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ORIGINAL ARTICLE

Antioxidant System-Inducing Effects of Jeju Ground Water in C57BL/6 Mice against Gamma-ray Radiation

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Abstract Recently, we reported that Jeju ground water contains vanadium components and exerts antioxidant effects *in vitro* and *in vivo* via the scavenging of reactive oxygen species and enhancement of antioxidant enzyme activities. In the present study, the antioxidant actions of Jeju ground water were compared with those of tap water against gamma-ray radiation in mice. C57BL/6 mice were irradiated with gamma-ray at a dose rate of 2 Gy. The mice were then given tap water or Jeju ground water for 90 days. Jeju ground water compared with tap water enhanced the activities and levels of superoxide dismutase, catalase, and glutathione peroxidase in irradiated liver tissues. Jeju ground water also enhanced the levels of intracellular reduced glutathione, which is vital for normal liver function and repair. These results suggest that vanadium-containing Jeju ground water can safeguard against the harmful actions of gamma-ray radiation through the support of hepatic antioxidant processes.

Keywords antioxidant enzymes · gamma-ray radiation · Jeju ground water · reactive oxygen species · vanadium

Introduction

Ionizing radiation provokes the decomposition reaction of water, producing a variety of reactive oxygen species (ROS) (Sadani and Nadkarni, 1997). The overproduction of ROS in various tissues leads to significant alterations in cellular oxidant activity causing oxidative damage of lipids, proteins, and DNA (Navratil et al., 2008). The resultant cellular damage in turn leads to apoptosis, necrosis, cell dysfunction and/or mitotic cell death (Shimizu and Tsujimoto, 2000; Nair et al., 2001).

A search for chemical agents that can safeguard humans and other living organisms against ionizing radiation is an important pursuit in radiation biology (Nair et al., 2001). Recent efforts have focused on radio-protective chemicals and herbal extracts that have the capacity to modify immune and radiation responses (Goel et al., 2004; Arora et al., 2005; Maurya et al., 2006).

Organisms can adapt to increased ROS production by up-regulating their antioxidant defenses, including the levels and activities of antioxidant enzymes (e.g., superoxide dismutase (SOD), catalase (CAT)) (Livingstone, 2003). Enzymes involved in glutathione metabolism (e.g., glutathione peroxidase (GPx) and glutathione reductase) are also up-regulated in response to elevated ROS levels (Galeotti et al., 1991; Portakal et al., 2000). In this regard, reduced glutathione (GSH) is a important antioxidant that provides crucial reducing equivalents for GPx (Winterbourn et al., 1993; Sheikh et al., 1998).

Vanadium is chemical element that exists in many oxidation states, from −1 to +5. This enables the element to function as an electron transfer agent in a wide variety of reactions (Badmaev et al., 1999). Pharmacological applications of vanadium encompass the treatment of diabetes, hypertension, obesity, and inflammatory disorders, as well as cancer therapy (Hopfner et al., 1998; Bakhtiar and Ochiai, 1999; Thompson, 1999; Thompson and Orvig, 2001; Chakraborty et al., 2007). Recently, we reported that Jeju ground water containing the vanadium components, S1, S2, and S3 exhibited antioxidant effects via the scavenging of ROS such as superoxide anion and hydroxyl radical (Kim et al., 2010a). The S3 also enhanced the activities of SOD, CAT, GPx, and heme oxygenase-1 (HO-1) *in vitro* and *in vivo* (Kim et al., 2010b; 2011). In the present study, S1-, S2-, and S3-containing ground water preparations were compared with tap water for antioxidant system in gamma-ray irradiated C57BL/6 mice.

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Materials and Methods

Jeju ground water preparation. Jeju ground water preparations containing the vanadium components, S1 (8.0±0.9 μg/L), S2 (24.0±2.0 μg/L), and S3 (26.0±2.0 μg/L) were provided by the Jeju Special Self-Governing Province Development Corporation (Jeju, Korea).

Mice. C57BL/6 mice were purchased from Orientbio Inc. (Sungnam, Korea). The mice used for the experiments were of 6–8 weeks of age and weighed 18–25 g. They were housed in conventional animal facilities at a constant temperature (23±1°C) and provided with an NIH-07-approved diet and drinking water *ad libitum,* according to the Guidelines for the Care and Use of Laboratory Animals set forth by the Institutional Ethics Committee. The tap water was given throughout the course of the experiment (90 days) and corresponded to either Jeju ground water S1, S2, or S3.

Radiation with ^{60}Co gamma-ray. Each mouse was placed in a separate plastic container (3×3×11 cm) and given a single dose of gamma-ray radiation at a dose rate of 2.0 Gy/min. The source-surface distance was 150 cm from a ^{60}Co irradiator (Applied Radiological Science Institute, Jeju National University, Jeju, Korea).

SOD activity. SOD activity was measured in mouse liver tissue using a colorimetric assay kit (Abcam, Cambridge, MA) 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) 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, Rochester Hills, MI) 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 blot analysis. Liver tissues were lysed in 0.5 mL of a lysis buffer consisting of 120 mM NaCl, 40 mM 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 poly-acrylamide gel. The gels were transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Nitrocellulose membranes were subsequently incubated with primary antibodies against SOD, CAT, or GPx. The membranes were further incubated with secondary anti-immunoglobulin-G horseradish peroxidase conjugate (Pierce, Rockford, IL), 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.

Intracellular GSH measurement. The intracellular GSH content was measured using the GSH-400 colorimetric assay kit (OXIS International, Portland, OR), 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 mM.

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.

Results and Discussion

Radiation exposure attenuates the expression and activity of endogenous antioxidant enzymes, which are considered to function as a first line of defense to maintain cellular redox balance and normal biochemical processes. Thus, supplementation of antioxidants to improve the efficacy of radiotherapy is currently proposed as a strategy to counteract radiation-induced damage (Barker et al., 2005). Antioxidants are capable of scavenging free radicals (e.g., ROS) that are generated from the radiolysis of water, thereby providing protection to cells and tissues against harmful rays (Mansour et al., 2008). Indeed, most of the toxic effects of ionizing radiation to normal cells and tissues can be attributed to the generation of ROS, given that ROS trigger the formation of several reactive intermediates. Healthy cells are equipped with a comprehensive and integrated endogenous antioxidant enzymatic system that includes SOD, CAT, GPx, and the GSH substrate, GSH. In addition, cells are equipped with non-enzymatic antioxidant systems (Karbownik and Reiter, 2000). However, as noted above, these antioxidant system must be augmented following exposure to ionizing radiation in order to overcome radiation-induced oxidative stress.

Recently, we reported that vanadium-containing Jeju ground water exerted antioxidant effects via the enhancement of antioxidant enzyme activities *in vivo* (Barker et al., 2005). Furthermore, Jeju ground water sustained immune activities that were suppressed by

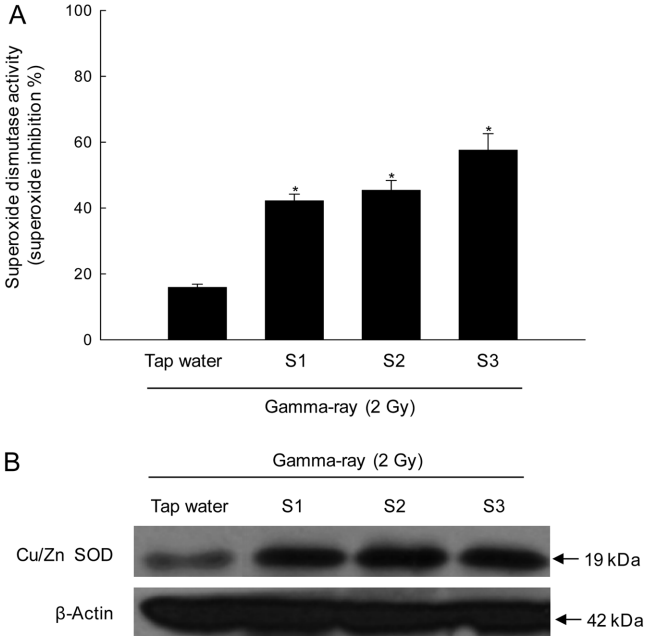

Fig. 1 Effect of S1, S2 and S3 on SOD activity in gamma-ray irradiated mice. (A) SOD activity was measured using a SOD activity colorimetric assay kit. All values are represented as the mean ± SE. * indicates significantly different from tap water (p <0.05). (B) Liver tissue lysates were electrophoresed on an SDS polyacrylamide gel. The gel was transferred to a nitrocellulose membrane, and the Cu/Zn SOD protein was detected using Cu/Zn SOD specific antibody.

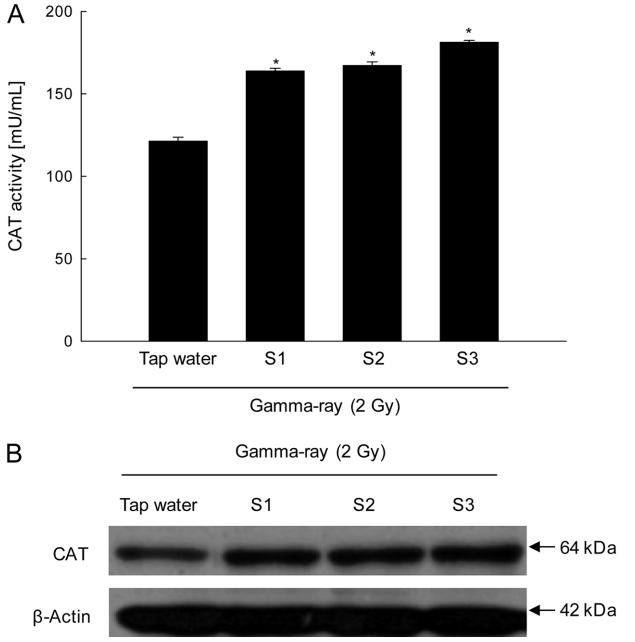

Fig. 2 Effect of S1, S2 and S3 on CAT activities in gamma-ray irradiated mice. (A) CAT activity was measured using a CAT assay kit. All values are represented as the mean ± SE. * indicates significantly different from tap water (p <0.05). (B) Liver tissue lysates were electrophoresed on an SDS polyacrylamide gel. The gel was transferred to a nitrocellulose membrane, and the CAT protein was detected using CAT specific antibody.

gamma ray radiation (Ha et al., 2011). The current study was designed to evaluate the antioxidant properties of vanadium-containing Jeju ground water against gamma-ray-induced damage in C57BL/6 mice. To do so, mice were provided with drinking water that corresponded to either tap water or Jeju ground water containing the vanadium components S1, S2, or S3. The activity and expression of various antioxidant enzymes in mouse liver tissue, including SOD, CAT, and GPx were then evaluated.

SOD dismutates the superoxide radical into hydrogen peroxide and molecular oxygen (Singh et al., 2008). As shown in Fig. 1A, S1, S2, and S3 preparations displayed 42, 45, and 57% inhibition of the superoxide anion, respectively, compared with 15% inhibition by tap water. These results are consistent with the expression level of the Cu/Zn SOD protein following the different treatments; notably, Cu/Zn SOD expression was elevated following the administration of ground water S1, S2, and S3 as compared with tap water (Fig. 1B).

CAT is located at the peroxisome and converts hydrogen peroxide into molecular oxygen and water. Thus, CAT plays important roles in cellular protection from oxidative stress-induced cellular damages (Forstrom et al., 1978; Ceballos-Picot et al., 1992; Kawakami et al., 2006). S1, S2, or S3 all increased the activity of CAT. CAT exhibited activities of 163, 167, and 168 mU/mL following the administration of ground water S1, S2, and S3, respectively (Fig. 2A). The corresponding value following the administration of tap water was 121 mU/mL (Fig. 2A). Moreover, S1, S2, or S3 all increased CAT protein expression compared with tap water (Fig. 2B).

GPx uses GSH to catalyzes the reduction of hydroperoxides, including hydrogen peroxide, and functions to protect cells from oxidative damage (Guemouri et al., 1991). S1, S2, and S3 also augmented the activity of GPx. GPx showed activities of 67, 73, and 85 mU/mL following the administration of S1, S2, and S3, respectively, and 38 mU/mL following the administration of tap water (Fig. 3A). Western blot results confirmed that S1, S2, and S3 enhanced the protein expression of GPx compared with tap water (Fig. 3B).

As described above, GSH is an antioxidant protein that helps to slow the molecular oxidation. GSH therefore provides protection from harmful effects of free radicals. Chemically, GSH contains three basic amino acids; cysteine, glycine, and glutamic acid. GSH exists in two forms, namely the reduced-form (GSH, monomer) and the oxidized-form (GS-SG, dimer). The former represents active glutathione, whereas the latter is acted upon by reductase, converting it into active GSH (Ulusu et al., 2003). Increased cellular GSH levels are suggested to result from the activation of protective tissue responses that regulate physiological mechanisms within cells through redox balancing reactions (Pathak et al., 2007). As shown in Fig. 4, S1, S2, and S3 increased intracellular GSH content as determined by a colorimetric assay. The GSH content was 97, 69, and 105 μM following the administration of S1, S2, and S3, respectively, versus 73 μM

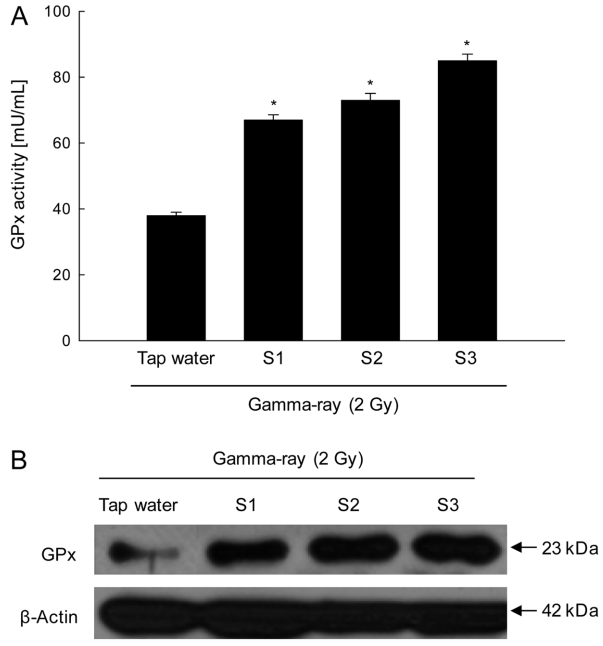

Fig. 3 Effect of S1, S2 and S3 on GPx activities in gamma-ray irradiated mice. (A) GPx activity was measured using GPx assay kit. All values are represented as the mean ± SE. * indicates significantly different from tap water (p <0.05). (B) Liver tissue lysates were electrophoresed on an SDS polyacrylamide gel. The gel was transferred to a nitrocellulose membrane, and the GPx protein was detected using GPx specific antibody.

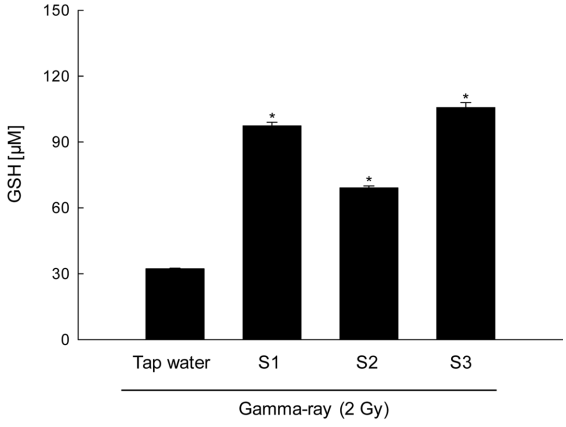

Fig. 4 Effect of S1, S2 and S3 on GSH level in gamma-ray irradiated mice. The intracellular GSH content was measured in liver tissue lysates using a colorimetric assay kit. All values are represented as the mean ± SE. * indicates significantly different from tap water (p <0.05).

following the administration of tap water.

In conclusion, the results of this study indicate that vanadium-containing Jeju ground water exhibits antioxidant effects against gamma-ray irradiation in C57BL/6 mice by enhancing the expression and activity of hepatic antioxidant enzymes, and by enhancing the content of GSH.

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