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Modulation of Nrf2/ARE and Inflammatory Signaling Pathways by *Hericium erinaceus* Mycelia Extract

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Abstract *Hericium erinaceus* is an edible mushroom used as a medicinal food in Asian countries. In this study, the chemopreventive effects of *H. erinaceus* mycelia hot water extract (HEW) were evaluated. HEW remarkably induced the luciferase activity of the antioxidant response element (ARE), located in the promoter region of phase 2 and antioxidant genes and regulated by nuclear factor E2-related factor 2 (Nrf2). The up-regulation of ARE activity by HEW corresponded with the induction of Nrf2 and the antioxidant enzyme, hemeoxygenase-1. The inhibition of cyclooxygenase-2 (COX-2) activity is a promising effective approach in cancer chemoprevention, and HEW prominently suppressed COX-2 protein expression in HepG2 cells. Furthermore, HEW showed anti-inflammatory activity by modulating inflammatory mediators such as nitric oxide (NO), inducible NO synthase, tumor necrosis factor-α, interleukin-1β, and the transcription factor, nuclear factor-κB, in lipopolysaccharide-stimulated RAW 264.7 cells. These results suggest that *H. erinaceus* possessed anti-tumor and anti-inflammatory effects via the modulation of Nrf2/ARE and inflammatory signaling pathways, and may therefore have potential use as a natural chemopreventive agent.

Keywords: *Hericium erinaceus* mycelia, antioxidant response element (ARE), nuclear factor E2-related factor 2 (Nrf2), anti-inflammation, chemoprevention

Introduction

A great number of mushrooms and their extracts are being used as dietary supplements, and increasingly as remedies for diseases. *Hericium erinaceus* is an edible mushroom used as a traditional medicinal food in oriental countries. It is called '*yamabushitake*' and '*houtou*' in Japan and China, respectively (1,2). Polysaccharides including β-D-glucan are its main compounds having anti-tumor and immuno-stimulating properties (3,4). Previous reports on the physiological activities of *H. erinaceus* have focused primarily on the treatment of stomach and intestinal disorders such as chronic gastritis, gastric ulcers, duodenal ulcers, stomach cancer, and colon cancer (2,5). Improvement effects by *H. erinaceus* on nerve cells or neural dysfunctions (6-8) and hypoglycemic effects via the reduction of blood lipid and glucose levels (9) have also been reported. Although *H. erinaceus* has chemopreventive properties comprising anti-tumor, anti-inflammation, and anti-microbial effects, its precise mechanisms have been poorly studied.

Nuclear factor E2-related factor 2 (Nrf2) is an important cytoprotective transcription factor that has recently received much attention. Nrf2 has been shown to regulate the induction of cellular defensive phase 2 detoxifying and antioxidant enzymes, including glutathione *S*-transferase (GST), NAD(P)H:quinine oxidoreductase 1 (NQO1), and hemeoxygenase-1 (HO-1). These enzymes protect cells and/or tissues from carcinogens that can initiate carcinogenic processes (10-12). Several dietary phytochemicals possess cancer chemopreventive functions by stimulation of the Nrf2-antioxidant response element (ARE) pathway and the resulting induction of phase 2 and antioxidant enzymes (13).

An intimate connection between inflammation and cancer has been established and chronic inflammation may be a key predisposing factor to cancer. Some chemopreventive agents possess anti-inflammatory effects through the Nrf2 dependent pathway (14-16). Chemopreventive agents activate Nrf2 and phase 2/antioxidant defense enzymes sequentially, potentially leading to the inhibition of pro-inflammatory cytokines and other mediators (17).

There are several important inflammatory mediators that are precisely related to inflammation and cancer. Cyclooxygenase-2 (COX-2) is an inducible enzyme overexpressed in various inflammation-associated cancers, and helps to maintain a persistent inflammatory state in premalignant and malignant lesions. It also accelerates angiogenic processes by triggering prostaglandin E2 signaling (18). The induction of COX-2 has been shown to promote cell growth, inhibit apoptosis, and enhance cell motility (19,20). Many chemopreventive agents have a suppressive effect on COX-2 expression in human hepatocarcinoma HepG2 cells (21,22).

Inflammation induces oxidative stress by the production of oxidants, such as nitric oxide (NO), produced by inducible NO synthase (iNOS) (23-25). The expression of iNOS is closely related to the up-regulation of nuclear factor (NF)-κB (26). NF-κB binding sites have been identified in the promoter region of the genes of iNOS as well as COX-2 (27, 28). Interleukin (IL)-1β, IL-6, and

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tumor necrosis factor (TNF)-α are known as inflammatory cytokines that also play important roles in the regulation of the immune system. They activate pro-inflammatory signaling mediated via Jak-Stat and NF-κB, and help to maintain inflammatory tumor microenvironments by stimulating cell proliferation and inhibiting apoptosis (18).

To elucidate the chemopreventive effects of *H. erinaceus* mycelia hot water extract (HEW), growth inhibition, the modulation of the Nrf2-ARE pathway, and COX-2 suppression were investigated in human hepatocarcinoma HepG2 cells. In addition, pro-inflammatory mediators, transcription factor, and cytokines such as NO, iNOS, COX-2, NF-κB, TNF-α, and IL-1β were studied in murine macrophage RAW264.7 cells. The current results will be useful data in the production of a function food material using *H. erinaceus* mycelia.

Materials and Methods

Materials The cell culture facilities were obtained from Corning Life Sciences (Corning, NY, USA). The cell culture reagents were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Antibodies against Nrf2, COX-2, iNOS, IL-1β, and actin were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA); HO-1 from Calbiochem (Darmstadt, Germany); and TNF-α, p-p65, and p-inhibitory κBα (IκBα) from Cell Signaling Technology (Beverly, MA, USA). Bovine anti-rabbit immunoglobulin (Ig) G and bovine anti-goat IgG were purchased from Santa Cruz Biotechnology Inc. All other reagents were obtained from commercial sources.

Cultivation and extraction of *Hericum erinaceus* mycelia The *H. erinaceus* mycelia were supplied by the Rural Development Administration (Suwon, Gyeonggi, Korea), and were cultivated for 2 weeks at 25°C on solid medium containing 2% glucose, 2% soy peptone, 1% yeast extract, and 0.5% agar. After lyophilization of the mycelia, HEW was prepared by extraction using 20 fold hot distilled water for 12 hr at 100°C. After concentration by a rotary evaporator (Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) up to a 10th, the HEW was stored at 4°C until use. The concentration of solid materials in the HEW was 0.31 g/mL calculated after lyophilization.

Measurement of glucan content The contents of α- and β-glucan were analyzed using a mushroom and yeast β-glucan kit (Megazyme Int., Wicklow, Ireland) according to the manufacturer's instruction and previous researches (29). To measure total glucan content, 5 mg of lyophilized HEW and 75 μL of 10 N HCl were added in a tube and stirred vigorously on a vortex mixer frequently during incubation time in a water bath at 30°C for 45 min. The material was mixed with 500 μL of distilled water and then, the tube was boiled at 100°C for 2 hr. After cooling at room temperature, 500 μL of 2 N KOH was added to the tube and then, the sample was washed and adjusted to 5 mL volume with 200 mM sodium acetate buffer (pH 5.0). After centrifugation at 1,500×g for 10 min, 50 μL of the supernatant was combined with 50 μL of a mixture of exo-1,3-β-(1→3)-D-glucanase (20 U/mL) and β-glucosidase (4 U/mL) in 200 mM sodium acetate buffer (pH 5.0) and incubated at 40°C for 60 min. A 1.5 mL of glucose peroxidase reagent was added and incubated at 40°C for 20 min and the absorbance was analyzed at 510 nm. To measure α-glucan content, 5 mg of lyophilized HEW and 100 μL of 2 N KOH was added to a tube and stirred vigorously on a vortex mixer for 20 min. The suspension was added to 400 μL of 1.2 M sodium acetate buffer (pH 3.8), mixed with amyloglucosidase (1,630 U/mL) and invertase (500 U/mL), and incubated at 40°C for 30 min. Distilled water was added up to 5 mL to the sample and centrifuged at 1,500×g for 10 min. Fifty μL of the supernatant was combined with 50 μL of 200 mM sodium acetate buffer (pH 5.0) and 0.5 mL of glucose peroxidase reagent, incubated at 40°C for 20 min and the absorbance was analyzed at 510 nm. The β-glucan content was determined by subtracting the α-glucan from the total glucan content.

Cell culture and treatments The HepG2 human hepatoma cells and RAW 264.7 murine macrophage cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in F12 (for HepG2) and Dulbecco's modified Eagle medium (DMEM, for RAW 264.7) supplemented with 10% fetal bovine serum (FBS), 1.17 (for F12) or 3.7 g/L (for DMEM) of sodium bicarbonate, 100 U/mL of penicillin, 100 μg/mL of streptomycin, 1% essential amino acids (for F12), and 0.1% insulin (for F12) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The HepG2 cells were seeded in multi-well plates (24-well for cell viability, 12-well for luciferase activity, and 6-well for Western blotting) and allowed to grow for 24 hr (about 80 to 90% confluency). Then, the cells were starved overnight with serum-free F12 media prior to further treatments with HEW. The RAW 264.7 cells were seeded in 6-well plates and allowed to grow for 24 hr. After HEW treatment for 2 hr, lipopolysaccharide (LPS) (1 μg/mL) was added and the cells were incubated for 20 additional hr. Cells not treated with LPS served as the negative control (untreated, NT), while cells treated with LPS alone served as the positive control (control, Con).

Cell viability determined by MTS assay Cell viability was assessed with the CellTiter 96® aqueous one solution cell proliferation assay (Promega Corp., Madison, WI, USA) based on the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, according to the manufacturer's instructions. Briefly, the cells were seeded in 24-well plates, and after 24 hr of incubation, were starved overnight with serum-free media prior to treatment with HEW for 24 hr. After treatment, the media were removed, and fresh serum-free media containing MTS were added. After incubation for 1 hr at 37°C, the absorbance was measured at 490 nm with a microplate reader (PowerWave XS; BioTek Instrument, Inc., Winooski, VT, USA)

ARE-luciferase activity assay A HepG2-ARE-C8 (HepG2-C8) cell line was kindly donated by Dr. Ah-Ng Tony Kong (Rutgers University, Piscataway, NJ, USA), which was established by the stable transfection of HepG2 cells with the p-ARE-TI-luciferase reporter gene as previously described (30). The HepG2-C8 cells were treated with

HEW for 24 hr and sulforaphane (SFN, 25 μM) was used as a positive control for ARE induction according to previous studies (31). The ARE-luciferase activity was determined using a Luciferase assay system from Promega, according to the manufacturer's instructions. Briefly, after treatment, the cells were washed twice with ice-cold 1× phosphate buffered saline (PBS, pH 7.4) and harvested in 1×cell culture lysis buffer. After centrifugation at 13,000×g for 10 min, a 20 μL aliquot of the supernatant was assayed for luciferase activity with a GloMax™ 20/20 luminometer (Promega). The luciferase activity was normalized against the amount of protein, determined by a BCA protein assay (Pierce Biotechnology, Inc., Rockford, IL, USA), and expressed as fold-induction over the luciferase activity of the no treatment control.

NO measurement (Nitrite assay) Nitrite accumulation in the culture medium is an indicator of NO production and was measured according to the Griess reaction (32). One-hundred μL of each medium supernatant was mixed with 50 μL of sulphanilamide (1% in 5% phosphoric acid) and 50 μL of naphthylenediamine dihydrochloride (0.1%) and then incubated at room temperature for 10 min. The absorbance at 550 nm was measured against a sodium nitrite ($NaNO_2$) serial dilution standard curve and nitrite production was determined.

Western blot analysis After treatment, the cells were washed with ice-cold 1×PBS and harvested with 200 μL of cell lysis buffer (pH 7.4). The cell lysates were left on ice for 30 min and then the homogenates were centrifuged at 13,000×g for 10 min at 4°C. The supernatants were collected and equal amounts of total protein from each sample, as determined by the BCA protein assay (Pierce Biotechnology, Inc.), were mixed with 2×loading buffer, and boiled at 100°C for 5 min. The samples were then separated on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel at 100 to 150 V and transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA) for 1.5 hr, using a semi-dry transfer system (Bio-Rad). The membranes were blocked with 5% nonfat dry milk in 1×PBST buffer (0.1% Tween 20 in PBS) for 1 hr at room temperature and incubated with primary antibodies in 5% nonfat dry milk overnight at 4°C. After hybridization with the primary antibodies, the membranes were washed 3 times with 1×PBST, followed by incubation with anti-rabbit-IgG or anti-goat-IgG with horseradish peroxidase (Santa Cruz Biotechnology Inc.) for 1 hr at room termperature, and then washed with 1×PBST 3 times. Final detection was performed with Western blotting chemiluminescence luminol reagent (Santa Cruz Biotechnology Inc.). The image data shown are representative of 3 independent experiments with similar results. All data were quantified by Quantity One 4.5.0 software (Bio-Rad Laboratories, Inc.) and described as fold induction of the target protein expression ratio per actin against control.

Statistical analysis The data were expressed as mean± standard deviations (SD). The values were compared to the control using analysis of variance (ANOVA) followed by unpaired Student's *t*-tests. A p values <0.05 were considered significant.

Results and Discussion

Composition of glucans in HEW The composition of α- and β-D-glucan in the HEW was analyzed prior to performing the biological assays (Table 1). The mushroom polysaccharides have been recognized as the primary components containing antitumor and immuno-stimulating properties. These polysaccharides mainly belong to the β-D-glucan group (3). *H. erinaceus* is known to possess at least 3 fold higher amount of β-D-glucan than other well-known medicinal mushrooms such as *Agaricus* (2). In this study, the content of β-D-glucan (2.97%) was much higher than that of α-D-glucan (0.09%) in the total glucans (3.06%). Contents of total glucans were known about 23.3 g in 100 mg of *H. erinaceus* mushroom (fruit body). Although we could not compare the yield of glucans in mycelia extracts with other researches directly because of the lack of information, we could deduce that the yields of total glucans and β-D-glucan were significantly high in this study following the comparison with the amount of crude polysaccharide might contain glucans in *Agaricus blazei* indirectly. The content of crude polysaccharide was 3.03% in dried mycelia of *A. blazei* murrill (33).

Cytotoxicity on HepG2 cells One of the common markers of antitumor activity is the trigger of cancer cell death. Many chemopreventive agents induce cancer cell death by cell cycle arrest and apoptosis induction (34). The effect of HEW on HepG2 cell viability was determined by a colorimetric MTS assay after 24 hr treatments. The data were expressed as percent cell viability compared to that of the 0% treatment control (Fig. 1). The HEW treatments at concentrations below 3% did not affect cell viability, while the 4 and 5% treatments prominently decreased cell viability up to 34% at the 5% concentration. The IC_{50} of HEW was calculated as 4.47%, which was slightly higher than that of a crude polysaccharide from *A. blazei* Murill (35). Since the treatment doses of HEW up to at 3% did not affect the cell viability, we used concentrations below 3% for further biological assays.

ARE-reporter gene activity To evaluate the effects of HEW on ARE-mediated gene expression, ARE-luciferase activity was determined in a HepG2-C8 cell line that was stably transfected with the pARE-luciferase reporter gene. The ARE-reporter gene assay can be a useful tool to examine unknown chemopreventive agent candidates, due to the critical region of ARE sequence in phase 2 and antioxidant enzymes for cellular defense mechanisms (10). The ARE-luciferase activity of the treated groups was expressed as a fold-induction over the 0% treatment control. As shown in Fig. 2, HEW highly induced ARE activity in a concentration-dependent manner, up to about 20 fold at 3% which was much higher induction than that of the positive control SFN, a known strong ARE inducer

Table 1. The composition of glucans in HEW

Component	Content (%)
Total glucan	3.06±0.05
α-D-Glucan	0.09±0.01
β-D-Glucan	2.97±0.04

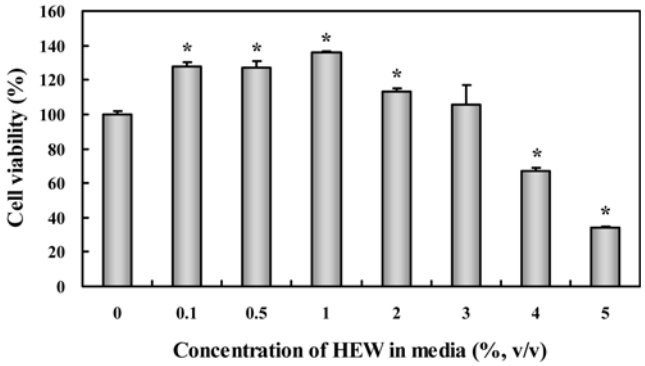

Fig. 1. Effects of HEW on cell viability of HepG2 cells. Values are mean±SD; n=3, *p<0.05 vs. 0% control.

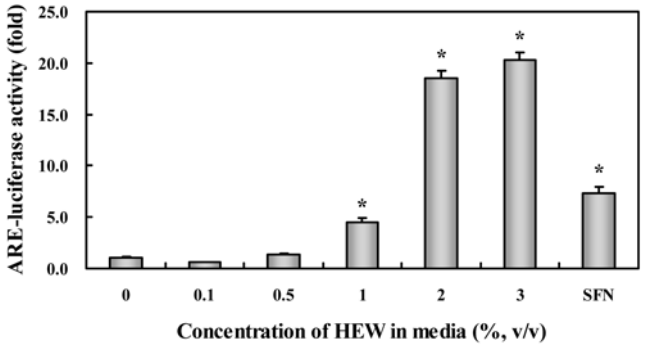

Fig. 2. ARE-luciferase activity by HEW in HepG2-ARE-C8 cells. Values are mean±SD; n=3, *p<0.05 vs. 0% control.

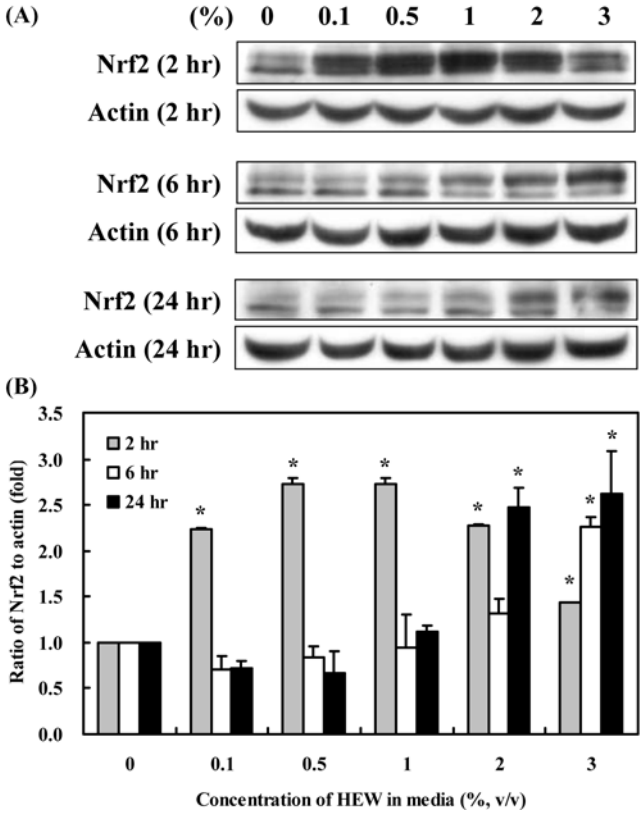

Fig. 3. Nrf2 protein expression by HEW in HepG2 cells. The image data shown are representative of 3 independent experiments with similar results (A) and described as fold-induction in (B). (n=3, *p<0.05 vs. 0% control)

(31). Through this result we could predict that HEW might trigger phase 2 enzyme expression via ARE induction on the promoter sequence by Nrf2 regulation.

Induction of Nrf2 and phase 2 enzyme expression To confirm the mechanism of ARE induction, we investigated the regulatory role of HEW on Nrf2 expression. Nrf2 is a transcription factor that is known to bind to the ARE sequence and thereby transactivates the cellular defense genes containing the ARE. After 2 hr of HEW treatment, Nrf2 protein expression was induced in HepG2 cells, independent of the HEW concentration (Fig. 3). After 6 and 24 hr of treatment, Nrf2 was highly induced in a concentration dependent manner, implying sustainable inductions of Nrf2 expression at higher concentrations of HEW. These results helped support the ARE-luciferase activity data shown in Fig. 2. Furthermore, protein expression of the antioxidant enzyme, HO-1, was also highly induced after 24 hr of treatment in a concentration dependent manner, and up to 6.7 fold at 3% (Fig. 4). This suggests that HEW induced Nrf2 expression to stimulate ARE on the promoter sequence of HO-1. Nrf2 shows an early response to stimulate and trigger HO-1 induction sequentially. HO-1 is an important antioxidant enzyme that plays a pivotal role in cellular adaptation and protection in response to various stimuli. Furthermore, HO-1 induction has been associated with the prevention of various diseases, including acute inflammation and carcinogenesis (36,37).

Inhibition of COX-2 expression Recently, many researches have focused on the cross-talk between inflammation and cancer (18). In this study, the modulation of COX-2 protein expression by HEW was investigated. After 2 or 24 hr of treatment, COX-2 expression was suppressed effectively by HEW (Fig. 5). COX-2 is a key mediator linking inflammation and cancer. It is an inducible enzyme related to inflammation and overexpressed in various types of cancer. It is involved in cellular proliferation, anti-apoptotic activity, angiogenesis, and metastasis (38). Therefore, the inhibition of COX-2 could be a meaningful approach for cancer prevention and treatment. In the present study, HEW suppressed COX-2 protein expression effectively, suggesting that HEW possessed chemopreventive effects via COX-2 inhibition. Furthermore, according to previous reports, the modulation of COX-2 expression can be controlled by the Nrf2 signaling pathway (14). In the future, we intend to study more precisely whether HEW-induced COX-2 suppression might be controlled by the Nrf2 signaling pathway.

Inhibition of NO synthesis Several studies have suggested that elevated NO production and oxidative stress in activated macrophages is closely related to inflammation (39). It has been documented that the addition of LPS to cells significantly elevates the generation of nitrite, the oxidative product of NO, compared to LPS-untreated negative controls (28,40,41). In this study, HEW treatment notably suppressed NO production in a concentration-

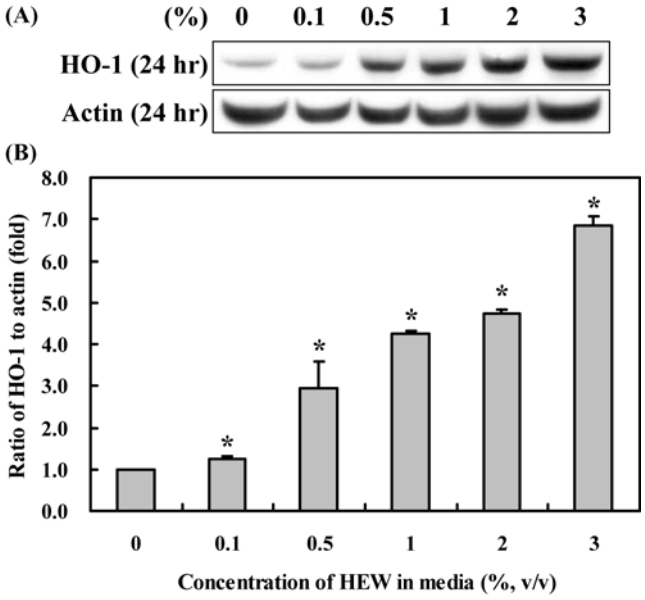

Fig. 4. HO-1 protein expression by HEW in HepG2 cells. The image data shown are representative of 3 independent experiments with similar results (A) and described as fold-induction in (B). (n=3, *p<0.05 vs. 0% control)

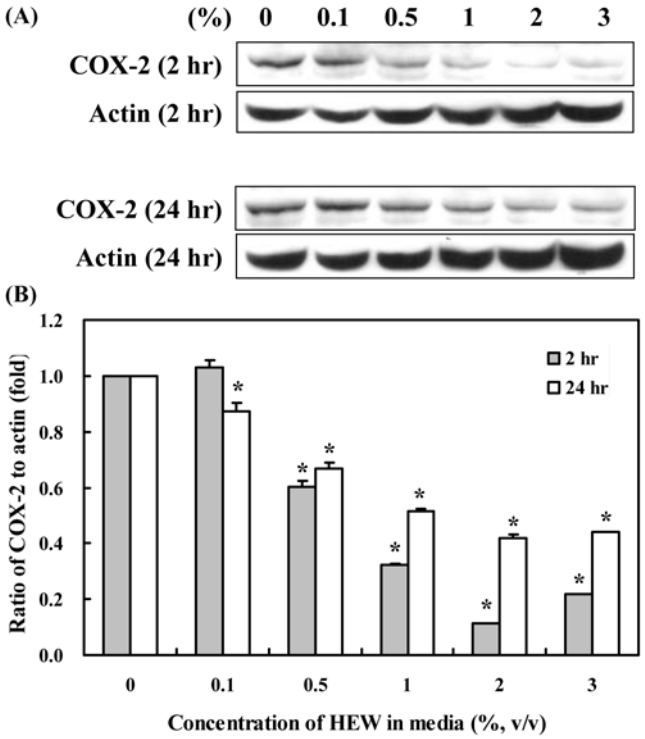

Fig. 5. COX-2 protein expression by HEW in HepG2 cells. The image data shown are representative of 3 independent experiments with similar results (A) and described as fold-induction in (B). (n=3, *p<0.05 vs. 0% control)

dependent manner at concentrations greater than 1% (Fig. 6). LPS (1 μg/mL) significantly increased the production of NO in RAW 264.7 cells from the basal level of 1.3±0.5 to 52.5±0.5 μM over 20 hr of incubation, and HEW (1-3%) remarkably inhibited this NO overproduction in a

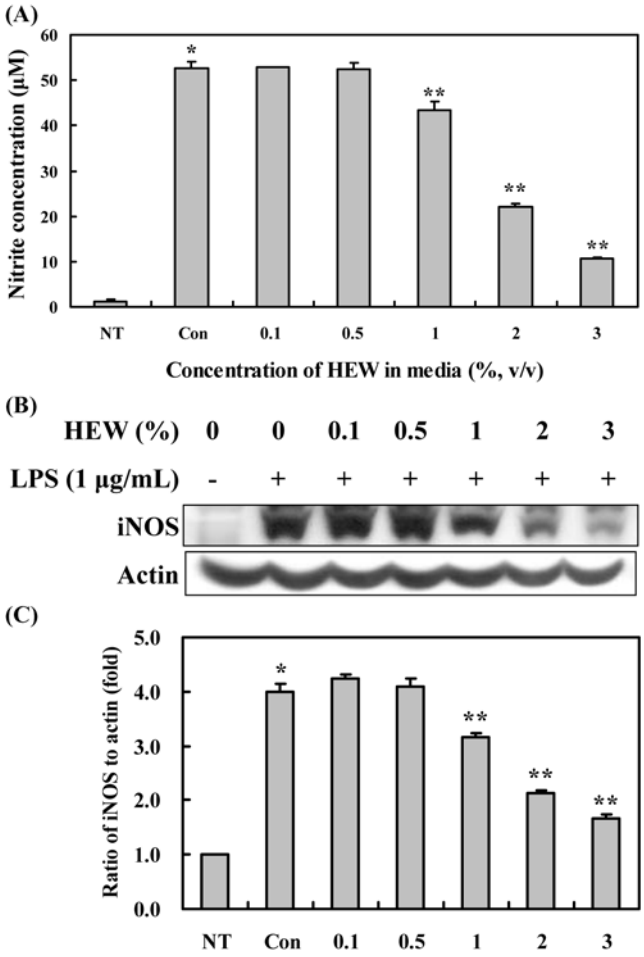

Fig. 6. Effects of HEW on NO production (A) and iNOS protein expression (B, C) in LPS-stimulated RAW 264.7 macrophages. Data represent the mean±SD of triplicate experiments. *, **Significantly different from the no treatment control (NT) and LPS treated control (Con), respectively (p<0.05).

concentration-dependent manner (Fig. 6A). NO is an important inflammatory mediator and produced endogenously during arginine metabolism by NOS. Among the 3 kinds of NOS, iNOS in macrophage cells leads to NO production, and an overproduction of NO occurs during inflammation (18). iNOS protein expression was investigated in order to elucidate the mechanism of the suppressive effect of HEW on NO production. HEW treatment remarkably decreased LPS-induced iNOS protein expression (3.9 fold) in a concentration-dependent manner at concentrations greater than 1%, and up to 1.6 fold at 3% (Fig. 6B). These results correspond with the results for NO suppression by HEW.

Inhibition of inflammatory mediators Pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, play key roles in inflammatory processes. In response to LPS, gene expression and pro-inflammatory cytokine production increases and their overproduction induces other inflammatory mediators such as iNOS and COX-2. Thus, controlling pro-inflammatory cytokines might be a tool to prevent various inflammatory diseases (42,43). In this study, LPS-induced NF-κB p65 expression was suppressed by HEW

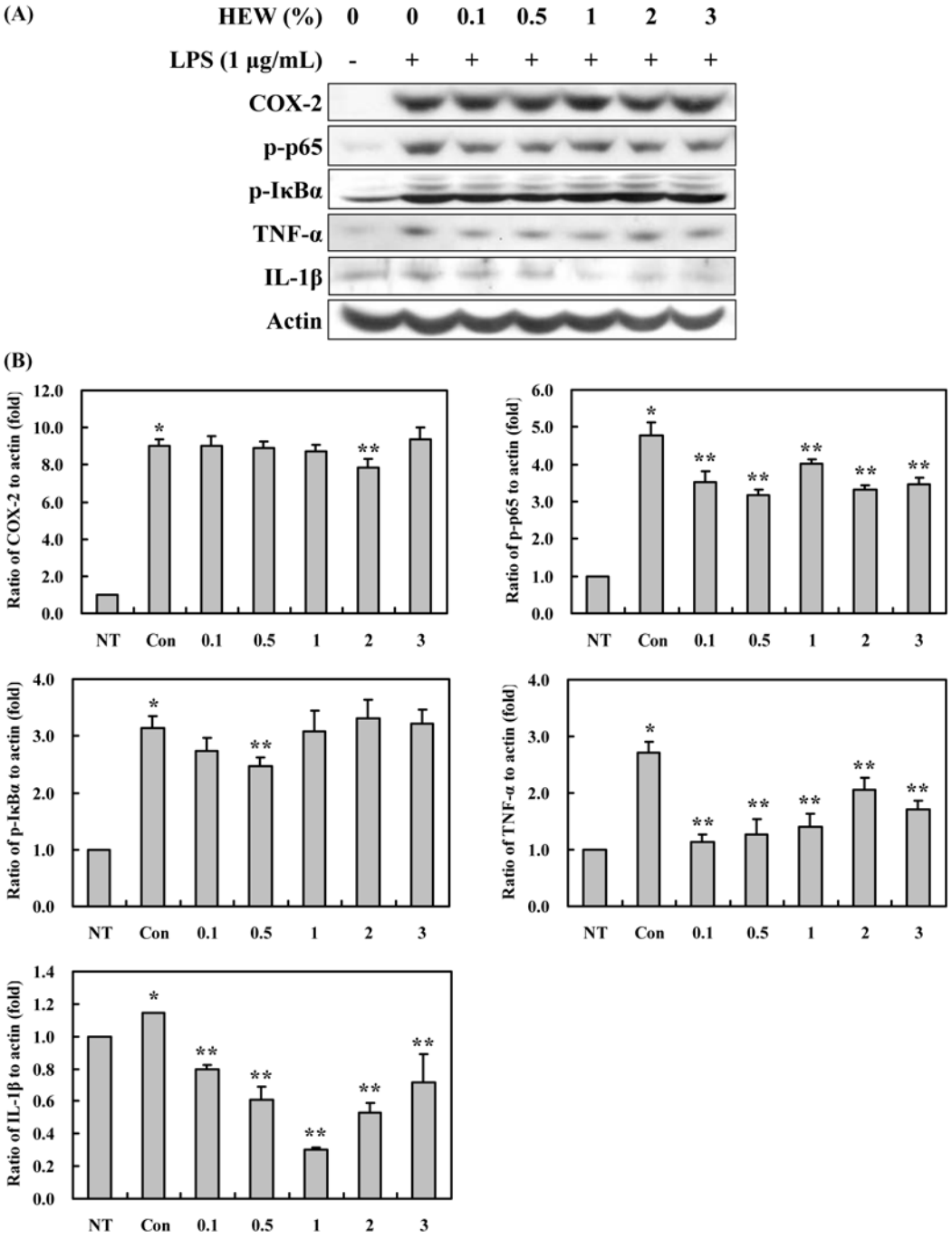

Fig. 7. Effects of HEW on pro-inflammatory transcription factor and cytokines in LPS-stimulated RAW 264.7 macrophages. The image data shown are representative of 3 independent experiments with similar results (A) and described as fold-induction in (B). *, **Significantly different from the no treatment control (NT) and LPS treated control (Con), respectively ($p<0.05$).

(Fig. 7). NF-κB is a transcription factor that increases the expression of pro-inflammatory mediators and amplifies inflammatory signal transduction (18). The suppression of pro-inflammatory pathways can be mediated by NF-κB signaling (44). HEW inhibited the protein expression of pro-inflammatory cytokines such as TNF-α and IL-1β in LPS-stimulated murine macrophages (Fig. 7). Therefore, these findings indicate that HEW might have anti-inflammatory activity by controlling the production of pro-inflammatory cytokines.

In the present study, the chemopreventive and anti-inflammatory effects of HEW on the Nrf2/ARE pathway and NO/iNOS synthesis were investigated. HEW showed cytotoxic effects in the human hepatocarcinoma cell line, HepG2, and prominently activated the Nrf2/ARE pathway. The activation of Nrf2 also stimulated HO-1 induction. In murine macrophage RAW 264.7 cells, HEW effectively inhibited NO synthesis and iNOS protein expression induced by LPS. Furthermore, HEW inhibited the expression of pro-inflammatory transcription factor NF-κB and cytokines

such as TNF-α and IL-1β. At this moment, it is not clear which components are responsible for the cellular defensive and anti-inflammatory activities of HEW. Through these results, the potential of HEW as an effective chemopreventive and anti-inflammatory food source was confirmed. Given the high content of β-D-glucan in HEW (Table 1), the beneficial properties of HEW might be driven from the high content of β-D-glucan in HEW. Yang *et al.* (45) showed the anti-inflammatory effect of pure β-D-glucan from barley at concentrations of 50 to 200 μg/mL. The contents of β-D-glucan in HEW were calculated between 92 and 276 μg/mL (92, 184, and 276 μg/mL of β-D-glucan in 1, 2, and 3% HEW, respectively). β-D-Glucans from different sources have been shown various biological activities such as lipid lowering effect, blood sugar reduction, weight reduction, immune modulator, and anticarcinogenic effects (46). Since the biological activities of β-D-glucan might vary between the sources of β-D-glucan (46), however, it would be important to analyze the size and the structure of β-D-glucan in HEW. More detailed analyses on the chemical structure of HEW along with the analyses on other bioactive ingredients are under investigation and to be reported separately.

Acknowledgments

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References

1. Yang BK, Park JB, Song CH. Hypolipidemic effect of an exo-biopolymer produced from a submerged mycelial culture of *Hericium erinaceus*. Biosci. Biotech. Bioch. 67: 1292-1298 (2003)
2. Mizuno T, Wasa T, Ito H, Suzuki C, Ukai N. Antitumor-active polysaccharides isolated from the fruiting body of *Hericium erinaceum*, an edible and medicinal mushroom called *yamabushitake* or *houtou*. Biosci. Biotech. Bioch. 56: 347-348 (1992)
3. Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biot. 60: 258-274 (2002)
4. Borchers AT, Keen CL, Gershwin ME. Mushrooms, tumors, and immunity: An update. Exp. Biol. Med. 229: 393-406 (2004)
5. Xu CP, Liu WW, Liu FX, Chen SS, Liao FQ, Xu Z, Jiang LG, Wang CA, Lu XH. A double-blind study of effectiveness of *Hericium erinaceus* pers therapy on chronic atrophic gastritis. A preliminary report. Chinese Med. J. Peking 98: 455-456 (1985)
6. Mori K, Inatomi S, Ouchi K, Azumi Y, Tuchida T. Improving effects of the mushroom *yamabushitake* (*Hericium erinaceus*) on mild cognitive impairment: A double-blind placebo-controlled clinical trial. Phytother. Res. 23: 367-372 (2008)
7. Park YS, Lee HS, Won MH, Lee JH, Lee SY, Lee HY. Effect of an exo-polysaccharide from the culture broth of *Hericium erinaceus* on enhancement of growth and differentiation of rat adrenal nerve cells. Cytotechnology 39: 155-162 (2002)
8. Mori K, Obara Y, Hirota M, Azumi Y, Kinugasa S, Inatomi S, Nakahata N. Nerve growth factor-inducing activity of *Hericium erinaceus* in 1321N1 human astrocytoma cells. Biol. Pharm. Bull. 31: 1727-1732 (2008)
9. Wang JC, Hu SH, Wang JT, Chen KS, Chia YC. Hypoglycemic effect of extract of *Hericium erinaceus*. J. Sci. Food Agr. 85: 641-646 (2004)
10. Jeong WS, Jun M, Kong AN. Nrf2: A potential molecular target for cancer chemoprevention by natural compounds. Antioxid. Redox. Sign. 8: 99-106 (2006)
11. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem. Bioph. Res. Co. 236: 313-322 (1997)
12. Kong AN, Yu R, Hebbar V, Chen C, Owuor E, Hu R, Ee R, Mandlekar S. Signal transduction events elicited by cancer prevention compounds. Mutat. Res. 480-481: 231-241 (2001)
13. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat. Rev. Cancer 3: 768-780 (2003)
14. Lin W, Wu RT, Wu T, Khor TO, Wang H, Kong AN. Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. Biochem. Pharmacol. 76: 967-973 (2008)
15. Khor TO, Yu S, Kong AN. Dietary cancer chemopreventive agents - targeting inflammation and Nrf2 signaling pathway. Planta Med. 74: 1540-1547 (2008)
16. Gonzalez-Gallego J, Sanchez-Campos S, Tunon MJ. Anti-inflammatory properties of dietary flavonoids. Nutr. Hosp. 22: 287-293 (2007)
17. Osburn WO, Kensler TW. Nrf2 signaling: An adaptive response pathway for protection against environmental toxic insults. Mutat. Res. 659: 31-39 (2008)
18. Kundu JK, Surh YJ. Inflammation: Gearing the journey to cancer. Mutat. Res. 659: 15-30 (2008)
19. Soh JW, Weinstein IB. Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. Prog. Exp. Tumor Res. 37: 261-285 (2003)
20. Oshima M, Taketo MM. COX selectivity and animal models for colon cancer. Curr. Pharm. Design 8: 1021-1034 (2002)
21. Park SE, Lee SW, Hossain MA, Kim MY, Kim MN, Ahn EY, Park YC, Suh H, Kim GY, Choi YH, Kim ND. A chenodeoxycholic derivative, hs-1200, induces apoptosis, and cell cycle modulation via egr-1 gene expression control on human hepatoma cells. Cancer Lett. 270: 77-86 (2008)
22. Bae SJ, Choi YH. Methanol extract of the seaweed *Gloiopeltis furcata* induces G2/M arrest and inhibits cyclooxygenase-2 activity in human hepatocarcinoma HepG2 cells. Phytother. Res. 21: 52-57 (2007)
23. Prabhu KS, Zamamiri-Davis F, Stewart JB, Thompson JT, Sordillo LM, Reddy CC. Selenium deficiency increases the expression of inducible nitric oxide synthase in RAW 264.7 macrophages: Role of nuclear factor-kappaB in up-regulation. Biochem. J. 366: 203-209 (2002)
24. Luoma JS, Yla-Herttuala S. Expression of inducible nitric oxide synthase in macrophages and smooth muscle cells in various types of human atherosclerotic lesions. Virchows Arch. 434: 561-568 (1999)
25. Leiro JM, Alvarez E, Arranz JA, Cano E, Orallo F. Antioxidant activity and inhibitory effects of hydralazine on inducible NOS/COX-2 gene and protein expression in rat peritoneal macrophages. Int. Immunopharmacol. 4: 163-177 (2004)
26. Lo AH, Liang YC, Lin-Shiau SY, Ho CT, Lin JK. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-kappaB in mouse macrophages. Carcinogenesis 23: 983-991 (2002)
27. Lee AK, Sung SH, Kim YC, Kim SG. Inhibition of lipopolysaccharide-inducible nitric oxide synthase, TNF-α, and COX-2 expression by sauchinone effects on I-κBα phosphorylation, C/EBP, and AP-1 activation. Brit. J. Pharmacol. 139: 11-20 (2003)
28. Hwang YH, Kim MS, Song IB, Lim JH, Park BK, Yun HI. Anti-inflammatory effects of talosin A via inhibition of NF-kappaB activation in lipopolysaccharide-stimulated RAW264.7 cells. Biotechnol. Lett. 31: 789-795 (2009)
29. Park HG, Shim YY, Choi SO, Park WM. New method development for nanoparticle extraction of water-soluble beta-(1,3)-D-glucan from edible mushrooms, *Sparassis crispa* and *Phellinus linteus*. J. Agr. Food Chem. 57: 2147-2154 (2009)
30. Yu R, Lei W, Mandlekar S, Weber MJ, Der CJ, Wu J, Kong AN. Role of a mitogen-activated protein kinase pathway in the induction

of phase II detoxifying enzymes by chemicals. J. Biol. Chem. 274: 27545-27552 (1999)
31. Jeong WS, Keum YS, Chen C, Jain MR, Shen G, Kim JH, Li W, Kong AN. Differential expression and stability of endogenous nuclear factor E2-related factor 2 (Nrf2) by natural chemopreventive compounds in HepG2 human hepatoma cells. J. Biochem. Mol. Biol. 38: 167-176 (2005)
32. D'Agostino P, Ferlazzo V, Milano S, La Rosa M, Di Bella G, Caruso R, Barbera C, Grimaudo S, Tolomeo M, Feo S, Cillari E. Anti-inflammatory effects of chemically modified tetracyclines by the inhibition of nitric oxide and interleukin-12 synthesis in J774 cell line. Int. Immunopharmacol. 1: 1765-1776 (2001)
33. Lin JH, Yang SS. Mycelium and polysaccharide production of *Agaricus blazei* murrill by submerged fermentation. J. Microbiol. Immunol. 39: 98-108 (2006)
34. Pan MH, Ho CT. Chemopreventive effects of natural dietary compounds on cancer development. Chem. Soc. Rev. 37: 2558-2574 (2008)
35. Hong JH, Youn KS, Choi YH. Characteristics of crude protein-bound polysaccharide from *Agaricus blasei* murill by extraction and precipitation conditions and its antitumor effect. Korean J. Food Sci. Technol. 36: 586-593 (2004)
36. Prawan A, Kundu JK, Surh YJ. Molecular basis of heme oxygenase-1 induction: Implications for chemoprevention and chemoprotection. Antioxid. Redox Sign. 7: 1688-1703 (2005)
37. Farombi EO, Surh YJ. Heme oxygenase-1 as a potential therapeutic target for hepatoprotection. J. Biochem. Mol. Biol. 39: 479-491 (2006)
38. Prescott SM, Fitzpatrick FA. Cyclooxygenase-2 and carcinogenesis. Biochim. Biophys. Acta 1470: M69-M78 (2000)
39. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol. Cancer Res. 4: 221-233 (2006)
40. Lim KM, Lee JY, Lee SM, Bae ON, Noh JY, Kim EJ, Chung SM, Chung JH. Potent anti-inflammatory effects of two quinolinedione compounds, OQ1 and OQ21, mediated by dual inhibition of inducible NO synthase and cyclooxygenase-2. Brit. J. Pharmacol. 156: 328-337 (2009)
41. Jin JQ, Li CQ, He LC. Down-regulatory effect of usnic acid on nuclear factor-kappaB-dependent tumor necrosis factor-alpha and inducible nitric oxide synthase expression in lipopolysaccharide-stimulated macrophages RAW 264.7. Phytother. Res. 22: 1605-1609 (2008)
42. Fujiwara N, Kobayashi K. Macrophages in inflammation. Curr. Drug Targets Inflamm. Allergy 4: 281-286 (2005)
43. Ghezzi P, Cerami A. Tumor necrosis factor as a pharmacological target. Mol. Biotechnol. 31: 239-244 (2005)
44. Li W, Khor TO, Xu C, Shen G, Jeong WS, Yu S, Kong AN. Activation of Nrf2-antioxidant signaling attenuates NFκB-inflammatory response and elicits apoptosis. Biochem. Pharmacol. 76: 1485-1489 (2008)
45. Yang JL, Jang JH, Radhakrishnan V, Kim YH, Song YS. β-Glucan suppresses LPS-stimulaged NO production through the down-regulation of iNOS expression and NF-βB transactivation in RAW 264.7 macrophages. Food Sci. Biotechnol. 17: 106-113 (2008)
46. Kim SY, Song HJ, Lee YY, Cho K-H, Roh YK. Biomedical issues of dietary fiber β-glucan. J. Korean Med. Sci. 21: 781-789 (2006)