Potential inhibitory effects of low-dose thoron inhalation and ascorbic acid administration on alcohol-induced hepatopathy in mice

Takahiro Kataoka1, Tsuyoshi Ishida1, Shota Naoe1, Norie Kanzaki2, Akihiro Sakoda2, Hiroshi Tanaka2, Fumihiro Mitsunobu3 and Kiyonori Yamaoka1,*

1Graduate School of Health Sciences, Okayama University, 5-1 Shikata-cho 2-chome, Kita-ku, Okayama-shi, Okayama 700-8558, Japan
2Ningyo-toge Environmental Engineering Center, Japan Atomic Energy Agency, 1550 Kamisaihara, Kagamino-cho, Tomata-gun, Okayama 708-0698, Japan
3Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Okayama University, 5-1 Shikata-cho 2-chome, Kita-ku, Okayama-shi, Okayama 700-8558, Japan
*Corresponding author. Graduate School of Health Sciences, Okayama University, 51 Shikata-cho, 2-chome, Kita-ku, Okayama 700-8558, Japan. Phone: +81-86-235-6852; E-mail: yamaoka@md.okayama-u.ac.jp
(Received 23 March 2022; revised 6 May 2022; editorial decision 19 June 2022)

ABSTRACT

Although thoron inhalation exerts antioxidative effects in several organs, there are no reports on whether it inhibits oxidative stress-induced damage. In this study, we examined the combined effects of thoron inhalation and ascorbic acid (AA) administration on alcohol-induced liver damage. Mice were subjected to thoron inhalation at 500 or 2000 Bq/m3 and were administered 50% ethanol (alcohol) and 300 mg/kg AA. Results showed that although alcohol administration increased the levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in the serum, the combination of thoron inhalation (500 Bq/m3) and AA administration 24 h after alcohol administration effectively inhibited alcohol-induced liver damage. The combination of thoron inhalation (500 Bq/m3) and AA administration 24 h after alcohol administration increased catalase (CAT) activity. Alcohol administration significantly decreased glutathione (GSH) levels in the liver. The GSH content in the liver after 2000 Bq/m3 thoron inhalation was lower than that after 500 Bq/m3 thoron inhalation. These findings suggest that the combination of thoron inhalation at 500 Bq/m3 and AA administration has positive effects on the recovery from alcohol-induced liver damage. The results also suggested that thoron inhalation at 500 Bq/m3 was more effective than that at 2000 Bq/m3, possibly because of the decrease in GSH content in the liver. In conclusion, the combination of thoron inhalation at 500 Bq/m3 and AA administration promoted an early recovery from alcohol-induced liver damage.

Keywords: alcohol-induced liver damage; oxidative stress; antioxidative function; ascorbic acid (AA); thoron

INTRODUCTION

Radon (222Rn) inhalation activates antioxidative functions and inhibits oxidative stress-induced damage in mice [1]. For example, radon inhalation inhibits alcohol-induced acute hepatopathy [2], which is induced by free radicals or reactive oxygen species (ROS).

Treatment of some pain-related diseases, such as rheumatoid arthritis [3, 4], may involve radon therapy, where inhalation treatment using radon gas is applied [5]. There are two types of radon sources: hot springs [6] and galleries [7, 8]. However, the application of radon therapy is limited owing to the scarcity of natural radon sources. Therefore, thoron (220Rn; Tn), a radioisotope of radon, may be useful for reproducing an artificial radon treatment environment. Interestingly, we previously reported that thoron inhalation also has antioxidative effects [9]. Our findings suggested that thoron inhalation could be used for the treatment of ROS-related diseases.

The bioactive characteristics of thoron differ from those of radon. One of the probable reasons for this is the difference in physical characteristics. For example, the half-life of radon is 3.8 days, whereas...
that of thoron is 55.6 seconds. These differences may affect the distribution of radon or thoron in the body. Another difference is in the $\alpha$-particle energy emitted; the $\alpha$-particle energy emitted by radon is 5.490 MeV, whereas that emitted by thoron is 6.288 MeV. In addition, the radioactive characteristics of radon progenies differ from those of thoron progenies. Moreover, the dose conversion factors of radon and thoron progenies are 16.8 and 107 nSv (Bq m$^{-1}$ h$^{-1}$), respectively [10]. Thus, their biological effects are different.

Previously, we reported that artificial thoron hot springs show beneficial effects for human health [11]. To enhance the health effects and reduce the exposure dose, a combination of thoron inhalation and antioxidant treatment may be useful, based on our previous findings [12]. Ascorbic acid (AA), or vitamin C, may be one of choices, as it is abundant in some foods, including lemons.

Several attempts have been made to reproduce radon therapy conditions using monazite [9], which contains relatively high levels of thorium. Thus, according to the availability of monazite, it may be used for practical medical applications without depending on natural radon sources. Our previous animal study using materials including a small amount of thorium series showed that thoron concentration in the air was sufficient to activate antioxidative functions in mice [9]. However, there are no reports of thoron inhalation inhibiting oxidative stress-induced damage. Based on our previous research, hepatopathy may be a possible indication for radon therapy [1]. Moreover, another study reported that thoron has a stronger physiological effect than radon [9]. Therefore, to further investigate and confirm the underlying mechanisms in mice, we adopted an alcohol administration method that can easily induce hepatopathy.

In this study, we aimed to examine whether thoron inhalation inhibits oxidative stress-induced damage. We used a mouse model of alcohol-induced oxidative damage for the following reasons: antioxidative effects induced by radon inhalation in the liver have already been reported [2]; effects of radon on alcohol-induced hepatopathy in terms of alleviating oxidative stress have also been reported [13]. Thoron concentrations were determined based on our previous study [9]. Despite the lack of epidemiological evidence for the cancer risk induced by thoron inhalation [10], we used low thoron doses in combination with AA, which is a widely used antioxidant.

### MATERIALS AND METHODS

#### Animals

Male C57BL/6 N mice (8 weeks old) were purchased from CLEA Japan (Tokyo, Japan). This experimental protocol was approved by the Animal Care and Use Committee of Okayama University. Mice were housed at room temperature (22 ± 2°C) during the experiment.

#### Experimental protocol

The mice were divided into 24 groups. There were 5–7 mice in each group. The details of the experimental conditions are shown in Fig. 1. The mice were exposed to thoron at 500, or 2000 Bq/m$^3$ for 24 h. Next, AA and alcohol or alcohol only were administered to the mice. After 6 h (Fig. 1A) or 24 h (Fig. 1B) of incubation, the mice were euthanized using CO$_2$.

The mice were first administered AA and then exposed to thoron at concentrations of 500, or 2000 Bq/m$^3$ for 24 h. Subsequently, the mice were administered alcohol. At 24 h after administration, the mice were euthanized with CO$_2$ (Fig. 1C).

#### Thoron inhalation

Mice were exposed to thoron at concentrations of 500, or 2000 Bq/m$^3$ for 1 d using our exposure system [9]. The sham inhalation group was exposed to ambient air using an exposure system. The coefficients of variance for thoron concentrations during the exposure were 10% or lower.

#### Alcohol and ascorbic acid administration

The mice were intraperitoneally administered AA (Kanto Chemica. Co. Inc, Tokyo, Japan) at a dose of 300 mg/kg body weight. Immediately after AA administration, the mice were intraperitoneally administered 50% ethanol at a concentration of 5.0 g/kg body weight. The concentration of AA administered was determined in our previous study [12]. The livers were removed quickly after euthanasia using CO$_2$. Blood was collected from the heart and serum was separated by centrifugation at 3000 × g for 5 min at 4°C. The supernatant and liver were preserved at −80°C until the assay.

#### Biochemical assays

The levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in the serum and the level of triglyceride (TG) in the liver were assayed using an assay kit (Wako Pure Chemical Industry, Co., Ltd., Osaka, Japan).

Superoxide dismutase (SOD), catalase (CAT), total glutathione (t-GSH) and total protein levels in the liver were assayed using assay kits (SOD: Dojindo molecular Technologies, Inc., Kumamoto, Japan; CAT: Cayman Chemical, MI, USA; t-GSH: OXIS Health Products, Inc., Portland, OR, USA; total protein: Dojindo Molecular Technologies, Inc., Kumamoto, Japan). They were measured following the method described previously [14, 15].

#### Statistical analyses

Each data point is a ratio of the activity or content compared with that of the control group. The data are presented as the mean ± standard error of the mean. Statistical significance of the differences was determined using Tukey’s test for multiple comparisons, followed by a one-way analysis of variance. Data were considered statistically significant at $P < 0.05$.

### RESULTS

#### Effects of thoron inhalation followed by AA administration on hepatic functions and TG accumulation in the liver at 6 h after alcohol administration

GOT activities in the serum of all groups and GPT activities of Sham + Alco (500–6, 2000–6), Tn + Alco (500–6, 2000–6) and Tn + AA + Alco (500–6, 2000–6) groups were significantly higher than those in the control group. GOT activity in the serum of the Tn + AA + Alco (2000–6) group was significantly higher than that in the serum of the Sham + AA + Alco (2000–6) group (Fig. 2A). The TG levels in the livers of the Sham + AA + Alco (2000–6) group were significantly higher than those in the livers of the control (2000–6) group (Fig. 2B).
Fig. 1. Experimental procedure. Mice were treated with thoron (Tn), Alcohol (Alco), AA. (A) AA administration after Tn inhalation at a concentration of 500 or 2000 Bq/m³ for 6 h; (B) AA administration after Tn inhalation at a concentration of 500 or 2000 Bq/m³ for 24 h; (C) AA administration before Tn inhalation at a concentration of 500 or 2000 Bq/m³ for 24 h.
Fig. 2. Effects of thoron inhalation followed by AA administration on hepatic functions and TG accumulation in the liver at 6 h after alcohol administration (Fig. 1A). Mean ± standard error of mean (SEM), n = 5–7, *P < 0.05, **P < 0.01, ***P < 0.001.
Fig. 3. Effects of thoron inhalation followed by AA administration on hepatic functions and TG accumulation in the liver at 24 h after alcohol administration (Fig. 1B). Mean ± standard error of mean (SEM), n = 6–7, *P < 0.05, **P < 0.01.

**Effects of thoron inhalation followed by AA administration on hepatic functions and TG accumulation in the liver at 24 h after alcohol administration**

GOT activities in the serum of Sham + Alco (500–24), Tn + Alco (500–24) and Sham + AA + Alco (2000–24) groups were significantly higher than those in the serum of the control group. GPT activities in the serum of Sham + Alco (2000–24), Sham + AA + Alco (2000–24) and Tn + AA + Alco (2000–24) groups were significantly higher than those in the serum of the control (2000–24) group (Fig. 3A).

The TG levels in the livers of the Sham + Alco (2000–24) group were significantly higher than those in the livers of the control (2000–24) group (Fig. 3B).
Fig. 4. Effects of thoron inhalation following AA administration on hepatic functions and TG accumulation in the liver at 24 h after alcohol administration (Fig. 1B Control, Sham + Alco, Tn + Alco, Fig. 1C). Mean ± standard error of mean (SEM), n = 6–7, *P < 0.05, **P < 0.01.

Effects of thoron inhalation following AA administration on hepatic functions and TG accumulation in the liver at 24 h after alcohol administration

The activities of GOT and GPT in the serum of Sham + Alco (500–24) and AA + Tn + Alco (2000–24) groups were significantly higher than those in the serum of the control group, while GOT activities in the serum of AA + Tn + Alco (500–24) group were significantly lower than those in the serum of the Sham + Alco (500–24) group (Fig. 4A).

Effects of thoron inhalation followed by AA administration on antioxidative functions in the liver at 6 h after alcohol administration

SOD activity in the liver of the Tn + AA + Alco (2000–6) group was significantly lower than that in the liver of the Sham + Alco (2000–6) and Sham + AA + Alco (2000–6) groups. CAT activity in the Tn + AA + Alco (500–6) group was significantly higher than that in the other groups. The t-GSH contents in the liver of Sham + Alco (500–6, 2000–6), Tn + Alco (500–6, 2000–6), Sham + AA + Alco
Thoron and ascorbic acid inhibit liver damage

500 Bq/m³

2000 Bq/m³

Fig. 5. Effects of thoron inhalation followed by AA administration on antioxidative functions in the liver at 6 h after alcohol administration (Fig. 1A). Mean ± standard error of mean (SEM), n = 6–7, *P < 0.05, **P < 0.01, ***P < 0.001.

(500–6, 2000–6) and Tn + AA + Alco (500–6, 2000–6) groups were significantly lower than those in the control group. In addition, the t-GSH content in the liver of Tn + AA + Alco (2000–24) group was significantly lower than that in the liver of the Sham + Alco (2000–24) group (Fig. 5).

Effects of thoron inhalation followed by AA administration on antioxidative functions and TG accumulation in the liver at 24 h after alcohol administration

The t-GSH levels in the liver of Tn + Alco (2000–24), Sham + AA + Alco (2000–24) and Tn + AA + Alco (2000–24) groups were significantly lower than those in the liver of the control (2000–24) group. In addition, the t-GSH content in the liver of Tn + AA + Alco (2000–24) group was significantly lower than that in the liver of the Sham + Alco (2000–24) group (Fig. 6).

Effects of thoron inhalation following AA administration on antioxidative functions and TG accumulation in the liver at 24 h after alcohol administration

CAT activity in the liver of the AA + Sham + Alco (500–24) group was significantly lower than that in the liver of the control (500–24) and Sham + Alco (500–24) groups. The t-GSH content in the liver of
Fig. 6. Effects of thoron inhalation followed by AA administration on antioxidative functions in the liver at 24 h after alcohol administration (Fig. 1B). Mean ± standard error of mean (SEM), n = 7, ∗ P < 0.05, ∗∗ P < 0.01, ∗∗∗ P < 0.001.

the Tn + Alco (2000–24) group was significantly lower than that in the liver of the control (2000–24) group (Fig. 7).

**DISCUSSION**

We reported that although thoron inhalation at 500 or 2000 Bq/m³ for 24 h significantly increased t-GSH content in the normal mouse liver, lipid peroxidation (LPO) levels in the liver were significantly increased by thoron inhalation at 2000 Bq/m³ [9]. This suggests that thoron inhalation at 2000 Bq/m³ results in a redox imbalance in the liver. A high thoron concentration of approximately 530 kBq/m³ decreased the activity of GSH peroxidase, an antioxidant enzyme, within 30 days of exposure, in the lung and heart and increased LPO levels [16]. We also reported that the combination of radon inhalation (2000 Bq/m³) for 24 h and AA administration after radon inhalation effectively inhibited acute alcohol-induced liver damage [12]. In comparison, in the present study, we observed that the combination of thoron inhalation (500 Bq/m³) and AA administration 24 h after alcohol administration effectively inhibited alcohol-induced liver damage (Figs 3 and 4). These results were similar to those of our previous study [12]. However, the combination of thoron inhalation (2000 Bq/m³) and AA administration 24 h after alcohol administration did not inhibit alcohol-induced liver damage (Figs 3 and 4). The results showed a tendency similar to that in our previous study [9]. These findings
Thoron and ascorbic acid inhibit liver damage

Fig. 7. Effects of thoron inhalation following AA administration on antioxidative functions in the liver at 24 h after alcohol administration (Fig. 1B Control, Sham + Alco, Tn + Alco, Fig. 1C). Mean ± standard error of mean (SEM), n = 6–7, * P < 0.05, ** P < 0.01.

suggest that the optimal concentration for thoron inhalation with AA administration may be lower than that for radon inhalation.

AA is a widely used antioxidant. Non-enzymatic ascorbate reactions are involved in the detoxification of ROS, such as superoxide, hydroxyl, alkoxyl and peroxyl radicals [17]. This antioxidative property leads to the inhibition of hepatopathy induced by carbon tetrachloride [18], cypermethrin [19], organophosphate [20] and alcohol [21]. We also compared the effects of AA administration with pre-treatment or post-treatment of thoron inhalation on acute alcohol-induced liver damage because our previous study suggested that manganese SOD (Mn-SOD) activity is induced by moderate oxidative stress induced by radon [22] and inhibits alcohol-induced liver damage [23]. Therefore, pretreatment with AA may not induce antioxidative activities via thoron inhalation. Interestingly, the mitigation effects on alcohol-induced liver damage in the AA + Sham + Alco group seemed to be more effective than those in the Sham + AA + Alco group (Figs 3 and 4). It has been previously reported that the peak AA concentration in the liver after oral administration is approximately 3 hours, while the concentration is 25% of the peak at 24 hours [24]. Although the administration method was different from that used...
in the present study, pre-treatment with AA may result in beneficial effects.

Ethanol is metabolized via three pathways which involve alcohol dehydrogenase, the microsomal ethanol oxidation system and CAT [25]. These pathways induce ROS production during ethanol metabolism. For example, GSH utilization increases whereas GSH synthesis decreases after a high-dose ethanol treatment [26]. Other reports have suggested that alcohol administration depletes GSH by inhibiting the expression of mitochondrial GSH transporter [27–29]. Thus, antioxidants play a critical role in the inhibition of alcohol-induced liver damage. An increase in the ratio of reduced GSH to oxidized GSH (GSSG) is an indicator of oxidative stress. On the other hand, intracellular GSH is usually present in the reduced form in most cases [30]. Therefore, in this study, we focused on t-GSH, which is the sum of the two. We previously reported that radon inhalation inhibits alcohol-induced liver damage [2] and that the combination of AA administration and radon inhalation exerts more beneficial effects against alcohol-induced liver damage [12]. In the present study, 6 h after alcohol administration, the GSH content in the liver significantly decreased (Fig. 5). In addition, GSH content in the livers of mice that inhaled thoron at 2000 Bq/m³ with or without AA administration was lower than that in the livers of mice that inhaled thoron at 500 Bq/m³ (Fig. 5). These results indicate differences in the inhibitory effects of thoron inhalation. Moreover, CAT activity in the liver of the Tn + AA + Alco (500–6) group was significantly higher than that in the livers of other groups (Fig. 5). CAT plays a role not only in the detoxification of ROS but also in alcohol metabolism, thereby resulting in the production of ROS [25]. This could also explain why the GPT activity in the Tn + AA + Alco (500–6) group increased. Another possible reason may be the state in which alcohol is not sufficiently digested naturally at 6 h after alcohol administration. As a result, both GOT and GPT activities increased. Figures 3 and 4 show that at 24 h spontaneous digestion had progressed after alcohol administration. By this time, the inhalation of 500 Bq/m³ Tn had promoted decreases in both GOT and GPT activity, as compared to the sham + Alco group. Moreover, the GSH content in the liver after AA administration before thoron inhalation at 2000 Bq/m³ was closer to the control level than that after thoron inhalation (Figs 6 and 7). These findings suggest that pre-treatment with AA is more effective than post-treatment with AA.

In conclusion, the combination of thoron inhalation at 500 Bq/m³ and AA administration resulted in the early recovery from alcohol-induced liver damage in mice. The results also suggest that thoron inhalation at 500 Bq/m³ was more effective than that at 2000 Bq/m³. Although the absorbed dose in the liver following radon inhalation was estimated in our previous study [31], we could not estimate the absorbed dose following thoron inhalation. In addition, since we did not examine concentration dependence, we cannot conclude whether or not these results depend on the concentration of AA. However, AA dose–response experiments may yield new insights. Furthermore, we also plan to conduct a more detailed study to include other indications and to determine the extent to which the dose of a therapeutic drug can be reduced if thoron inhalation is used as an add-on treatment. Estimation of the absorbed dose may provide clues to clarify the differences between radon and thoron inhalation.

ACKNOWLEDGMENTS
The authors are grateful to Mr. Junki Yano and Ms. Hina Shuto for their technical support.

CONFLICT OF INTEREST
No conflicts of interest.

FUNDING
This work was supported by Okayama University and Japan Atomic Energy Agency.

REFERENCES
1. Kataoka T. Study of Antioxidative effects and anti-inflammatory effects in mice due to low-dose X-irradiation or radon inhalation. J Radiat Res 2013;54:587–96.
2. Toyota T, Kataoka T, Nishiyama Y et al. Inhibitory effects of pretreatment with radon on acute alcohol-induced hepatopathy in mice. Mediat Inflamm 2012;2012:382801.
3. Franke A, Reiner L, Franke PH et al. Long-term efficacy of radon therapy in rheumatoid arthritis-a randomized, sham-controlled study and follow-up. Rheumatology 2000;39:894–902.
4. Franke A, Reiner L, Resch KL. Long-term benefit of radon spa therapy in the rehabilitation of rheumatoid arthritis: a randomised, double-blinded trial. Rheumatol Int 2007;27:703–13.
5. Maier A, Wiedemann J, Rapp F et al. Radon exposure-therapeutic effect and cancer risk. Int J Mol Sci 2021;22:316.
6. Mitsuonobu F, Yamaoka K, Hanamoto K et al. Elevation of antioxidid enzymes in the clinical effects of radon and thermal therapy for bronchial asthma. J Radiat Res 2003;44:95–9.
7. Modr A, Dobias H, Ritter M. Effects of Low-Dose Radon Therapy Applied Under Hyperthermic Conditions (RnHT) on Inflammatory and Non-Inflammatory Degenerative Disease Conditions. In: Huilgol N (ed). Hyperthermia. London: Intech, 2013, 185–92.
8. Tempfer H, Hofmann W, Schober A et al. Deposition of radon progeny on skin surfaces and resulting radiation doses in radon therapy. Radiat Environ Biophys 2010;49:249–59.
9. Kobashi Y, Kataoka T, Kanzaki N et al. Comparison of antioxidative effects between radon and thoron inhalation in mouse organs. Radiat Environ Biophys 2020;59:473–82.
10. Tokonami S. Characteristics of thoron (220Rn) and its progeny in the indoor environment. Int J Environ Res Public Health 2020;17:8769.
11. Kataoka T, Aoyama Y, Sakoda A et al. Basic study on biochemical mechanism of thoron and thermal therapy. Physiol Chem Phys Med NMR 2006;38:85–92.
12. Etani R, Kataoka T, Nishiyama Y et al. Combined effects of radon inhalation and antioxidant vitamin administration on acute alcohol-induced hepatopathy in mice. J Nucl Sci Technol 2015;52:1512–8.
13. Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. Arch Toxicol 2009;83:519–48.
14. Kataoka T, Shuto S, Naoe S et al. Radon inhalation decreases DNA damage induced by oxidative stress in mouse organs via
14. Kataoka T, Shuto H, Yano J et al. X-irradiation at 0.5 Gy after the forced swim test reduces forced swimming-induced immobility in mice. *J Radiat Res* 2020;61:517–23.
15. Menon A, Indu R, Chacko T et al. Low-dose ionising radiation effects: effects of thoron inhalation. *Int J Low Radiat* 2014;9:252–65.
16. Njus D, Kelley PM, Tu YJ et al. Ascorbic acid: the chemistry underlying its antioxidant properties. *Free Radic Biol Med* 2020;159:37–43.
17. Ademuyiwa O, Adesanya O, Ajuwon OR. Vitamin C in CCl4 hepatotoxicity - a preliminary report. *Hum Exp Toxicol* 1994;13:107–9.
18. Grajeda-Cota P, Ramirez-Mares MV, Gonzalez de Mejia E. Vitamin C protects against in vitro cytotoxic-ity of cypermethrin in rat hepatocytes. *Toxicol in Vitro* 2004;18:13–9.
19. Suresh MV, Kumar CVS, Lal JJ et al. Impact of massive ascorbic acid supplementation on alcohol induced oxidative stress in guinea pigs. *Toxicol Lett* 1999;104:221–9.
20. Kataoka T, Etani R, Kanzaki N et al. Radon inhalation induces manganese-superoxide dismutase in mouse brain via nuclear factor-κB activation. *J Radiat Res* 2017;58:887–93.