exposure to radon increases SOD activity in most organs, but SOD activity transiently returns to normal levels at around 2 days. When mice were exposed to radon at 2000 day•Bq/m³ (0.5 day–4000 Bq/m³, 1 day–2000 Bq/m³, 2 days–1000 Bq/m³), the peak in SOD activity occurred around 0.5 days after radon inhalation, suggesting that activation of SOD activity induced by radon inhalation has a dose-rate effect under these exposure conditions. In addition, our data suggested some new indications for radon treatment. Specifically, radon inhalation is very likely to inhibit brain disorders induced by ROS.

Radon inhalation protects mice from CCl₄-induced hepatic and renal damage

Our previous study demonstrated that low-dose X-irradiation inhibits CCl₄-induced hepatopathy [32] and that radon inhalation activates antioxidative functions in liver and kidney in mice [52–53]. Although hepatic and renal damage are not the main indication for radon therapy, radon therapy may mitigate liver and kidney damage due to activation of antioxidative functions in liver and kidney. In this study, we assessed whether radon inhalation provided protection from CCl₄-induced hepatic and renal damage in mice. We also attempted to shorten the inhalation time because radon inhalation for 24 or 48 h is unsuitable for medical treatment.

Therefore, mice inhaled a higher concentration of radon (18 000 Bq/m³, 6 h) than in our previous study [54]. Results showed that radon inhalation inhibited CCl₄-induced hepatic and renal damage in mice due to activation of antioxidative functions in liver and kidney, similar to low-dose X-irradiation. The data presented in this study provide a substantial basis for future studies aimed at assessing new radon-based therapies for treatment of hepatic and renal damage in humans.

Inhibitory effects of pretreatment with radon on acute alcohol-induced hepatopathy in mice

We previously reported that CCl₄ induces hepatopathy [54]; however, humans are unlikely to suffer from CCl₄-induced hepatopathy. In contrast, alcohol-induced hepatopathy is a common contemporary disease. Therefore, we assessed whether pretreatment with radon inhibits acute alcohol-induced hepatopathy in mice [55]. Results showed that pretreatment with radon inhibits the depression of hepatic functions and antioxidative functions. These findings suggested that radon inhalation activates antioxidative functions in the liver and inhibits acute alcohol-induced hepatopathy in mice.

Comparative study of the inhibitory effects of radon inhalation before and after CCl₄-induced oxidative damage in mouse organs

Although radon therapy is performed for treatment rather than preventive purposes, the effects of radon inhalation for alleviating oxidative damage have never been examined; therefore, there is no comparative study on the effect of radon inhalation before and after oxidative damage. In this study, we compared the differences between radon inhalation before and after CCl₄-induced oxidative damage in brain, heart, lung, liver and kidney of mice [56]. Our results showed that CCl₄ administration increases lipid peroxide levels in brain, heart, lung, liver and kidney, suggesting oxidative damage. We expected that radon inhalation before CCl₄ treatment would be more effective than radon inhalation after CCl₄ treatment, because antioxidants react with ROS before organs are subjected to oxidative stress; however, no significant differences in lipid peroxide levels in the brain, heart, lung, liver and kidney were found between radon-inhalation before CCl₄ treatment of mice and radon-inhalation after CCl₄ treatment of mice. In contrast, radon inhalation increased the total glutathione (t-GSH; GSH + GSSG) content in brain, heart, lung, liver and kidney, suggesting activation of antioxidative functions. These findings suggest that radon inhalation after CCl₄ treatment has the same effects as radon inhalation before CCl₄-induced oxidative damage in the brain, heart, lung, liver and kidney, possibly because the GSH-redox cycle could have been an important factor in the recovery from CCl₄-induced oxidative damage.

Comparative study of the inhibitory effects of antioxidant vitamins and radon on CCl₄-induced hepatic and renal damage

We previously reported that radon inhalation activates antioxidative functions in liver and kidney in mouse and inhibits oxidative damage [54–56]. However, to date, no quantitative reports on the anti-oxidative effects of radon have been reported, and thus, the optimal conditions for radon therapy remain unknown. The purpose of this study was to compare the antioxidative effects of radon (1000 or 2000 Bq/m³) and antioxidant vitamins such as ascorbic acid (vitamin C) and α -tocopherol (vitamin E) [57]. To assess the antioxidative effects of radon, we used a CCl₄-induced liver injury model. We estimated the inhibitory effects on CCl₄-induced hepatopathy based on hepatic function-associated parameters (GOT, GPT, triglyceride, total cholesterol), oxidative damage-associated parameters (lipid peroxide level), and histological changes (necrosis, fatty liver). The results revealed that the activities of SOD, catalase and GPx in the liver were significantly higher in mice exposed to radon than in mice treated with CCl₄ alone. In contrast, treatment with ascorbic acid or α -tocopherol did not result in an increase in SOD, catalase, GPx or t-GSH in the liver. The therapeutic effects of radon inhalation were almost equivalent to treatment with ascorbic acid at a dose of 500 mg/kg or α -tocopherol at a dose of 300 mg/kg due to activation of antioxidative functions.

In addition, we also evaluated the quantitative effects of the activation of antioxidative activities in kidney induced by radon inhalation on CCl₄-induced renal damage [58]. The activities of SOD and catalase in kidneys were significantly higher in mice exposed to radon compared to mice treated with CCl₄ alone. In contrast, treatment with α -tocopherol did not result in an increase in SOD or catalase in the kidney. In the case of renal function, radon inhalation at a concentration of 2000 Bq/m³ has inhibitory effects similar to α -tocopherol treatment at a dose of 300–500 mg/kg body weight.

Radon inhalation-induced suppression of Streptozotocin-induced type I diabetes in mice

Radon hot springs have long been used to treat diabetes. However, few reports have been published of a protective effect of radon inhalation on diabetes. One report suggested that radon inhalation increases blood insulin levels in rabbits and thus promotes glycogen synthesis [59]. However, the increase in blood insulin levels after radon inhalation does not provide direct evidence because the rabbits do not have diabetes. Streptozotocin (STZ) has been used as a model for type I diabetes in mice. STZ is a nitric oxide (NO) donor, and NO partially restricts adenosine triphosphate (ATP) generation in mitochondria and increases xanthine oxidase, which catalyzes the synthesis of

superoxide anion radicals [60–61]. As a result, H₂O₂ and \cdot OH are formed [61]. These radicals attack β cells that produce insulin, which increases blood glucose. To clarify whether radon inhalation suppresses type I diabetes associated with ROS, we examined the protective effect of radon inhalation on STZ-induced type I diabetes in mice [62]. Results showed that STZ administration induced characteristics of type I diabetes such as hyperglycemia and hypoinsulinemia; however, pretreatment with radon at doses of 1000 and 5500 Bq/m³ significantly suppressed the elevation of blood glucose. Serum insulin was significantly higher in mice pretreated with radon at a dose of 1000 Bq/m³ than in sham-treated mice. A significant decrease in the mean size of pancreatic islets was observed in mice pretreated with radon inhalation compared with the control mice; however, pretreatment with radon at doses of 1000 and 5500 Bq/m³ significantly suppressed pancreatic islet damage. In addition, SOD activities and t-GSH contents were significantly higher, and lipid peroxide was significantly lower in mice pretreated with radon at doses of 1000 and 5500 Bq/m³ compared with sham-treated mice. These findings suggest that radon inhalation suppresses STZ-induced type I diabetes. The possible mechanism of the inhibitory effect is that dismutation of ROS by activated antioxidative functions protected pancreatic islets and that insulin production was increased.

Radon inhalation has anti-inflammatory effects Protective effects of radon inhalation on carrageenan-induced inflammatory paw edema in mice

We previously reported that radon inhalation has antioxidative effects [52–58, 62]. However, no reports on anti-inflammatory effects of radon inhalation in mice have been published. The purpose of this study was to determine whether radon inhalation has anti-inflammatory effects in mice [63]. Radon inhalation significantly increased SOD and catalase activities and significantly decreased lipid peroxide levels in mouse paws, indicating that radon inhalation activates antioxidative functions. Carrageenan administration increased paw volume and significantly increased tumor necrosis factor-alpha (TNF- α) and NO in serum, suggesting that carrageenan induces inflammatory paw edema. However, radon inhalation significantly reduced carrageenan-induced paw edema. In addition, in mice injected with carrageenan, serum TNF- α levels were lower in the radon-treated mice than in sham-treated mice. Carrageenan triggers the expression of inducible NO synthase, a process that occurs, at least in part, via activation of nuclear factor κ B. NO combines with O₂ to yield peroxynitrite (ONOO⁻) [64]. Another mechanism of production of ROS is polymorphonuclear leukocyte infiltration and activation that induces O₂⁻ and H₂O₂ production [64]. The \cdot OH and ONOO⁻ radicals induce cellular injury. SOD and

catalase activities in paws were significantly higher in radon-treated mice than in sham-treated mice. These findings indicate that radon inhalation has anti-inflammatory effects via ROS dismutation.

Radon inhalation provides pain relief *Antinociceptive effects of radon inhalation on formalin-induced inflammatory pain in mice*

Our previous study demonstrated that radon inhalation has anti-inflammation effects [63]. However, no reports have been published on whether radon inhalation reduces the pain associated with formalin-induced inflammation. In the present study, we investigated the antinociceptive effects of radon inhalation in a mouse model of formalin-induced inflammatory pain [65]. Formalin injection induces a transient biphasic pain, namely the first phase and the second phase of the response. The first phase is a direct stimulation of sensory nerve endings by formalin, resulting in acute pain. The second phase is an inflammatory response, resulting in persistent pain [66–67]. We examined whether radon inhalation inhibits the second phase. Results showed that radon inhalation at a concentration of 2000 Bq/m³ significantly inhibited the licking response time in the second phase, but it was not effective in the first phase of the response. These

findings indicate that radon inhalation inhibits inflammatory pain. To clarify the mechanisms underlying the inhibitory effect of radon inhalation on formalin-induced inflammatory pain, TNF- α and NO in serum, leukocyte migration in paws, and antioxidant-associated substances such as SOD, catalase and t-GSH were examined. Formalin administration significantly increased TNF- α and NO in serum and leukocyte migration in paws, suggesting that formalin administration induces inflammation. In mice injected with formalin, serum levels of TNF- α and NO and leukocyte migration in paws were lower in the radon-pretreated mice (2000 Bq/m³) than in sham-treated mice, suggesting that radon inhalation has anti-inflammatory effects. In mice injected with formalin, SOD activity and t-GSH content were increased by radon pretreatment, especially at a concentration of 2000 Bq/m³. These results suggest that radon inhalation has anti-inflammatory, antinociceptive, and antioxidative effects in the periphery, including serum and the paws.

Preventive and curative effects of radon inhalation on chronic constriction injury-induced neuropathic pain in mice

Pain-related diseases are the main indications for radon therapy. However, no studies have been reported regarding

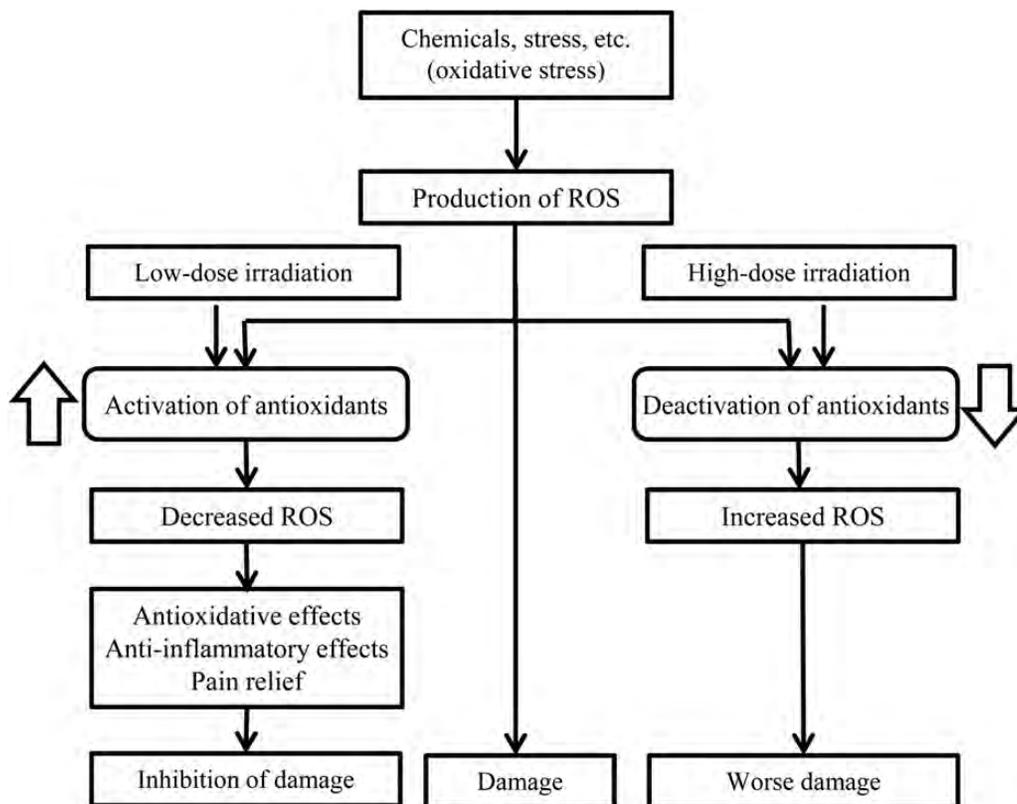


Fig. 1. Mechanisms of inhibition of low-dose X-irradiation or radon inhalation on oxidative damage, inflammation and pain induced by ROS.

the effects of radon on neuropathic pain in any organism, and the mechanisms by which radon mediates pain relief have not been fully clarified. Therefore, the purpose of this study was to determine whether radon inhalation before or after chronic constriction injury (CCI) produces remission of neuropathic pain [68]. Results showed that pretreatment with radon inhalation at a concentration of 2000 Bq/m³ significantly inhibited CCI-induced neuropathic pain on the third day, and the effect remained on the seventh day after CCI surgery. In contrast, radon inhalation at a concentration of 2000 Bq/m³ after CCI treatment inhibited neuropathic pain on the third day after surgery, but these effects lasted only until the fifth day after radon inhalation. In summary, pretreatment with radon had longer-lasting effects on neuropathic pain than post-treatment. To clarify the mechanism underlying the preventive and curative effects of radon inhalation on CCI-induced neuropathic pain, we examined plasma levels of norepinephrine (NE), TNF- α , and NO, as well as paw histology. Numerous studies have suggested that nerve injury-induced inflammatory responses, such as increased TNF- α levels, migration of inflammatory leukocytes [69–70], and expression of NE, play an important role in neuropathic pain [71]. Our results showed that the plasma NE level with both pretreatment and post-treatment with radon inhalation was significantly reduced compared with sham inhalation, suggesting that radon inhalation

inhibited central sensitization of NE, or increased the extracellular concentration of NE via a descending inhibitory system such as the serotonin/norepinephrine reuptake inhibitor. Moreover, CCI surgery increased both plasma TNF- α levels and the migration of inflammatory leukocytes in paws. This increase in TNF- α was significantly reduced after pretreatment with 2000 Bq/m³ radon, whereas leukocyte migration was decreased by both pre- and post-treatment with radon at concentrations of 1000 and 2000 Bq/m³. Furthermore, O₂⁻ production has been suggested to play a major role in the development of pain in peripheral nerves and to induce the release of various cytokines and ONOO⁻ [72]. Therefore, we also examined antioxidative functions such as SOD activity and t-GSH content. Our results showed that plasma SOD activity was reduced by CCI surgery and tended to increase with radon inhalation before or after CCI surgery. These findings suggest that dismutation of ROS by activation of antioxidative functions results in reduction of CCI-induced neuropathic pain in mice.

Other reports of preventive effects of low-dose irradiation on several types of damage

Several recent reports have suggested that low-dose irradiation inhibits several types of diseases. For example, low dose-rate γ -irradiation ameliorates type II diabetes by

Table 1. Summary of our studies on antioxidative effects, anti-inflammatory effects and pain relief by radon inhalation in mice

Organs	Antioxidants	Effect	Experimental model	References
Brain	SOD \uparrow , Catalase \uparrow , t-GSH \uparrow	Antioxidative effect	CCl ₄	[52, 53, 56]
Lung	SOD \uparrow , Catalase \uparrow , t-GSH \uparrow	Antioxidative effect	CCl ₄	[52, 53, 56]
Thymus	SOD \uparrow	Antioxidative effect	–	[53]
Heart	SOD \uparrow , Catalase \uparrow , t-GSH \uparrow	Antioxidative effect	CCl ₄	[53, 56]
Liver	SOD \uparrow , Catalase \uparrow , t-GSH \uparrow , GPx \uparrow , GR \uparrow	Antioxidative effect	CCl ₄ Alcohol	[52–54, 56, 57] [55]
Stomach	No change	–	–	[53]
Pancreas	SOD \uparrow , Catalase \uparrow , t-GSH \uparrow	Antioxidative effect	STZ	[53, 62]
Kidney	SOD \uparrow , Catalase \uparrow , t-GSH \uparrow , GPx \uparrow , GR \uparrow	Antioxidative effect	CCl ₄	[52–54, 56, 58]
Small intestine	SOD \uparrow	Antioxidative effect	–	[53]
Plasma	SOD \uparrow	Antioxidative effect	–	[53, 63, 65]
Paw	SOD \uparrow , t-GSH \uparrow	Antioxidative effect	Carageenan	[63, 65]
		Anti-inflammatory effect		
		Anti-inflammatory effect	Formalin	[65]
		Pain relief		
		Pain relief	CCI	[68]

SOD = superoxide dismutase, t-GSH = total glutathione, GPx = glutathione peroxidase, GR = glutathione reductase, CCl₄ = carbon tetrachloride, STZ = streptozotocin, CCI = chronic constriction injury.

maintaining insulin secretion. The possible mechanism of the inhibitory effect indicates that low dose-rate γ -irradiation increases SOD activity in the pancreas [73]. Moreover, continuous low dose-rate γ -irradiation ameliorates diabetic nephropathy and increases the life span in db/db mice through the activation of renal antioxidants [74]. In addition, low-dose γ -ray irradiation has been reported to attenuate collagen-induced arthritis by suppressing pro-inflammatory cytokines and autoantibody production and by inducing regulatory T cells [75]. Furthermore, repeated γ -ray irradiation attenuates collagen-induced arthritis by up-regulating regulatory T cells [76].

CONCLUSION

Fig. 1 shows the possible mechanism of the inhibition of several types of damage by low-dose irradiation. Low-dose irradiation including radon inhalation activates antioxidative functions in several organs in mice and inhibits ROS-induced damage. In contrast, high-dose irradiation deactivates the antioxidative functions, resulting in worse damage. As shown in Table 1, radon inhalation activates some types of antioxidant-associated molecules, such as SOD, catalase, t-GSH, GPx and GR, in many organs. These findings suggest that radon therapy is applicable in many diseases. In particular, pretreatment with radon inhalation is useful for protecting against ROS-related damage.

Radiation is routinely used in medical care, such as diagnostic imaging and radiation therapy. Low-dose irradiation is used in diagnosis because of its transmission and photographic effects. In contrast, high-dose irradiation is used in radiation therapy. Our recent studies have demonstrated that radon inhalation activates antioxidative functions in mice and inhibits several types of damage. These findings suggest that other possible applications for low-dose or low-dose rate irradiation may exist in medicine. In particular, radon inhalation is highly likely to be beneficial in liver diseases.

Estimating the radiation dose for radon treatment is important. We have reported the doses absorbed by organs due to inhalation of radon itself [77] and lung dosimetry of inhaled radon in progeny in mice [78]. From these data, we next need to evaluate whether radon therapy is beneficial to patients with various diseases. In addition, further studies on the molecular mechanisms of radon therapy are needed.

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