Detoxifying effect of fermented black ginseng on H_2O_2-induced oxidative stress in HepG2 cells

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Abstract. Fermented black ginseng (FBG) is prepared by repeated steaming and drying processes with fresh ginseng followed by fermentation with *Saccharomyces cerevisiae*. It has recently been shown to have several bioactivities. FBG contains crude saponin (1,440 μg/ml), ginsenoside Rg2 (2.86 μg/ml), ginsenoside Rg3 (24.52 μg/ml), ginsenoside Rh1 (12.64 μg/ml), ginsenoside Rh2 (0.63 μg/ml) and ginsenoside Rf (1.32 μg/ml). The present study investigated the antioxidant defense properties of FBG against hydrogen peroxide (H_2O_2)-mediated oxidative stress in HepG2 human hepatocellular carcinoma cells. The increased production of reactive oxygen species (ROS) induced by H_2O_2 was attenuated in a dose-dependent manner when the cells were pre-treated with FBG (10-50 μg/ml). FBG induced both the expression and activity of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase in the H_2O_2-treated HepG2 cells. The inhibitory effects of FBG on the phosphorylation of upstream mitogen-activated protein kinases (MAPKs), such as c-Jun N-terminal kinase, extracellular signal-regulated kinase and p38 were also observed. Overall, our results demonstrate that FBG protects HepG2 cells from oxidative stress through the induction of antioxidant enzyme activity and the inhibition of MAPK pathways.

Introduction

Reactive oxygen species (ROS), including superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) have been implicated in the development of a variety of diseases, such as carcinogenesis, inflammation, aging and atherosclerosis. The accumulation of ROS induces lipid peroxidation, the inactivation of proteins and DNA damage in cells (1,2). Protective enzymatic and non-enzymatic antioxidant defense mechanisms reduce oxidative stress by scavenging ROS. To protect themselves against toxic free radicals and ROS, cells have developed a variety of antioxidant defenses (3). These include antioxidant enzymes, such as superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anions to hydrogen peroxide; catalase (CAT), which converts H_2O_2 into molecular oxygen and water; glutathione peroxidase (GPx), which catalyzes the degradation of H_2O_2 and hydroperoxides. On the non-enzymatic level, certain vitamins and other antioxidant compounds scavenge free radicals and delay the oxidation of molecules (4,5). Phytochemicals provide further protection against oxidative damage from free radicals. A large number of studies have indicated that phytochemicals present in fruits, vegetables and herbs, exert their antioxidant effects against oxidative stress through the induction or activation of these endogenous antioxidant enzymes (6-9). In addition, increased ROS production not only directly damages cells by oxidizing macromolecules, such as DNA and lipids, but also indirectly by triggering mitogen-activated protein kinase (MAPK) signaling pathways (10). MAPKs, such as extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK) are involved in crucial signaling pathways in cell proliferation, differentiation and cell death in response to various signals produced by growth factors, hormones and cytokines, as well as genotoxic and oxidative stressors (11). Studies have demonstrated that MAPK signaling pathways can also be modulated by the production of ROS and antioxidant enzymatic activity and expression (12,13).

Ginseng (*Panax ginseng* C.A. Meyer) is considered as one of the most popular medicinal herbs, and has well known pharmacological activities which include anticancer (14), anti-aging (15), anti-diabetic (16), anti-stress (17) and neuroprotective effects (18). Black ginseng is a processed ginseng produced by a nine-time steaming at approximately 85˚C and a nine-time drying process (repetitive steaming and drying) using fresh ginseng, at which point the ginseng becomes black in color (19). Fermented black ginseng (FBG) is processed further by incubating black ginseng with *Saccharomyces cerevisiae* for 24 h to produce more active ginsenosides. Black ginseng

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has been shown to enhance biological activities possibly due to the enrichment of the bioactive chemical constituents during the heat and drying processing stage (20). However, to the best of our knowledge, studies on the antioxidant properties and the underlying molecular mechanisms of FBG are limited. In the present study, we investigated the antioxidant defense properties of FBG at the enzymatic and cellular levels and its ability to inhibit ROS production; we demonstrate that FBG induces both the activity and the expression of antioxidant enzymes and modulates upstream protein kinases, including MAPKs.

Materials and methods

Reagents. FBG was obtained from Ginseng-By-Pharm Co. (Wonju, Korea). The main composition of FBG was determined using the liquid chromatography-mass spectrometry (LC/MS) method (Joongbu University, Geumsan, Korea) and is shown in Table I. KCN (potassium cyanide, 60178), H_2O_2, sulfanilamide (S9251), N-(1-naphthyl)ethylenediamine dihydrochloride (P7626), nitroblue tetrazolium salt, xanthine, copper chloride, glutathione, xanthine oxidase from bovine milk (0.1-0.4 U/mg of protein), glutathione reductase from baker's yeast (*Saccharomyces cerevisiae*; 100-300 U/mg of protein) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, inner salt (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2',7'-Dichlorohydrofluorescein diacetate (DCF-DA) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). Antibodies against CAT (14097), phosphorylated ERK (9101), phosphorylated JNK (9251), phosphorylated p38 (9211) and horseradish peroxidase-conjugated anti-rabbit IgG (7074) were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). Antibodies against SOD (sc-18504), GPx (sc-30147), β-actin (sc-1616) and horseradish peroxidase-conjugated anti-goat IgG (sc-2350) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other reagents were of the highest quality generally available.

Cell culture. HepG2 human hepatocellular carcinoma cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were maintained in Dulbecco's minimum essential medium with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin (all from HyClone, Logan, UT, USA), 1% essential amino acids and 1% glutamax (both from Gibco, Grand Island, NY, USA) at 37˚C in a humidifying incubator containing 5% CO_2. For subculture, the HepG2 cells were harvested at 80-90% confluence.

Cell viability assay. MTT assay was used to measure cell viability. The HepG2 cells were seeded in 24-well plates at a rate of $1x10^5$ cells/well. After 24 h of incubation, the cells were treated with distilled water (DW; control) or different doses of FBG for 24 h. Subsequently, 50 μl of 1 mg/ml MTT were added and the plates were incubated for 4 h. Following incubation at 37˚C for 4 h, the MTT medium was removed by aspiration and 200 μl of dimethyl sulfoxide (DMSO) were added to each well. After reacting for 10 min at room temperature, formazan production was detected by the measurement of the optical density (OD) at 570 nm using a PowerWave XS microplate reader (BioTek Instruments, Winooski, VA, USA). The data are expressed as the percentage cell viability compared to the vehicle-treated control.

Table I. Saponin and main ginsenosides found in fermented black ginseng.

Ginsenosides	Concentration (μg/ml)
Crude saponin	1,440
Ginsenoside Rg2	2.86
Ginsenoside Rg3	24.52
Ginsenoside Rh1	12.62
Ginsenoside Rh2	0.63
Ginsenoside Rf	1.32

ROS formation assay. Intracellular ROS levels were determined by the DCF-DA assay. Briefly, the cells were seeded in 96-well dark plate at a rate of $1x10^4$ cells/well and pre-incubated with 20 μM DCF-DA (dissolved in DMSO) for 1 h at 37˚C in the dark. After washing out the excess probe using 1X ice-cold phosphate-buffered saline (PBS), the cells were treated with the vehicle (DW for control), vitamin C (positive control), or FBG in the presence or absence of 1 mM H_2O_2 for 12 h and then washed twice with 1X ice-cold PBS. Fluorescence was detected by excitation at 485 nm and emission at 535 nm using a fluorescence multi-detection reader (BioTek Instruments). Vitamin C (100 μg/ml) was used as positive control for current study. Vitamin C is well known as a strong antioxidant and is widely used as a positive control in antioxidant studies.

Assays for antioxidant enzymes. The cells were seeded in 6-well plates at a rate of $2x10^5$ cells/well. After 24 h of incubation, the cells were treated with DW (control), vitamin C (positive control), or different doses of FBG for 24 h. For the CAT and GPx enzyme assays, the cells were homogenized with 1 ml of 50 mM potassium phosphate buffer (pH 7.0), and then centrifuged at 12,000 rpm for 20 min at 4˚C. For the manganese-superoxide dismutase (Mn-SOD) assay, the cells were homogenized with 1 ml of 65 mM phosphate buffer (pH 7.8), and then centrifuged at 12,000 rpm for 20 min at 4˚C. The cell lysis supernatant was analyzed to determine CAT and GPx activity, while the cell pellet was used to detect Mn-SOD activity. The protein concentration was determined using the BCA protein assay (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's instructions. The results are expressed as the enzyme activity per milligram protein compared with the corresponding control cultures.

Mn-SOD activity. The activity of Mn-SOD was measured according to the method described in the study by Oyanagui (21). The remaining pellet (i.e., the mitochondrial fraction) was dissolved in 0.1% Triton X-100 and used for the determination of Mn-SOD activity. For the Mn-SOD activity assay, 60 μl of 4 mM of KCN solution were added to the assay mixture to inhibit copper- and zinc-containing superoxide dismutase (Cu/Zn-SOD). The samples were pre-incubated with 15 μl of 75 mM Na-xanthine and 15 μl of 10 mM hydroxylamine hydrochloride at 37˚C for 10 min. Subsequently, 0.1 units of xanthine oxidase were added and the samples were incubated at 37˚C for an additional 20 min. The reaction was terminated by the addition of 1% sulphanilamide and 0.02% ethylenediamine

dihydrochloride. After standing at room temperature for 20 min, the absorbance of the final mixture was measured at 450 nm. Enzyme activity was expressed as units per milligram protein.

CAT activity. The activity of CAT was measured as previously described (22). The reaction mixture contained 12 μl of 3% (vol/vol) H_2O_2 and 20 μg of cell lysates in 50 mM potassium phosphate buffer (pH 7.0) at a final volume of 1.0 ml. The samples were incubated for 5 min at 37˚C and the absorbance of the samples was monitored for 5 min at 240 nm. The change in absorbance is proportional to the breakdown of H_2O_2.

GPx activity. The activity of GPx was measured by as previously described (23). A total of 20 μg of supernatant, containing cytosolic fraction, was incubated with 1 mM EDTA, 1 mM sodium azide (NaN_3), 5 mM GSH, 1 mM NADPH and 1 unit glutathione reductase at room temperature for 5 min. The reaction was initiated by the addition of 25 μl of 2.5 mM H_2O_2. GPx activity was measured as the rate of NADPH oxidation at 340 nm.

Western blot analysis. The cells were pre-treated with the vehicle (DW for control), vitamin C (positive control), or FBG (10, 25 or 50 μg/ml) for 1 h and then challenged with H_2O_2 for another 1 h. Subsequently, the cells were washed 3 times with ice-cold PBS (pH 7.4) and harvested with 200 μl of whole cell lysis buffer (pH 7.4) containing 10 mM Tris-HCl, 50 mM sodium chloride, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 100 μM sodium orthovanadate, 2 mM iodoacetic acid, 5 mM $ZnCl_2$, 1 mM phenylmethylsulfonyl fluoride and 0.5% Triton X-100. The cell lysates were vigorously vortexed, homogenized in a sonicator for 10 sec and left on ice for 1 h. The homogenates were centrifuged at 13,000 x g for 10 min at 4˚C. The supernatants were collected and equal amounts of total protein, as determined by BCA protein assay (Pierce Biotechnology), were mixed with 2X loading buffer and heated at 95˚C for 5 min. An equal amount (30 μg) of protein from each cell lysate was separated by 12% SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat dry milk in 1X PBST buffer (0.1% Tween-20 in PBS) for 1 h at room temperature and then incubated overnight with the appropriate primary antibody. Following hybridization with the primary antibody, the membranes were washed 3 times with PBST, and then incubated with anti-rabbit and anti-goat antibodies with horseradish peroxidase for 1 h at room temperature and washed with PBST 3 times. Final detection was performed with enhanced chemiluminescence (ECL™) western blotting reagents (Santa Cruz Biotechnology, Inc.).

Statistical analysis. The data are expressed as the means ± standard deviation (SD) values using Microsoft Excel. The values were compared with those of the control using analysis of variance, followed by Bonferroni's test (GraphPad Prism software version 5.01; GraphPad Software, San Diego, CA, USA). The significance level was defined at P-values <0.05.

Results

Effects of FBG on cell viability. The cytotoxic effects of FBG on the HepG2 cells were evaluated by MTT assay. The percentage of viable cells was determined by MTT assay and compared to that of the control cells. The cells were treated with FBG at the concentration range of 0-200 μg/ml for 24 h. Treatment with 10-200 μg/ml FBG inhibited cell viability in a dose-dependent manner (Fig. 1). The survival rate of the cells treated with 50 μg/ml of FBG was approximately 70% compared to that of the control cells. Based on the cell viability data, subsequent experiments were performed using concentrations of FBG below 50 μg/ml of FBG.

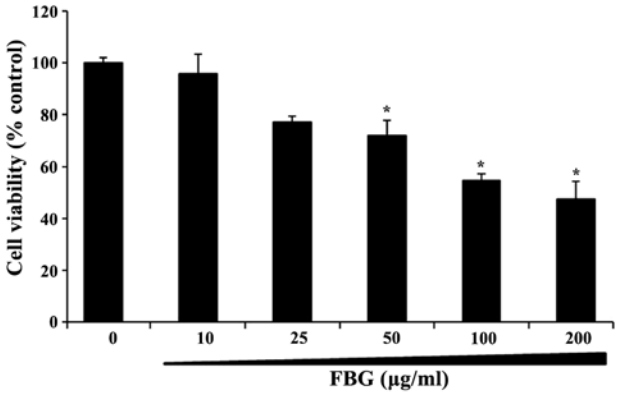

Figure 1. Cytotoxic effects of fermented black ginseng (FBG) on HepG2 cells. Cells were treated with DW or FBG (10-200 μg/ml) for 24 h, and cell viability was determined by MTT assay. Data are the means ± standard deviation (SD) values of 6 individual experiments. The values were compared with those of the control using analysis of variance, followed by Bonferroni's test. *P<0.05, significantly different from the control group.

Effects of FBG on ROS production. We examined whether FBG exerts inhibitory effects on the production of ROS using DCF-DA in H_2O_2-treated HepG2 cells. When the cells were treated with 1 mM H_2O_2, a >2.1-fold increase in the generation of ROS compared to the vehicle-treated controls was observed. Pre-treatment with FBG decreased the H_2O_2-mediated production of ROS in a dose-dependent manner and vitamin C (100 μg/ml) also markedly decreased ROS formation in the presence and/or absence of H_2O_2 in the HepG2 cells (Fig. 2). The dose-dependent effects of FBG were observed even in the absence of H_2O_2. These results suggest that FBG acts as an antioxidant which can directly scavenge excessive ROS generation in cells.

Effects of FBG on the activity of antioxidant enzymes. In order to investigate whether the antioxidant activity of FBG is mediated by its ability to increase the activity of cellular antioxidant enzymes, we measured the activity of antioxidant enzymes, including CAT, GPx and Mn-SOD in the H_2O_2-treated HepG2 cells. When the cells were treated with H_2O_2 (1 mM) alone, the activity of CAT, GPx and SOD-2 significantly decreased below the basal level of the vehicle-treated controls (Fig. 3). However, treatment with FBG increased the activity of these enzymes; the increased activity levels of CAT and GPx following treatment with 50 μg/ml FBG were much higher than the basal control (DW) levels and were even higher than the levels observed following treatment with vitamin C (positive control; 100 μg/ml), a well known antioxidant.

Effects of FBG on the expression of antioxidant enzymes. In addition to its stimulatory effects on enzyme activity, we determined

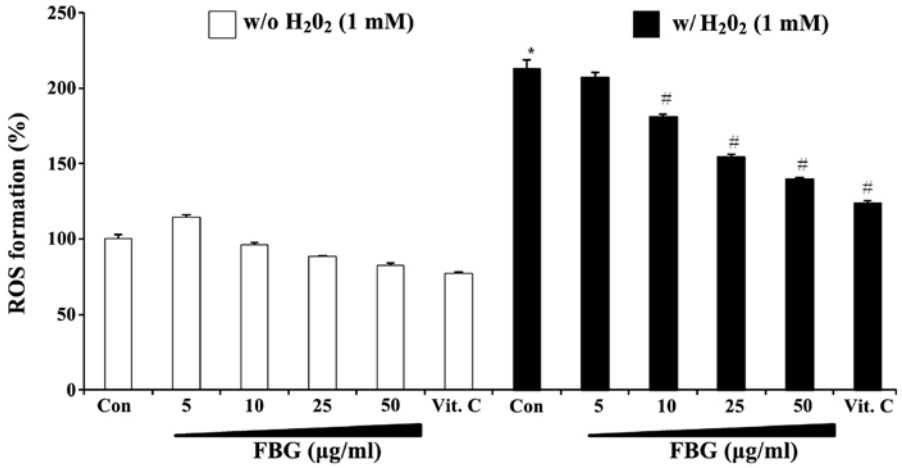

Figure 2. Effect of fermented black ginseng (FBG) on hydrogen peroxide (H_2O_2)-induced reactive oxygen species (ROS) production in HepG2 cells. Cells was pre-treated with DCF-DA for 1 h and exposed to DW (control) or FBG for another 6 h with or without H_2O_2. Data are the means ± standard deviation (SD) values of 3 individual experiments. The values were compared with those of the control using analysis of variance, followed by Bonferroni's test. *P<0.05, significantly different from the vehicle control in the absence of H_2O_2; #P<0.05, significantly different from the vehicle control in the presence of H_2O_2.

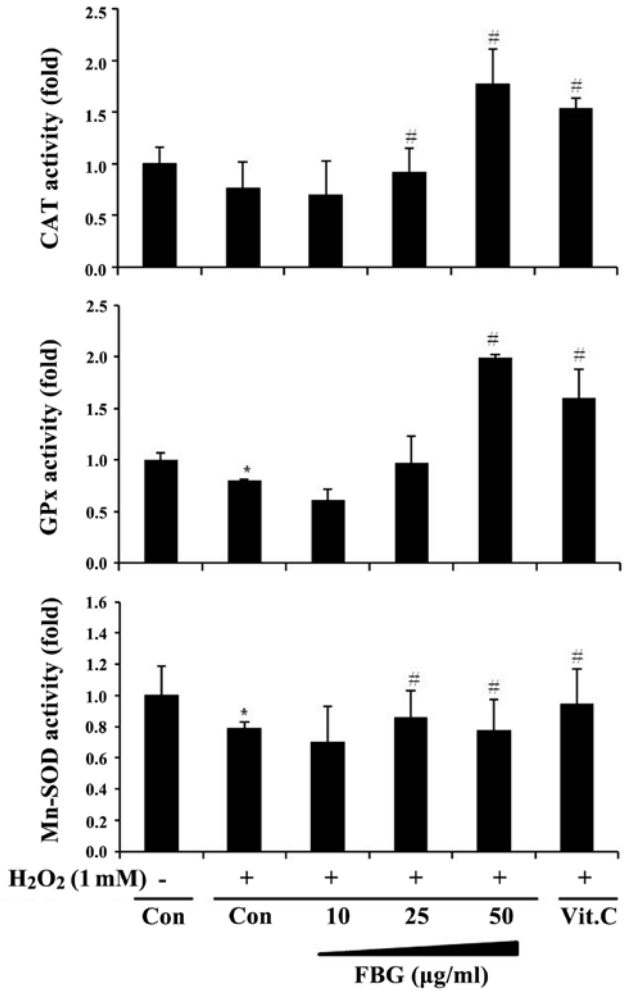

Figure 3. Effects of fermented black ginseng (FBG) on catalase (CAT), glutathione peroxidase (GPx) and manganese-superoxide dismutase (Mn-SOD) activity in hydrogen peroxide (H_2O_2)-treated HepG2 cells. Treatment with FBG showed a tendency to increase the activity of CAT, GPx and Mn-SOD in the H_2O_2-treated HepG2 cells. Data are the means ± standard deviation (SD) values of 3 individual experiments. The values were compared with those of the control (DW) using analysis of variance, followed by Bonferroni's test. *P<0.05, significantly different from the control group; #P<0.05, significantly different from the H_2O_2-treated group.

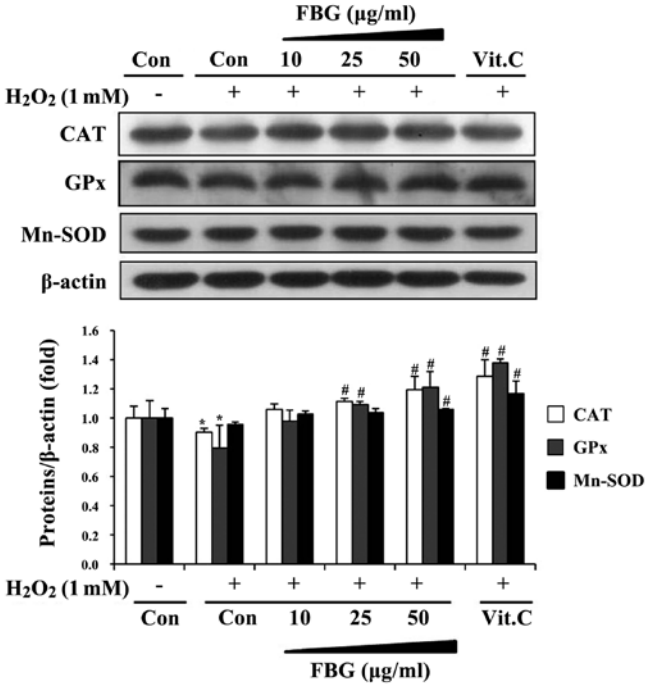

Figure 4. Effects of fermented black ginseng (FBG) on catalase (CAT), glutathione peroxidase (GPx) and manganese-superoxide dismutase (Mn-SOD) protein expression in hydrogen peroxide (H_2O_2)-treated HepG2 cells. Cells were pre-treated with the vehicle (DW) or FBG (10, 25 or 50 μg/ml) for 1 h and then challenged with H_2O_2 for 6 h. The bands shown are representative of 3 independent experiments with similar results. The values were compared with those of the control using analysis of variance, followed by Bonferroni's test. *P<0.05, significantly different from the control group; #P<0.05, significantly different from the H_2O_2-treated group.

the effects of FBG on the protein expression of these antioxidant enzymes in HepG2 cells. When the cells were treated with H_2O_2 alone, CAT, GPx and Mn-SOD protein expression levels were diminished compared with the vehicle-treated controls. However, treatment with FBG restored and upregulated the protein expression of these enzymes in a dose-dependent manner and vitamin C significantly induced the protein level of antioxidant enzymes compared to the H_2O_2-treated group (Fig. 4).

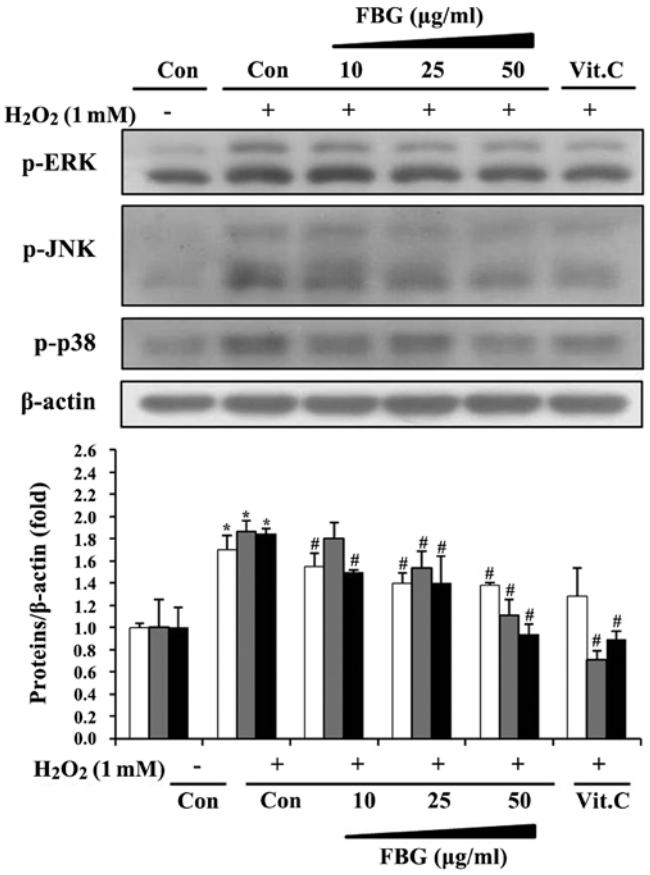

Figure 5. Effects of fermented black ginseng (FBG) on mitogen-activated protein kinase (MAPK) phosphorylation in hydrogen peroxide (H_2O_2)-treated HepG2 cells. Cells were pre-treated with the vehicle (DW) or FBG (10, 25 or 50 μg/ml) for 1 h and then challenged with H_2O_2 for 1 h. The bands shown are representative of 3 independent experiments with similar results. The values were compared with those of the control using analysis of variance, followed by Bonferroni's test. *P<0.05, significantly different from the unstimulated control group; $^{\#}$P<0.05, significantly different from the H_2O_2-treated group.

Effects of FBG on the phosphorylation of MAPKs. In order to determine whether antioxidant enzyme expression induced by FBG was associated with the MAPK pathway, we examined the phosphorylation levels of MAPK subfamilies, such as ERK, JNK and p38. The results revealed that H_2O_2 stimulated the phosphorylation of all MAPKs (Fig. 5). The H_2O_2-stimulated phosphorylation of MAPKs was decreased following treatment with FBG in a dose-dependent manner and vitamin C inhibited the phosphorylation levels of MAPKs in the presence of H_2O_2. This confirmed that protective effects of vitamin C against H_2O_2 by the induction of antioxidant enzymes through the inhibition of MAPK phosphorylation.

Discussion

The induction of antioxidant enzyme activity may be considered as a frontline defense strategy to protect human health against various oxidative stress-related diseases. Accordingly, numerous bioactive plant materials have been investigated for their antioxidant potential (4,6-8). In the present study, to the best of our knowledge, we demonstrate for the first time that FBG protects HepG2 cells against H_2O_2-induced oxidative stress through the regulation of ROS production and antioxidant enzymes, and signaling pathways including MAPKs.

ROS are known to play a central role in mediating various metabolic disorders related to several diseases. Thus, inhibiting ROS production and enhancing the scavenging ability of antioxidants may prove to be a useful strategy in the treatment of diseases related to oxidative stress (24). ROS-induced oxidative DNA damage has been implicated in mutagenesis and carcinogenesis and has attracted extensive attention in recent years. In addition, H_2O_2 is a major component of ROS produced intracellularly during a number of physiological and pathological processes, and causes oxidative damage (25,26). For this reason, H_2O_2 has often been used as an experimental model to investigate the mechanisms of cell injury induced by oxidative stress (27-29). In the present study, when HepG2 cells pre-treated with FBG were challenged with H_2O_2, ROS formation decreased. A previous study demonstrated that Korean red ginseng extract exerted antioxidant and chemopreventive effects by decreasing ROS production in HepG2 cells treated with arachidonic acid and iron (30). These results suggest that ginsengs may have an antioxidant capacity by directly scavenging radicals. Maintaining the balance between free radicals (and/or ROS) and antioxidants is an essential part of biological homeostasis (31).

Antioxidant enzymes, including SOD, CAT and GPx are regarded as the firstline of the antioxidant defense system against ROS generation during oxidative stress. Recently, white ginseng has been reported to prevent oxidative stress by enhancing the intracellular activity of antioxidant enzymes and decreasing ROS formation (32). In addition, red ginseng has been reported to exhibit a variety of antioxidant and hepatoprotective effects on ethanol-induced oxidative injury in rat liver and TIB-73 cells (33,34). Jun and Chang (35), reported that red ginseng extract increased SOD, CAT and GPx activity after ICR male mice were γ-irradiated. Furthermore, a ginseng extract has been shown to induce hepatic SOD, CAT and GPx activity in Sprague-Dawley rats (36). Our results are in accordance with those of these studies, suggesting that FBG may prove useful against oxidate stress by reducing ROS levels and increasing antioxidant enzymes activity and expression. According to previous studies, a variety of ginsengs has shown antioxidant properties in maintaining cellular function against free radicals *in vivo*, as well as *in vitro* (4,7,37). In addition, the activity of intracellular antioxidant enzymes, such as SOD, CAT and GPx plays an important role in protecting cells against oxidative stress. Since the changes occurring in the activities of these enzymes can be considered a biomarker of antioxidant response under conditions of oxidative stress, the increased activity of these enzymes in FBG-treated HepG2 cells strongly suggests that FBG has antioxidant properties that function by simulating the activity of antioxidant enzymes in addition to directly scavenging ROS/free radicals. Lee *et al* (38), revealed that black ginseng has a protective effect on ethanol-induced teratogenesis through the augmentation of antioxidant activity in embryos.

Heat processing has been reported to increase the free radical scavenging activity of ginseng and stimulate the protective effects of ginseng against oxidative damage caused by oxidative stress (20,39). Black ginseng is known to contain different ginsenosides (Rg3, Rg4, Rg6, Rk3, Rs3 and Rs4)

which are not present in white ginseng (40), and exhibits more potent pharmacological activities than white ginseng and red ginseng (41,42). Black ginseng is prepared by steaming at 85˚C for 8 h and then drying until the water content decreases below 20%. This steaming and drying process is repeated 9 times. This process makes white ginseng black, and from this point on it is known as 'black ginseng'. For the preparation of FBG, black ginseng is grinded and extracted with distilled water at 80˚C for 72 h. Subsequently, this water extract is fermented with *Saccharomyces cerevisiae* at 35˚C for 24 h.

The activity and expression of antioxidant enzymes may be modulated by upstream protein MAPKs, such as JNK, ERK and p38. The phosphorylation of these proteins has been shown to be mediated through H_2O_2-induced oxidative stress (11). Although FBG inhibits the phosphorylation of MAPK, as observed in this study, its effects on the downstream targets, such as antioxidant enzymes may be selective, at least in the current model system. Dong *et al* (30), indicated that red ginseng extract attenuates oxidative stress by reducing ROS formation through the LKB1-AMPK pathway in the HepG2 cell line, but not through the MAPK signaling pathway. It has been reported that the effects of dietary compounds on antioxidant enzyme expression and activity are mediated by the modification of several different signal transduction pathways (43). In addition, different agents can play one or more roles at different targets, and the cellular events may depend on the types and concentrations of the agents, as well as on the cell or tissue types. For example, Fan *et al* (44), revealed that ginseng pectin exerts protective effects against H_2O_2 through the ERK1/2 and Akt pathway in U87 neuronal cells. In addition, red ginseng and its primary ginsenosides inhibit ethanol-induced oxidative injury by reducing ROS production and lipid peroxidation through MAPK pathways in TIB-73 mouse hepatocytes (34). To the best of our knowledge, in this study, we report for the first time the modulatory role of FBG on upstream MAPKs. However, further studies are required using animals in order to determine the optimal dose, duration and method of administration.

In conclusion, FBG has the ability to protect cells against oxidative damage by scavenging ROS and inducing both the activity and the expression of cellular antioxidant enzymes possibly through the inhibition of MAPK signaling pathways. Therefore, FBG may be a potential natural agent for cellular defense, at least in liver cells. However, further *in vivo* studies using FBG are warranted.

Acknowledgements

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