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Biochemical Comparison between Radon Effects and Thermal Effects on Humans in Radon Hot Spring Therapy

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Radon therapy/Radon effect/Thermal effect/Antioxidant/Immunity.

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 superoxide dismutase (SOD) and catalase, which inhibit lipid peroxidation and total cholesterol produced in the body. Moreover the therapy enhanced concanavalin A (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. Furthermore, the therapy increased the levels of α atrial natriuretic polypeptide (αANP), β endorphin, adrenocorticotropic hormone (ACTH), insulin and glucose-6-phosphate dehydrogenase (G-6-PDH), and it decreased the vasopression level. 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.

INTRODUCTION

Radon is a radioactive gaseous element that mainly emits α-rays. If radon is inhaled, the lungs will be subjected to the actions 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.[1)] Another known effect of a radon spring is to promote the effects of such tissue perfusion agents as adrenaline in plasma; that is, the level of plasma adrenaline is increased by radon inhalation.[2,3)] So far, no epidemiologic data exists on the hazardous effects of radon.[4)]

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Abbreviations: SOD, superoxide dismutase; Con A, concanavalin A; CD, cell differentiation; αANP, α atrial natriuretic polypeptide; ACTH, adrenocorticotropic hormone; G-6-PDH, glucose-6-phosphate dehydrogenase; RIA, radioimmuno assay.

We have reported earlier a decrease in the lipid peroxide (thiobarbituric acid reaction substances [TBARS]) level and an increase in the SOD activity in the brain, liver and various organs of the immune system in rats after general low-dose X-irradiation.[5)] We also administered radon to rabbits by inhalation and examined changes in the lipid peroxide level, SOD activity, and membrane fluidity in various organs to clarify the therapeutic effects of radon. The results suggest that the inhalation of radon at radon springs contributes to the prevention of brain disorders related to peroxidation reactions by promoting these beneficial physiologic changes.[6)]

The therapy using radon gas, which is volatilized from radon-enriched water, is performed for various diseases, such as hypertension, osteoarthritis, asthma, and diabetes.[7)] Several attempts have been made to clarify its mechanism, but there have been only a few studies on radon therapy in humans.[8,9)] Because most diseases to which radon therapy and the thermal therapy is applied are related to activated oxygen, it is important to compare the radioactive effects of radon and the thermal effects under a sauna room or hot spring conditions with the similar chemical component. Therefore, in this study, we compared the radon effect with the thermal effect by using as parameters, the antioxidant-, immune-, vasoactive-, pain-, and diabetes-associated substances, which are the causes of

life-style-related diseases, to elucidate the mechanism of the diseases in which the radon hot spring therapy is used as a treatment, and most of which are called activated oxygen-related diseases.

MATERIALS AND METHODS

Subjects

The subjects were 15 males 20 to 40 years of age who were divided into 3 groups: radon, thermal, and control. The radon group went to a hot bathroom (the room) with a high concentration of radon at the Misasa Medical Center of the Okayama University Medical School. The temperature was 36°C (equivalent to body temperature), the radon concentration was 2,080 Bq/m^3 (equivalent to about 40-fold higher than that in a local sauna (background level)[10]). The thermal group went to a local sauna in the region. The temperature there was 48°C, and the radon concentration was 54 Bq/m^3.

The control group went to the other local sauna. The temperature there was 36°C, and the radon concentration was 54 Bq/m^3. No subjects took a bath; they stayed only at the 3 saunas and lived under the same conditions. On days 1, 3, 5, 8, and 10, the inhalation of vapor from each hot spring in these rooms through the nose was performed for 40 min once a day under a condition of high humidity (about 90%). Blood samples from the subjects were collected on days 5 and 10 at 2 hr after each treatment, and before they ate a meal, a blood sample was collected before the first treatment (at body temperature and background radon level). To prevent any effects from a change in climate, the subjects were all residents of the region. Nasal inhalation was used, since the intake of radon is most efficient by this method. The study protocol was approved by the ethics committee of our institution, and written informed consent was obtained from all subjects.

Assays

As antioxidant-associated substances, the SOD activity was measured by the nitroblue tetrazolium (NBT) method, catalase activity by the colorimetric method, lipid peroxide by the thiobarbituric acid (TBA) method, and total cholesterol by the enzyme method. As immune-associated substances, Con A, which stimulated and blast-transformed lymphocyte, was measured by the DNA quantitative method by using nucleic acid/fluorescent probe and CD8/CD4 by the monoclonal antibody assay. Vasoactive substances such as αANP and vasopression were analyzed by radioimmuno assay (RIA). As pain-associated substances, plasma β endorphin were analyzed by RIA, ACTH by the immuno radiometric assay (IRMA), and uric acid by the enzymatic assay (F-DAOS method). As diabetes- associated substances, serum insulin was measured by the RIA method, blood glucose by the glucose enzyme method, pancreatic glucagon by the RIA method, and G-6-PDH by the ultraviolet ray (UV) method.

Data values are presented as the mean ± the standard error of mean (SEM). The statistical significance of differences was determined by using Student's *t*-test to for comparison between two groups or two-way repeated measures analysis of variance (ANOVA). The values of $*p$, $**p$, or $***p$ of less than 0.05, 0.01, or 0.001, respectively, were considered significant.

RESULTS

Temporal changes in control group

On days 5 and 10 in the control group, there were no significant changes in antioxidant-associated substances, immune-associated substances, vasoactive substances, pain-associated substances, and diabetes-associated substances compared to the activities and levels before the first treatment.

Table 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 ± 0.9	12.7 ± 1.3	13.7 ± 0.8*
	Thermal	12.0 ± 0.7	11.1 ± 0.9	13.2 ± 0.4*
	No (Control)	12.3 ± 1.0	11.5 ± 1.2	11.9 ± 0.9
Catalase activity [U/I]	Radon	0.52 ± 0.10	0.44 ± 0.06	0.72 ± 0.08*
	Thermal	0.64 ± 0.09	0.48 ± 0.09	0.84 ± 0.10*
	No (Control)	0.54 ± 0.14	0.50 ± 0.11	0.57 ± 0.12
Lipid peroxide level [nmol/ml]	Radon	1.00 ± 0.10	0.88 ± 0.05*	0.73 ± 0.07**
	Thermal	1.03 ± 0.08	0.83 ± 0.06*	0.87 ± 0.07*
	No (Control)	0.98 ± 0.09	0.96 ± 0.11	0.92 ± 0.10
Total cholesterol [mg/dl]	Radon	105 ± 4	102 ± 6	84 ± 6**
	Thermal	113 ± 11	99 ± 9	94 ± 7*
	No (Control)	108 ± 7	102 ± 9	106 ± 12

Each value represents the mean ± SEM. The number of subjects per each experiment is five. Significance: $*p < 0.05$, $**p < 0.01$ vs. before treatment.

Table 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 ± 64	484 ± 20*	496 ± 38*
	Thermal	406 ± 47	451 ± 26	490 ± 22*
	No (Control)	419 ± 45	422 ± 34	434 ± 36
CD8 positive cells [%]	Radon	35.5 ± 1.9	31.3 ± 1.1*	30.7 ± 1.3*
	Thermal	33.3 ± 1.5	30.9 ± 1.4	31.7 ± 2.1
	No (Control)	34.7 ± 2.8	32.2 ± 1.7	33.5 ± 1.9
CD4 positive cells [%]	Radon	39.4 ± 1.6	44.9 ± 1.1**	43.4 ± 1.0*
	Thermal	38.0 ± 1.9	41.7 ± 1.7*	40.2 ± 2.4
	No (Control)	40.4 ± 1.7	41.6 ± 1.5	41.8 ± 2.1

Each value represents the mean ± SEM. The number of subjects per each experiment is five. Significance: *$p < 0.05$, **$p < 0.01$ vs. before treatment.

Table 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 ± 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 ± 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 ± SEM. The number of subjects per each experiment is five. Significance: **$p < 0.01$ vs. before treatment.

Table 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 ± 0.9**	18.6 ± 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
Adrenocorticotropic hormone [pg/ml]	Radon	18.6 ± 1.6	22.8 ± 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 ± SEM. The number of subjects per each experiment is five. Significance: *$p < 0.05$, **$p < 0.01$ vs. before treatment.

Temporal changes in antioxidant-associated substances

On days 5 and 10, the lipid peroxide level was significantly decreased in both groups compared to the level before the first treatment. On day 10, the activities of SOD and catalase were significantly increased, and the total cholesterol level was significantly decreased in both groups compared to the activities and level before the first treatment (Table 1).

Temporal changes in immune-associated substances

On day 5, the percentage of CD4 positive cells was significantly increased in the thermal group compared to the level before the first treatment. On days 5 and 10, the ConA-induced proliferation and the percentage of CD4 positive cells were significantly increased, and the percentage of CD8 positive cells was significantly decreased in the radon group compared to the proliferation and levels before the first treatment. On day 10, the ConA-induced proliferation was significantly increased in the thermal group compared to the proliferation before the first treatment (Table 2). The percentage of CD4 positive and CD8 positive cells and the percentage of CD4 negative and CD8 negative cells showed no significant changes.

Table 5. Emporal changes in fiabetes-associated dubstances in blood of humans at rach radon inhalation- or thermal-treatment after first treatment.

	Treatment	Before	After first treatment	
			5 days	10 days
Insulin [μU/ml]	Radon	32.2 ± 6.1	33.5 ± 3.6	49.2 ± 4.8***
	Thermal	31.6 ± 4.9	32.8 ± 3.9	41.8 ± 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 ± 9*	102 ± 10
	Thermal	115 ± 12	135 ± 17*	114 ± 6
	No (Control)	108 ± 11	111 ± 14	107 ± 5

Each value represents the mean ± SEM. The number of subjects per each experiment is five. Significance: $*p < 0.05$, $***p < 0.001$ vs. before treatment.

Temporal changes in vasoactive substances

On day 10, the αANP level was significantly increased and the vasopression level was significantly decreased in the radon group compared to the levels before the first treatment, but in the thermal group, there were no significant changes (Table 3).

Temporal changes in pain-associated substances

On day 5, the ACTH level in the radon group was significantly increased compared to the level before the first treatment. On days 5 and 10, the β endorphin level in the radon group was significantly increased compared to the level before the first treatment (Table 4). The uric acid level in the radon group showed no significant change. There were no significant changes in the thermal group.

Temporal changes in diabetes-associated substances

On day 5, the G-6-PDH level was significantly increased in both groups compared to the level before the first treatment. On day 10, the insulin level was significantly increased in both groups compared to the level before the first treatment (Table 5). The levels of blood glucose and pancreatic glucagon showed no significant changes.

DISCUSSION

It has been known that the activity of SOD, which is a scavenger of superoxide radicals, is increased in cultured cells[11] and in various organs of rats[12] and rabbits[6] by exposure to radon. In the present study, similar results were obtained with human blood. In the thermal group, the increase in the activities of SOD and catalase were considered to be due to heat shock protein (HSP) induced by the high temperature of 48°C.[13] The change in the membrane lipids including lipid peroxidation was a direct cause of the decrease of the membrane fluidity. The increase in the membrane fluidity may be caused by an alteration of cell functions associated with structural changes, such as a decrease in membrane cholesterol and an increase in unsaturated bonds in lipids as well as by systemic changes such as a alteration in the Ca^{2+} concentration in response to external stimulation.[14] The decrease in the levels of lipid peroxide and total cholesterol may be linked to the radon effects on membrane structure. These possibilitlies, however, were not evaluated in this study. On the other hand, the induction by a low dose of α-ray irradiation with treatment inhalation of enzymes such as SOD and catalase, which inhibit lipid peroxidation in the body, i.e., an activation of the protective mechanisms of the body, may also be related to this decrease in total cholesterol level. These findings are important in understanding the mechanism of diseases to which radon therapy can be performed, and most of them are called activated oxygen-related diseases such as arteriosclerosis.

Radon inhalation enhanced ConA-induced mitogen response and increased the percentage of the level of CD4 positive cells (CD4; antigen, which is the marker of helper T cell); it decreased the percentage of the level of CD8 positive cells (CD8; antigen, which is the common marker of killer T cell and supresser T cell). The results were larger in the radon group than in the thermal group. The findings suggest that the radon therapy contributes to the prevention of life-style-related diseases, which relate to peroxidation reactions and immune suppression, by an enhancement of the antioxidation function and the immunity function.

Radon inhalation increases the level of αANP, which decreases blood pressure by relaxation of the vascular smooth muscle, and decreases the level of vasopression (a hormone that increases blood pressure). Radon therapy increases the αANP level and decreases the level of vasopression, which is an antidiuretic hormone, in the blood of osteoarthritis patients. These findings indicate what may be a part of the mechanism for the increase in tissue perfusion, namely, the decrease in blood pressure brought about radon inhalation. These findings were consistent with the inhibitory action of αANP on vasopression. The levels of β endorphin with morphine-like analgesic actions and ACTH significantly increased, suggesting a part of the mechanism of alleviation of pain by radon. On the other hand, no significant changes were observed in the levels of blood glucose and pancreatic glucagons, which promotes glucogen degradation during hypogly-

cemia. However, the increase in the levels of insulin, which promotes glycogen synthesis and G-6-PDH suggest that they may contribute to the mechanism underlying the alleviation of diabetic symptoms by radon treatment.

In previous studies, it has been known that the SOD activity and the membrane fluidity were increased, and the lipid peroxide level and the activity of aromatic-L-amino acid decarboxylase, which are key enzymes in the metabolism of biogenic amines, were decreased in the brains of rabbits by radon inhalation.[15] Moreover, we found that radon inhalation increased the level of β endorphin and αANP in the blood of rabbits.[16] These results of animal experiments support those in humans in this study.

Our other study showed no significant changes in blood pressure in normal Wistar Kyoto rats (WKY), but a significant decrease in blood pressure was noted in spontaneously hypertensive rats (SHR) after partial X-irradiation (5 Gy) on the chest. We speculated that hypertensionis are due to Cu/Zn-SOD deficiency in the chest aorta, but irradiation increased the activity of this enzyme, resulting in reduced blood pressure.[17] Furthermore, when an irradiation of 0.5 Gy γ-rays was performed on animals before the induction of diabetes mellitus by alloxan administration, significant changes in the direction of alleviation of diabetic symptoms were observed in the blood glucose level, namely, a degranulation in the pancreas, SOD activity and the peroxylipid level when compared with an induction of diabetes without irradiation.[18] Moreover, we performed 0.5 Gy γ-ray irradiation on nonobese diabetic (NOD) mice[19] to elucidate the mechanism of these diseases in which radon therapy is used as a treatment and found an alleviation of the symptoms of both diseases, thereby demonstrating the enhancement of the antioxidation function. We also obtained similar results with mice having disorders of the liver[20,21] or brain.[22] These findings suggest that an appropriate amount of active oxygen is produced in the body after radon treatment, and this contributes to the alleviation of the symptoms of active oxygen diseases after certain processes, such as an activation of the biological defense mechanism or a promotion of these physiologic changes such as tissue perfusion.[2]

The results in the present study were on the whole larger in the radon group than in the thermal group. The findings suggest that the radon therapy contributes more to the prevention of life-style-related diseases related to peroxidation reactions and immune suppression than thermal therapy. Moreover, they 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. The results obtained in this study, however, do not directly support the hypothesis that the radon therapy contibutes to the prevention of life-style-related diseases.

REFERENCES

1. Ooshima, Y. (1966) System of Current Internal Medicine II, pp. 122–144. Nakayama Shoten, Tokyo.
2. Komoto, Y., Kohmoto, T., Nakao, T., Sunakawa, M. and Yorozu, H. (1988) Tissue perfusion with radon bath in combination with CO_2. Z. Phys. Med. Baln. Med. Klim. **17**: 72–78.
3. Suzuka, I., Yamaoka, K. and Komoto, Y. (1991) Adrenal secretion of catecholamines by inhalation of radon water in relation to an increase of the tissue perfusion rate in rabbits. J. Jpn. Coll. Angiol. **31**: 1182.
4. Mifuna, M., Sobue, T., Arimoto, H., Komoto, Y., Kondo, S. and Tanaoka, H. (1992) Cancer mortality survey in a spa area (Misasa, Japan) with a high radon background. Jpn. J. Cancer Res. **83**: 1–5.
5. Yamaoka, K., Edamatsu, R. and Mori, A. (1991) Increased SOD activities and decreased lipid peroxides levels in rat organs induced by low dose X-irradiation. Free Radic. Biol. Med. **11**: 299–306.
6. Yamaoka, K., Komoto, Y., Suzuka, I., Edamatsu, R. and Mori, A. (1993) Effects of radon inhalation on biological function—Lipid peroxide, SOD activity and membrane fluidity—. Arch. Biochem. Biophys. **302**: 37–41.
7. Deetjen, P. (1997) Epidemiology and biological effects of radon. In: Radon in der Kur medizin, Eds. Pratzel, H. G. and Deetjen, P., pp. 32–38, ISMH, Verlag, Geretsried.
8. Mitsunobu, F., Yamaoka, K., Hanamoto, K., Kojima, S., Hosaki, Y., Ashida, K., Sugita, K. and Tanizaki, Y. (2003) Elevation of antioxidant enzymes in the clinical effects of radon and thermal therapy for bronchial asthma. J. Radiat. Res. **44**: 95–99.
9. Yamaoka, K., Mitsunobu, F., Hanamoto, K., Mori, S., Tanizaki, Y. and Sugita, K. Study on biological effects of radon and thermal therapy on osteoarthritis. J. Pain (in press).
10. Mifune, M. (1981) Radioactive spring and Misasa hot spring. Spa. Sci. **31**: 79–93.
11. Frick, H. and Pfaller, W. (1988) Die auswirkung niedriger a-strahlendosis auf epitheliale zellkulturen. Z. Phys. Med. Baln. Med. Klin. **17**: 23–30.
12. Ma, J., Yonehara, H., Ikebuchi, M. and Aoyama, T. (1996) Effect of radon exposure on superoxide dismutase (SOD) activity in rat. J. Radiat. Res. **37**: 12–19.
13. Hass, M. A. and Massaro, D. (1988) Regulation of the synthesis of superoxide dismutase in rat lungs during oxidant and hyperthermic stresses. J.B.C. **26**: 776–781.
14. Yamaoka, K., Edamatsu, R., Ito, T. and Mori, A. (1994) Effects of low dose X-irradiation on biomembrane in brain cortex of aged rats. Free Radic. Biol. Med. **16**: 529–534.
15. Yamaoka, K., Ishii, K., Ito, T., Komoto, Y., Suzuka, I., Edamatsu, R. and Mori, A. (1994) Changes in biogenic amine neurotransmitters in rabbits brain by inhalation of radon spring. Neurosciences **20**: 17–22.
16. Yamaoka, K. and Komoto, Y. (1996) Experimental study of alleviation of hypertation, diabetes and pain by radon inhalation. Physiol. Chem. Phys. Med. NMR **28**: 1–5.
17. Yamaoka, K. and Ishii, K. (1995) Effects of low-dose g-irradiation to the chest regions on the blood pressure of spontaneous hypertensive rat. Physiol. Chem. Phys. Med. NMR **27**: 161–165.
18. Takehara, Y., Yamaoka, K., Hiraki, Y., Yoshioka, T. and Utsumi, K. (1995) Protection against alloxan diabetes by low dose g-irradiation before alloxan administration. Physiol.

Chem. Phys. Med. NMR **27**: 149–159.
19. Takahashi, M., Kojima, S., Yamaoka, K. and Niki, E. (2000) Prevention of type I diabetes by low-dose g-irradiation in NOD mice. Radiat. Res. **154**: 680–685.
20. Yamaoka, K., Kojima, S. and Nomura, T. (2000) Inhibitory effects of post low dose g-ray irradiation on ferricnitrilotriacetate-induced mice liver damage. Free Radic. Res. **32**: 213–221.
21. Nomura, T. and Yamaoka, K. (1999) Low-dose g-ray irradiation reduces oxidative damage induced by CCl4 in mouse liver. Free Radic. Biol. Med. **27**: 1324–1333.
22. Kojima, S., Matsuki, O., Nomura, T., Yamaoka, K., Takahashi, M. and Niki, E. (1999) Elevation of antioxidants potency in the brain of mice by low-dose g-ray irradiation and its effect on 1-methyl-4-phenyl 1,2,3,6-tetrahydro-pyridine (MPTP)-induced brain damage. Free Radic. Biol. Med. **26**: 388–395.

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