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Consumption of Jeju Ground Water Containing Vanadium Components Enhances Hepatic Antioxidant Defense Systems in *ob/ob* Mice

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The present study examined the effects of consumption of Jeju ground water containing vanadium components on oxidative stress in obese (*ob/ob*) mice. Intake of Jeju ground water decreased the generation of oxidative stress induced-lipid peroxidation in the liver of *ob/ob* mice It also enhanced the enzymatic antioxidant defense system by increasing the protein expression and activity of superoxide dismutase, catalase, and glutathione peroxidase in liver tissues. Jeju ground water intake also upregulated the intracellular content of reduced glutathione. The induction of antioxidant enzyme expression by consumption of Jeju ground water was mediated by the erythroid transcription factor NF-E2 (Nrf2). Increased nuclear expression of phospho Nrf2 was observed in *ob/ob* mouse liver cells following intake of Jeju ground water. These results suggest that consumption of Jeju ground water stimulated the antioxidant defense system in the livers of *ob/ob* mice via induction of Nrf2.

Key words : Jeju ground water, *ob/ob* mice, antioxidant defense system, erythroid transcription factor NF-E2

Introduction

The liver plays a central role in the maintenance of systemic lipid homeostasis and is especially susceptible to damage caused by reactive oxygen species (ROS) [7]. ROS exert detrimental effects on hepatocytes by damaging DNA, lipid, and protein, leading to a disruption in cellular metabolic equilibrium and an aggravation of metabolic syndrome [17]. The latter is characterized by insulin resistance, hypertension, abnormalities in cholesterol levels, excessive blood clotting, and obesity. Mutant mice that cannot produce the appetite-suppressing hormone leptin (*ob/ob* mice) eat to the point of extreme obesity. These mice, as well as high-fat diet-induced obese mice, generate high amounts of ROS and demonstrate increased hepatic lipid peroxidation compared with healthy wild type mice [1,4,8,23]. As such, *ob/ob* mice were employed in the current study to explore the impact of Jeju ground water on the hepatic antioxidant system.

Recently, we reported that Jeju ground water possesses *in vitro* and *in vivo* antioxidant [9,10,11] and immuno-stimulant properties, as determined in peripheral immunocytes of gamma-ray-irradiated mice [6]. Jeju ground water contains trace amounts of vanadium components, which have been shown to exert antioxidant effects [15,18,22].

The present study investigated the effects of vanadium-containing Jeju ground water on oxidative stress in *ob/ob* mice, as assessed by the extent of lipid peroxidation; the levels and activities of antioxidant enzymes; the intracellular content of reduced glutathione (GSH); and the nuclear content of phospho erythroid transcription factor NF-E2 (Nrf2).

Materials and Methods

Reagents

Jeju ground water preparations S3 and S1, containing vanadium compounds at concentrations of 26.0±2.0 μg/l (S3) and 8.0±0.9 μg/l (S1), was provided by the Jeju Special Self-Governing Province Development Corporation (Jeju, South Korea). Primary antibodies directed against catalase (CAT) and glutathione peroxidase (GPx) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-copper/zinc superoxide dismutase (Cu/Zn SOD) antibody was purchased from Stressgen Corporation (Victoria, BC, Canada).

Animals and diet

The *ob/ob* mice were purchased from Jackson Laboratory

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(Bar Harbor, ME, USA). The *ob/ob* mouse breeding room was maintained under a constant 12 hr light/12 hr dark cycle with a temperature of 23±2℃ and a relative of humidity 55±5℃ throughout the experimental period (70 days). Mice were provided with a standard diet *ad libitum* and given free access to either tap water or Jeju ground water S3 or S1 during this period. The obesity status of each mouse was monitored by measuring the dietary intake and body weight. These experiments were approved by the Committee for Laboratory Animal Care and Use, Chosun University. In addition, all procedures were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health, Bethesda, MD, USA).

Lipid peroxidation assay

8-Isoprostanes, products of ROS-mediated peroxidation of arachidonic acid, were measured in mouse liver tissue by enzyme immunoassay (EIA). The 8-isoprostane EIA kit (Cayman Chemical, Ann Arbor, MI, USA) was used according to the manufacturer's instructions.

SOD activity

SOD activity was measured in mouse liver tissue using a colorimetric assay kit (Abcam, Cambridge, MA, USA) according to the manufacturer's protocol. The kit utilized the cell proliferation reagent WST-1, a tetrazolium salt, produces a water-soluble formazan dye that can be detected at 450 nm upon the reduction of WST-1 by superoxide anion. WST-1 reduction is inhibited by SOD, which catalyzes the dismutation of the superoxide anion to produce H_2O_2 and O_2. Therefore, SOD activity was calculated on the basis of the percent inhibition of WST-1 reduction, which in turn reflected the percent inhibition of the superoxide anion.

CAT activity

CAT activity was measured in mouse liver tissue using a CAT assay kit (Abcam, Cambridge, MA, USA) according to the manufacturer's protocol. CAT reacts with H_2O_2 to produce H_2O and O_2. Unconverted H_2O_2 reacts with the OxiRed probe provided in the kit to produce a product that can be detected at 570 nm. CAT activity was expressed in mU/ml.

GPx activity

GPx activity was determined in mouse liver tissue using the GPx assay kit (Oxford Biomedical Research, MI, USA) according to the manufacturer's protocol. The GPx enzyme reaction was indirectly assessed following the addition of tert-butyl hydroperoxide to the liver lysate. GPx catalyzes the reduction of tert-butyl hydroperoxide by GSH. Oxidized glutathione (GS-SG) is then reverted to GSH by a reaction between glutathione reductase and NADPH. The resultant oxidation of NADPH to $NADP^+$ yields a decrease in absorbance at 340 nm. The rate at which absorbance of 340 nm decreases is therefore directly proportional to the activity of GPx. GPx activity was expressed in mU/ml.

Western blotting analysis

Liver tissues were lysed in 0.5 ml of a lysis buffer consisting of 120mM NaCl, 40mM Tris (pH 8), and 0.1% NP-40. Aliquots of the lysates (40 μg protein) were boiled for 5 min and electrophoresed in a 10% SDS polyacrylamide gel. The gels were transferred onto nitrocellulose membranes. Nitrocellulose membranes were subsequently incubated with primary antibodies. The membranes were further incubated with secondary anti-immunoglobulin-G horseradish peroxidase conjugate (Pierce, Rockford, IL, USA), followed by exposure to X-ray film. The protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's instructions.

Measurement of intracellular reduced glutathione (GSH)

The intracellular GSH content was measured using the GSH-400 colorimetric assay kit (OXIS International, Portland, OR, USA), as follows. Liver tissues were homogenized in a metaphosphoric acid working solution. After centrifugation, 50 ml of a chromogenic reagent solution in HCl was added to 900 ml of supernatant, followed by gentle vortex mixing. Following the addition of 50 ml of 30% NaOH, the mixtures were incubated at 25±3°C for 10 min. After centrifugation, the absorbance of the clear supernatant was measured at 400 nm. GSH level was measured in μM [21].

Statistical analysis

All values are represented as the mean±the standard error (SE). Data were analyzed with analysis of variance (ANOVA) using the Tukey test. A $p<0.05$ value was considered statistically significant.

Result and Discussion

A recent study reported that obese mice showed increased hepatic lipid peroxidation, suggesting that the livers of these mice were under oxidative stress [14]. Additional data indicated that hepatic oxidative stress in high-fat diet-induced obesity animal model and *ob/ob* mutant mice stemmed from the excessive generation of ROS and/or the decreased capacity of antioxidant systems [5,8,19]. The present study therefore examined the impact of Jeju ground water preparations S3 or S1 on lipid peroxidation and the enzymatic antioxidant defense system in the livers of *ob/ob* mice.

Obesity is associated with metabolic alterations in lipid concentrations and lipid peroxidation. These metabolic abnormalities lead to detrimental effects, such as damage to cellular membranes and rupture of red blood cells, heart disease, and production of carcinogenic and/or mutagenic end product [24]. The isoprostanes provide an example of metabolic lipid abnormalities. These prostaglandin-like molecules comprise a family of eicosanoids of non-enzymatic origin that result from the random oxidation of phospholipids by ROS. Notably, the plasma concentration of isoprostane is increased in obese subjects. Indeed, the 8-isoprostane level has been used as a biomarker of lipid peroxidation under conditions of antioxidant deficiency and oxidative stress including those associated with obesity [20].

To investigate the antioxidant effects of Jeju ground water in *ob/ob* mice, the extent of lipid peroxidation and the activity and protein expression of SOD, CAT, and GPx were evaluated in *ob/ob* mouse liver tissue. Jeju ground water S3 and S1 decreased hepatic lipid peroxidation compared with tap water. The 8-isoprostane values were 122 pg/ml and 137 pg/ml following administration of S3- and S1-Jeju ground water, respectively and 155 pg/ml following administration of tap water (Fig. 1).

SOD dismutates the superoxide anion into hydrogen peroxide and oxygen. Hydrogen peroxide is in turn excreted as water based on the activity of CAT and GPx. These actions thereby protect the body from ROS toxicity. GPx has a broader protective spectrum than CAT, in that GPx uses reducing equivalents donated by GSH to catalyze the reduction of both hydrogen peroxide and other hydroperoxides, including lipid hydroperoxides [2,3].

It has been reported that hepatic Cu/Zn SOD, GPx and CAT activities are all reduced in the livers of *ob/ob* mice in comparison with the livers of wild type mice [16]. In this

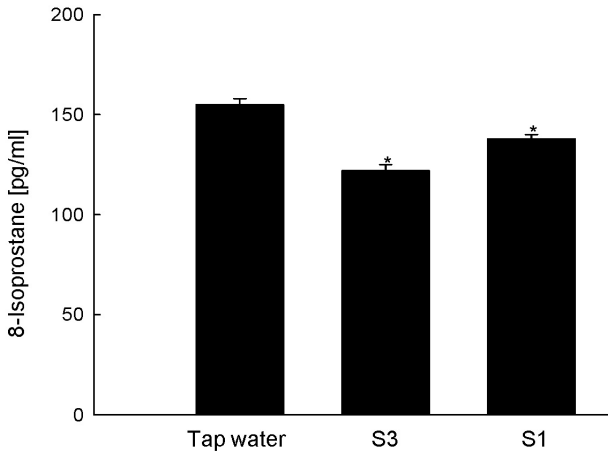

Fig. 1. Effects of Jeju ground water S3 or S1 on lipid peroxidation. The level of 8-isoprostane was measured by EIA using an 8-isoprostane EIA kit. * indicates significantly different from the tap water group (p<0.05).

regard, Jeju ground water S3 and S1 compared with tap water enhanced the protein expression and activity of SOD (Fig. 2A and B), CAT, and GPx (Fig. 3A and B) in *ob/ob* mouse liver tissue.

Next, the impact of Jeju ground water on intracellular

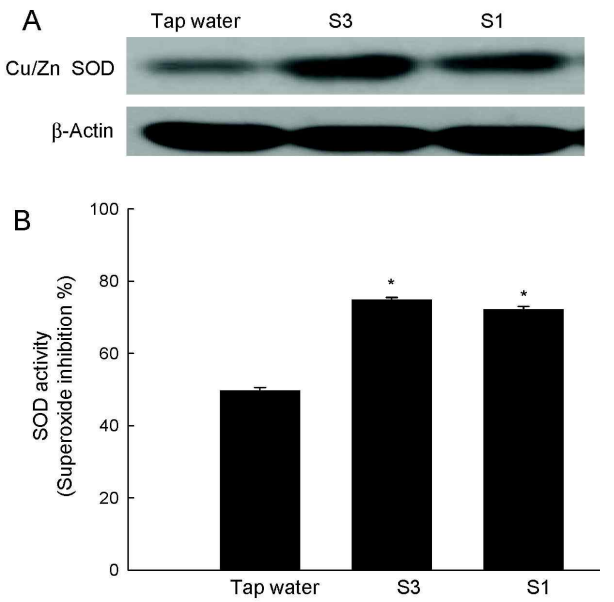

Fig. 2. Effects of Jeju ground water S3 or S1 on SOD expression and activity. (A) Cell lysates were electrophoresed on an SDS-polyacrylamide gel. Electrophoresed proteins were transferred onto a nitrocellulose membrane, and the Cu/Zn SOD protein was detected using a primary antibody specific for Cu/Zn SOD. (B) SOD activity was measured using a colorimetric assay kit. * indicates significantly different from the tap water group (p<0.05).

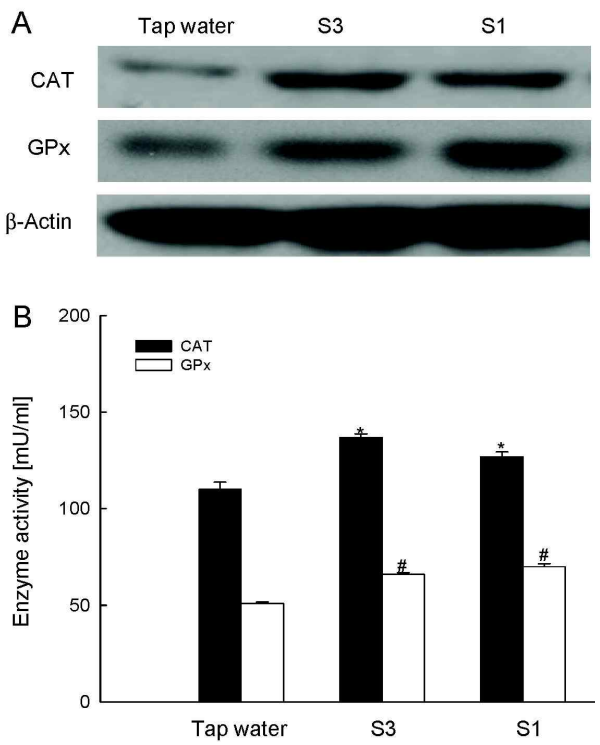

Fig. 3. Effects of Jeju ground water S3 or S1on CAT and GPx. (A) Cell lysates were electrophoresed on an SDS-polyacrylamide gel. Electrophoresed proteins were transferred onto a nitrocellulose membrane, and the CAT and GPx protein were detected using a primary antibody specific for CAT and GPx. (B) CAT and GPx activities were measured using assay kits as described in Materials and Methods. $^{*,\#}$ indicates significantly different from the tap water group (p<0.05).

GSH content was evaluated. GSH is an important non-enzymatic antioxidant tri-peptide that is responsible for maintaining cellular oxidation – reduction homeostasis. As noted above, GSH donates an electron in the GPx-catalyzed reduction of peroxides. Jeju ground water S3 and S1 increased hepatic intracellular GSH. Intracellular GSH content was 141 μM and 131 μM following administration of S3- and S1-Jeju ground water, respectively, and 90 μM following administration of tap water (Fig. 4).

Capel and Dorell demonstrated that *ob/ob* mice compared with wild type mice had reduced levels of hepatic GPx and GSH [1]. Consistent with this observation, Park *et al.*, showed that high-fat diet-induced obese mice also had lower hepatic GSH level [14]. Induction of GPx- and GSH-mediated antioxidant defenses has been reported to occur downstream from the transcriptional activation of Nrf2, which in turn occurs via the interaction of Nrf2 with the antioxidant response element [12]. Nrf2 protects cells from oxidative stress by regulating cytoprotective genes, including antioxidant enzymes and enzymes required for GSH synthesis [13]. Importantly, Jeju ground water S3 and S1 increased nuclear phospho Nrf2 protein expression in comparison with tap water (Fig. 5).

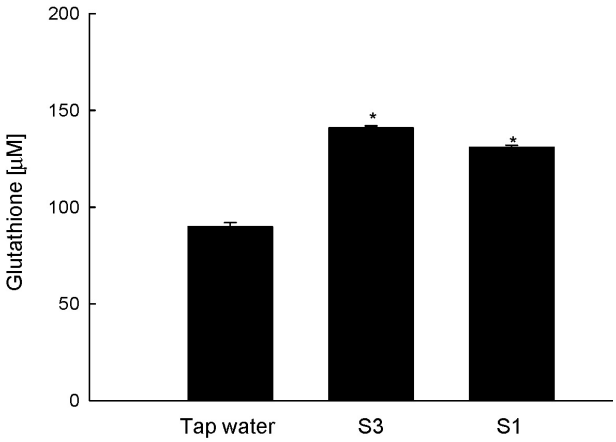

Fig. 4. Effects of Jeju ground water S3 or S1on intracellular GSH content. GSH levels were detected using a colorimetric assay kit. * indicates significantly different from the tap water group (p<0.05).

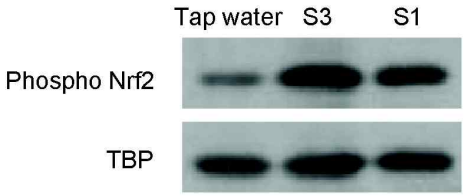

Fig. 5. Effects of Jeju ground water S3 or S1on Nrf2. Nuclear extracts were subjected to gel electrophoresis followed by Western blotting using an antibody specific for phospho Nrf2. TBP indicates TATA box-binding protein.

In conclusion, the present study demonstrates that Jeju ground water decreases oxidative hepatic stress by: 1) ameliorating lipid peroxidation, 2) increasing antioxidant enzyme expression and activity, and 3) augmenting the intracellular GSH content.

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초록 : 비만 마우스 간의 항산화시스템에 대한 바나듐 함유 제주지하수의 증강효과

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비만 마우스의 산화적 스트레스에 대해 바나듐 함유 제주 지하수에 대한 연구로서, 제주 지하수는 수도수 처리군보다 비만 마우스 간의 과산화 지질을 감소시켰으며, superoxide dismutase, catalase, 그리고 glutathione peroxidase의 단백질 발현 및 활성 그리고 환원형 glutathione 양을 증가 시켰다. 제주 지하수는 항산화 효소의 전사인자인 erythroid transcription factor NF-E2 (Nrf2)의 인산화형 단백질 발현을 증가시켰다. 이로서 제주 지하수는 Nrf2의 활성화를 통하여 항산화 효소시스템을 증가시켰다.