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Anti-inflammatory Effect of the Hot Water Extract from *Sasa quelpaertensis* Leaves

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Abstract Bamboo grass, *Sasa quelpaertensis*, is a native plant to Jeju Island, Korea. The leaves of *Sasa* plants are widely used in traditional Korean medicine to treat inflammation-related diseases. We investigated the effect of hot water extract from *Sasa quelpaertensis* leaves (HWE-SQ) on nitric oxide (NO) production and nuclear factor-κB (NF-κB) activation in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. HWE-SQ inhibited LPS-induced NO production and inducible NO synthase (iNOS) protein expression in a dose-dependent manner. Reporter gene assays indicated that HWE-SQ decreases LPS-induced NF-κB transcriptional activation. However, HWE-SQ did not affect the phosphorylation and degradation of inhibitory κBα (IκBα). HWE-SQ also directly inhibited iNOS enzyme activity in a dose-dependent manner. These results suggest that HWE-SQ suppresses NO synthesis in macrophages by attenuating NF-κB-mediated iNOS protein expression and inhibiting iNOS enzymatic activity, thereby implicating a mechanism by which HWE-SQ is able to ameliorate inflammation-related diseases by limiting excessive or prolonged NO production in pathological events.

Keywords: *Sasa quelpaertensis*, hot water extract, anti-inflammatory effect, inducible nitric oxide synthase, nuclear factor-κB

Introduction

Nitric oxide (NO) is known to participate in the physiological and pathological function of many organs and is synthesized by the NO synthase (NOS) family of enzymes (1). Three types of NOS have been identified: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (2). nNOS and eNOS are constitutively expressed in neuronal and endothelial cells, respectively. iNOS is not constitutively present and can be rapidly induced by inflammatory stimuli, including toxins such as lipopolysaccharide (LPS) and cytokines (3). Although NO plays an important physiological role as a defense molecule in the immune system (1), the excessive production of NO by iNOS in macrophages has been implicated in numerous pathological processes, including inflammation (4), and atherosclerosis (5, 6). Thus, the suppression of NO production by down-regulating iNOS expression and/or activity is an attractive therapeutic target.

Nuclear factor-κB (NF-κB) plays a critical role in the transcriptional regulation of the iNOS gene induced by LPS and cytokines (7). NF-κB is sequestered in the cytoplasm of most resting cells by inhibitory κB (IκB) proteins. In response to various stimuli, IκB is phosphorylated by the IκB kinase (IKK) complex and then rapidly degraded by the proteasome, allowing NF-κB nuclear translocation and gene activation (8, 9).

Recently there has been increased interest and efforts in the search for new functional food resources from herbal medicine through bioassay-guided evaluation (10-12). The bamboo grasses, including the genus *Sasa*, *Phyllostachys*, and *Bambusa*, are widely used to treat and prevent many diseases, including inflammation, cardiovascular disease, diabetes, and hypertension in traditional Korean, Chinese, and Japanese medicine. The leaves of *Sasa* bamboo grass were also reported to be used against cancer (13). Modern scientific studies have shown that bamboo leaves have anti-oxidant (14-17) and anti-tumor (18, 19) activities, supporting their usage in folk medicine. It has been reported that bamboo leaves contain a wide range of potent anti-inflammatory and anti-oxidant agents such as orientin, homoorientin, vitexin, isovitexin, quercetin, luteoline, rutin, tricin, caffeic acid, (-)-syringaresinol, chlorogenic acid, and *p*-coumaric acid (19-22).

Sasa quelpaertensis Nakai is a bamboo grass grown only on Jeju Island, Korea. These leaves have been used to make a leaf tea for the treatment of inflammatory diseases including diabetes, hypertension, gastritis, and cancer. In order to uncover the molecular basis of the anti-inflammatory effect of *S. quelpaertensis* leaves, we examined the inhibitory effect of hot water extract from *S. quelpaertensis* leaves (HWE-SQ) on NO production, iNOS protein expression, and NF-κB activation using an *in vitro* model system of RAW 264.7 cells activated with LPS. A macrophage RAW 264.7 cell produces proinflammatory mediators, including NO, upon stimulation with LPS. For this reason, the decreased production of proinflammatory mediators from these cells may serve as a direct indicator of the anti-inflammatory properties of nutraceuticals. We show that HWE-SQ suppresses NO synthesis in macrophages by inhibiting iNOS enzymatic activity and attenuating NF-κB-mediated iNOS protein expression.

Materials and Methods

Chemicals Dulbecco's modified Eagle's medium (DMEM),

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fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). Antibody to inducible NOS (iNOS) was purchased from Calbiochem (San Diego, CA, USA), antibodies to p38, phospho-p38, JNK1/2, phospho-JNK1/2, ERK1/2, and phosphor-ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA, USA), and antibodies to IκBα, and phospho-IκBα were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The lactate dehydrogenase (LDH) cytotoxicity detection kit was obtained from Takara Shuzo Co. (Otsu, Shiga, Japan). LPS (*Escherichia coli* 026:B6), sodium nitroprusside (SNP), and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO, USA). All other reagents were purchased from Sigma unless otherwise noted.

Preparation of hot water extract of *S. quelpaertensis* leaves Fresh leaves of *S. quelpaertensis* were collected from the region of Hallasan(Mt.) in Jeju Island, Korea. One hundred g of the dried crushed leaves was boiled with 1 L of distilled water at 120°C for 30 min in an autoclave. After filtration, the residue was freeze-dried into powder and dissolved in phosphate-buffered saline to a final concentration of 100 mg/mL. The hot water extract from *S. quelpaertensis* leaves is referred to as HWE-SQ.

Cell culture The RAW 264.7 murine macrophage cell line was obtained from the Korea Cell Line Bank (Seoul, Korea). Cells were grown in DMEM medium with 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and supplemented with 10% FBS in a humidified atmosphere with 5% CO_2 at 37°C.

Measurement of cytotoxicity Cell viability was determined by the MTT cell viability assay (23). Cells were seeded at a density of 5×10^4 cells/well in 96-well, flat-bottom culture plates in the presence or absence of HWE-SQ. Mitochondrial enzyme activity, an indirect measure of the number of viable respiring cells, was determined using the MTT reagent after 40 hr of HWE-SQ treatment. Absorbance was read using a μQuant microplate reader (Bio-Tek Instrument, Winooski, VT, USA). The effect of HWE-SQ on cell viability was evaluated as the relative absorbance compared with that of control cultures. The cytotoxic effect of LPS or LPS plus HWE-SQ in RAW 264.7 cells was also estimated by the measurement of LDH in culture supernatants (24). Leakage of LDH is a well-known marker of damage to the cellular membrane (25). Cytotoxicity was expressed as the percentage of LDH released (LDH released in medium/maximal LDH release × 100). Maximal LDH release was measured after lysis of cells with 0.5% Triton-X100.

Nitrite assay The amount of nitrite, the end product of NO generation by activated macrophages, was determined by a colorimetric assay (26). Briefly, 100 μL of cell culture medium was mixed with an equal volume of Griess reagent [1% sulfanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid] and incubated at room temperature for 10 min. The absorbance at 540 nm was read in a microplate reader. The nitrite concentration was determined by extrapolation from a sodium nitrite standard curve.

iNOS enzyme activity The iNOS activity in cell lysates was measured as the L-arginine- and NADPH-dependent generation of nitrite as described previously (27). The assay was performed by incubating 200 μg of cellular extract from LPS-stimulated cells in the absence or presence of HWE-SQ for 180 min at room temperature in 100 μL of reaction buffer containing 20 mM sodium phosphate buffer, 2 mM NADPH, 2 mM L-arginine, and 10 μm FAD at pH 6.7. The reaction was stopped by the addition of 10 U/mL LDH and 10 mM pyruvate. The reaction mixture was incubated with an equal volume of Griess reagent. The absorbance at 540 nm was read in a microplate reader. The nitrite concentration was determined by extrapolation from a sodium nitrite standard curve.

Western blot analysis Cells were incubated with LPS (100 ng/mL) in the absence or presence of HWE-SQ for various times. Cells were then washed with ice-cold phosphate buffered saline, collected, and centrifuged. The cell pellets were resuspended in lysis buffer [50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1 mM EGTA, 1.0 mM EDTA, 0.1% SDS in the presence of proteinase inhibitors] and incubated at 4°C for 20 min. Cell debris was removed by microcentrifugation and the protein concentration was determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA). Cell lysates were subjected to 7.5 or 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were blocked in a solution of 0.1% Tween 20/Tris buffered saline containing 5% nonfat dry milk for 1 hr at room temperature. After incubation overnight at 4°C with the primary antibody, membranes were incubated with the horseradish peroxidase-conjugated secondary antibody for 1 hr at room temperature. Immunodetection was done using enhanced chemiluminescence (Pierce, Rockford, IL, USA). The relative band density was determined by densitometry using image acquisition and analysis software (LabWorks, UVP, UK).

Transient transfection and luciferase assay Cells were cotransfected with or without 10 ng of NF-κB driven luciferase reporter gene plasmid (pNF-κB-Luc; Promega, Madison, WI, USA) and 4 ng of *Renilla* luciferase reporter plasmid (pRL-TK; Stratagene, La Jolla, CA, USA) which served as the internal standard, using the FuGENE 6 transfection reagent (Roche, Indianapolis, IN, USA). After 24 hr, cells were incubated with LPS (100 ng/mL) in the absence or presence of HWE-SQ. After 24 hr of incubation, luciferase activity in the cell lysate was determined using the Dual-Luciferase Reporter Assay kit (Promega). Luciferase activity was normalized to the transfection efficiency based on *Renilla* luciferase expression. The level of luciferase activity was determined relative to unstimulated cells.

Statistical analysis Results were expressed as means±

SD. Statistical analysis was performed by one-way analysis of variance (ANOVA). Probability ($p<0.05$) were considered significant.

Results and Discussion

HWE-SQ inhibits NO production in LPS-activated RAW 264.7 cells It is well known that iNOS from macro-phages is predominantly responsible for the overproduc-tion of NO in injured tissues and inflammatory processes (28). Thus, we chose to utilize the well-characterized murine macrophage cell line, RAW 264.7, to perform our studies. Treatment of cells with LPS (100 ng/mL) induced nitrite accumulation in the culture medium during the 24 hr observation period, indicative of NO production (Fig. 1A). Simultaneous treatment with HWE-SQ and LPS significantly and dose-dependently decreased the produc-tion of NO (Fig. 1A). Post-treatment more closely resembles an actual clinical treatment regimen than pretreatment or cotreatment. Thus, we examined whether HWE-SQ was able to inhibit NO production when added after 6 hr of stimulation with LPS. As shown in Fig. 1A, treatment with HWE-SQ after 6 hr of LPS treatment significantly decreased LPS-induced NO production. HWE-SQ alone did not cause NO production (Fig. 1A). The inhibitory effect of HWE-SQ on LPS-induced NO production was not attributable to its cytotoxicity because HWE-SQ at <2,000 μg/mL did not show significant cytotoxic effects toward RAW 264.7 cells (Fig. 1B).

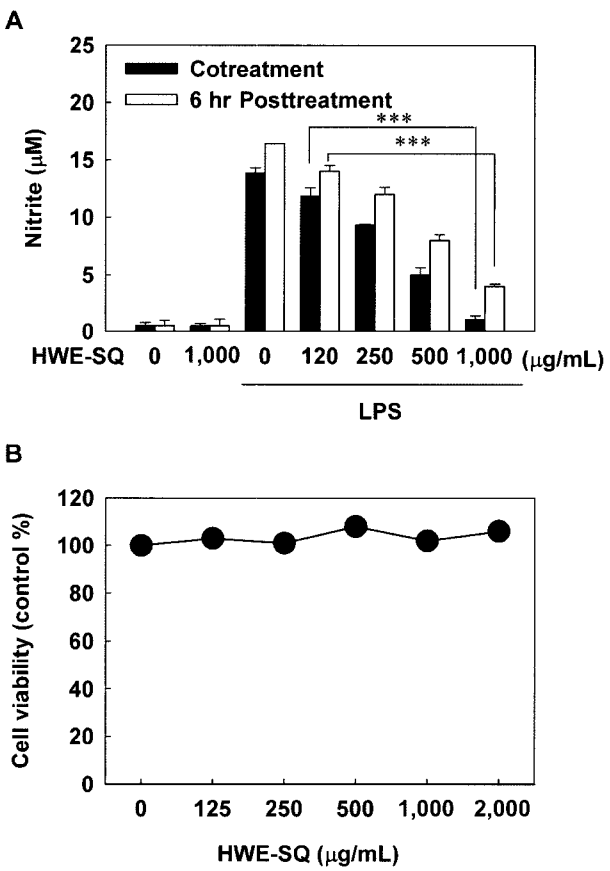

Fig. 1. Effect of HWE-SQ on LPS-induced NO production and cell viability in RAW 264.7 cells. (A) The levels of nitrite released in the culture medium were assayed using the Griess reagent. The data represent the mean±SD. *$p<0.01$ was considered significantly different from LPS alone-treated cells. (B) Cell viability was determined by the MTT cell viability assay. Results from a representative experiment are expressed as mean±SD (n = 4 in a single experiment).

HWE-SQ has a direct inhibitory effect on iNOS enzyme activity To investigate whether HWE-SQ directly inhibits iNOS enzyme activity, RAW 264.7 cells were stimulated with 100 ng/mL LPS for 20 hr and cell homogenates were prepared as a source of iNOS enzyme. iNOS enzyme activity in the presence or absence of HWE-SQ (0, 125-1,000 μg/mL) was measured. HWE-SQ inhibited the enzymatic activity of iNOS in a dose-dependent manner (Fig. 2). These results suggest that the direct inhibition of iNOS enzyme activity by HWE-SQ may be responsible, in part, for the suppression of NO production in murine macrophages. Although the mechanism of inhibition remains to be determined, other natural products and herbal medicines, such as *Ginkgo biloba* (29) and soy isoflavones (30), have previously been shown to selectively regulate the activity of the iNOS isoform.

HWE-SQ inhibits iNOS protein expression in LPS-activated RAW 264.7 cells To determine whether the altered synthesis of iNOS was responsible for the observed effect on NO production, we analyzed the expression of iNOS protein by Western blot analysis. LPS increased the levels of cellular iNOS protein after 24 hr of treatment (Fig. 3A). Simultaneous treatment with HWE-SQ and LPS dose-dependently attenuated LPS-induced iNOS protein levels in RAW 264.7 cells (Fig. 3A). Densitometric analysis (Fig. 3B) showed that LPS-induced iNOS protein expression was significantly reduced by HWE-SQ. Increased iNOS expression has been demonstrated in ulcerative colitis patient samples (31) and in a variety of human malignant tumors including breast (32) and prostate (33). High expression of iNOS was observed in each of these tumors compared to the adjacent normal

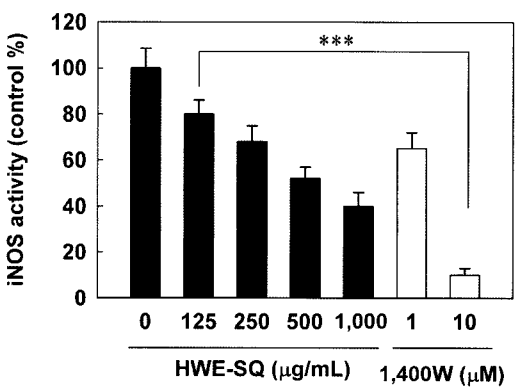

Fig. 2. Effect of HWE-SQ on iNOS enzyme activity. The data represent the mean±SD (n=3). *$p<0.01$ was considered significantly different from LPS alone-treated cells.

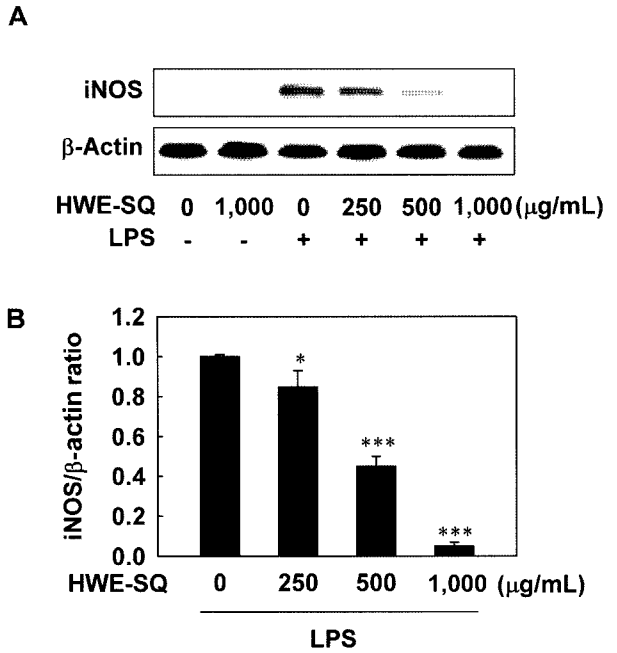

Fig. 3. Effect of HWE-SQ on iNOS protein expression in LPS-activated RAW 264.7 cells. (A) The figure shows a representative Western blot analysis from 3 independent experiments. (B) Graph representing the changes in the protein levels of iNOS normalized by β-actin. The data represent the mean±SD. *$p<0.01$ was considered significantly different from LPS alone-treated cells.

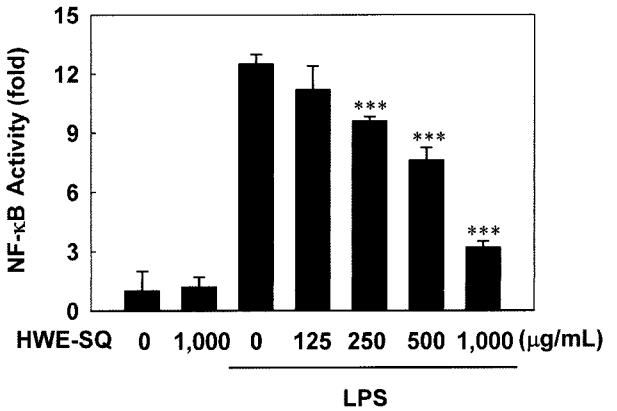

Fig. 4. Effect of HWE-SQ on LPS-induced NF-κB activation in RAW 264.7 cells. The luciferase activity was measured and data were normalized by Renilla luciferase expression. The data represent the mean±SD. *$p<0.01$ was considered significantly different from LPS alone-treated cells.

tissue (34). Therefore, inhibiting the overexpression of iNOS may serve as a therapeutic target for preventing chronic inflammatory disorders. In this respect, our results indicate that HWE-SQ contains phytochemicals that can effectively retard NO production in LPS-activated RAW 264.7 cells.

HWE-SQ inhibits NF-κB activation in LPS-activated RAW 264.7 cells We examined the effect of HWE-SQ on LPS-induced NF-κB activity. RAW 264.7 cells were transiently transfected with a commercially available plasmid designed for monitoring NF-κB activation (pNF-κB-Luc). When stimulated (100 ng/mL, 24 hr) with LPS, luciferase activity increased by 12.5±0.5-fold compared to unstimulated cells. Simultaneous treatment of HWE-SQ with LPS significantly decreased LPS-induced NF-κB transcriptional activity in a dose-dependent manner with significant inhibition (Fig. 4). The transcription factor NF-κB is important in inflammatory responses through the regulation of iNOS (35, 36). We have demonstrated that HWE-SQ inhibits the production of NO, expression of iNOS protein, and activation of NF-κB. These results together suggest that HWE-SQ inhibits NO production and iNOS protein expression by the attenuation of NF-κB activity in LPS-activated RAW 264.7 cells. Therefore, the results of this study demonstrate the underlying anti-inflammatory molecular mechanism of action of HWE-SQ in mouse macrophage cell lines.

HWE-SQ does not affect the phosphorylation and degradation of IκBα in LPS-activated RAW 264.7 cells We then examined cytosolic events involved in NF-κB activation. In unstimulated cells, NF-κB is sequestered in the cytoplasm in its inactive form, a result of being bound to inhibitors of κB (IκB) (37). Specific NF-κB-activating agents such as LPS promote the phosphorylation of IκBα in RAW 264.7 cells, causing its degradation by proteosomes. This proteolytic degradation activates NF-κB by releasing it from its inactive IκBα-bound state, allowing NF-κB to translocate to the nucleus and initiate gene transcription via κB motif binding in the promoter region of the iNOS gene. Although there are a number of IκB proteins, IκBα is the primary regulator of rapid signal-induced activation of NF-κB (38). Whether or not HWE-SQ inhibits NF-κB transcriptional activity by attenuating IκBα degradation was examined. LPS-stimulation induced phosphorylation of IκBα within 10 min and phosphorylation of IκBα had recovered to basal level by 30 min. HWE-SQ did not affect LPS-induced IκBα phosphorylation (Fig. 5A). We next determined the effect of HWE-SQ on LPS-induced IκBα degradation. IκBα degradation happened within 20 min upon exposure to LPS and HWE-SQ did not inhibit LPS-induced IκBα degradation (Fig. 5B). These results indicate that HWE-SQ may involve transcriptional regulation through the suppression of NF-κB trans-criptional activity without affecting IκBα degradation in LPS-activated RAW 264.7 cells. Further investigation will focus on the precise mechanism of HWE-SQ-dependent modulation of NF-κB transcriptional activation.

HWE-SQ does not affect the activation of ERK1/2, JNK1/2, and p38 MAPK in LPS-activated RAW 264.7 cells LPS activates mitogen-activated protein kinase (MAPKs) signal-transduction pathways in RAW 264.7 cells (39). Three well-defined MAPKs, extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun NH_2-terminal kinase (JNK), have been implicated in the transcriptional regulation of pro-inflammatory mediators because specific MAPK inhibitors suppress the expression of inflammatory genes (40-42). We investigated the effect of HWE-SQ on MAPK activation by examining the

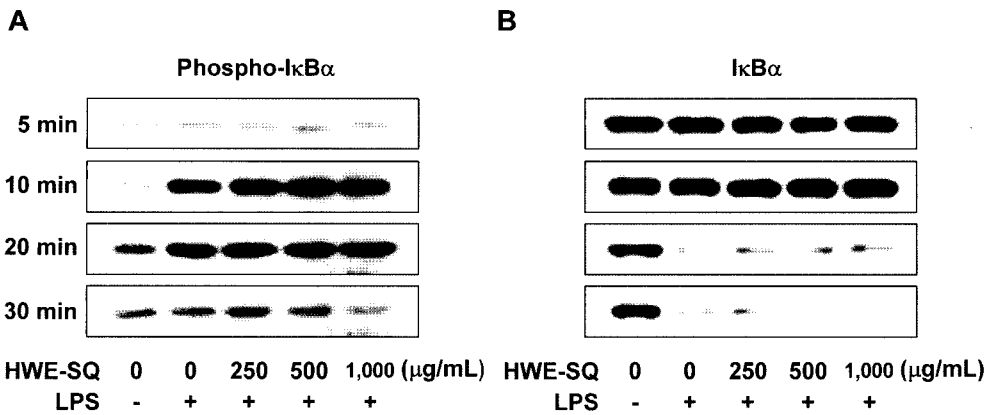

Fig. 5. Effect of HWE-SQ on the phosphorylation and degradation of IκBα in LPS-activated RAW 264.7 cells. Whole-cell lysates were prepared and subjected to Western blot analysis with (A) anti-phospho-IκBα antibody, and (B) anti-IκBα antibody.

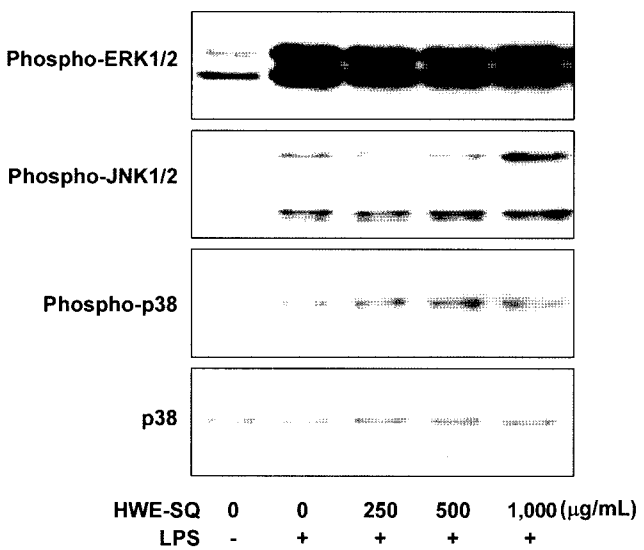

Fig. 6. Effect of HWE-SQ on the activation of ERK1/2, JNK1/2, and p38 MAPK in LPS-activated RAW 264.7 cells. Whole-cell lysates were prepared and subjected to Western blot analysis with antibodies against activated ERK1/2, JNK1/2, or p38 MAPK. Total p38 MAPK was measured to ensure equal loading of proteins.

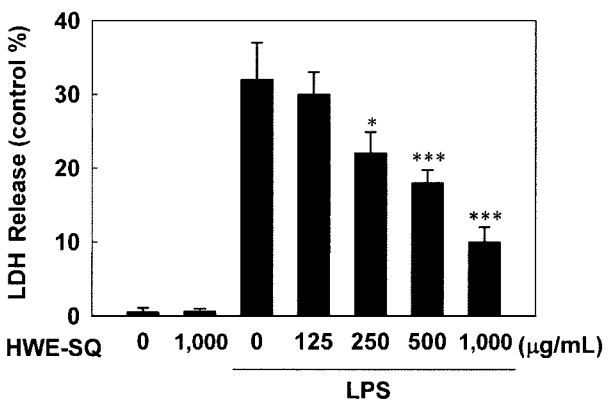

Fig. 7. Effect of HWE-SQ on LPS-induced cell death in RAW 264.7 cells. Data represent the mean±SD (n=3). *p<0.01 was considered significantly different from LPS alone-treated cells.

phosphorylation of ERK1/2, JNK1/2, and p38 MAPK with Western blot analysis (Fig. 6). Stimulation of cells with LPS increased the phosphorylation of all three MAPKs by 30 min. HWE-SQ did not affect the LPS-induced phosphorylation of ERK1/2, JNK1/2, and p38.

HWE-SQ inhibits LPS-induced cell death of RAW 264.7 cells LPS-induced cell death was evaluated by LDH release into the culture medium. The level of LDH release increased significantly to 32.5±5.2% after 24 hr of LPS stimulation, suggesting that LPS stimulation for 24 hr resulted in the loss of cell membrane integrity. HWE-SQ significantly reduced LPS-induced cell death of RAW 264.7 cells (Fig. 7).

In conclusion, HWE-SQ exhibits potent inhibitory effects on NO production by the activated macrophage cell line RAW 264.7. The mechanism of inhibition seems to be due to a reduction in iNOS expression and iNOS enzyme activity. Thus, in disease states where NO overproduction has been shown to play a role, we suggest that HWE-SQ may be effective as an iNOS inhibitor. Furthermore, since NF-κB is a ubiquitous transcription factor controlling the gene expression of numerous enzymes, cytokines, cell adhesion molecules, and growth factors involved in inflammation, carcinogenesis, and other immunological disorders, HWE-SQ may be an effective down-modulator of NF-κB and thus may have additional important therapeutic functions.

Acknowledgments

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References

1. Hobbs AJ, Higgs A, Moncada S. Inhibition of nitric oxide synthase as a potential therapeutic target. Annu. Rev. Pharmacol. 39: 191-220 (1999)

2. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function, and inhibition. Biochem. J. 357: 593-615 (2001)
3. Wang Y, Marsden PA. Nitric oxide synthases: gene structure and regulation. Adv. Pharmacol. 34: 71-90 (1995)
4. Bogdan C. Nitric oxide and the immune response. Nat. Immun. 2: 907-916 (2001)
5. Luoma JS, Stralin P, Marklund SL, Hiltunen TP, Sarkioja T, Yla-Herttuala S. Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic lesions: colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins. Arterioscl. Throm. Vas. 18: 157-167 (1998)
6. Dusting GS. Nitric oxide in coronary artery disease: roles in atherosclerosis, myocardial reperfusion, and heart failure. EXS 76: 33-55 (1996)
7. Xie Q, Kashiwabara Y, Nathan C. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. J. Biol. Chem. 269: 4705-4708 (1994)
8. Baeuerle PA, Henkel T. Function and activation of NF-κB in the immune system. Annu. Rev. Immunol. 12: 141-179 (1994)
9. Thanos D, Maniatis T. NF-kappa B: a lesson in family values. Cell 80: 529-532 (1995)
10. Choi SY, Hwang JH, Ko HC, Park SY, Kim GO, Kim DH, Chang IS, Kwon HM, Kim SJ. Inhibitory action of Tsunokaori tangor peel on the lipopolysaccharide-induced inflammatory response in RAW 264.7 macrophage cells. Food Sci. Biotechnol. 15: 270-276 (2006)
11. Khatib A, Kim MY, Chung SK. Anti-inflammatory activities of *Cinanamomum burmanni* Bl. Food Sci. Biotechnol. 14: 223-227 (2005)
12. Yeo EJ, Kim KT, Han YS, Nah SY, Paik HD. Antimicrobial, anti-inflammatory, and anti-oxidative activities of *Scilla scilloides* (Lindl.) Druce root extract. Food Sci. Biotechnol. 15: 639-642 (2006)
13. Namba T, Bae KH. Pharmacognostical studies on the crude drug '*Zhu-Ye*' and the Rambusaceous plants the botanical origins of the folk medicine '*Kuma-Zasa*' on the Japanese markets and the comparative anatomical studies of the leaves of the genus *Sasa* (Sections S). Shoyakugaku Zasshi 36: 43-54 (1982)
14. Lee MJ, Moon GS. Antioxidative effects of Korean bamboo trees, *Wang-dae*, *Som-dae*, *Maengjong-juk*, *Jolit-dae*, and *O-juk*. Korean J. Food Sci. Technol. 35: 1226-1232 (2003)
15. Zang Y, Wu XQ, Yu XY. Comparison study on total flavonoid content and anti-free radical activity of leaves of bamboo, *phyllostachys nigra*, and *Ginko biloba*. Zhongguo Zhong Yao Za Zhi 27: 254-257 (2002)
16. Hu C, Zhang Y, Kitts DD. Evaluation of antioxidant and prooxidant activities of bamboo *Phylloatachys nigra* var. *henonis* leaf extract *in vitro*. J. Agr. Food Chem. 48: 3170-3176 (2000)
17. Park HS, Lim JH, Kim HJ, Choi HJ, Lee IS. Antioxidant flavone glycosides from the leaves of *Sasa borealis*. Arch. Pharm. Res. 30: 161-166 (2007)
18. Jeong YH, Chung SY, Han AR, Sung MK, Jang DS, Lee J, Kwon Y, Lee HJ, Seo EK. P-glycoprotein inhibitory activity of two phenolic compounds, (-)-syringaresinol, and tricin from *Sasa borealis*. Chem. Biodivers. 4: 12-16 (2007)
19. Ren M, Reilly RT, Sacchi N. *Sasa* health exerts a protective effect on Her2/NeuN mammary tumorigenesis. Anticancer Res. 24: 2879-2884 (2004)
20. Kweon MH, Hwang HJ, Sung HC. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phylloatachys edulis*). J. Agr. Food Chem. 49: 4646-4655 (2001)
21. Lu B, Wu X, Shi J, Dong Y, Zhang Y. Toxicology and safety of antioxidant of bamboo leaves. Part 2: developmental toxicity test in rats with antioxidant of bamboo leaves. Food Chem. Toxicol. 44: 1739-1743 (2006)
22. Yoon KD, Kim CY, Huh H. The flavone glycosides of *Sasa borealis*. Korean J. Pharmacogn. 31: 224-227 (2000)
23. Ferrari M, Fornasiero MC, Isetta AM. MTT colorimetric assay for testing macrophage cytotoxic activity *in vitro*. J. Immunol. Methods 131: 165-172 (1990)
24. He Q, Riley RT, Sharma RP. Pharmacological antagonism of fumonisin B1 cytotoxicity in porcine renal epithelial cells (LLC-PK1): a model for reducing fumonisin-induced nephrotoxicity *in vivo*. Pharmacol. Toxicol. 90: 268-277 (2002)
25. Hsieh GS, Acosta D. Dithranol-induced cytotoxicity in primary cultures of rat epidermal keratinocytes. Toxicol. Appl. Pharm. 107: 16-26 (1991)
26. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [^{15}N] nitrate in biological fluids. Anal. Biochem. 126: 131-138 (1982)
27. Wang MJ, Huang HM, Chen HL, Kuo JS, Jeng KC. Dehydroepiandrosterone inhibits lipopolysaccharide-induced nitric oxide production in BV-2 microglia. J. Neurochem. 77: 830-838 (2001)
28. Wilson KT, Ramanujam KS, Mobley HL, Musselman RF, James SP, Meltzer SJ. *Helicobacter pylori* stimulates inducible nitric oxide synthase expression and activity in a murine macrophage cell line. Gastroenterology 111: 1524-1533 (1996)
29. Kobuchi H, Droy-Lefaix MT, Christen Y, Packer L. *Ginkgo biloba* extract (EGb 761): inhibitory effect on nitric oxide production in the macrophage cell line RAW 264.7. Biochem. Pharmacol. 53: 897-903 (1997)
30. Sheu F, Lai HH, Yen GC. Suppression effect of soy isoflavones on nitric oxide production in RAW 264.7 macrophages. J. Agr. Food Chem. 49: 1767-1772 (2001)
31. Goodman JE, Hofseth LJ, Hussain P, Harris CC. Nitric oxide and p53 in cancer-prone chronic inflammation and oxyradical overload disease. Environ. Mol. Mutagen. 44: 3-9 (2004)
32. Vakkala M, Kahlos K, Lakari E, Paakko P, Kinnula V, Soini Y. Inducible nitric oxide synthase expression, apoptosis, and angiogenesis in *in situ* and invasive breast carcinomas. Clin. Cancer Res. 6: 2408-2416 (2000)
33. Uotila P, Valve E, Martikainen P, Nevalainen M, Nurmi M, Harkonen P. Increased expression of cyclooxygenase-2 and nitric oxide synthase-2 in human prostate cancer. Urol. Res. 29: 25-28 (2001)
34. Lechner M, Lirk P, Rieder J. Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. Semin Cancer Biol. 15: 277-289 (2005)
35. Zhang X, Laubach VE, Alley EW, Edwards KA, Sherman PA, Russell SW, Murphy WJ. Transcriptional basis for hyporesponsiveness of the human inducible nitric oxide synthase gene to lipopolysaccharide/interferon-c. J. Leukocyte Biol. 59: 575-585 (1996)
36. Taylor BS, Geller DA. Molecular regulation of the human inducible nitric oxide synthase (iNOS) gene. Shock 13: 413-424 (2000)
37. Baeuerle PA, Baltimore D. IκB: a specific inhibitor of the NF-κB transcription factor. Science 242: 540-546 (1988)
38. Sun SC, Ganchi PA, Ballard DW, Greene WC. NF-κB controls expression of inhibitor IκBα: evidence for an inducible auto-regulatory pathway. Science 259: 1912-1915 (1993)
39. Rao KM, Meighan T, Bowman L. Role of mitogen-activated protein kinase activation in the production of inflammatory mediators: differences between primary rat alveolar macrophages and macrophage cell lines. J. Toxicol. Env. Heal. A 65: 757-768 (2002)
40. Kefaloyianni E, Gaitanaki C, Beis I. ERK1/2 and p38-MAPK signalling pathways, through MSK1, are involved in NF-kappaB transactivation during oxidative stress in skeletal myoblasts. Cell. Signal. 18: 2238-2251 (2006)
41. Palsson-McDermott EM, O'Neill LA. Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. Immunology 113: 153-162 (2004)
42. Wong WS. Inhibitors of the tyrosine kinase signaling cascade for asthma. Curr. Opin. Pharmacol. 5: 264-271 (2005)