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The Elevation of *p53* Protein Level and SOD Activity in the Resident Blood of the Misasa Radon Hot Spring District

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Radon hot spring/Misasa/Cancer-related mortality rate/*p53* protein level/Superoxide dismutase activity.

To clarify the mechanism by which radon hot springs prevent cancer or not, in this study, blood was collected from residents in the Misasa hot spring district and in a control district. The level of a representative cancer-suppressive gene, *p53*, and the activity of a representative antioxidant enzyme, superoxide dismutase (SOD), were analyzed as indices. The level of serum *p53* protein in the males in the Misasa hot spring district was found to be 2-fold higher than that in the control district, which is a significant difference. In the females in the Misasa hot spring district, SOD activity was approximately 15% higher than that in the control district, which is also statistically significant, and exceeded the reference range of SOD activity despite advanced age. These results suggested that routine exposure of the residents in the Misasa hot spring district to radon at a concentration about 3 times higher than the national mean 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.

INTRODUCTION

A hot spring in Misasa-cho in Tottori Prefecture (Misasa hot spring) is famous as a radon hot spring, where the indoor radon level is reported to be about 3 times (54 Bq/m^3) higher than the national mean in Japan.[1)] According to a survey, the standardized mortality ratios (cancer-related mortality rates) in males and females in the Misasa hot spring district between 1952 and 1988 were 0.538 and 0.463, respectively, regarding the national mean as 1.000. Among the residents in the control district in which environmental factors, except for the radon level similar to the national mean, resembled those of the Misasa hot spring district, the cancer-related mortality rates in males and females were 0.850 and 0.770, respectively.[2)] To clarify whether this was associated with the effects of general hot springs or with those of specific hot springs containing radon, a study investigated the cancer-related mortality rate in the Beppu hot spring district in which environmental factors, except for the radon level similar to the national mean, resembled those of Misasa, and reported that there was no significant difference in the cancer-related mortality rate between the hot spring district and the control district.[3)] The results suggest that radon reduces cancer-related mortality. However, the relative risk for stomach cancer was low in Misasa, as compared with the control district,[2)] and it was true for the standardized incidence ratio of males and the relative risk of females in Beppu. The relative risk for stomach cancer was also low in Beppu.[3)]

On the other hand, the mortality rates from all causes between 1976 and 1993 exhibited no difference between the elevated radon level area and the control area for both sexes. No difference was observed in the incidence of all-site cancers, while stomach cancer incidence seemed to decrease for both sexes and lung cancer incidence for males only seemed to increase in the elevated radon level area. Caution is needed in the interpretation of these findings, however, since the individual exposure level was not measured and major confounding factors, such as smoking and diet, could not be controlled in this study.[4)]

To clarify the mechanism by which the radon hot spring prevents cancer or not, in this study, blood was collected from the residents in the Misasa hot spring district and in the

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control district after obtaining informed consent, and the protein level of *p53*, which is a representative cancer-suppressive gene, and the activity of superoxide dismutase (SOD), which is a representative antioxidant enzyme, were analyzed as indices.

MATERIALS AND METHODS

Subjects

Misasa town was divided into an elevated radon level area and a control area, with mean indoor radon levels of about 54 and 16 Bq/m^3, respectively. Blood samples were collected from 6 males (53 to 81 years) and 8 females (55 to 92 years) in the Misasa hot spring district and from 7 males (73 to 93 years) and 7 females (66 to 81 years) in the control district. The study protocol was approved by the ethics committee of our institution, and written informed consent was obtained from all subjects.

Assays

The level of serum *p53* protein was assayed using the *p53* ELISA,[5] which could detect mutated *p53* in addition to wild-type *p53*. Namely, the microwell strips was washed twice with wash buffer. 100 μL sample diluent was added, in duplicate, to standard wells. 100 μL reconstituted *p53* standard was pipetted into the first wells and the standard dilutions ranging from 50 to 0.8 U/mL was created by transferring 100 μL from well to well. 100 μL from the last wells was discarded. 100 μL sample diluent was added, in duplicate, to the blank wells. 50 μL sample diluent was added to sample wells. 50 μL sample was added, in duplicate, to designated wells. 50 μL of diluted biotin-conjugate to all wells. The microwell strips was covered and incubated 2 hours at room temperature (18 to 25°C). The microwell strips was washed 3 times with wash buffer. 100 μL diluted streptavidin-HRP was washed to all wells. The microwell strips was washed and incubated 1 hour at room temperature. TMB substrate solution was prepared a few minutes prior to use. The microwell strips was washed 3 times with wash buffer. 100 μL mixed TMB substrate solution was added to all wells including blank wells. The microwell strips was incubated for about 10 to 20 minutes at room temperature. 100 μL stop solution was added to all wells including blank wells, and was measured color intensity at 450 nm.

SOD activity was assayed by employing an indirect inhibition assay, in which xanthine and xanthine oxidase serve as a superoxide generator, and nitroblue tetrazolium (NBT) is used as a superoxide indicator.[6] The assay mixture consisted of 960 μl of 50 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.025 mM NBT, and 0.1 mM EDTA, 20 μl of xanthine oxidase, and 20 μl of the serum. Changes in absorbance at 25°C were monitored spectrophoto-metrically at 560 nm for several minutes. Fifty percent inhibition of the reaction between NBT and superoxide anions is defined as one unit of enzymatic activity. Aliquots of the supernatant were chosen such that between 20 and 60% inhibition was attained and incubated in the carbonate buffer for 10 min at 25°C with/without 5 mM KCN. CN^- is known to inhibit Cu/Zn-SOD and thus to allow the measurement of Mn-SOD. The Mn-SOD activity was subtracted from the total SOD activity to obtain the Cu/Zn-SOD activity. The activity was expressed as units/min/mg protein.

Statistical analysis

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 for comparison between two groups or two-way repeated measures analysis of variance (ANOVA). The value of *P of less than 0.01 was considered significant, respectively.

RESULTS

Comparison of the level of serum p53 protein

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. The level of *p53* protein in the females in the Misasa hot spring district was higher than that in the control district; however, there was no significant difference (Fig. 1).

Comparison of the blood level of the SOD activity

SOD activity in the males in the Misasa hot spring district

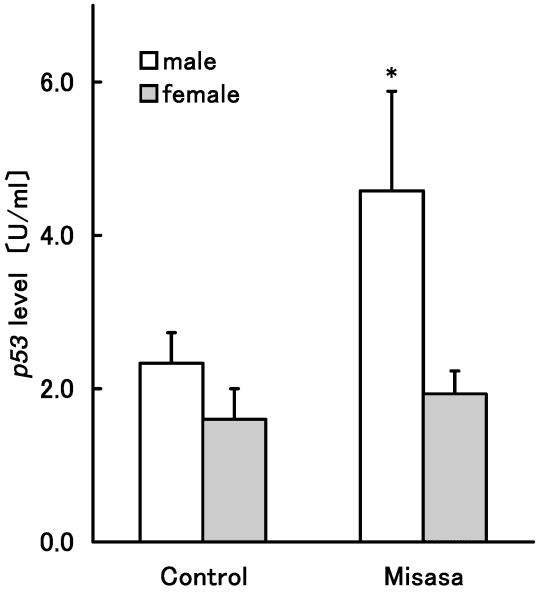

Fig. 1. Comparison of the level of serum *p53* protein between the residents in the Misasa hot spring district and those in the control district. The number of subjects per each experiment is six to eight. Each value represents the mean ± SEM. Significance: *P<0.01 by *t*-test, the residents in the Misasa hot spring district vs. those in the control district.

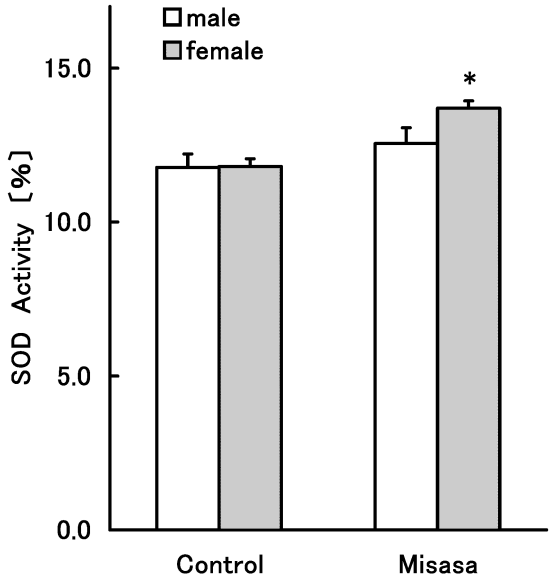

Fig. 2. Comparison of the blood level of the SOD activity between the residents in the Misasa hot spring district and those in the control district. The number of subjects for each experiment and significance are the same as in Fig. 1.

was higher than that in the control district; however, there was no significant difference. SOD activity in the females in the Misasa hot spring district was significantly higher than that in the control district, and the rate of increase was approximately 15%. In the females in the Misasa hot spring district, SOD activity exceeded the reference range of SOD activity (6.4 to 12.8%) (Fig. 2).

DISCUSSION

p53 gene and SOD are considered stress response proteins.[7,8] 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 cancer-suppressive gene protein and antioxidant function. Moreover, *p53* molecules plays a vital role in suppressing the development of cancer,[9,10] and is a tumor suppressor gene which induces apoptosis. As the *p53* level was high in the residents in the Misasa hot spring district, apoptosis of cancer cells may readily occur. SOD activity generally decreases with aging, however, in the females in the Misasa hot spring district, SOD activity exceeded the reference range of SOD activity despite advanced age. These factors may have led to the low cancer-related mortality rate.

We have reported 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 radon group went to a hot bathroom with a high concentration of radon at Misasa Medical Center of 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).[1] 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 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. 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. The results were on the whole larger in the radon group than in the thermal group.[11] 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.

On the other hand, the reports were that *p53* was accumulated by low-dose irradiation[12] or hyperthermia.[13] Moreover, other studies of ours suggested that an appropriate amount of active oxygen species, which is producedin the body after radon inhalation, enhanced the antioxidation functions, and this contributes to the alleviation of the symptoms of active-oxygen-related diseases such as osteoarthritis[14] and bronchial asthma.[15] These results support those in this study.

In the future, the mechanism should be further investigated in a larger number of subjects using immune function as an index.

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