Elevation of Antioxidant Enzymes in the Clinical Effects of Radon and Thermal Therapy for Bronchial Asthma

FUMIHIRO MITSUNOBU^{1*}, KIYONORI YAMAOKA², KATSUMI HANAMOTO², SHUJI KOJIMA³, YASUHIRO HOSAKI¹, KOZO ASHIDA¹, KATSUHIRO SUGITA² and YOSHIRO TANIZAKI¹

Asthma/Radon and thermal therapy/Superoxide dismutase (SOD)/Catalase (Cat)/Lipid peroxide.

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 thermal therapy on asthma in relation to antioxidant enzymes and lipid peroxide. 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; the room radon concentration was 2,080 Bq/m³. Blood samples were collected at 2 h, 14, and 28 days after the first therapy. A blood sample also was collected before the first therapy (at body temperature and background radon level) to be used as the control. The forced expiratory volume in one second (%FEV₁) was significantly increased 28 days after the first therapy. On day 28, the catalase (CAT) activity was significantly increased in comparison with the control. The superoxide dismutase (SOD) activity was significantly increased 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.

INTRODUCTION

Asthma is a disease characterized by an infiltration of the airways by inflammatory cells, the release of numerous mediators from the cells, and airway hyperresponsiveness¹⁾. It has been reported that an increased systemic production of oxygen-free radicals [including the superoxide anion (O_2^-), hydroxyl radicals (·OH), and hydrogen peroxide] by activated inflammatory cells is thought to be involved in the pathophysiology of asthma, such as bronchoconstriction, mucous secretion, and edema^{2,3)}. These harmful effects of the oxidants on airways will depend on the local antioxidant defenses available within the airway epithelial lining fluids⁴⁾. The antioxidant defenses in lung are widely distributed and include both enzymatic and nonenzymatic systems.

E-mail: fumin@cc.okayama-u.ac.jp

¹Department of Medicine, Misasa Medical Center, Okayama University Medical School, 827 Yamada, Misasa, Tottori 682-0192, Japan; ²Department of Radiological Technology, Okayama University Medical School, 2-5-1 Shikata, Okayama 700-8558, Japan; and ³Department of Pharmaceutical Sciences, Tokyo University of Science, 2669 Yamazaki ,Noda, Chiba 278-0022, Japan The major enzymatic antioxidants are SOD and CAT⁵).

Previous studies demonstrated that antioxidant capacity is reduced in patients with asthma⁵⁻⁹⁾. De Raeve et al.⁵⁾ demonstrated that the SOD activity in asthmatics not on inhaled corticosteroid was lower than asthmatics on inhaled corticosteroid and controls, though catalase and glutathione peroxidase in the bronchial epithelium of asthmatics were similar to control. Comhair et al.⁶ showed that a loss of SOD activity in bronchoalveolar lavage fluid (BALF) occurs within minutes of an acute asthmatic response to segmental antigen instillation into the lung of individuals with atopic asthma. Tekin et al.7) found that erythrocyte SOD activity of asthmatics was significantly lower than that of the controls, but there was no significant difference in CAT activity between asthmatics and controls. Shanmugasundaram et al.8) represented that the activities of SOD and CAT in blood were significantly lower in children with asthma, even during resting conditions. They also found that the lipid peroxide levels in plasma and erythrocytes were significantly elevated in asthma.

Therapy using radon (222 Rn), which is volatilized from radon enriched water and mainly emits α -rays, is performed for various diseases such as osteoarthritis and asthma¹⁰). Most of the diseases on which radon therapy is used are related with activated oxygen. Several attempts have been made to clarify its mecha-

^{*}Corresponding author: Phone: +81-858-43-1211, Fax: +81-858-43-1305,

nism, but only a few studies have been made on radon therapy in humans¹¹⁾. We and Ma *et al.* reported the effect of radon inhalation, which enhanced SOD activities in the organs of rabbits and rats^{12,13)}. Moreover, we reported that the antioxidation function was more enhanced by radon therapy than by thermotherapy¹⁴⁾.

In the present study, radon and thermal effects on bronchial asthma were examined in relation to the activities of antioxidant enzymes and the lipid peroxide level.

MATERIALS AND METHODS

Subjects

Nine asthmatics (4 females and 5 males; mean age 59, range 23-79) were recruited from the Misasa Medical Center (Table 1). Asthma was diagnosed according to the definition proposed by the American Thoracic Society¹⁵⁾. All subjects with asthma showed episodic symptoms of wheezing and coughing and experienced symptomatic relief and reversible airway response with an accompanying increase in FEV₁ that exceeded 15% upon treatment with β_2 -adrenergic agonists. Asthmatics were controlled with no changes in medication for at least 1 month, except for the use of short-acting inhaled β_2 -agonists. The severity of asthma was diagnosed according to the guidelines of the National Institutes of Health/World Health Organization (NIH/ WHO)¹⁶⁾. All subjects were nonsmokers and atopic patients. The geometric mean of serum IgE was 582 IU/ml (range 161-2192 IU/ml). No subjects had a history of upper respiratory tract infection within the month before entry.

Atopy was evaluated by a combination of the histories of allergies and skin tests and the presence of serum IgE antibodies specific to the 12 common aeroallergens, including dust mites, pollens, molds, and animal danders. Serum-specific IgE was measured by using the Pharmacia CAP[®] System (Pharmacia Diagnostics AB, Uppsala, Sweden). Atopic patients were defined as those who had positive skin tests and/or the presence of allergen-specific IgE.

Informed consent was obtained from all subjects. The study protocol was approved by the ethics committee of our institution

Pulmonary function tests

Pulmonary function tests were performed with a Chestac 33 (Chest Co., Tokyo, Japan). The following ventilatory parameters were measured in all subjects: forced vital capacity (FVC), FEV₁, and FEV₁/FVC. The FVC and FEV₁ were expressed as a percentage of their predicted values (%FVC and %FEV₁) according to the prediction equations of the Japanese Society of Chest Diseases¹⁷⁾. The ratio of FEV₁ to FVC (FEV₁/FVC) was expressed as a percentage.

Therapy

Therapy was performed once a week. All subjects went to a hot bathroom that had a high concentration of radon at Misasa Medical Center of Okayama University Medical School. The room temperature was 48°C; the room radon concentration was 2,080 Bq/m³ (equivalent to about 40-fold higher than background level)¹⁸⁾. On days 1, 7, 14, 21, and 28, the nasal inhalation of vapor from a hot spring in the bathroom was performed for 40 min once a day under a condition of high humidity. Nasal inhalation was used, since the uptake of radon is most efficient by this method.

Antioxidant assays

Blood samples were collected at 2 h, and at 14 and 28 days after the first therapy; a blood plasma sample was also collected before the first therapy (at body temperature and a radon level background) to be used as the control. The SOD activity was measured by the nitroblue tetrazolium (NBT) method, CAT activity by the spectrophotometric method, and lipid peroxide by the thiobarbituric acid (TBA) method^{19–21)} from blood plasma.

Statistical Analysis

The results were expressed as the means \pm SEM. Serum IgE level was given as the geometric mean and range. A Student's paired *t* test was used to show the change of the means. A *p* value of < 0.05 was regarded as statistically significant.

RESULTS

Pulmonary function tests

The %FEV₁ was 70.2 ± 3.3% (range 61.1–78.9%) before the radon and thermal therapy and 77.7 ± 4.9% (range 64.8–90.5%) 28 days after the first therapy. Namely, the %FEV₁ was significantly increased 4 weeks by radon and thermal therapy (p < 0.05) (Fig. 1). On the other hand, the %FVC was increased to 95.6 ± 4.4%, from 87.1 ± 5.1%, and the FEV₁/FVC was increased to 61.6±5.0%, from 56.3±4.9%. However, no significant changes were noted (Fig. 2).

CAT activities

On day 1, there was no significant change in the CAT activity compared to the control $(1.03 \pm 0.10 \text{ U/ml})$ after the first therapy.



Fig. 1. Changes in %FEV₁ in asthmatics 28 days after the first radon and thermal therapy. Each value presents the mean \pm SEM.

RADON AND THERMAL THERAPY ON ASTHMA

Fig. 4.



Fig. 2. Changes in %FVC in asthmatics 28 days after the first radon and thermal therapy. Each value presents the mean \pm SEM.



Fig. 3. Time-dependent changes in CAT activities in the blood of asthmatics after the first radon and thermal therapy. Each value presents the mean \pm SEM. The shaded area on the graph indicates control range (the value before therapy). Significant difference from the control value with **p* < 0.05.

On day 14, no significant change was observed in the CAT activity. However, on day 28 this activity was significantly increased (238%) in comparison with the control (p < 0.05) (Fig. 3).

SOD activities

On day 1, the SOD activity was significantly increased (121%) compared to the control (10.4 \pm 0.8%) after the first therapy (p < 0.05). On days 14 and 28, no significant changes in the SOD activity were noted (Fig. 4).

Lipid peroxide levels

On day 1, there was no significant change in the lipid peroxide level compared to the control $(0.53 \pm 0.06 \text{ nmol/ml})$ after the first inhalation. On days 14 and 28, the lipid peroxide level was significantly decreased by 43% in comparison with the control (p < 0.05) (Fig. 5).



Time-dependent changes in SOD activities in the blood of

asthmatics after the first radon and thermal therapy. All details are as those described in Fig. 2.



Fig. 5. Time-dependent changes in lipid peroxide level in the blood of asthmatics after the first radon and thermal therapy. All details are as those described in Fig. 2.

DISCUSSION

To clarify the clinical effects of radon and thermal therapy on asthma, the pulmonary function, the activities of SOD and CAT, and the lipid peroxide level were examined in asthmatics before and 28 days after the first therapy. At 28 days, the %FEV₁ (the measurement of airflow limitation) was significantly increased in association with increased CAT activity and decreased lipid peroxide level.

Radon is an inert gas and as such does not react with any chemical component of the body. Upon entry through the lungs or the skin, it reaches the bloodstream and is then distributed throughout the body. Being a lipid soluble, radon tends to accumulate in organs with rich fat, such as the endosecretory glands, and also in nerve fibers, which are surrounded and protected by a lipid-containing layer. Retention time in the body is short, 50% having disappeared after only 15–30 min. During this short period, however, radon launches the beneficial effects while it is in contact with the tissue. Radon is a source of α rays, and it can travel only a distance of about 20 μ m through body tissues. The

Table 1. Clinical characteristics of the patients.

Subject No.	Sex	Age (years)	Asthma duration (years)	Serum IgE (IU/ml)	Atopy	Asthma severity
1	М	50	40	1,256	+	moderate
2	Μ	65	7	396	+	moderate
3	Μ	61	44	2,192	+	severe
4	Μ	63	59	483	+	severe
5	Μ	79	24	196	+	moderate
6	F	23	15	1,020	+	moderate
7	F	50	12	475	+	moderate
8	F	67	10	953	+	moderate
9	F	77	10	161	+	moderate

large transfer of energy associated with the absorption of α particles causes a series of complicated reactions within the tissues. As yet, the molecular processes involved are still poorly understood. It is difficult to directly investigate the effect of radon therapy on alveolar cells in vivo and to take much blood sample. It is safe, however, to assume that radiolytic radicals are released, and these stimulate detoxification processes and may also stimulate such processes as cell metabolism and energy conversion within mitochondria as well as a biosynthesis of enzymes and other proteins or bioactive peptides¹⁰. It was suggested that SOD activity was temporarily elevated because low-dose superoxide anion produced by α -ray transmission induced the synthesis of enzymes.

We examined whether the radon and thermal therapy could improve the reduced enzymatic antioxidant activities in this study. It was observed that the CAT activity was significantly increased and that the lipid peroxide level was significantly decreased 28 days after the first therapy. In contrast, the SOD activity was significantly increased only 2 h after the first therapy, but not after 28 days. Although the reason that the difference between changes in the activities of SOD and CAT was unclear, we speculated that it may be due to the difference between two enzymes as an antioxidant in asthma. Clinical effects were observed even after the level of SOD activities decreased to the initial level. We speculate that the clinical effects may appear in association with more than SOD activity and other antioxidant activities.

Although several epidemiological studies have been reported to determine an association between residential radon exposure and the risk of lung cancer, it is still controversial. In a recent study, Barros-Dios *et al.*²²⁾ demonstrated that residential radon exposure may lead to a 2.5-fold rise in the risk of lung cancer by a population-based case-control study in northwest Spain. But another study, one performed in Misasa Town, Japan, by Sobue *et al.*²³⁾, failed to show the relation between residential radon and lung cancer risk. The dose used in the present study (2,080 Bq/m³) was almost 100 times more than Japanese residential radon levels and about 5 times more than the countermeasure level of radon activity (200–600 Bq/m³ in publication 65 of the International Commission on Radiological Protection), but the radon exposure time is shorter. We considered that radon therapy will

be safe because of the results of the epidemiological study in Misasa Town, where our present study was carried out.

Radon therapy was performed under conditions of high temperature and high humidity in the present study. The hot-spring therapy has been reported to have beneficial effects on pulmonary functions in patients with asthma^{24,25)}. We speculate that the improvement of pulmonary function and the elevation of antioxidant enzymes after the radon and thermal therapy depend on both radon and thermotherapy with humid conditions.

In conclusion, this study has shown that radon and thermal therapy improved the pulmonary function of asthmatics by increasing the reduced activities of antioxidant enzymes. This therapy may be a useful tool to elucidate the contribution of decreased antioxidant protection to the pathogenesis of asthma. This is the pilot study. Further investigation is necessary to clarify the radon and thermal effects on serum IgE levels and the airway inflammation of asthma in association with antioxidant activities, including GPx activities.

REFERENCES

- Barnes, P. J., Chung, K. F. and Page, C. P. (1988) Inflammatory mediators and asthma. Pharmacol. Rev. 40: 49–84.
- Bast, A., Haenen, G. R. and Doelmanm, C. J. (1991) Oxidants and antioxidants: state of the art. Am. J. Med. 91(Suppl 3C): 2S–13S.
- Ryrfeldt, A., Bannenberg, G. and Moldeus, P. (1993) Free radicals and lung disease. Br. Med. Bull. 49: 588–603.
- Heffner, J. E. and Repine, J. E. (1989) Pulmonary strategies of antioxidant defense. Am. Rev. Respir. Dis. 140: 531–554.
- De Raeve, H. R., Thunnissen, F. B., Kaneko, F. T., Guo, F. H., Lewis, M., Kavuru, M. S., Secic, M., Thomassen, M. J. and Erzurum, S. C. (1997) Decreased Cu, Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo. Am. J. Physiol. **272**: L148–154.
- Comhair, S. A., Bhathena, P. R., Dweik, R. A., Kavuru, M. and Erzurum, S. C. (2000) Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. Lancet **355**: 624.
- Tekin, D., Sin, B. A., Mungan, D., Misirligil, Z. and Yavuzer, S. (2000) The antioxidative defense in asthma. J. Asthma **37**: 59–63.
- 8. Shanmugasundaram, K. R., Kumar, S. S. and Rajajee, S. (2001)

Excessive free radical generation in the blood of children suffering from asthma. Clin. Chim. Acta **305**: 107–114.

- Smith, L. J., Shamsuddin, M., Sporn, P. H., Denenberg, M. and Anderson, J. (1997) Reduced superoxide dismutase in lung cells of patients with asthma. Free Radic. Biol. Med. 22: 1301–1307.
- Deetjen, P. (1997) Epidemiology and biological effects of radon. In: Radon in der kurmedizin, Eds. H. G. Pratzel and P. Deetjen, pp. 32–38, I.S.M.H., Verlag Geretsried.
- Gastl, G.D., Egg, M., Herold, A.M., Fodinger, C.H. and Gunhter, R. (1988) Influence of finish bath and radon balneotherapy on the frequency and activity of natural killer cells in peripheral blood. Z. Phys. Med. Baln. Med. Klin. 17: 47–53.
- Yamaoka, K., Komoto, Y., Suzuka, I., Edamatsu, R. and Mori, A. (1993) Effects of radon inhalation on biological functionlipid peroxide level, superoxide dismutase activity, and membrane fluidity. Arch. Biochem. Biophys. **302**: 37–41.
- Ma, J., Yonehara, H., Ikebuchi, M. and Aoyama, T. (1996) Effect of radon exposure on superoxide dismutase (SOD) activity in rats. J. Radiat. Res. 37: 12–19.
- Yamaoka, K., Mifune, T., Mitsunobu, F., Kojima, S., Mori, S., Shibuya, K., Tanizaki, Y. and Sugita, K. (2001) Basic study on radon effects and thermal effects on humans in radon therapy. Physiol. Chem. Phys. Med. N. M. R. 33: 133–138.
- American Thoracic Society (1987) Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am. Rev. Respir. Dis. 136: 225–244.
- National Institutes of Health. National Heart, Lung, and Blood Institute (1997) Guidelines for the diagnosis and management of asthma. National Institutes of Health, Washington, DC.; Publication No. 97–4051.
- 17. Japanese Society of Chest Diseases (1993) Standards of pulmo-

nary function tests for Japanese. The Japanese Journal of Thoracic Diseases **31**: appendix.

- Hopke, T. (1984) Radon and its decay products occurrence. Am. Chem. Soc. pp. 103–106.
- Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276–287.
- Aebi, H. (1984) Catalase in vitro. In: Methods in Enzymology, Vol. 105. pp. 121–126, Academic Press, New York.
- Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay of lipid peroxide in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95: 351–358.
- Barros-Dios, J.M., Barreiro, M.A., Ruano-Ravina, A. and Figueiras, A. (2002) Exposure to residential radon and lung cancer in Spain: a population-based case-control study. Am. J. Epidemiol. 156: 548–555.
- Sobue, T., Lee, V.S., Ye, W., Tanooka, H., Mifune, M., Suyama, A., Koga, T., Morishima, H. and Kondo, S. (2000) Residential radon exposure and lung cancer risk in Misasa, Japan: a case-control study. J. Radiat. Res. 41: 81–92.
- Kurabayashi, H., Kubota, K., Machida, I., Tamura, K., Take, H. and Shirakura, T. (1997) Effective physical therapy for chronic obstructive pulmonary disease. Pilot study of exercise in hot spring water. Am. J. Phys. Med. Rehabil. **76**: 204–207.
- Tanizaki, Y., Kitani, H., Okazaki, M., Mifune, T., Mitsunobu, F. and Honke, N. (1993) Clinical effects of complex spa therapy on patients with steroid-dependent intractable asthma (SDIA). Arerugi 42: 219–227.

Received on September 2, 2002 1st Revision on November 27, 2002 Accepted on January 27, 2003