J. Microbiol. Biotechnol. (2000), **10**(6), 851–857

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Enhancement of Phase II and Antioxidant Enzymes in Mice by Soybeans Fermented with Basidiomycetes

SHON, YUN-HEE, SO-YEUN KIM[1], JAE-SUNG LEE[1], AND KYUNG-SOO NAM*

Department of Pharmacology, College of Medicine and Intractable Disease Research Center, Dongguk University, Kyongju 780-714, Korea
[1]Department of Food Science and Technology, Yeungnam University, Kyongsan 712-749, Korea

Received: September 15, 2000
Accepted: October 23, 2000

Abstract The activities of phase II and antioxidant enzymes in the liver, lung, kidney, stomach, and colon of mice were examined following intragastric application of polysaccharides extracted from soybeans fermented with either *Agrocybe cylindracea* (AC) or *Phellinus igniarius* (PI). The intragastric application of the extracts to mice for 14 days significantly increased the activities of quinone reductase (QR) and glutathione S-transferase (GST) in the liver and kidney, glutathione (GSH) and superoxide dismutase (SOD) in the liver, kidney, lung, and stomach, and glutathione peroxidase (GSH-Px) in the liver, lung, and kidney. In general, the elevation of the phase II and antioxidant enzymes activities was more pronounced in the liver and kidney as compared to the lung, stomach, and colon. Accordingly, these findings suggest that polysaccharides extracted from soybeans fermented with *A. cylindracea* or *P. igniarius* have a cancer chemopreventive potential in various target organs.

Key words: Phase II enzyme, antioxidant enzyme, soybeans fermented with *Phellinus igniarius*, soybeans fermented with *Agrocybe cylindracea*

One of the most promising areas in cancer research is chemoprevention. The chemoprevention of cancer is a means of cancer control in which the occurrence of the disease is prevented by the administration of dietary or pharmaceutical agents. Chemopreventive agents function by a variety of mechanisms, directed at all major stages of carcinogenesis. The induction of phase II metabolizing enzymes such as quinone reductase (QR) or glutathione S-transferase (GST) is considered a major mechanism of protection against the initiation of carcinogenesis and chemical stress [21]. Prochaska and Fernandes [19] reported that dietary treatment of mice with 2(3)-*tert*-butyl-4-hydroxyanisole increased hepatic specific activities of QR and GST, and phase II enzyme induction results in the reduction of cancer risk via protective foodstuffs. Daily dietary intake of glucosinolate-containing Brussels sprouts leads to an increase of α-class GST levels in human blood plasma [2]. The induction of glutathione (GSH) is also used to test potential chemopreventive agents. GSH has a variety of cellular functions, and one role of particular importance is the protection of cellular macromolecules from reactive intermediates.

Reactive oxygen species produced by a free radical chain reaction and metabolic processes have been suggested as causative factors in mutagenesis, carcinogenesis, and tumor promotion and have been implicated in the etiology and pathophysiology of many human diseases [25]. However, living systems are protected from activated oxygen species by enzymes such as GST, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD). Nonenzymatic protection is also given by antioxidative nutrients (vitamins A, C and E, β-carotene) and materials (methionine and glutathione) [26]. The levels of antioxidant defense enzymes are known to be lower in transformed cells and/or tumors [18, 32]. Reiners *et al.* [23] have shown depleted levels of antioxidant enzymes in 7,12-dimethylbenz(a)anthracene-12-*O*-tetradecanoylphorbol-13-acetate-treated skin and chemically induced skin tumors.

There have been a number of studies which demonstrate the anticarcinogenic, antioxidant, or immunostimulating activities of basidiomycetes mushrooms [14, 17, 30]. The polysaccharides isolated from several basidiomycetes such as *Lentinus edodes* [5], *Coriolus versicolor* [34], *Ganoderma lucidum* [33], and *Agaricus blazei* [9] have exhibited antitumor activities in allogenic, syngenic, and even autologous tumor systems. Many synthetic chemicals such as phenolic compounds also exhibit a strong antioxidant activity, however, they usually have side effects [38]. Several reports have

*Corresponding author
Phone: 82-054-770-2412; Fax: 82-054-770-2477;
E-mail: namks@mail.dongguk.ac.kr

suggested that natural dietary plants may play an antioxidative role in the prevention of aging and carcinogenesis *in vitro* and *in vivo* [34, 36]. Among various naturally occurring substances, polysaccharide extracts from mushrooms appear to be useful candidates for effective and non-toxic substances with antioxidant activity. Polysaccharide extracts from *Ganoderma lucidum* and PSK, a protein-bound polysaccharide from *Coriolus versicolar*, have been reported as having antioxidant effects [24]. Kim and Kim [11] also reported that the water-soluble polysaccharide isolated from the fruit body of *Ganoderma lucidum* was effective in protecting against hydroxyl radical-induced DNA strand breaks.

The cultivated fruit bodies of mushrooms have already been used medicinally, and the mycelia produced in a liquid tank culture are used to develop medicines and for drinking products. However, some medicinal mushrooms can not be artificially cultivated, because the cost of the isolation and purification of useful materials in a liquid mass culture is expensive. Therefore, it is necessary to develop a fermentation method using solid materials (e.g. soybeans or various cereals) for culturing the mycelia of medicinal mushrooms.

Accordingly, the present study investigated the effect of soybeans fermented with *A. cylindracea* or *P. igniarius*, administered intragastrically to mice, on the activities of phase II and antioxidant enzymes QR, GST, GSH-Px, and SOD in the liver, lung, kidney, stomach, and colon.

MATERIALS AND METHODS

Preparation of Inoculum

The strains *P. igniarius* 26005 and *A. cylindracea* were obtained from the National Agricultural Science and Technology Institute, Korea. The strains were preserved on an MYG (malt:yeast:glucose=1.0:0.4:0.4) plate. Five (5 mm) pieces of the mycelium grown in the solid MYG media were put into 250 ml of an MYG broth in a 500-ml Erlenmeyer flask. The broth with the *P. igniarius* was grown for 10 days, whereas that with the *A. cylindracea* was grown for 7 days at 28°C in a shaking incubator (Hanback Scientific Co., HB201S, Korea) and then homogenized at 10,000 rpm for 30 s. The resulting homogenates were used as the inoculum.

Soybeans Fermented with Basidiomycetes

The soybeans (malt soybeans) were soaked with 10 volumes of cold water [18]. The water on the surface of the soaked soybeans was removed after 12 h (hydration time), and the hydrated soybeans (500 g) were autoclaved. Next, either the *A. cylindracea* or the *P. igniarius* homogenate was inoculated and then fermented for 15 days at 28°C in an incubator (Ki-woo Trading, LBI-400, Korea). The fermented soybeans with *P. igniarius* or *A. cylindracea* were dried for 48 h at 40°C and stored at - 20°C before use.

Extraction of Polysaccharides

The polysaccharides from the soybeans fermented with *A. cylindracea* or *P. igniarius* were extracted according to the method of Shon *et al.* [27] with the following modifications. The samples were extracted with 10 volumes of boiling water for 3 h at 100°C. After filtration, the filtrates were first precipitated by adding 3 volumes of 95% ethanol overnight at 4°C and then collected by centrifugation (10,000 rpm for 30 min). The precipitates were dissolved in distilled water and centrifuged at 8,000 rpm for 20 min. Finally, the supernatant (crude polysaccharides) was freeze-dried.

Treatment of Mice and Tissue Preparation

Four-week-old male ICR mice were purchased from the Dae-Han Laboratory Animal Research Center (Eumsung, Korea). Six animals were housed per cage. After a one-week acclimation period, the soybeans fermented with *P. igniarius* or *A. cylindracea* or the vehicle alone were administered for 14 consecutive days (intragastric application at doses of 100, 500, or 10,000 mg/ kg body wt). At the end of the application regimen, the mice were killed by cervical dislocation. The livers were perfused with cold 0.15 M KCl buffer (pH 7.0), and the tissues (lung, kidney, stomach, and colon) were removed and immediately placed in 0.25 M sucrose. The tissues were homogenized in 0.25 M sucrose (5.0 ml/g of tissue) and centrifuged at 9,000 ×g for 20 min. The supernatant was treated with 0.2 vol of 0.1 M $CaCl_2$ in 0.25 M sucrose for 30 min at 0°C and centrifuged at 27,000 ×g for 20 min to remove the microsomes. The clear cytosol fractions were then used for determination of the enzyme activities.

Determination of QR and GST Activities and GSH Levels in Mouse Tissues

The QR specific activity was measured by the addition of 200 µl of a mixture [0.5 M Tris-HCl (pH 7.4), bovine serum albumin, 1.5% Tween-20, 7.5 mM FAD, 150 mM glucose-6-phosphate, 50 mM NADP, yeast glucose-6-phosphate dehydrogenase, dimethythiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT), and 50 mM menadione in distilled water] to 50 µl of a suitable dilution of the tissue cytosol fraction according to the method of Prochaska [20]. The protein content was determined by the crystal violet staining of an identical set of test plates.

The GST activity was measured using a modification of the procedure developed by Habig *et al.* [8] with 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. The protein content was monitored in duplicate plates using a bicinchoninic protein assay kit (Sigma, St. Louis, U.S.A.) with bovine serum albumin as the standard. The GST activity was expressed as the slope/min/mg of protein.

The GSH concentration was measured using an enzymatic recycling procedure in 96-well plates [7]. The total GSH

levels were determined in 40 µl of a suitable dilution of the tissue cytosol fraction. A freshly prepared reaction mixture (170 µl) consisting of 6 mM 5,5'-dithiobis-(2-nitrobenzoic acid) in solution A [125 mM sodium phosphate buffer (pH 7.4) containing 6.3 mM EDTA], a glutathione reductase solution (50 units in 10 ml of solution A), and NADPH-generating system (solution B) was then added to each well to initiate the reaction. Solution B was composed of 0.5 M Tris-HCl (pH 7.4), 150 mM glucose-6-phosphate, 50 mM $NADP^+$, and glucose-6-phosphate dehydrogenase. The plates were shaken at room temperature for 5 min on a microtiter plate shaker. The optical density at 405 nm was determined using a microtiter plate reader 10 min after the initiation of the reaction. The GSH content was calculated by comparing with a GSH standard curve. The protein content was determined in duplicate plates prepared and treated as described above, using a bicinchoninic protein assay kit (Sigma, St. Louis, U.S.A.) with bovine serum albumin as the standard. The GSH levels were expressed as nmol/mg of protein. The induction of the enzyme (QR and GST) activities and GSH levels were calculated from the ratio of the specific enzyme activities and GSH levels of the sample-treated mouse organs by comparing with the solvent control. All data were expressed as mean±SD (standard deviation). The means were compared using the Student's t-test with n=3.

Determination of GSH-Px and SOD Activities

The GSH-Px activity was determined using the coupled assay developed by Paglia and Lawrence [13, 16] with hydrogen peroxide as the substrate. The reaction was started by the addition of 2.2 mM hydrogen peroxide as the substrate. The change in absorbance at 340 nm was measured for 1 min, and the activity was expressed as the µmol of NADPH oxidized/min/mg of protein. The protein content was determined by a bicinchoninic acid protein assay kit (Sigma, St. Louis, U.S.A.) with bovine serum albumin (Sigma, St. Louis, U.S.A.) as the standard. The SOD activity was determined by recording the inhibition of ferricytochrome *c* reduction with xanthine, xanthine oxidase, and EDTA [15]. The amount of enzyme in the sample sufficient to inhibit the rate of cytochrome *c* reduction by 50% was defined as 1 unit of activity and expressed as unit/mg of protein. The means of the specific enzyme activities were compared using the Student's t-test with n=3.

RESULTS AND DISCUSSION

Induction of Phase II Enzymes in Mice

The polysaccharides extracted from the soybeans fermented with either *P. igniarius* or *A. cylindracea* and administered to mice were evaluated for their potential to induce QR and GST activities and their effect on the GSH levels in the liver, lung, kidney, stomach, and colon. The data in Table 1 show the activities of QR and GST in the extract-administered (at doses of 100, 500, and 1,000 mg/kg body weight) experimental groups of mice. The QR activity in the liver and kidney was significantly ($p<0.05$) elevated with the treatment of the extracts from soybeans fermented with *A. cylindracea* or *P. igniarius* at doses of 500 and 1,000 mg/kg body weight. The intragastric application of soybeans fermented with *A. cylindracea* or *P. igniarius* resulted in an increase in the QR activity in the colon but not in the lung and stomach.

A greater increase was observed in the GST activity than in the QR activity. The increase in the activity of these two enzymes, however, was found to be markedly higher in the liver and kidney as compared to the lung, stomach, and colon (Table 1). In addition to the induction of QR and

Table 1. Induction of QR and GST activities by intragastric application of soybeans fermented with *A. cylindracea* (AC) or *P. igniarius* (PI) in cytosols of ICR mice organs.

Organ	Samples (mg/kg body wt)		Ratio of specific activities (treated/control) QR	GST
Liver	Soybean fermented with AC	100	1.24±0.11	2.42±0.17**
		500	1.45±0.08*	2.78±0.15**
		1000	1.53±0.13*	3.43±0.14**
	Soybean fermented with PI	100	1.29±0.21	3.24±0.23**
		500	1.41±0.09*	4.01±0.20**
		1000	1.65±0.21*	4.13±0.18**
Kidney	Soybean fermented with AC	100	1.25±0.11	2.31±0.25*
		500	1.40±0.15*	2.68±0.23**
		1000	1.58±0.16*	2.87±0.19**
	Soybean fermented with PI	100	1.32±0.15	2.60±0.21**
		500	1.59±0.13*	2.98±0.20**
		1000	1.63±0.19*	3.24±0.18**
Lung	Soybean fermented with AC	100	1.10±0.06	1.00±0.12
		500	1.09±0.15	1.00±0.07
		1000	1.12±0.10	1.11±0.16
	Soybean fermented with PI	100	1.00±0.12	1.00±0.15
		500	1.00±0.16	1.04±0.09
		1000	1.00±0.09	1.10±0.10
Stomach	Soybean fermented with AC	100	1.13±0.26	1.10±0.17
		500	1.26±0.31	1.26±0.21
		1000	1.34±0.18	1.41±0.25
	Soybean fermented with PI	100	1.20±0.25	1.12±0.22
		500	1.35±0.21	1.21±0.24
		1000	1.40±0.30	1.43±0.19
Colon	Soybean fermented with AC	100	1.20±0.15	1.02±0.20
		500	1.35±0.19	1.23±0.23
		1000	1.44±0.15*	1.38±0.17
	Soybean fermented with PI	100	1.27±0.03**	1.20±0.09
		500	1.42±0.20	1.36±0.19
		1000	1.45±0.21	1.47±0.17

The results are expressed as the ratios of the specific activities of the organ cytosols from the treated animals to those of the controls. The values are mean±SD (standard deviation). The values of each group are statistically significant as compared with the control group (*: $p<0.05$; **: $p<0.01$).

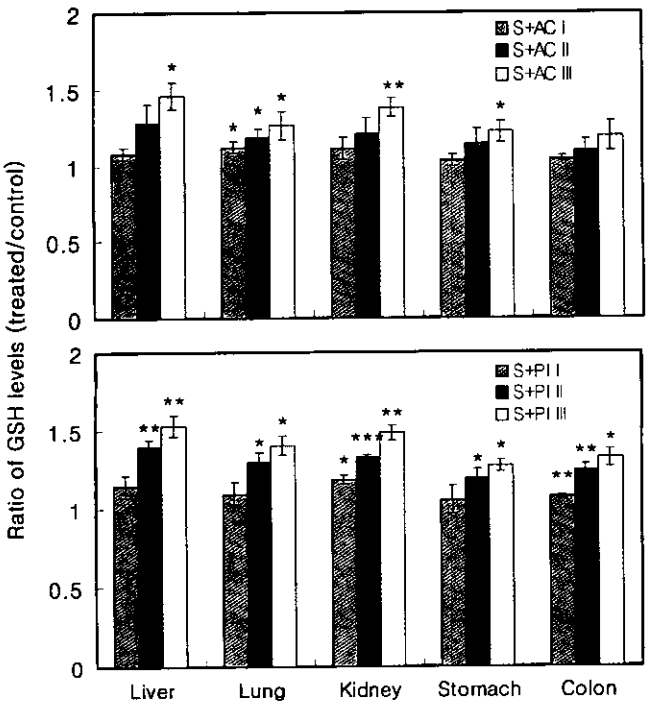

Fig. 1. Induction of GSH levels by soybeans fermented with *A. cylindracea* (AC) or *P. igniarius* (PI) in cytosols of ICR mouse organs.

The induction was calculated by comparing the treatment groups with the control group. S+AC I, II, and III represent 100, 500, and 1,000 mg/kg body weight of soybeans fermented with AC, respectively. S+PI I, II, and III represent 100, 500, and 1,000 mg/kg body weight of soybeans fermented with PI, respectively. The values are mean±SD (standard deviation). The mean is significantly different from the control (*$p<0.05$, **$p<0.01$, ***$p<0.001$) using the Student's t-test with n=3.

GST activities, the GSH levels were also increased with soybeans fermented with basidiomycetes (Fig. 1). At the highest concentration tested (1,000 mg/kg body weight), the soybeans fermented with *A. cylindracea* or *P. igniarius* elevated the GSH level in the liver 1.54- and 1.45-fold, respectively. The effect of the soybeans fermented with *A. cylindracea* or *P. igniarius* on the induction of GSH levels was dose-dependent within a concentration range of 100–1,000 mg/kg body weight in the various mouse organs. When the animals were treated with soybeans fermented with *P. igniarius* or *A. cylindracea*, no toxic effects were observed at the concentrations tested.

Previous studies suggested that the modulation of phase II detoxification enzyme activities is responsible for cancer chemopreventive activity [1, 6, 31, 37]. Many types of compounds, such as dithiolethione, sulforaphane, ethoxyquin, and phenolic antioxidants, have been reported to protect against carcinogens and the toxic effects of a variety of chemical agents at multiple sites in animal models, via the induction of phase II detoxification enzymes [3, 6, 10, 37], such as QR and GST. Dithiolethione induces GST and NAD(P)H:QR activities in the liver of rodents [31]. The colon tumor-inhibitory effect of organosulfur compounds is associated with increase of GST, NAD(P)H:QR, and UDP-glucuronosyl transferase activities in the colon [1]. Kim *et al.* [12] showed that Korean vegetables have a cancer protective activity by inducing QR activity in both cell cultures and *in vivo* model systems. Singh *et al.* [28] also found that chlorophyllin significantly elevated GST activity and GSH levels in the liver. Spencer *et al.* [31] demonstrated that the addition of dimethyl fumarate to the diet of mice and rats elevated cytosolic GST and QR activities in the liver, stomach, small intestine, colon, and spleen. These results are consistent with our current results which show increased QR and GST activities and GSH levels in various organs of mice with the administration of soybeans fermented with *A. cylindracea* or *P. igniarius*. These inducible enzymes facilitate the metabolic detoxification of xenobiotics in mammals and can achieve chemopreventive activity through increased carcinogen excretion and decreased carcinogen-DNA interactions.

Phase II drug-metabolizing enzymes such as QR or GST play a critical role in protecting tissues from xenobiotics and carcinogens through a variety of reactions. A one-electron reduction of quinone leads to the formation of semiquinones and these semiquinones are able to react rapidly with oxygen to form a superoxide anion radical, singlet molecular oxygen, hydrogen peroxide, and possibly other reactive chemical species. These various forms of reactive oxygen have been shown to damage enzymes and cell membranes and act as mutagens and carcinogens. A two-electron reduction of quinone to form the corresponding hydroquinone represents a detoxification pathway for quinones, and terminates the ability of quinone to participate in redox-cycling. Quinone reductase functions as an inducible protective device against quinone toxicity by reducing quinones to relatively stable hydroquinones. These resulting hydroquinones can be conjugated and excreted through mercapturic acid pathways. GST catalyzes the conjugation of electrophilic compounds with glutathione, resulting in soluble complexes that are generally more hydrophilic and less cytotoxic. Therefore, it is reasonable to assume that the increased activities of GST and QR in various organs of mice fed with soybeans fermented with *A. cylindracea* or *P. igniarius* may play an important role in relation to cancer chemopreventive effects.

Induction of Antioxidant Enzymes

The effect of intragastrically administrated soybeans fermented with *P. igniarius* or *A. cylindracea* on antioxidant enzymes (GSH-Px and SOD) activities in various organs of mice (liver, lung, kidney, stomach, and colon) was tested. The intragastric application of extracts from soybeans fermented with *A. cylindracea* for 14 days resulted in a significant increase in the GSH-Px activity in the liver, lung, and kidney. In contrast, the application of soybeans fermented with *P. igniarius* did not result in any significant change in

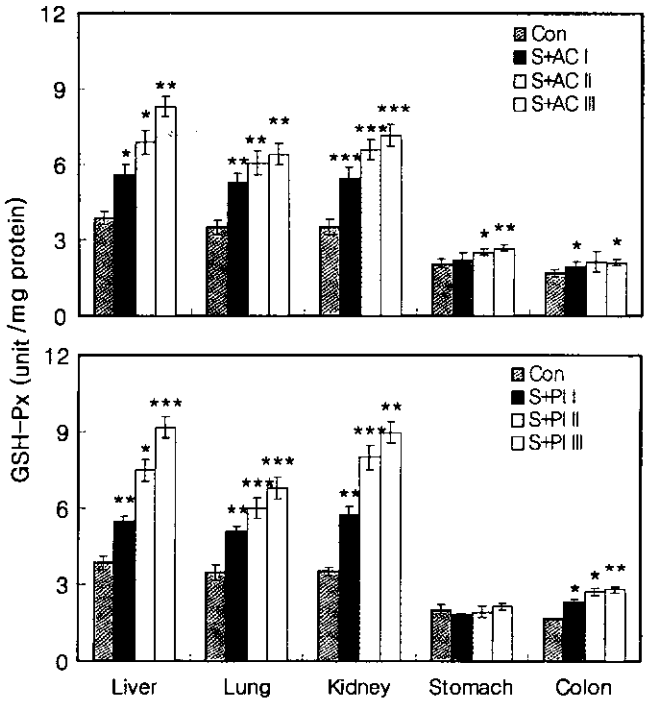

Fig. 2. Effect of intragastric application of soybeans fermented with *A. cylindracea* (AC) or *P. igniarius* (PI) to ICR mice on the activity of cytosolic GSH-Px in liver, lung, kidney, stomach, and colon.
Con: control group received intragastric application of distilled water. S+AC I, II, and III represent 100, 500, and 1,000 mg/kg body weight of soybeans fermented with AC, respectively. S+PI I, II, and III represent 100, 500, and 1,000 mg/kg body weight of soybeans fermented with PI, respectively. The values are mean±SD (standard deviation). The mean is significantly different from the control (*p<0.05, **p<0.01, ***p<0.001) using the student's t-test with n=3.

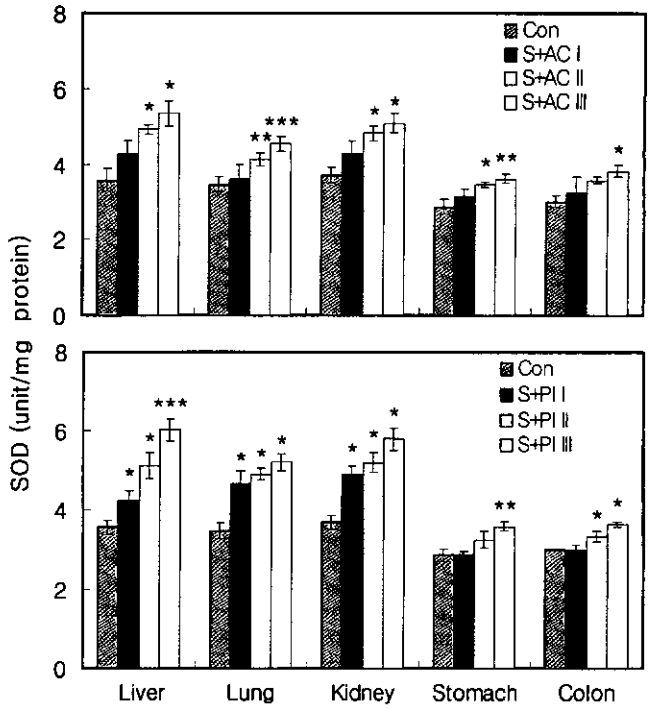

Fig. 3. Effect of intragastric application of soybeans fermented with *A. cylindracea* (AC) or *P. igniarius* (PI) to ICR mice on the activity of cytosolic SOD in liver, lung, kidney, stomach, and colon.
Con: control group received intragastric application of distilled water. S+AC I, II, and III represent 100, 500, and 1,000 mg/kg body weight of soybeans fermented with AC, respectively. S+PI I, II, and III represent 100, 500, and 1,000 mg/kg body weight of soybeans fermented with PI, respectively. The values are mean±SD (standard deviation). The mean is significantly different from control (*p<0.05, **p<0.01, ***p<0.001) using the student's t-test with n=3.

GSH-Px activity in the stomach; however, a significant increase in the activity of this enzyme was observed in the liver, lung, kidney, and colon (Fig. 2). There was significant increase in the SOD activity in the liver, lung, kidney, stomach, and colon with the application of soybeans fermented with *A. cylindracea* or *P. igniarius* at a concentration of 1,000 mg/kg body weight (Fig. 3).

GSH-Px plays a role in the detoxification of peroxides from cells and/or tissues [22]. The source of H_2O_2 in cells/tissues is mainly from the superoxide dismutase-mediated dismutation of O_2^- [4]. Several previous reports have suggested the effects of peroxides in producing cytotoxicity/genotoxicity in cellular systems [4, 29]. In addition, highly reactive OH generated from H_2O_2 is known to damage macromolecules, specifically DNA, to produce a pathological alteration [18, 32]. In view of these facts, the enhancement in the activity of both GSH-Px and SOD in various organs through the application of soybeans fermented with *A. cylindracea* or *P. igniarius* seem to suggest that such a treatment could protect cells/tissues against the cytotoxic/genotoxic effects of peroxides and OH.

The generation of reactive oxidants in biological systems, either by normal metabolic pathways or as a consequence of exposure to chemical carcinogens, has been extensively studied as a contributing factor to the multistage process of carcinogenesis [18, 32]. The collective action of both phase II and antioxidants enzymes, such as GST and QR, is to offer protection against the adverse effects of oxidants or reactive metabolites of precarcinogens [18, 32]. The depletion of antioxidant enzymes following exposure to carcinogens and/or tumor promoters is already known [18, 32]. In contrast, cancer chemoprevention studies showed that following the administration of chemopreventive agents, the levels of antioxidant enzymes were elevated in various organs of test animals [35].

In conclusion, the current study indicated an enhanced activity of phase II enzymes like QR and GST and antioxidant enzymes such as GSH-Px and SOD in various organs of mice after intragastric application of soybeans fermented with *A. cylindracea* or *P. igniarius*, and suggested a possible mechanism for cancer chemoprevention. These findings encourage us to further study the chemopreventive activity of solid-fermented basidiomycetes.

Acknowledgments

This study was supported by a Highly Advanced National (HAN) project grant from the Korean Ministry of Science and Technology.

REFERENCES

1. Bandaru, S. R., V. R. Chinthalapally, R. Abraham, and K. Gary. 1993. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res.* **53:** 3493-3498.
2. Bogaards, J. J. P., H. Verhagen, M. I. Willems, G. van Poppel, and P. J. van Bladeren. 1994. Consumption of Brussels sprouts results in elevated α-class glutathione S-transferase levels in human blood plasma. *Carcinogenesis* **15:** 1073-1075.
3. Cabral, J. R. P. and G. E. Neal. 1983. The inhibitory effect of ethoxyquin on the carcinogenic action of aflatoxin B_1 in rats. *Cancer Lett.* **19:** 125-132.
4. Chang, W.-S. and J.-S. So. 1999. Characterization of superoxide dismutase in *Lactococcus lactis*. *J. Microbiol. Biotechnol.* **9:** 732-736.
5. Chihara, G., J. Hamaro, Y. Maeda, Y. Arai, and F. Furuoka. 1970. Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) Sing. (an edible mushroom). *Cancer Res.* **30:** 2776-2781.
6. De Long, M. J., H. J. Prochaska, and P. Talalay. 1985. Tissue-specific induction patterns of cancer-protective enzymes in mice by tert-butyl-4-hydroxyanisole and related substituted phenols. *Cancer Res.* **45:** 546-551.
7. Griffith, O. W. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* **106:** 207-212.
8. Habig, W. H., M. H. Pabst, and W. B. Jacoby. 1974. Glutathione S-transferase: The first enzymatic step to mercapturic acid formation. *J. Biol. Chem.* **249:** 7130-7139.
9. Hirokazu, K., I. Ryuichi, K. Tetro, and M. Takashi. 1989. Fraction and antitumor activity of the water-insoluble residue of *Agaricus blazei* fruiting bodies. *Carbohydrate Res.* **186:** 267-273.
10. Kensler, T. W., J. D. Groopman, and B. D. Roebuck. 1992. Chemoprevention by oltipraz and other dithiolethiones. pp. 205-225, *In* Wattenberg, L., M. Lipkin, C. Boone, and G. Kelloff (eds.), *Cancer Chemoprevention*. CRC Press, Boca Raton, FL, U.S.A.
11. Kim, K. C. and I. G. Kim. 1999. *Ganoderma lucidum* extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. *Int. J. Mol. Med.* **4:** 273-277.
12. Kim, S. M., S. H. Ryu, H. D. Choi, S. S. Kim, J. H. Kim, and J. S. Kim. 1998. Screening for Korean vegetables with anticarcinogenic enzyme inducing activity using cell culture system. *Korean J. Food Sci. Nutr.* **3:** 277-281.
13. Lawrence, R. A. and R. F. Burk. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.* **71:** 952-958.
14. Lee, J.-H., S.-M. Cho, H.-M. Kim, N.-D. Hong, and I.-D. Yoo. 1997. Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. *J. Microbiol. Biotechnol.* **7:** 52-55.
15. Oyanagui, Y. 1984. Reevalution of assay methods and establishment of kit for superoxide dismutase activity. *Anal. Biochem.* **42:** 290-296.
16. Paglia, D. E. and W. N. Valentine. 1967. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Cli. Med.* **70:** 158-169.
17. Park, S.-S. and S.-M. Hwang. 1999. Purification and characterization of iron-containing superoxide dismutase from *Lentinus edodes*. *J. Microbiol. Biotechnol.* **9:** 854-860.
18. Perchellet, J. and E. M. Perchellet. 1989. Antioxidants and multistage carcinogenesis in mouse skin. *Free Radical Biol. Med.* **7:** 377-408.
19. Prochaska, H. J. and C. L. Fernandes. 1993. Elevation of serum Phase II enzymes by anticarcinogenic enzyme inducers: Markers for a chemoprotected state? *Carcinogenesis* **14:** 2441-2445.
20. Prochaska, H. J. and A. B. Santamaria. 1988. Direct measurement of NAD(P)H:Quinone reductase from cells cultured in microtiter wells: A screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* **169:** 328-336.
21. Prochaska, H. J. and P. Talalay. 1988. Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res.* **48:** 4776-4782.
22. Raes M., C. Michiels, and J. Remale. 1987. Comparative study of the enzymatic defense systems against oxygen-derived free radicals: The key role of glutathione peroxidase. *Free Radical Biol. Med.* **3:** 3-7.
23. Reiners, J. J. Jr., G. Thai, T. Rupp, and A. R. Cantu. 1991. Assessment of the antioxidant/prooxidant status of murine skin following topical treatment with 12-*O*-tetradecanoylphorbol-13-acetate and throughout the ontogeny of skin cancer. Part I: Quantitation of superoxide dismutase, catalase, glutathione peroxidase and xanthine oxidase. *Carcinogenesis* **12:** 2337-2343.
24. Sakagami, H., S. Kohno, M. Takeda, K. Nakamura, K. Nomoto, I. Ueno, S. Kanegasaki, T. Naoe, and Y. Kawazoe. 1992. O_2^- scavenging activity of lignins, tannins and PSK. *Anticancer Res.* **12:** 1995-2000.
25. Sarafian, T. A. and D. E. Bredesen. 1994. Is apoptosis mediated by reactive oxygen species? *Free Radic. Res.* **21:** 1-8.
26. Seifter, E., J. Mendecki, S. Holtzman, J. D. Kanofsky, E. Freidenthal, L. Davis, and J. Weinzweig. 1988. Role of vitamin A and in radiation protection; relation to antioxidant properties. *Pharmacol Ther.* **39:** 357-365.
27. Shon, Y. H., J. S. Lee, H. W. Lee, and K. S. Nam. 1999. Antimutagenic potential of *Phellinus igniarius*. *J. Microbiol. Biotechnol.* **9:** 525-528.
28. Singh, A., S. P. Singh, and R. Bamezai. 1996. Modulatory influence of chlorophyllin on the mouse skin papollomagenesis and xenobiotic detoxification system. *Carcinogenesis* **17:** 1459-1463.

29. Song, C.-H., E.-K. Park, H. J. Suh, Y. S. Lee, J. W. Choi, and K. S. Ra. 1999. Purification and characterization of manganese superoxide dismutase from *Staphylococcus sciuri*. *J. Microbiol. Biotechnol.* **9:** 271-275.
30. Song, C.-H., Y.-J. Jeon, B.-K. Yang, K.-S. Ra, and J.-M. Sung. 1998. The anti-complementary activity of exo-polymers produced from submerged mycelial cultures of higher fungi with particular reference to *Cordyceps militaris*. *J. Microbiol. Biotechnol.* **8:** 536-539.
31. Spencer, S. R., C. A. Wilczak, and P. Talalay. 1990. Induction of glutathione transferase and NAD(P)H:quinone reductase by fumaric acid derivatives in rodent cells and tissues. *Cancer Res.* **50:** 7871-7875.
32. Sun, Y. 1990. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radical Biol. Med.* **8:** 583-599.
33. Toshio, M. and N. Motohro. 1981. Studies on fungal polysaccharides. XXVII. Structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum*. *Chem. Pharm. Bull. (Tokyo)* **29:** 3611-3616.
34. Tsukagoshi, S. and F. Ohashi. 1974. Protein-bound polysaccharide preparation, PS-K, effective against mouse sarcoma 180 and rat ascites hepatoma AH-13 by oral use. *Gann* **65:** 557-558.
35. Wattenberg, L. W. 1990. Inhibition of carcinogenesis by naturally occurring and synthetic compounds. pp. 155-166. *In* Kuroda, Y., D. M. Shankel, and M. D. Waters (eds.) *Antimutagenesis and Anticarcinogenesis, Mechanisms II*. Plenum Publishing Corp., New York, U.S.A.
36. Zhang, Y. S., P. Talalay, C. G. Cho, and G. H. Posner. 1992. A major inducer of anticarcinogenic protective enzymes from broccoli - Isolation and elucidation of structure. *Proc. Natl. Acad. Sci. USA* **89:** 2399-2403.
37. Zhang, Y., T. W. Kensler, C. G. Cho, G. H. Posner, and P. Talalay. 1994. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc. Natl. Acad. Sci. USA* **91:** 3147-3150.
38. Zhou, Y. C. and R. L. Zheng. 1991. Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. *Biochem. Pharmacol.* **22:** 1177-1179.