Recent studies on anti-inflammatory effects of radon Inhalation in mice

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Introduction

Large numbers of patients are treated with traditional spa therapy in various countries. Specifically, radon spa therapy is used to treat respiratory diseases [1,2,3] or pain-related diseases [2,3,4] at Misasa Medical Center, Okayama University Hospital. The treatments with radon spa therapy are to drink radon hot spring water, to take radon bath, to walk in radon pool, and to inhale radon gas. The radon concentration in the room for radon inhaled treatment is around 2,000 Bq/m³ and, the room is hot (around 45°C) and humid (around 90%) [1,4]. A similar treatment is performed using health mines in Montana, United States [5]. Patients can sleep, sit, read, and talk with one another during their visit to the mine. The radon concentration in the mine is approximately 50,000 Bq/m³, the temperature is around 15 °C, and the humidity is

Radon therapy, which has been performed in Misasa (Japan) and Badgastein (Austria), was found to have a beneficial effect on pain-related diseases. Although several clinical studies of the curative effects on pain related diseases have been reported, the mechanism remains to be incompletely elucidated. In order to further clarify the mechanism, we developed radon exposure systems for small animals. In the present paper, we review several studies on the anti-inflammatory effects of radon inhalation in mice. We first examined whether radon inhalation inhibits carrageenan-induced inflammatory paw in mice. Radon concentration in mouse cage was approximately 2000 Bq/m³, which is similar to the level of radon therapy at Misasa Medical Center, Okayama University Hospital. Although carrageenan administration into right hind paw of mice induced paw edema, radon inhalation inhibited the edema. Antioxidants, such as superoxide dismutase (SOD) in paw, of radon inhaled mice were significantly higher than sham inhaled mice. Since the development of carrageenan-induced inflammation is mediated by reactive oxygen species (ROS), the inhibition of paw edema by radon inhalation is probably due to activation of antioxidant functions. We next examined the effects of radon inhalation on dextran sulfate sodium (DSS)-induced colitis in mice. Results showed that radon inhalation inhibited the damage caused by DSS-induced colitis. In addition, the mediators of inflammatory response such as tumor necrosis factor-alpha (TNF-α) were inhibited, and antioxidants such as SOD were increased by radon inhalation. Next, we examined the effects of radon inhalation on formalin-induced inflammatory pain in mice. Results showed that radon inhalation inhibited the licking response time. TNF-α, activated by formalin-induced inflammation, was lower in the radon inhaled mice than in the sham inhaled mice. Antioxidant activities such as SOD activity were increased in the mice that inhaled radon. These findings indicated that radon inhalation inhibits several types of inflammation in mice due to activation of antioxidant functions.

Keywords: radon therapy; anti-inflammation; reactive oxygen species; antioxidant function

not high. However, the expert advice of the Environmental Protection Agency in the United States is that the amount of exposure to radon should be reduced to as low as possible because it is considered to be the second leading cause of lung cancer and the leading environmental cause of cancer mortality. Although the American Medical Association, the Environmental Protection Agency, and the medical profession in general do not recommend radon therapy, the therapy is recognized as an evidence-based treatment in Europe. Concerns about the cancer risks of indoor radon contamination in Europe are similar to those in the United States. Badgastein is situated at the northern rim of the Hohe Tauern National Park in Austria [5]. In the mine, the concentration of radon is approximately 45,000 Bq/m³, hot (around 40ºC) and humid (up to 95%). The combination of heat, humidity and radon is particularly effective for chronic rheumatic conditions of the muscles, tendons and joints. Moreover, health insurance in Austria covers up to 90% of the cost [5].

In any of these cases, the radon therapy treatment is for pain-related diseases such as rheumatism [5,6,7]. Although several clinical studies on the curative effects for pain related diseases have been reported, the mechanism is not well understood. To clarify the mechanism, some radon exposure systems have been developed [8,9]. The objective of this work was to review the latest knowledge regarding the anti-inflammatory effects of radon inhalation.

**Physiological Effects of Radon**

Radon is an inert, noble gas. Radon is dissolved into blood, and is distributed to cells and organs. Radon enters blood by gas exchange in lung, by absorption through skin, and by drinking water containing radon. α-rays (5.49MeV), which are emitted by the radioactive decay of radon, induce the production of reactive oxygen species (ROS) and cause DNA damage in the body [10]. The effective half-life of radon is approximately 30 min [11,12].

The absorbed dose rate of radon through lung to the main organs and tissues was calculated 0.04 - 1.4 nGy/(Bq/m³)/day in mice, rats and humans. Specifically, it is estimated that red bone marrow receives a higher dose than other tissues because radon has lipid solubility. There is not much difference in the absorbed dose among other organs [13].

**Effects of Antioxidant and Anti-inflammatory by Radon Inhalation**

1) **Inflammation and Oxidative Stress**

Inflammation is an immune reaction against foreign substances from outside the body such as viruses. Activated neutrophils roll and adhere to the vascular endothelial wall, and infiltrate outside the blood vessel. Activated neutrophils and macrophages eat pathogens voraciously at inflamed areas. Tumor necrosis factor-alpha (TNF-α) and ROS which are produced by macrophages activate nuclear factor-kappa B (NF-κB) [14]. NF-κB is an activated gene associated with inflammation. TNF-α is a kind of cytokine related inflammation and biological defense system, and is produced from activated macrophages by various stimuli. Cytokines are proteins that condition physiology, such as immunity and

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**Figure 1. Activated Neutrophil Infiltrate to Outside Blood Vessel.** Activated neutrophils and macrophages consume pathogens voraciously at the inflamed area. TNF-α and ROS are produced by macrophages, and activated NF-κB. NF-κB is an activated gene associated with inflammation.
wound healing. TNF-α is produced in quantity at the inflammation focus, and becomes a factor in the deteriorating condition\cite{14,15} (Fig. 1).

Likewise, ROS are produced when phagocytosis cells, such as neutrophils and macrophages, consume pathogens voraciously. ROS constantly play important roles in a variety of normal biochemical functions, but excess ROS damages healthy organs and cells\cite{16}. ROS, such as superoxide anion (O\textsuperscript{2−}), hydroxyl radicals (·OH) and nitric oxide (NO), are defined as short-lived oxygen species and have high oxidative-related reactivity. ROS are generally removed by antioxidants, such as superoxide dismutase (SOD), and maintain a delicate balance between ROS and antioxidants. Antioxidants inhibit damage to healthy organs and cells induced by excess ROS. SOD is an enzyme that catalyzes the disproportionation reaction of superoxide into hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). Hydrogen peroxide is rapidly detoxified by catalase (CAT) and glutathione (GSH) (Fig. 2) \cite{17,18,19}. Excess ROS produced by inflammatory responses cause oxidation of lipid, protein and nucleic acid, and lead to cell dysfunctions. Therefore, inflammation is closely linked to oxidative stress.

2) Possible Mechanisms of Anti-inflammatory Effect of Low-dose Irradiation

As shown in Fig. 1, activated neutrophils cause inflammation and oxidative stress, and various stimuli in a vicious circle, while also rolling on the vascular endothelial wall. Low-dose irradiation gives a small stimulus and enhances antioxidant effects\cite{20}. If the antioxidant effects are enhanced by radon inhalation, excess ROS are detoxified. This reduces the activated neutrophils by NF-κB. NF-κB is an activated gene associated with inflammation. Enhanced antioxidative effects inhibit the activation of ROS and NF-κB. Finally, it inhibits the inflammatory response.

Studies on Anti-inflammatory Effects of Radon Inhalation in Mice

1) Protective Effect of Radon Inhalation on Carrageenan-induced Inflammatory Paw Edema\cite{21}

It has been generally recognized that cutaneous injection of cagrrageenan induces inflammation. To assess the effects of radon inhalation for carrageenan induced-inflammatory paw edema, which is considered to be useful as an inflammatory model, mice were treated with sham or radon inhalation at a concentration of 2,000 Bq/m\textsuperscript{3} for 24 hours. Then, carrageenan was injected into right hind paw of each mouse.

Results showed that the paw volume increased after carrageenan injection. However, the paw volumes of mice which had inhaled radon significantly decreased at all points (1, 2, 3, 4 or 5 hours after carrageenan administration) after carrageenan injection. To assess the effects of radon inhalation on anti-inflammatory responses and antioxidant functions, we examined the levels of nitric oxide (NO), TNF-α, SOD, CAT and lipid peroxide in serum and paw. Not only SOD activity in serum, but also the activities of SOD and CAT in paw, were significantly higher in radon-exposed mice than in control mice. In addition, the lipid peroxide levels in paws were significantly lower in radon-exposed mice than in control mice. These findings suggested that radon inhalation activates antioxidant functions. Moreover, the levels of NO and TNF-α in serum were significantly higher in carrageenan administered mice than in control mice, indicating inflammation. The levels of TNF-α and NO in serum were higher in carrageenan administered mice than in radon and carrageenan treated mice. These findings suggested that radon inhalation inhibits carrageenan-induced inflammatory paw edema in mice. Moreover, the levels of SOD, CAT and total-GSH (t-GSH) in paws were significantly lower in sham mice than in control mice. The activities of SOD and CAT in paws and the SOD activities in serum were significantly higher in radon and carrageenan treated mice than in carrageenan administered mice. In addition, migration of inflammatory leukocytes in paw of mice was induced by carrageenan administration in the presence of absence of radon inhalation. However, the migration of inflammatory leukocytes was inhibited by radon inhalation. These results suggested that radon inhalation has a protective effect on carrageenan-induced inflammatory paw edema in mice due to the activation of antioxidant functions following radon inhalation.

2) Suppression of Dextran Sulfate Sodium-induced Colitis by Radon Inhalation\cite{22}
On account of having similarities to human ulcerative colitis, dextran sulfate sodium (DSS) induced colitis is recognized as a good experimental model of colitis. We examined the inhibitory effects of radon inhalation on DSS-induced colitis. Mice were divided into 4 groups: sham inhalation (control group), radon inhalation (Rn group), DSS administration without radon inhalation, and DSS administration with radon inhalation. Mice in each group were treated with air only or radon of 2,000 Bq/m$^3$ for 8 days. In mice of DSS administration with or without radon inhalation that were administrated 3% DSS replacing normal drinking water for the last 7 days.

Results showed that the disease activity index (DAI) score was almost 0 in the control and Rn groups, indicating that radon inhalation does not induce DSS-induced colitis. In the DSS administration with or without radon inhalation, the DAI scores significantly elevated at the first day. However, the elevation of the DAI scores on day 3 to 7 were inhibited by radon inhalation. Although DSS administration significantly decreased body weight gain at the first day, radon inhalation significantly inhibited the decrease of the rates on day 3 to 7. In the DSS administration with or without radon inhalation, the colon length was significantly shorter in the DSS administration with or without radon inhalation than in the control group. However, the length was significantly longer in the DSS administration with radon inhalation than in the DSS administration without radon inhalation. The histological damage score of mice in the DSS administration with or without radon inhalation was significantly higher than the score of mice in the control group. However, radon inhalation significantly inhibited the increase of the score. Although the number of goblet cells per unit was decreased by DSS administration, radon inhalation inhibited the decrease of the number. These results indicated that radon inhalation inhibits DSS-induced colitis. In addition, the anti-inflammatory effects and antioxidant effects of radon inhalation were characterized by assaying the mediators of the inflammatory response, such as TNF-α and NO, an enzyme involved in inflammation response related neutrophils, such as MPO, antioxidants, such as SOD, CAT, and t-GSH, and an indicator of oxidative damage. We revealed that radon inhalation enhanced the antioxidant effects in colon and suppressed DSS-induced colitis.

3) Anti-nociceptive Effect of Radon Inhalation on Formalin-induced Inflammatory Pain [23]

Formalin injection induces characteristic biphasic pain response; the first phase and the second phase responses. The first phase is an acute pain, which is caused by direct activation of primary afferent sensory neurons, and the second is an inflammatory response indicating persistent pain. To assess the anti-nociceptive effect of radon inhalation on formalin-induced inflammatory pain, the mice were divided into 3 groups. Mice were treated with radon inhalation at background (BG) levels (atmospheric air, 19 Bq/m$^3$), at a concentration of 1,000 Bq/m$^3$, and at a concentration of 2,000 Bq/m$^3$ for 24 hours. Immediately after radon inhalation, 20 µl of 1.35% formalin (0.5% formaldehyde in saline) was injected subcutaneously into the plantar surface of one hind paw.

Results showed that licking response time was significantly inhibited in the 2,000 Bq/m$^3$ radon inhalation group from 25 to 30 min. There were no significant differences in the levels of TNF-α and NO in serum, and inflammatory leukocytes in paws between the control and two radon inhalation groups. The activities of SOD in serum and paw and CAT were higher in the radon inhalation groups than in the control group. In contrast, the lipid peroxide levels were significantly reduced by 2,000 Bq/m$^3$ radon inhalation compared with the other groups. These findings suggested that radon inhalation activates antioxidant functions. The levels of TNF-α and NO in serum were significantly increased by formalin injections (BG vs. control), and were significantly lower in the radon inhalation groups than in the BG group. Formalin administrations increased the leukocyte migration in paw irrespective of the presence or absence of radon inhalation (BG vs. control). The leukocyte migration in paws was significantly lower in the radon inhalation groups than in the BG group. These findings indicated the effects of radon inhalation on anti-inflammatory and anti-nociceptive functions following formalin administration. SOD activities and t-GSH contents in serum and paw, and CAT activities in paw were significantly lower in mice following formalin administration than in the control mice (BG vs. control). On the other hand, these were significantly increased in the radon inhalation groups compared to the BG group. The lipid peroxide level in paw was increased by formalin administration (BG vs. control) and inhibited by 2,000 Bq/m$^3$ radon inhalation (2,000 Bq/m$^3$ vs. BG). These findings indicated that formalin-induced pain was significantly reduced by radon treatment. Therefore, these findings suggested that radon inhalation had anti-inflammatory and anti-nociceptive effects.

Conclusions

As can be seen from the previously described results, it is possible that radon inhalation inhibits several types of inflammation in mice due to the activation of antioxidant functions. The possible mechanism of the inhibitory effects is detoxification of ROS induced by inflammation. Radon therapy is performed for pain related diseases. Specifically, radon inhalation probably inhibits rheumatic pain due to
anti-inflammatory effects mediated by the detoxification of ROS. However, the mechanisms of the activation of antioxidant functions by radon inhalation have yet to be confirmed. They provide a substantial basis for future studies aimed at assessing the detailed mechanisms of the inhibitory effects of inflammation.

References


