Activation of Antioxidant System by Low Dose Radiation and Its Applicable Possibility for Treatment of Reactive Oxygen Species-Related Diseases

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Summary We clarified that adequate oxygen stress induced by low dose irradiation activates not only chemical biological protective function, such as induction of the synthesis of superoxide dismutase, glutathione peroxidase, and heat shock protein 70, but also the biomembrane function, such as enhanced membrane fluidity and ATPase activity. It is possible that activation of these mechanisms alleviates *in vivo* oxidation injuries resulting in alleviation of pathologic condition, such as symptoms of hepatopathy and diabetes mellitus. Namely, adequate activation of the functions of the living body by low dose irradiation can contribute to suppressing aging and to preventing or reducing reactive oxygen species related diseases which are thought to involve peroxidation and have been regarded as the diseases for which radon spring water is an effective treatment. Clarification in detail of the mechanisms of these phenomena is required to understand the effects of low dose irradiation or radon inhalation on the functions of the living body, including adaptive response.

Key Words: antioxidant system, low dose irradiation, radon inhalation, reactive oxygen species related diseases, adaptive response

Introduction

Excessive reactive oxygen species (ROS) produced *in vivo* by various causes, such as excessive stress, are toxic. Accumulation of oxidation injuries due to excessive active oxygen causes cell and tissue injuries, inducing various pathologic conditions such as aging and carcinogenesis. While, there are chemical defense mechanisms in the body that eliminate ROS or repair damaged molecules, defending against resultant injury [1]. It is interesting reports that appropriate oxidative stress activates the chemical biological

defense mechanisms. In this study, to elucidate these phenomena and its mechanism by low dose irradiation, we examined on the effects of low dose irradiation on chemical biological defense mechanisms, structure and function of biomembranes, and vital oxidative injury.

Radon (^{222}Rn) is a radioactive gaseous element that emits α -particle. If radon is inhaled, the lung will be subjected to the action of free radicals created by the radiation and may suffer inflammation. Although radon inhalation has been thought to be hazardous in general, radon springs have been reported to have therapeutic effects on senile brain disorders and hypertension [2]. Another known effect of radon spring is to promote the effects of such tissue perfusion agents as adrenaline in plasma, that is to say the level of plasma adrenaline is increased by radon inhalation [3]. So far there are no epidemiologic data on the hazardous effects of radon [4]. In this study, we also examined as will be seen later to clarify the mechanisms of therapeutic effects (e.g. clinical

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indications for Misasa hot spring, a radon spring, include hypertension, diabetes mellitus and pain) of low dose radon.

These representative results and discussion are outlined in the following.

Activation of Chemical Biological Defense Mechanisms by Low Dose Irradiation

Effects of low dose Irradiation on SOD activity, lipid peroxide level, and membrane fluidity

Rats were irradiated with low dose X-ray over their wholebodies. The following results were obtained. Unlike high dose X-ray irradiation, the superoxide dismutase (SOD) activity was elevated, suggesting that X-ray irradiation at doses of 0.25–0.5 Gy activate the host defensive function. These changes were particularly marked in the organs related to immune functions of the animals which received low dose X-ray. Moreover, low dose X-ray irradiation reduced lipid peroxide (thiobarbituric acid (TBARS)) levels and made the state of the SH-group on membrane-bound proteins closer to that of juvenile animals, although the sensitivity to radioactivity varied depending on the age of the animals and among different organs and tissues (Fig. 1) [5, 6]. It was also found that these changes continued for longer periods after 0.25 Gy X-ray irradiation. Namely, the SOD activities in the spleen showed a persistent radiation-induced increase for at least 12 weeks, livers for 8 weeks, brains and thymuses for 4 weeks, and bone marrows for about 1 week (Fig. 2-1). The TBARS levels in the brain and thymus showed persistent decreases due to irradiation for at least 12 weeks, and those in bone marrows for 8 hr (Fig. 2-2) [7].

In the same manner, we examined the effects of vitamin E (vit.E) and low dose X-ray irradiation on lipid peroxide (TBARS) level in human embryonic cells under various doses of irradiation. As a result, in comparison with controls, the TBARS levels in cells decreased by 10% under addition of vit.E and non irradiation. The TBARS levels in cells, like some organs and tissues of rats, decreased by 20–60% at least for 1–24 hr after irradiation at doses of 0.1–1 Gy under non addition of vit.E, but conversely increased under addition of vit.E [8].

Induction of two species of SOD and glutathione synthesis-related proteins by low dose irradiation

Four hours after 0.25 Gy X-ray irradiation, the Cu/Zn-SOD activity in the spleen of mature rats showed a significant quantitative increase; this was accompanied by a marked increase in the mRNA for this enzyme as compared to the control (sham irradiated) group. Irradiation had no effect on the Mn-SOD activity. Moreover, the expression of the mRNA for Mn-SOD was similar to that of the unirradiated control group. On the other hand, the activity of both Cu/Zn-SOD and Mn-SOD in the liver of fetal rats showed a

Fig. 1. Dose- and aging-dependent changes in SOD activity, lipid peroxide level, and membrane fluidity (W/S ratio) in brain cortex of wistar rats at 4 hr after X-ray irradiation. ■ shows the data from sham irradiated 7 weeks-old control, and each value indicates the mean $\pm$ SEM. Significance: * p <0.05 and ** p <0.01 vs sham irradiated 65 or 91 week-old control by t -test. The number of rats per experimental point (N) = 10–15. Reprinted with permission [5].

significant quantitative increase, the expression of the mRNA for the two species of SOD increased at 4 hr after 1 Gy X-ray irradiation as compared to the control group. These findings suggested that the increase in SOD mRNA level is due to novel transcription of SOD mRNA by low

Fig. 2. Time-dependent changes in 1) SOD activity and 2) lipid peroxide level in organs of F344/N Slc (Fischer) rats after 0.25 Gy X-ray irradiation. Each value indicates the mean $\pm$ SEM. * p < 0.05 vs sham irradiated control by t -test. N = 10–15. Reprinted with permission [7].

dose irradiation [9].

In the same manner, we found that 0.25 Gy γ -ray irradiation induced the mRNAs for glutathione synthesis-related proteins in mouse liver [10] and brain [11], and γ -glutamylcysteine synthetase (γ -GCS) in mouse liver [12]. Furthermore, it was also found that 0.25 Gy γ -ray irradiation elevated glutathione in Raw 264.7 cells [13, 14] and this cells acquired the radio-resistance [14, 15].

Change of GPx synthesis along with that of SOD synthesis by low dose irradiation

Since SOD is an enzyme that mediates the dismutation of O_2^- to H_2O_2 , the question as to whether the resultant H_2O_2 is further detoxicated into H_2O and O_2 or not must still be evaluated. Hence, we studied the effect of low dose X-ray irradiation on the synthesis of glutathione peroxidase (GPx), which is an antioxidant that catalyzes this reaction. The results suggest that H_2O_2 produced by increased SOD activity can be detoxicated into H_2O and O_2 due to simultaneous enhancement of the GPx activity by X-ray irradiation at

0.2 Gy, in contrast to irradiation at 4 Gy (Fig. 3-1). The results also show the enhancement in enzyme activities by induction of their synthesis shortly after 0.2 Gy irradiation (Fig. 3-2). Moreover, as this phenomenon was observed in BALB/c mice (which are more radiation-sensitive compared to other mouse strains) and radiation-resistant C57BL/6NJel

mice, it was considered to be a common phenomenon on in the spleen of mouse or rat [16].

In the same manner, to test whether low dose X-ray irradiation induces mRNA for heat shock protein 70 (HSP70) or heme oxygenase (HO), reverse transcriptase polymerase chain reaction (RT-PCR) method was performed in adrenal and stomach of Wistar rat. Half an hour after 0.5 Gy irradiation, HSP70 mRNA in stomach was induced. HO mRNA in adrenal and stomach was markedly induced by radiation in dose dependent manner [17].

Adjustment function among antioxidant substances in acatalasemic mouse and its enhancement by low dose irradiation

The catalase activities in blood and organs of the acatalasemic (C3H/AnLC^sbC^s^b) mouse of the C3H strain are lower than those of the normal (C3H/AnLC^saC^s^a) mouse. We conducted a study to examine changes in the activities of antioxidant enzymes, such as, catalase, SOD and GPx, the total glutathione content, and the lipid peroxide level in the brain, which is more sensitive to oxidative stress than other organs, at 3, 6, or 24 hr following X-ray irradiation at doses of 0.25, 0.5, or 5.0 Gy to the acatalasemic and normal mice. Under non irradiation conditions, no significant differences in the activities of SOD and GPx, and lipid peroxide level were observed between the acatalasemic mouse and the normal mouse. But the acatalasemic mouse showed a significantly lower catalase activity by 80% and a significantly higher total glutathione content by 50% compared with those in the normal mouse. However, the acatalasemic mouse brain was more damaged than the normal mouse brain by excessive oxygen stress, such as a high dose (5.0 Gy) X-ray. On the other hand, unlike 5.0 Gy X-ray, a relatively low dose (0.5 Gy) irradiation significantly increased the activities of catalase and GPx (Fig. 4-1A, 4-2A) and significantly decreased the total glutathione content (Fig. 4-3A) and lipid peroxide level in acatalasemic mouse brain making the activities, the content, and the level closer to those in the normal mouse brain (Fig. 4-1B, 4-2B, 4-3B). These findings may indicate that the free radical reaction induced by the lack of catalase is more properly neutralized by low dose irradiation [18].

Fig. 3. 1) Dose- and time-dependent changes in the activities of both A) GPx and B) SOD in BALB/c mice spleens after X-ray irradiation. The ratios of measured values at various intervals after irradiation as compared with the value at the same intervals after sham irradiation are shown. Each value indicates the mean $\pm$ SEM. * $p < 0.05$ by t -test, irradiated group values vs sham-irradiated group value at same intervals. N = 8–12. 2) Dose- and time-dependent changes of the mRNAs for GPx in spleens of BALB/c mice after X-ray irradiation. '0 hr' means 'immediately'. Reprinted with permission [16].

Elevation of glutathione induced by low dose irradiation and its involvement in increased natural killer activity

We examined the relationship between the induction of an increase in the level of glutathione and the elevation of natural killer (NK) activity in mouse splenocytes by a low dose of γ -rays. The glutathione level in mouse splenocytes increased significantly between 2 hr and 6 hr after wholebody γ -ray irradiation at 0.5 Gy, peaked at 4 hr, and then decreased almost to the level before irradiation by 12 hr post irradiation. A significant enhancement of NK activity was found in

Fig. 4. Time dependent changes in the activities of 1) catalase and 2) GPx, and 3) total glutathione content in brain of A) acatalasemic mice or B) normal mice after sham-irradiation and X-ray irradiation at a dose of 0.25, 0.50, or 5.0 Gy. Each value indicates the mean $\pm$ SEM. * p <0.05, ** p <0.01, and *** p <0.001 by t -test, each irradiated (acatalasemic mice or normal mice) group value vs sham irradiated group value. *** p <0.001 by t -test, non irradiated acatalasemic mice group value vs normal mice group value. $N = 6$. Reprinted with permission [18].

the splenocytes obtained from wholebody irradiated mice between 4 hr and 6 hr post irradiation (Fig. 5A, 5B). Reduced glutathione added exogenously to splenocytes obtained from normal mice enhanced both the total cellular glutathione content and the NK activity in a dose-dependent manner. Other precursors of *de novo* glutathione synthesis, such as cysteine, *N*-acetylcysteine and oxidized glutathione, also

increased the activity. These enhancements were completely blocked by buthionine sulfoximine, an inhibitor of *de novo* glutathione synthesis. We conclude that the induction of endogenous glutathione in living cells immediately after low dose γ -ray irradiation is at least partially responsible for the appearance of enhanced NK activity [19].

Activation of the other biological defense mechanisms by low dose irradiation

To elucidate the stimulative effect of whole body low dose X-ray irradiation on the immune system, *in vivo*, we studied its effects on some immune functions of mouse splenocytes. Results show that concanavalin A (Con A) and phytohemagglutinin (PHA) responses of splenocytes were significantly increased by irradiation of 0.025 Gy and 0.05 Gy, whereas lipopolysaccharide (LPS) response was significantly depressed by irradiation of 0.05 Gy. By irradiation of 0.025 Gy, Con A response was significantly accelerated at each concentration of Con A, but the optimum concentration of Con A shifted to a higher value of 4 $\mu\text{g/ml}$ from 2 $\mu\text{g/ml}$ in the control group. When blood plasma obtained from 0.025 Gy irradiated mice was added into the medium at

0.05–1%, the Con A response of splenocytes in another unirradiated mouse was significantly accelerated over that where the plasma added came from the sham irradiated control mice. Furthermore, 0.025 Gy irradiation also enhanced the biological activity of intracellular interleukin-1 (IL-1) of LPS-stimulated splenocytes [20]. Moreover, it was also found that 0.2 Gy X-ray irradiation stimulates production of prostanooids related to the inflammatory response in mice [21].

Changes of Structure and Function of Biomembranes by Low Dose Irradiation

Influence of low dose irradiation on structure and transport function of cell membranes

The concentration of cysteine (Cys) significantly increased at doses of 0.25–1 Gy and the concentration of cystine (Cys-Cys) significantly decreased at a dose of 0.25 Gy. It showed no dose dependent changes in tyrosine (Tyr), phenylalanine (Phe) and glycine (Gly). Similarly phospholipid and cholesterol levels were unchanged. Na^+, K^+ -ATPase activities significantly decreased at a dose of 1 Gy or higher but significantly increased at doses of 0.25 and 0.5 Gy (Fig. 6). These findings suggested that unlike high dose irradiation which promotes membrane damage, low dose irradiation stimulates the SH group of membrane proteins and enhances the ability to control the membrane transport mechanism as reflected by an increase in Na^+, K^+ -ATPase activity [22].

Fig. 5. Changes in A) levels of total glutathione and B) NK activity in mice splenocytes after 0.5 Gy γ -ray irradiation. Each value indicates the mean $\pm$ SD. * $p < 0.5$, ** $p < 0.1$, and *** $p < 0.01$ vs unirradiated control group value. N = 6. Reprinted with permission [19].

Fig. 6. Dose-dependent changes in Na^+, K^+ -ATPase activity in brain cortex of wistar rats at 4 hr after X-ray irradiation. Each value indicates the mean $\pm$ SEM. * $p < 0.05$ vs sham irradiated control group by *t*-test. The dotted line indicates the control values, i.e., the concentration in the cortex of rats not exposed to irradiation. N = 10–15. Reprinted with permission [22].

Effects of low dose irradiation on purine metabolism

This study examines the influence of low dose X-ray irradiation on purine nucleotide metabolites such as adenosine, inosine, hypoxanthine, xanthine and uric acid, and hence generation of ATP-mediated energy in mouse splenocytes. It was found that, unlike high dose irradiation which promotes membrane damage, low dose irradiation enhances the ability to regulate the energy metabolisms as reflected by the increase in Na^+, K^+ -ATPase activity and the adequate activation of the above salvage pathway. Namely, the levels of adenosine, inosine and uric acid significantly increased, while the levels of xanthine and hypoxanthine decreased significantly. Moreover, the cysteine level and SOD activity significantly increased at a dose of 0.2 Gy [23].

Remission of Vital Oxidative Injury by Low Dose Irradiation

Protection against alloxan diabetes before alloxan administration and delay of the onset of type I diabetes in nonobese diabetic mice by low dose irradiation

We evaluated the protective effects of a single low dose wholebody ^{60}Co γ -ray irradiation against alloxan-induced hyperglycemia in rats. In rats that did not receive alloxan, the SOD activity in the pancreas significantly increased after irradiation at doses of 0.5 or 1 Gy. In rats that received alloxan, pancreatic lipid peroxide (TBARS) level and blood glucose were increased. However, the increase in pancreatic

TBARS level was prevented by irradiation at doses of 0.5 or 1 Gy and the increase in blood glucose was also prevented by irradiation at a dose of 0.5 Gy (Fig. 7). After alloxan administration, degranulation was observed in β cells, but this was prevented by low dose irradiation at a dose of 0.5 Gy (Photo. 1) [24].

In the same manner, we examined the effect of low dose

Fig. 7. Dose-dependent effects of γ -ray irradiation on elevation of blood glucose induced by alloxan in rats. Rats were sacrificed at 48 hr after i.v. injection of alloxan (40 mg/kg body weight). * $p < 0.05$ and ** $p < 0.01$ vs sham irradiated no-allowan group. ## $p < 0.01$ vs sham irradiated alloxan group. $N = 8$. Reprinted with permission [24].

Photo. 1. Cell injury by alloxan and effects of low dose γ -ray irradiation evaluated by staining of pancreas. Reprinted with permission [24].

γ -ray irradiation on the progression of type I diabetes (IDDM) using nonobese diabetic (NOD) mice. Elevated level of urine glucose was first detected at 15 weeks of age in control NOD mice, whereas it was delayed as long as 7 weeks in 0.5 Gy γ -ray irradiated mice. Greatest effect was observed in mice irradiated at 13 weeks of age, e.g. 2 weeks prior to the onset of disease. Detection of apoptotic cells by TUNEL staining revealed much less incidence of apoptosis in pancreas from γ -ray irradiated mice compared with that of control mice. Both increase in glucose and decrease in insulin level in the blood at 24 weeks of age were effectively suppressed by irradiation at 13 weeks of age. One week after the irradiation the specific activity of SOD in pancreas was found to be increased twice as much. The results indicate that low dose irradiation delays the onset of IDDM in NOD mice by suppressing apoptotic cell death in pancreas, probably through enhancing antioxidant defense [25].

Inhibitory effects of prior or post low dose irradiation on Fe^{3+} -NTA-induced hepatopathy

It was found that when the iron-complex, Fe^{3+} -NTA was administered to rats, transient hepatopathy occurred, a response resembling excessive iron disease in humans [26].

Blood activities of hepatocellular enzymes such as lactate dehydrogenase (LDH), glutamic pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT) peaked at 12 hr after a single intraabdominal injection of ferric nitrilotriacetate (Fe^{3+} -NTA) in rats. Enzymes such as alkaline phosphatase (ALP) and leucin amino peptidase (LAP) originating in the capillary bile ducts or bile secretory liver cells were also released into the blood between 6–24 hr after intraabdominal injection of Fe^{3+} -NTA in rats. Furthermore, hyperoxidation of lipids occurred in rat hepatic cell membranes, reaching a peak at 6 hr after intraabdominal injection of Fe^{3+} -NTA. It was found that a single prior 0.5 Gy wholebody X-ray irradiation significantly increased SOD activities and suppressed above-mentioned symptoms of transient hepatopathy in rats [27].

In the same manner, the post 0.5 Gy γ -ray irradiation accelerated the rate of recovery from Fe^{3+} -NTA-induced mice liver damage. Based on the changes in GOT activities, GPT activities and lipid peroxide (malondialdehyde (MDA)) levels, it was shown that hepatopathy was improved by low dose irradiation at 3 hr after Fe^{3+} -NTA administration (Fig. 8). This may be because of the enhancement of antioxidant agents such as total glutathione (GSH + GSSG), GPx, glutathione reductase (GR) and γ -GCS by low dose irradiation (Fig. 9). These findings suggest that low dose irradiation relieved functional disorders at least in the livers of mice with ROS-related diseases [28].

Elevation of antioxidant potency in brain by low dose irradiation and its effect on Fe -NTA-induced brain damage

The increase in lipid peroxide levels in mice brain

Fig. 8. Time dependent changes in activities of GOT and GPT in C57BL/6 mice serum treated with Fe^{3+} -NTA after 0.5 Gy γ -ray irradiation. Each value indicate the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by t -test, each sham irradiated or irradiated group value vs the control group value (3 hr after Fe^{3+} -NTA administration). # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ by t -test, each group value at various intervals after irradiation vs the values at the same intervals after sham irradiation. N = 4–6. Reprinted with permission [28].

following Fe^{3+} administration was about 50% of that when 1-methyl-4-phenyl 1,2,3,6-tetra-hydro-pyridine (MPTP) was administered. This may be due to excessive oxidation by Fe^{3+} , and was supported by the decreases in activities of antioxidant enzymes, such as SOD, catalase and GPx, Na^+, K^+ -ATPase activity and membrane fluidity after Fe^{3+} administration. Relatively 0.5 Gy X-ray irradiation inhibited lipid peroxidation associated with Fe^{3+} administration and restored the decreased activities of the above antioxidant enzymes and Na^+, K^+ -ATPase (Fig. 10A), and membrane fluidity (Fig. 10B) to the levels in the Fe^{3+} oxidation by non Fe^{3+} administered group. In the purine metabolism system, uric acid decreased after Fe^{3+} administration, which may be due to transient impairment of the system for production of uric acid from xanthine by excessive oxidation by Fe^{3+} .

Fig. 9. Time dependent changes in lipid peroxide level, total glutathione content, GPx activity, and GR activity in mice livers treated with Fe^{3+} -NTA after 0.5 Gy γ -ray irradiation. The data, numbers of mice and significance are as described in Fig. 8. Reprinted with permission [28].

However, 0.5 Gy irradiation inhibited this decrease in uric acid, increasing its level to that in the non Fe^{3+} administered group. This may be due to factors such as rapid recovery of the activities of above antioxidant enzymes and Na^+, K^+ -ATPase, and membrane fluidity after 0.5 Gy irradiation. In addition, since no changes were observed in xanthine and uric acid, increased inosine and hypoxanthine may have advanced to a salvage pathway leading to not xanthine but to inosine 5'-monophosphate (IMP) [29].

Inhibitory effects of prior or post low dose irradiation on carbon tetrachloride-induced hepatopathy in acatalasemic mice

Carbon tetrachloride (CCl_4) is frequently used as a chemical inducer of experimental liver cirrhosis. Transient hepatocellular damage such as degeneration and necrosis after the administration of CCl_4 is thought to be induced by trichloromethyl radical ($\cdot\text{CCl}_3$). The radical induces an adverse reaction by forming radicals after its administration in the early stage between intracellular uptake and transformation into storage types. Thus many biological substances such as membrane lipids, proteins, nucleic acids, and microsome are injured by $\cdot\text{CCl}_3$ [30].

We examined the effects of prior or post 0.5 Gy X-ray irradiation, which reduced the oxidative damage under CCl_4 -induced hepatopathy in the acatalasemic or normal mice. In the former, prior low dose irradiation increased the catalase activity in the acatalasemic mouse liver to a level similar to that of the normal mouse liver. Pathological examinations and analyses of the activities of GOT and GPT in blood and lipid peroxide levels showed that CCl_4 -induced hepatopathy was inhibited by low dose irradiation (Fig. 11, Photo. 2). These findings may indicate that the free radical reaction induced by the lack of catalase and the administration of CCl_4 is more properly neutralized by high GPx activity and low dose irradiation in the acatalasemic mouse liver [31].

In the latter, the 0.5 Gy post irradiation after CCl_4 administration decreased the activities of GOT and GPT in the acatalasemic mouse blood to a level similar to that of the acatalasemic mouse blood not treat with CCl_4 , this is in contrast to a high dose (15 Gy) irradiation. In the same manner, pathological disorder was improved by 0.5 Gy irradiation (Table 1). The fat degeneration in normal mice quickly reduced, in contrast to acatalasemic mice. These findings suggest that low dose irradiation after CCl_4

Fig. 10. Time dependent changes in A) Na⁺,K⁺-ATPase activities and B) membrane fluidities in mice brains treated with Fe³⁺-NTA after 0.5 Gy X-ray irradiation. Each value indicates the mean $\pm$ SEM. #p<0.05 and ##p<0.01 by *t*-test, each sham irradiated (Fe³⁺-NTA administrated and sham irradiated) or irradiated (Fe³⁺-NTA administrated and irradiated) group value vs the control (saline administrated and sham irradiated) group value. *p<0.05 and **p<0.01 by *t*-test, each group value at various intervals after irradiation vs the values at the same intervals after sham irradiation. N = 8–12. Reprinted with permission [29].

Fig. 11. Differences in the activities of A) GOT and B) GPT in serum between normal (open bars) and acatalasemic (hatched bars) mice, six to seven weeks of age, under non treated control, CCl₄ administration, 0.5 Gy irradiation, or 0.5 Gy irradiation prior to CCl₄ administration. Each value indicates the mean $\pm$ SEM. +++p<0.001 by *t*-test, acatalasemic mice value vs. normal mice value under no treatment. ***p<0.001, **p<0.01, and *p<0.05 by *t*-test, each normal mice value or acatalasemic mice value under CCl₄ administration or 0.5 Gy X-ray irradiation vs under no treatment, respectively. ###p<0.001, ##p<0.01, and #p<0.05 by *t*-test, each acatalasemic mice value or normal mice value under CCl₄ administration after 0.5 Gy X-ray irradiation vs under CCl₄ administration. N = 6–12. Reprinted with permission [31].

administration accelerated the rate of recovery and that catalase played an important role in the recovery from hepatopathy induced by CCl₄, in contrast to high dose irradiation [32].

In the same manner, 0.5 Gy γ -irradiation elevated the chemical biological defense mechanisms, such as glutathione level, in mouse liver and reduced CCl₄-induced hepatopathy [33, 34].

Photo. 2. Differences in liver histopathology between A) acatalasemic and B) normal mice under non treated control a), CCl₄ administration b), 0.5 Gy X-ray irradiation c), or 0.5 Gy irradiation prior to CCl₄ administration d). All figures under lower magnification ($\times 100$) are stained with Sudan Black B (black-colored) for the detection of fatty degeneration surrounding the central vein (C) and the portal vein (P). The areas of cell necrosis surrounding the central vein in the CCl₄ administrated mice after 0.5 Gy irradiation were smaller in both mice in comparison with the CCl₄ administrated mice. No obvious difference was noted in the extent of the centrilobular hepatocyte damage by non treatment or 0.5 Gy irradiation in both mice. Reprinted with permission [31].

Inhibitory effects of low dose irradiation on MPTP-induced brain damage

The elevation of endogenous thiol-related antioxidants and free radical scavenging enzymes in the brain of C57BL/6 female mice after low dose γ -ray irradiation and its inhibitory effect on MPTP-induced brain damage were investigated. The brain level of the reduced form of glutathione increased soon after irradiation with 0.5 Gy of γ -rays, reached a

maximum at 3 hr post treatment, and remained elevated until 12 hr. Thioredoxin (TRX) was also transiently increased after irradiation. The activities of free radical scavenging enzymes, including Cu/Zn-SOD, catalase and GPx, were significantly induced after irradiation as well. Cerebral lipid peroxide (MDA) level was remarkably elevated by MPTP treatment, and this elevation was suppressed by prior irradiation at a dose of 0.5 Gy (Fig. 12). The contents of

Table 1. Temporal changes in aminotransferases in blood of acatalasemic or normal mice at each sham, 0.5 Gy, or 15 Gy X-ray irradiation following CCl₄ administration

	Treatment	Mice	
		Normal	Acatalasemic
GOT activity [kU/L]	Control	0.84 ± 0.09	2.20 ± 0.15 ⁺⁺⁺
	CCl ₄ + Sham	5.62 ± 0.47	7.74 ± 0.56
	CCl ₄ + 0.5 Gy	4.57 ± 0.43*	6.61 ± 0.51*
	CCl ₄ + 15 Gy	7.52 ± 0.55*	9.65 ± 0.62*
GPT activity [kU/L]	Control	0.97 ± 0.10	2.17 ± 0.15 ⁺⁺⁺
	CCl ₄ + Sham	7.36 ± 0.54	9.14 ± 0.60
	CCl ₄ + 0.5 Gy	5.27 ± 0.46**	7.62 ± 0.55**
	CCl ₄ + 15 Gy	7.98 ± 0.55	10.14 ± 0.64*

Each value indicates the mean ± SEM. ⁺⁺⁺P<0.001 vs. normal mouse. ^{**}P<0.01, ^{*}P<0.05 vs control. N = 5. Reprinted with permission [32].

Fig. 12. Effects of γ -rays on MPTP-induced elevation of lipid peroxide level in the whole brain. Mice were prior irradiated with 0.5 Gy of γ -rays 1 hr before MPTP treatment. Mice were killed 4 hr after MPTP administration (30 mg/kg body weight). ^{***}p<0.001 vs control group. [¶]p<0.001 vs MPTP alone-treated group. N = 5. Reprinted with permission [35].

glutathione and TRX were significantly decreased by MPTP treatment in comparison with those of the control group. These reductions both seemed to be attenuated by prior irradiation with γ -rays. These results suggest that low dose γ -ray irradiation induces endogenous antioxidative potency in the brain of mice and might be effective for the prevention and/or therapy of various reactive oxygen species-related neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease [35].

Moreover, it was found that 5 Gy γ -ray irradiation to the chest regions inhibited the blood pressure of spontaneous hypertensive rat [36].

Effects of Radon Inhalation on Physiology and Disorders in Rabbits

The radon therapy uses radon gas, which mainly emits α -rays, and induces a small amount of active oxygen in the body. To clarify the mechanisms of therapeutic effects of radon, we administered sprained radon (7–18 kBq/l) to rabbits by inhalation and examined the changes in lipid peroxide (TBARS) level, SOD activity and membrane fluidity in various organs, biogenic amine neurotransmitters in brain, adrenal secretion of catecholamines and blood components such as vasoactive substances.

Changes in lipid peroxide level, SOD activity, and membrane fluidity by radon inhalation

The lipid peroxide (TBARS) level of the brain was significantly decreased immediately after radon inhalation for 90 min in both the low concentration group (7–10 kBq/l) and the high concentration group (14–18 kBq/l) as compared with that in the control group. It further decreased in the low concentration group but slightly recovered in the high concentration group 2 hr after inhalation. The TBARS level of the lung showed no change immediately after inhalation but decreased significantly in both groups 2 hr after inhalation. With regard to SOD activity in the brain and lung, only that in the brain showed significant increase in the high concentration group immediately after inhalation; no other change was observed. Membrane fluidity, especially the fluidity of membrane protein, was significantly increased in the brains of both groups immediately after inhalation. In the lung, the membrane fluidity was significantly increased 2 hr after inhalation in both groups (Fig. 13). These findings suggest that the inhalation of radon at radon springs contributes to the prevention of brain disorders related to peroxidation reactions by promoting these physiologic changes[37].

Changes in biogenic amine neurotransmitters by radon inhalation

In rabbit brain immediately after radon inhalation, nor-adrenaline (NA), serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) levels decreased significantly by inhalation of radon spring of 13 kBq/l or over. Changes in tyrosine, dopamine (DA) and homovanillic acid (HVA) levels did not depend on the concentrations of radon inhaled. The turnover ratios for these amines were evaluated. The results suggested possible decrease in the activities of aromatic-L-amino acid decarboxylase, which are key enzymes of the metabolism of biogenic amines [38].

Changes in adrenal secretion of catecholamines in relation to increase of tissue perfusion rate by radon inhalation

In the radon inhalation group, plasma adrenaline and NA levels were significantly higher, while adrenaline and NA

Fig. 13. Time-dependent changes in lipid peroxide level, SOD activity, and membrane fluidity in the brain and lungs of rabbits after radon inhalation. Each indicate the mean $\pm$ SEM. "□" immediately after inhalation, "■" 2 h after inhalation. *p<0.05 and **p<0.01 vs control by *t*-test. Reprinted with permission [37].

levels were significantly lower than those in the control group. In the no medication and phentolamine subgroups, tissue perfusion rates in the radon group were significantly higher than those in the control group. It is suggested that catecholamines are secreted from the adrenal glands by inhalation of radon water and that the β -action of catecholamines contributes to the increase in tissue perfusion (Table 2) [39].

Changes in blood components indicated for hypertension, diabetes, and pain by radon inhalation

Significant dose-dependent increases were observed in histamine and α atrial natriuretic polypeptide (α ANP) and

significant decrease in vasopressin in both high and low concentration groups compared with the control group. Angiotensin II and prostaglandin E_2 (PGE_2) showed no significant changes. Insulin significantly increased in the high concentration group, and glucose-6-phosphate dehydrogenase (G-6-PDH) activity and glucagon significantly increased in both high and low concentration groups. The blood glucose level decreased slightly. β -endorphin and M-enkephalin dose-dependently increased with significant differences between the high concentration group and control group (Table 3). Namely, vasodilation, alleviation of diabetic symptoms and morphine-like analgesic effects were observed, suggesting that these changes constitute part of the mechanisms of the

Table 2. Changes of catecholamines and tissue perfusion rate by radon inhalation

Rn [kBq/l]	control	14–18
plasma catecholamines [pg/mg protein]		
adrenaline	4.2 ± 1.7	22.9 ± 12.1**
noradrenaline	10.9 ± 5.6	18.8 ± 8.2*
adrenal catecholamines [ng/mg protein]		
adrenaline	825 ± 544	249 ± 129**
noradrenaline	134 ± 67	23.6 ± 14.9**
tissue perfusion rate [ml/100 g/min]		
no medication	15.8 ± 1.8	21.4 ± 2.4**
phentolamine	18.4 ± 1.7	22.6 ± 2.2**
propranolol	17.4 ± 2.7	19.3 ± 2.9
atenolol	16.6 ± 2.6	18.4 ± 3.0

Each value indicates the mean ± SEM. The number of rabbits per experiment was 8–14 at control and 8–15 at 14–18 kBq/l. *P<0.05 and **P<0.01 vs control. Reprinted with permission [39].

Table 3. Dynamic changes in vasoactive, diabetes-associated, and pain-associated substances of rabbit blood by radon inhalation

Rn [kBq/l]	control	7–10	14–18
histamine [μg/dl]	67 ± 12	146 ± 31**	152 ± 62**
α atrial natriuretic polypeptide [pg/ml]	1660 ± 240	2170 ± 170*	330 ± 520**
vasopression [pg/ml]	13.2 ± 3.6	3.2 ± 0.6**	5.4 ± 0.8**
angiotensin II [pg/ml]	34 ± 1	33 ± 1	32 ± 1
prostaglandin E2 [pg/ml]	26 ± 4	33 ± 10	18 ± 5
insulin [U/ml]	4.3 ± 0.4	4.3 ± 0.6	8.5 ± 1.8**
glucose-6-phosphate dehydrogenase [IU/37°C]	1.9 ± 0.2	2.8 ± 0.2**	2.6 ± 0.3**
pancreatic glucagon [104 × pg/ml]	1.6 ± 0.1	1.9 ± 0.1*	2.4 ± 0.3*
blood glucose [mg/dl]	218 ± 21	195 ± 22	191 ± 19
β endorphin [ng/wet-g]	16.2 ± 2.5	19.0 ± 1.9	22.4 ± 3.5*
M-enkephalin [ng/wet-g]	6.1 ± 1.0	6.5 ± 1.1	11.8 ± 1.9**

Each value indicates the mean ± SEM. The number of rabbits per experiment was ten at control, eight at 7–10 kBq/l and nine at 14–18 kBq/l. *P<0.05 and **P<0.01 vs control. Reprinted with permission [40].

radon spring therapy include hypertension, diabetes mellitus, and pain [40].

Effects of Radon Inhalation on Physiology and Disorders in Humans

Therapy using radon gas, which is volatilized from radon enriched water and mainly emits α-rays, and induces a small amount of active oxygen in the body, is performed for various diseases such as osteoarthritis and bronchial asthma [41]. Several attempts have been made to clarify its mechanism but there have been only a few studies on radon therapy in humans. In our studies, radon and thermal therapy were performed once a week. All subjects went to a hot bathroom with a high concentration of radon, and nasal inhalation of

vapor from a hot spring was performed for 40 min once a day under conditions of high humidity. The room temperature was 48°C and the room radon concentration was 2,080 Bq/m³.

Biochemical comparison between radon effects and thermal effects on humans in radon and thermal therapy

The radioactive and thermal effects of radon hot spring were biochemically compared under a sauna room or hot spring conditions with a similar chemical component, using the parameters that are closely involved in the clinic for radon therapy. The results showed that the radon and thermal therapy enhanced the antioxidation functions, such as the activities of SOD and catalase, which inhibit lipid peroxidation and total cholesterol produced in the body (Table 4-1). Moreover the therapy enhanced ConA-induced

mitogen response and increased the percentage of CD4 positive cells, which is the marker of helper T cells, and decreased the percentage of CD8 positive cells, which is the common marker of killer T cells and suppressor T cells, in the white blood cell differentiation antigen (CD8/CD4) assay (Table 4-2). Furthermore, the therapy increased the levels of α ANP, β -endorphin, adrenocorticotrophic hormone (ACTH), insulin and G-6-PDH, and it decreased the vasopression level (Table 4-3, 4-4, 4-5). The results were on the whole larger in the radon group than in the thermal group. The findings suggest that radon therapy contributes more to the prevention of life-style-related diseases related

to peroxidation reactions and immune suppression than to thermal therapy. Moreover, these indicate what may be a part of the mechanism for the alleviation of hypertension, osteoarthritis (pain), and diabetes mellitus brought about more by radon therapy than by thermal therapy [42].

Biological effects of radon and thermal therapy on osteoarthritis

To elucidate the mechanism of the osteoarthritis in which radon therapy is used as a treatment, we examined the temporal changes in antioxidants, immune, vasoactive, and pain-associated substances in human blood by the therapy.

Table 4-1. Temporal changes in antioxidant-associated substances in blood of humans at each radon inhalation- or thermal-treatment after first treatment

	Treatment	Before	After First Treatment	
			5 days	10 days
SOD activity [%]	Radon	11.8 $\pm$ 0.9	12.7 $\pm$ 1.3	13.7 $\pm$ 0.8*
	Thermal	12.0 $\pm$ 0.7	11.1 $\pm$ 0.9	13.2 $\pm$ 0.4*
	No (Control)	12.3 $\pm$ 1.0	11.5 $\pm$ 1.2	11.9 $\pm$ 0.9
catalase activity [U/I]	Radon	0.52 $\pm$ 0.10	0.44 $\pm$ 0.06	0.72 $\pm$ 0.08*
	Thermal	0.64 $\pm$ 0.09	0.48 $\pm$ 0.09	0.84 $\pm$ 0.10*
	No (Control)	0.54 $\pm$ 0.14	0.50 $\pm$ 0.11	0.57 $\pm$ 0.12
lipid peroxide level [nmol/ml]	Radon	1.00 $\pm$ 0.10	0.88 $\pm$ 0.05*	0.73 $\pm$ 0.07**
	Thermal	1.03 $\pm$ 0.08	0.83 $\pm$ 0.06*	0.87 $\pm$ 0.07*
	No (Control)	0.98 $\pm$ 0.09	0.96 $\pm$ 0.11	0.92 $\pm$ 0.10
total cholesterol [mg/dl]	Radon	105 $\pm$ 4	102 $\pm$ 6	84 $\pm$ 6**
	Thermal	113 $\pm$ 11	99 $\pm$ 9	94 $\pm$ 7*
	No (Control)	108 $\pm$ 7	102 $\pm$ 9	106 $\pm$ 12

Each value represents the mean $\pm$ SEM. N = 5. Significance: *P<0.05 and **P<0.01 vs before treatment. Reprinted with permission [42].

Table 4-2. Temporal changes in immune-associated substances in blood of humans at each radon inhalation- or thermal-treatment after first treatment

	Treatment	Before	After First Treatment	
			5 days	10 days
ConA-induced proliferation [S.I.]	Radon	392 $\pm$ 64	484 $\pm$ 20*	496 $\pm$ 38*
	Thermal	406 $\pm$ 47	451 $\pm$ 26	490 $\pm$ 22*
	No (Control)	419 $\pm$ 45	422 $\pm$ 34	434 $\pm$ 36
CD8 positive cells [%]	Radon	35.5 $\pm$ 1.9	31.3 $\pm$ 1.1*	30.7 $\pm$ 1.3*
	Thermal	33.3 $\pm$ 1.5	30.9 $\pm$ 1.4	31.7 $\pm$ 2.1
	No (Control)	34.7 $\pm$ 2.8	32.2 $\pm$ 1.7	33.5 $\pm$ 1.9
CD4 positive cells [%]	Radon	39.4 $\pm$ 1.6	44.9 $\pm$ 1.1**	43.4 $\pm$ 1.0*
	Thermal	38.0 $\pm$ 1.9	41.7 $\pm$ 1.7*	40.2 $\pm$ 2.4
	No (Control)	40.4 $\pm$ 1.7	41.6 $\pm$ 1.5	41.8 $\pm$ 2.1

Each value represents the mean $\pm$ SEM. N = 5. Significance: *P<0.05 and ** P<0.01 vs before treatment. Reprinted with permission [42].

Table 4-3. Temporal changes in vasoactive substances in blood of humans at each radon inhalation- or thermal-treatment after first treatment

	Treatment	Before	After First Treatment	
			5 days	10 days
α atrial natriuretic polypeptide [pg/ml]	Radon	7.0 ± 1.3	7.6 ± 1.6	$11.1 \pm 1.5^{**}$
	Thermal	8.4 ± 1.2	7.2 ± 1.0	6.8 ± 0.8
	No (Control)	7.9 ± 1.5	7.3 ± 1.1	8.1 ± 0.9
vasopression [pg/ml]	Radon	1.50 ± 0.18	1.32 ± 0.22	$0.95 \pm 0.22^{**}$
	Thermal	1.52 ± 0.39	1.16 ± 0.20	1.25 ± 0.21
	No (Control)	1.42 ± 0.22	1.47 ± 0.31	1.41 ± 0.27

Each value represents the mean $\pm$ SEM. N = 5. Significance: *P<0.05 and **P<0.01 vs before treatment. Reprinted with permission [42].

Table 4-4. Temporal changes in pain-associated substances in blood of humans at each radon inhalation- or thermal-treatment after first treatment

	Treatment	Before	After First Treatment	
			5 days	10 days
β endorphin [pg/ml]	Radon	16.0 ± 0.7	$19.8 \pm 0.9^{**}$	$18.6 \pm 1.2^*$
	Thermal	20.4 ± 1.9	18.2 ± 0.8	21.6 ± 1.4
	No (Control)	19.9 ± 1.3	18.4 ± 1.5	18.7 ± 1.8
adrenocorticotrophic hormone [pg/ml]	Radon	18.6 ± 1.6	$22.8 \pm 1.8^*$	18.4 ± 2.3
	Thermal	17.2 ± 1.5	15.5 ± 2.2	16.3 ± 1.6
	No (Control)	18.8 ± 1.9	17.1 ± 1.7	18.2 ± 2.2

Each value represents the mean $\pm$ SEM. N = 5. Significance: *P<0.05 and ** P<0.01 vs before treatment. Reprinted with permission [42].

Table 4-5. Temporal changes in diabetes-associated substances in blood of humans at each radon inhalation- or thermal-treatment after first treatment

	Treatment	Before	After First Treatment	
			5 days	10 days
insulin [μ U/ml]	Radon	32.2 ± 6.1	33.5 ± 3.6	$49.2 \pm 4.8^{***}$
	Thermal	31.6 ± 4.9	32.8 ± 3.9	$41.8 \pm 5.1^*$
	No (Control)	32.5 ± 4.3	33.4 ± 5.0	34.1 ± 4.2
G-6-PDH [mU/RBC]	Radon	103 ± 4	$124 \pm 9^*$	102 ± 10
	Thermal	115 ± 12	$135 \pm 17^*$	114 ± 6
	No (Control)	108 ± 11	111 ± 14	107 ± 5

Each value represents the mean $\pm$ SEM. N = 5. Significance: *P<0.05 and *** P<0.001 vs before treatment. Reprinted with permission [42].

Results show that radon inhalation enhanced the antioxidation and immune function, and the findings suggest that the radon therapy contributes to the prevention of osteoarthritis related to peroxidation reactions and immune depression. Moreover the changes of vasoactive and pain-associated substances indicate increase in tissue perfusion brought about by radon therapy and suggesting that radon inhalation plays a role in

alleviating pain (Fig. 14-1, 14-2). The findings suggest that an appropriate amount of active oxygen is produced in the body after radon inhalation, and this contributes to the alleviation of the symptoms of ROS related diseases such as osteoarthritis [43].

Fig. 14. Temporal changes in 1) vasoactive substances (A) atrial natriuretic polypeptide and B) vasopressin) and 2) pain-associated substances (A) β endorphine and B) adrenocorticotrophic hormone) of osteoarthritis patient blood after first radon and thermal therapy. Each value represents the mean ± SEM. Normal is the mean of 20 healthy persons lived in Misasa area. The number of patients experiment was 20 (11 males and 9 females). *, **, *** Significantly different from the no therapy control (Before) group at $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively. Reprinted with permission [43].

Elevation of antioxidant enzymes in the clinical effects of radon and thermal therapy for bronchial asthma

An increased systemic production of oxygen free radicals by activated inflammatory cells is thought to be involved in the pathophysiology of asthma. The aim of this study is to evaluate the clinical effects of radon and involved therapy on asthma in relation to antioxidant enzymes and lipid peroxide. The forced expiratory volume in one second (%FEV₁) was significantly increased 28 days after the first therapy (Fig. 15). On day 28, the catalase activity was significantly increased in comparison with the control.

The SOD activity was significantly increased in compared to the control after first inhalation. On days 14 and 28, the lipid peroxide level was significantly decreased in comparison with the control. In conclusion, the present pilot study has shown that radon and thermal therapy improved the pulmonary function of asthmatics by increasing the reduced activities of antioxidant enzymes [44].

Elevation of p53 protein level and SOD activity in the resident blood of the misasa radon hot spring district

To clarify the mechanism by which the radon hot spring

Fig. 15. Changes in %FEV₁ in asthmatic 28 days after the first radon and thermal therapy. Each value presents the mean $\pm$ SEM. Reprinted with permission [44].

prevents cancer or not, in this study, blood was collected from the residents in the Misasa hot spring district and in the control district, and the level of a representative cancer-suppressive gene, *p53*, and the activity of a representative antioxidant enzyme, SOD, were analyzed as indices. The level of serum *p53* protein in the males in the Misasa hot spring district was 2-fold higher than that in the control district, with a significant difference (Fig. 16A). In the females in the Misasa hot spring district, SOD activity was approximately 15% higher with statistical significance than that in the control district, and exceeded the reference range of SOD activity despite advanced age (Fig. 16B). These results suggested that routine exposure to radon at a concentration about 3 times higher than the national mean by the residents in the Misasa hot spring district induces trace active oxygen *in vivo*, potentiating products of cancer-suppressive gene and antioxidant function. As the *p53* protein level was high in the residents in the Misasa hot spring district, apoptosis of cancer cells may readily occur [45].

Conclusions

These findings suggest that an appropriate amount of ROS is produced in the body after low dose irradiation or radon inhalation, and this contributes to the alleviation of the symptoms of ROS-related diseases such as diabetes after certain processes such as activation of the biological defense mechanism, or promoting these physiologic changes such as tissue perfusion, in contrast to the toxic effects of high dose irradiation. In future, clarification in detail of the mechanisms of these phenomena is required to understand the effects of low dose radiation on the functions of the living body, including adaptive response [46].

Fig. 16. Comparison of A) the level of serum *p53* protein and B) the blood level of the SOD activity between the residents in the Misasa hot spring district and those in the control district. N=6–8. Each value represents the mean $\pm$ SEM. Significance: * $p < 0.01$ by *t*-test, the residents in the Misasa hot spring district vs those in the control district. Reprinted with permission [45].

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