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Isoegomaketone Upregulates Heme Oxygenase-1 in RAW264.7 Cells via ROS/p38 MAPK/Nrf2 Pathway

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Abstract

Isoegomaketone (IK) was isolated from *Perilla frutescens*, which has been widely used as a food in Asian cuisine, and evaluated for its biological activity. We have already confirmed that IK induced the HO-1 expression via Nrf2 activation in RAW264.7 cells. In this study, we investigated the effect of IK on the mechanism of HO-1 expression. IK upregulated HO-1 mRNA and protein expression in a dose dependent manner. The level of HO-1 mRNA peaked at 4 h after 15 μM IK treatment. To investigate the mechanisms of HO-1 expression modulation by IK, we used pharmacological inhibitors for the protein kinase C (PKC) family, PI3K, and p38 MAPK. IK-induced HO-1 mRNA expression was only suppressed by SB203580, a specific inhibitor of p38 MAPK. ROS scavengers (N-acetyl-L-cysteine, NAC, and glutathione, GSH) also blocked the IK-induced ROS production and HO-1 expression. Furthermore, both NAC and SB203580 suppressed the IK-induced Nrf2 activation. In addition, ROS scavengers suppressed other oxidative enzymes such as catalase (CAT), glutathione S-transferase (GST), and NADH quinone oxidoreductase (NQO-1) in IK-treated RAW264.7 cells. Taken together, it can be concluded that IK induced the HO-1 expression through the ROS/p38 MAPK/Nrf2 pathway in RAW264.7 cells.

Key Words: Isoegomaketone, Heme oxygenase-1, p38 MAPK, ROS scavenger

INTRODUCTION

Heme oxygenase-1 (HO-1), the inducible isoform of heme oxygenase that catalyzes the degradation of heme into biliverdin, iron, and carbon monoxide (CO), is a stress-responsive protein. HO-1 has anti-inflammatory, antioxidant, and antiproliferative effects (Yamada *et al*., 2000; Gabunia *et al*., 2012). HO-1 is an antioxidant enzyme induced by oxidative stress. However, recent studies have demonstrated that overexpression of HO-1 prior to stimulation with LPS markedly inhibited the production of subsequent inflammatory mediators such as nitric oxide (NO), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) (Tsoyi *et al*., 2008; Park *et al*., 2009a; Jin *et al*., 2010). Moreover, the deficiency of HO-1 resulted in severe inflammation in mice (True *et al*., 2007). Therefore, based on previous findings, the regulation of HO-1 expression may be a potential target for the treatment of inflammatory disease.

Perilla frutescens (L.) Britt. is an annual herbaceous plant belonging to the Lamiaceae family. Its leaves are used as food in Asian cuisines, and its seeds are used to make edible oil in Korea. It is also used in traditional Chinese medicine. The pharmacological activities of *P. frutescens* have been investigated in many studies (Brochers *et al*., 1997; Ueda and Yamzaki, 1997). Several compounds, such as rosmarinic acid, luteolin, apigenin, fefulic acid, (+)-catechin, caffeic acid, and isoegomaketone, have been isolated from *P. frutescens*. Anti-inflammatory activities of rosmarinic acid (Huang *et al*., 2009), luteolin (Kim and Jobin, 2005), and apigenin (Zhang *et al*., 2014) have been demonstrated in previous reports.

IK, an essential oil component in *P. frutescens*, has been shown to have numerous biological activities. It has been shown to have inhibitory activity on NO production in LPS-treated RAW264.7 cells (Jin *et al*., 2010), and IK induced apoptosis in several cancer cells through caspase-dependent and -independent pathways (Cho *et al*., 2011; Kwon *et al*., 2014). Furthermore, IK has the potential for increasing the effectiveness of prostate cancer therapy with TRAIL (Lee *et al*., 2014). Previously, our group has shown that IK induced the HO-1 expression in RAW264.7 cells (Jin *et al*., 2010), how-

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ever, the detailed mechanism was yet to be elucidated. In this study, we investigated the mechanisms of HO-1 induction by IK in RAW264.7 cells.

MATERIALS AND METHODS

Reagents

DMEM and fetal bovine serum (FBS) were purchased from Hyclone (Logan, UT, USA). LPS, phenylmethylsulfonyl fluoride, sodium nitrite, DMSO, Griess reagent, Rottlerin (RO), GF102903X (GF), and a protease inhibitor cocktail were purchased from Sigma (St. Louis, MO, USA). Goat anti-rabbit IgG HRP-conjugated antibody and Lipofectamine 2000 were purchased from Invitrogen (Carlsbad, CA, USA). The RNeasy kit was purchased from QIAGEN (Valencia, CA, USA). The EZ-Cytox Cell Viability assay kit was purchased from DAEIL lab (Seoul, Korea). The Advantage RT-for-PCR kit was purchased from Clontech (Mountain view, CA, USA). SYBR premix was purchased from Takara Bio Inc (Shiga, Japan). NP40 cell lysis buffer was purchase from Biosource (San Jose, CA, USA). SB203580 (SB) was purchased from Cell Signaling Technology (Danvers, MA, USA). LY294002 (LY) and Go6976 (GO) were purchased from Calbiochem (La Jalla, CA, USA). Rabbit polyclonal antibodies against β-tubulin, HO-1, laminB, and Nrf2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell culture

RAW264.7 macrophage cells were cultured in DMEM supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) and incubated at 37°C with 5% carbon dioxide.

Cytotoxicity assay

To measure cell viability, we used the EZ-Cytox cell viability assay kit. The cells were cultured in a 96-well flat-bottom plate at a density of 2.0×10^5 cells/mL for 24 h. The cells were subsequently treated with various concentrations of kinase inhibitor for an additional 24 h. After the incubation period, EZ-Cytox 10 μL was added to each well and incubated for 4 h at 37°C and 5% CO_2. The index of cell viability was determined by measuring formazan production at an absorbance of 480 nm, using an ELISA reader. The reference wavelength was 650 nm.

Determination of NO concentration

Nitrite in the cellular media was measured by the Griess method (Khan *et al*., 2009). The cells were cultured in a 96-well plate and treated with LPS (1 μg/mL) for 18 h. At the end of the culture period, the cellular media was collected for the determination of nitrite production. Equal volumes of Griess reagent and cellular supernatant were mixed, and the absorbance was measured at 540 nm. The concentration of nitrite (μM) was calculated using a standard curve produced from known concentration of sodium nitrite dissolved in DMEM. The results are presented as the mean ± SD of four replicates of one representative experiment.

Preparation of cell extracts and Western analysis

Cells were washed once with cold PBS and harvested by pipetting. For whole-cell extract preparation, the cells were lysed on ice, in a NP40-based cell lysis buffer containing a protease inhibitor cocktail (Sigma) and phenylmethylsulfonyl fluoride (Sigma) for 30 min. Nuclear and cytosolic extracts were prepared using nuclear and cytosolic extraction reagents (Thermo Scientific, Rockford, IL, USA). The protein concentration of the cell lysate was determined using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA, USA). Fifty μg of protein was loaded and electrophoresed on a 10% SDS-polyacrylamide gel, following which it was transferred onto a nitrocellulose membrane (Hybond ECL Nitrocellulose, GE Healthcare, Chandler, AZ, USA). The membranes were washed once with a wash buffer (PBS with 0.05% Tween 20) and blocked with a blocking buffer (PBS with 5% skim milk and 0.05% Tween 20) for 1 h. After blocking, the membranes were incubated with the rabbit anti-HO-1 or anti-β-tubulin primary antibody overnight at 4°C. Rabbit anti-HO-1 polyclonal antibody was diluted at 1:1000, and the rabbit anti-β-tubulin polyclonal antibody was diluted at 1:200 in blocking buffer. After incubation, the membranes were washed and subsequently incubated for 1 h at room temperature with the goat anti-rabbit IgG HRP-conjugated secondary antibody diluted to 1:5000 in blocking buffer. The membranes were washed and the protein bands were detected by a chemiluminescence system (GE Healthcare).

Quantitative real-time PCR

The cells were cultured in a 100-mm petri dish for 24 h (2×10^5 cell/mL). Total RNA was isolated using the RNeasy Kit according to the manufacturer's instructions. The Advantage RT-for-PCR kit was used for reverse transcription according to the manufacturer's protocol. A Chromo4 real-time PCR detection system (Bio-Rad) and iTaq™ SYBR® Green Supermix (Bio-Rad) were used for the RT-PCR amplification of HO-1 and β-actin using the following conditions: 50 cycles at 94°C for 20 s, 60°C for 20 s and 72°C for 30 s. All the reactions were repeated independently at least three times to ensure the reproducibility of the results. Primers for HO-1 and β-actin were purchased from Bioneer Corp. (Daejeon, Korea) and the following sense and anti-sense primers were used: HO-1, forward primer 5`-TTACCTTCCCGAACATCGAC-3`, reverse primer 5`-GCATAAATTCCCACTGCCAC-3`; β-actin, forward primer 5`-TGAGAGGGAAATCGTGCGTGAC-3`, reverse primer 5`-GCTCGTTGCCAATAGTGATGACC-3`.

Statistical methods

Data are expressed as the mean ± SEM of results obtained from the total number of replicate treatments. Difference between data sets were assessed by one-way analysis of variance (ANOVA) followed by Newman-Keuls tests. A $p<0.05$ was considered to be significant for all analyses.

RESULTS

Effect of IK on HO-1 expression in RAW264.7 cell

First, we examined IK induced the HO-1 expression in RAW 264.7 cells was examined. As shown in Fig. 1A, IK treatment markedly increased the expression of HO-1 mRNA in a dose-dependent manner. The maximum induction of HO-1 mRNA was at 4 h after treatment with 15 μM IK and the maximum induction level was approximately 60 fold greater than at 0 h treatment with IK (Fig. 1B). IK also induced the HO-1 protein expression in a dose-dependent manner (Fig. 1C). The maximum level of HO-1 protein expression was reached at 8

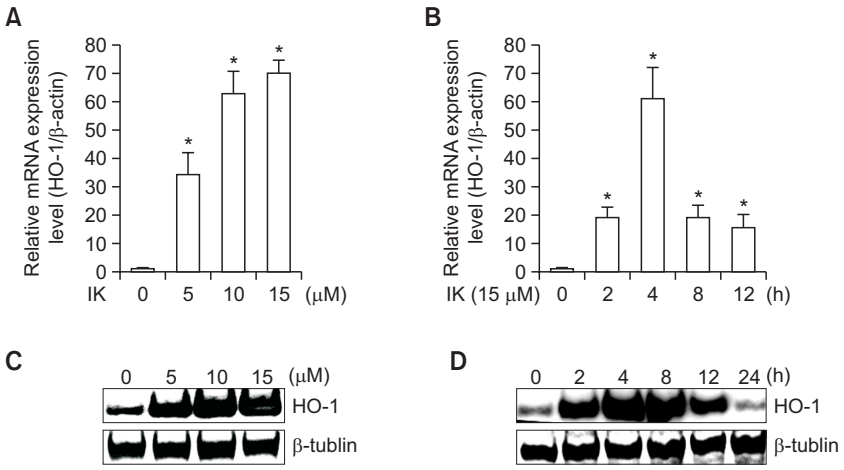

Fig. 1. The expression of HO-1 by IK treatment in RAW264.7 cells. (A, B) Total RNA was isolated, and the expression level of HO-1 mRNA was measured by quantitative real-time PCR. Data shown are mean ± SD (n=4). **p*<0.05 vs. the control group. (C, D) The whole cell lysates (50 μg) were used for Western blot analysis.

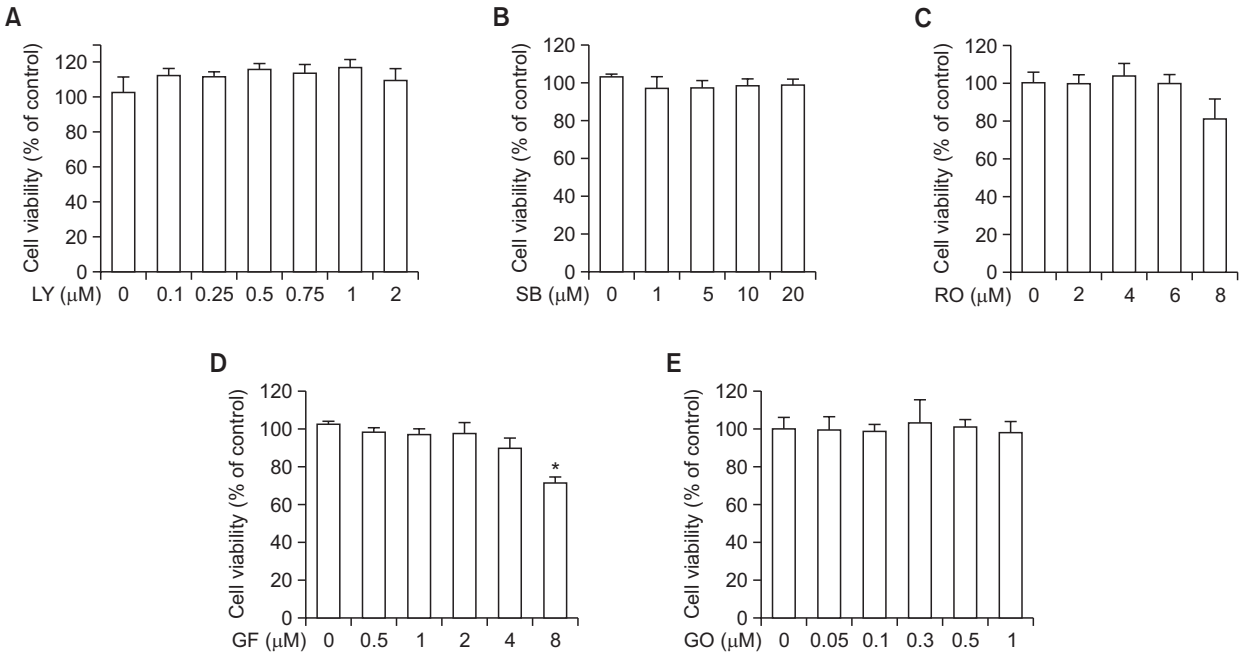

Fig. 2. Effect of specific kinase inhibitors on cell viability. Cell viability was determined by the EZ-Cytox cell viability assay kit. Data shown are mean ± SD (n=4). **p*<0.05 vs. the control group. (LY:LY294002, SB:SB203580, RO:rottlerin, GF:GF109203X, GO:Gö6976).

h after treatment with 15 μM IK. The induction of HO-1 protein was equal to basal level at 24 h (Fig. 1D).

Effect of kinase inhibitors on HO-1 expression by IK treatment

Previous studies have reported that the expression of HO-1 was mediated through activation of PKC, PI3K, Nrf2, and p38 MAPK (Shih *et al*., 2011; Lee *et al*., 2012). Previously, we have shown that IK increased the translocation of Nrf2 into the nucleus without affecting Nrf2 expression in RAW264.7 cells (Jin *et al*., 2010). We further examined whether the induction of HO-1 by IK treatment is mediated through activation of other kinases using relative specific inhibitors. Before the experiment, we determined the non-toxic concentration of kinase inhibitors in RAW264.7 cells via the cell viability assay (Fig. 2). According to the cytotoxicity result, RAW264.7 cells were treated with 15 μM IK, along with various specific kinase inhibitors. As shown in Fig. 3, only SB, a specific p38 MAPK inhibitor, suppressed the HO-1 induction in IK-treated RAW264.7 cells, whereas the other kinase inhibitors such as LY, RO, GF, and GO had no effect on HO-1 expression. Therefore, p38 MAPK seemed to play an important role in HO-1 induction by IK treatment.

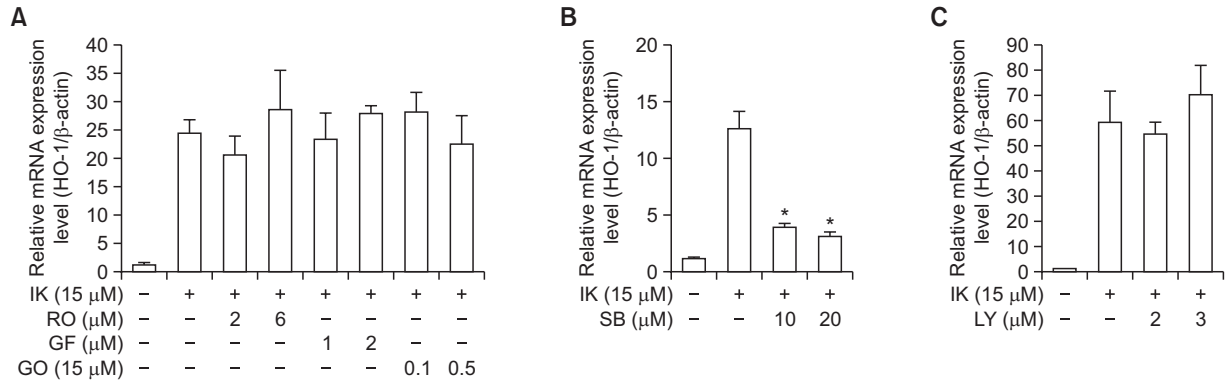

Fig. 3. Effect of specific kinase inhibitors on HO-1 expression in IK-treated RAW274.7 cells. IK was added with and without various concentration of kinase inhibitors for 4 h. Total RNA was isolated, and the expression of HO-1 mRNA was measured by quantitative real-time PCR. Data shown are mean ± SD (n=4). **p*<0.05 vs. the cells treated with only IK.

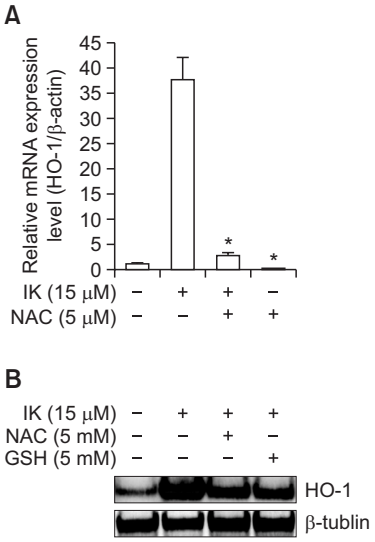

Fig. 4. Effect of ROS scavengers on HO-1 expression in IK-treated RAW264.7 cells. (A) Total RNA was isolated, and the expression of HO-1 mRNA was measured by quantitative real-time PCR. Data shown are mean ± SD (n=4). **p*<0.05 vs. the cells treated with only IK. (B) The whole cell lysates (50 μg) were used for Western blot analysis.

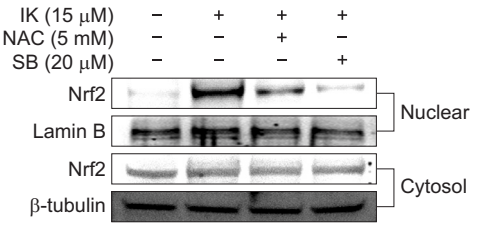

Fig. 5. Effect of NAC and p38 MAPK inhibitor on Nrf2 activation in IK-treated RAW264.7 cells. RAW264.7 cells were treated with IK for 2 h with NAC and SB. Nuclear extracts (30 μg) and the cytosolic fraction (50 μg) were used for Western blot analysis.

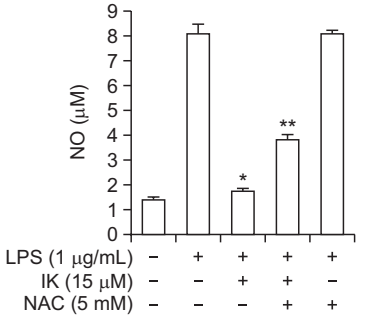

Fig. 6. Effect of NAC on NO production in LPS-treated RAW264.7 cells. RAW264.7 cells were treated with IK for 2 h prior to LPS addition (1 μg/ml) and were incubated for an additional 18 h. Cellular media (100 μl) were mixed with equal volumes of Griess reagent. Nitrite level were measured as an indicator of NO production as described in the Materials and Methods. Data shown are the mean ± SD (n=4). **p*<0.05 vs. the cells treated with only LPS, ***p*<0.05 vs. the cells treated with LPS and IK without NAC.

Effect of ROS scavengers on HO-1 expression by IK treatment

In follow-up experiments, the upstream signaling pathway that stimulates HO-1 expression was investigated. Since reactive oxygen species (ROS) have been implicated in the activation of Nrf2 (Alam and Cook, 2003), the involvement of oxidative stress was examined. Addition of glutathione (GSH) or the glutathione donor N-acetyl-L-cysteine (NAC) suppressed the generation of reactive oxygen in cells. Cells were treated with GSH or NAC to test whether the expression of HO-1 mRNA by IK was mediated via ROS generation. As shown in Fig. 4A, IK-induced expression of HO-1 mRNA was significantly reduced when GSH or NAC was added to the culture. The protein level of HO-1 was also reduced by GSH or NAC in IK-treated RAW264.7 cells (Fig. 4B). Therefore, ROS generation might be involved in the IK-induced expression of HO-1 in RAW264.7 cells.

Effect of ROS scavenger and p38 MAPK inhibitor on Nrf2 activation by IK treatment

Up to this point, we could not determine whether IK induces the HO-1 expression through two parallel pathways (p38 MAPK and ROS-Nrf2 pathway) or a single connected pathway, such as the ROS/p38 MAPK/Nrf2 pathway. To solve this question, we examined the effect of ROS scavenger (NAC) and specific p38 MAPK inhibitor (SB203580) on the subcel-

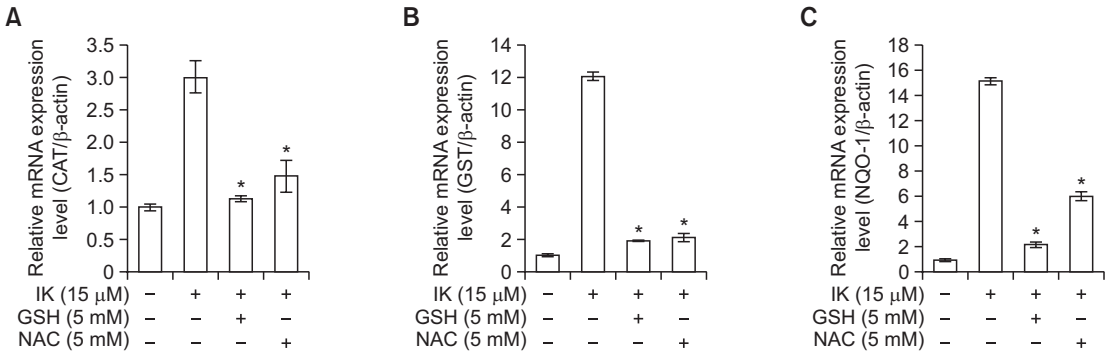

Fig. 7. Effect of ROS scavengers on the production of antioxidant enzymes such as catalase (CAT), glutathione S-transferase (GST), and NADH quinone oxidoreductase (NQO-1) in IK-treated RAW264.7 cells. Total RNA was isolated, and the expression level of HO-1 mRNA was measured by quantitative real-time PCR. Data shown are mean ± SD (n=4). *p<0.05 vs. the cells treated with only IK.

lular localization of Nrf2 in IK-treated RAW264.7 cells. As shown in Fig. 5, IK increased the Nrf2 protein levels in nuclear extracts. However, this increase was significantly reduced by NAC and SB203580 treatment. Therefore, IK induced the HO-1 expression through ROS/p38 MAPK/Nrf2 pathway.

Effect of NAC on NO production in LPS-treated RAW264.7 cells

Many previous reports have shown that HO-1 expression mediates the NO production in LPS-treated RAW264.7 cells (Park *et al*., 2009a; Kim *et al*., 2014). Whether HO-1 expression by IK treatment is involved in inhibition of NO production was examined in LPS-treated RAW264.7 cells using NAC. Cells were treated with LPS and IK in the presence of NAC, and the resultant NO levels were measured. As shown in Fig. 6, NAC restored the IK-mediated inhibition of NO production by 33%, while NAC alone had no effect on the LPS-stimulated NO production. Therefore, HO-1 induction plays an important role in the inhibitory effect of IK on LPS-induced NO production.

Effect of ROS scavengers on the production of antioxidant enzymes

Besides HO-1, the activation of Nrf2 is a major determinant of phase II enzyme induction, such as catalase (CAT), glutathione S-transferease (GST), and NADH quinone oxidoreductase (NQO-1) (Zhang *et al*., 2013). IK treatment induced the expression of HO-1 through ROS/Nrf2/p39 MAPK in RAW264.7 cells. Therefore, we examined whether IK could induce antioxidant enzymes CAT, GST, and NQO-1, and whether ROS scavengers could abolish the effects of IK on these enzymes. As shown in Fig. 7, phase II enzymes were induced by IK treatment; however, the induction levels of these were smaller than that of HO-1. The levels of CAT, GST, and NQO-1 mRNA in cells treated with IK were significantly reduced when cells were cultured in the presence of GSH or NAC.

DISCUSSION

Previously, we isolated isoegomaketone (IK) in *Perilla frutescens* (L.) Britt. cv. Chookyoupjaso. *Perilla frutescens* (L.) Britt. cv. Chookyoupjaso was mutated by gamma radiation (Park *et al*., 2009b) as mutant *P. frutescens* (L.) Britt., and contained about 10 times more IK than wild type species. IK is biosynthesized from egomaketone (EK), and this reaction is inhibited by gene *I* in *P. frutescens* (Nishizawa *et al*., 1989). It is possible that gene *I* was affected by gamma radiation and consequently resulted in an increased IK content in the mutant. Recently, we found that IK induced the expression of HO-1 in RAW264.7 cells (Jin *et al*., 2010). However, the molecular mechanism underlying IK-induced HO-1 expression was not completely understood. In this study, we examined the detailed mechanism for HO-1 expression by IK treatment in RAW264.7 cells.

Quantitative real-time PCR of the cells with specific kinase inhibitors revealed that IK-induced HO-1 expression was mediated by activation of the p38 MAPK pathway (Fig. 3). The western blot analysis of the cells with NAC and GSH suggested that IK-induced HO-1 expression was regulated through ROS generation (Fig. 4). To our knowledge, our report is the first that describes the mechanism of HO-1 induction by IK in RAW264.7 cells.

Heme oxygenase-1 (HO-1) expression is induced in response to oxidative stress and inflammatory stimuli in macrophages. HO-1 catalyzes the degradation of heme into equimolar amounts of carbon monoxide (CO), iron and biliverdin. Biliverdin is further converted to bilirubin, which is a potent endogenous anti-oxidant (Ryter *et al*., 2006). CO, one of the catabolic products of heme, exerts anti-inflammatory effects (Park *et al*., 2009a). Recent studies have demonstrated that HO-1 induction was mediated by the activation of PI3K, PKC, and p38 MAPK (Rojo *et al*., 2006; Shih *et al*., 2011; Lee *et al*., 2012). Signaling mechanisms of HO-1 expression may depend on cell types and inducers. Crotonaldehyde induces HO-1 expression in endothelial cells via PKC-δ and p38 MAPK activation (Lee *et al*., 2011). However, PKC-δ and p38 inhibitors did not affect the crotonaldehyde-induced HO-1 expression in RAW264.7 cells and A549 human lung epithelial cells. In this study, we investigated the contribution of PI3K, PKC, and p38 MAPK on IK-induced HO-1 expression using respective specific inhibitors. Among these inhibitors, only the specific p38 MAPK inhibitor attenuated HO-1 induction in IK-treated RAW264.7 cells. We have previously confirmed that IK increased the translocation of Nrf2 into the nucleus without affecting Nrf2 expression in RAW264.7 cells (Jin *et al*., 2010). The specific p38 MAPK inhibitor also suppressed the IK-induced translocation of Nrf2 into the nucleus (Fig. 5). Therefore, our results show that the p38 pathway is required for IK-stimulated expression of HO-1 and IK-induced translo-

cation of Nrf2 into the nucleus. Until now, there have been no reports showing the activation of p38 pathway by IK.

Reactive oxygen species (ROS) have been implicated in the induction of HO-1 expression (Liu *et al*., 2011; Shih *et al*., 2011). Cigarette smoke extract upregulated the HO-1 induction via ROS production in mouse brain endothelial cells (Shih *et al*., 2011), and curcumin induced the HO-1 expression by generation of ROS in human hepatoma cells (McNally *et al*., 2007). According to these previous reports, ROS generation is upstream of p38 MAPK. HO-1 expression by IK also seemed to be dependent on oxidative stress. IK-mediated induction of HO-1 was markedly suppressed by co-treatment of GSH or NAC (Fig. 4). Furthermore, IK-induced translocation of Nrf2 into the nucleus was inhibited by NAC (Fig. 5). It has been reported that IK induced apoptosis in B16 melanoma cells was through ROS generation (Kwon *et al*., 2014), in which ROS production by IK was measured by flow cytometry. However, the level of IK used for the treatment was 100 μM, which was high enough concentration to induce cytotoxicity in RAW264.7 cells (Jin *et al*., 2010). In this study, the level of IK was 15 μM, which was enough to induce ROS generation without toxicity. Even if NAC markedly suppressed the IK-mediated induction of HO-1, some amount of HO-1 protein still remained (Fig. 4B), along with Nrf2 activation (Fig. 5). Therefore, there may be another minor pathway involved, along with the ROS/p38 MAPK/Nrf2.

Upregulation of HO-1 is mediated by activation of nuclear factor E2-related factor 2 (Nrf2) (Otterbein and Choi, 2000). Under unstressed condition, Nrf2 remains inactive in the cytoplasm. Under oxidative stress, Nrf2 dissociates from Keap1, translocates into the nucleus and binds to the antioxidant response element (ARE) in the promoter region of phase II antioxidant enzymes such as HO-1, CAT, NQO-1, and GST (Kaspar *et al*., 2009). We have seen that IK induces the translocation of Nrf2 into the nucleus in RAW264.7 cells (Jin *et al*., 2010). In the present study, we investigated and confirmed that IK also induced antioxidant enzymes other than HO-1, such as CAT, NQO-1, and GST (Fig. 7). The expression levels of these enzymes were lesser than that of HO-1, and their expression was also blocked by the treatment with ROS scavengers. CAT and NQO-1 have been suggested to have anti-inflammatory activity (Turdi *et al*., 2012; Thapa *et al*., 2014). However, HO-1 is the most important enzyme for anti-inflammation among the phase II antioxidant enzymes. The expression level of HO-1 was much higher than that of CAT and NQO-1 in IK-treated RAW264.7 cell. Therefore, blocking HO-1 expression mainly resulted in decreased IK-mediated inhibition of NO production in LPS-treated RAW264.7 cells (Fig. 6).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alam, J. and Cook, J. L. (2003) Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway. *Curr. Pharm. Des*. **9**, 2499-2511.

Brochers, A. T., Hackman, R. M., Keen, C. L., Stern, J. S. and Gershwin, M. E. (1997) Complementary medicine: a review of immunomodulatory effects of Chinese herbal medicines. *Am. J. Clin. Nutr.* **66**, 1303-1312.

Cho, B. O., Jin, C. H., Park, Y. D., Ryu, H. W., Byun, M. W., Seo, K. I. and Jeong, I. Y. (2011) Isoegomaketone induces apoptosis through caspase-dependent and caspase-independent pathways in human DLD1 cells. *Biosci. Biotechnol. Biochem*. **75**, 1306-1311.

Gabunia, K., Ellison, S. P., Singh, H., Datta, P., Kelemen, S. E., Rizzo, V. and Autieri, M. V. (2012) Interleukin-19 (IL-19) induces heme oxygenase-1 (HO-1) expression and decreases reactive oxygen species in human vascular smooth muscle cells. *J. Biol. Chem.* **287**, 2477-2484.

Huang, N., Hauck, C., Yum, M. Y., Rizshsky, L., Widrlechner, M. P., McCoy, J. A., Murphy, P. A., Dixon, P. M., Nikolau, B. J. and Birt, D. F. (2009) Rosmarinic acid in *Prunella vulgaris* ethanol extract inhibits LPS-induced prostaglandin E2 and nitric oxide in RAW264.7 mouse macrophage. *J. Agric. Food Chem*. **57**, 10579-10589.

Jin, C. H., Lee, H. J., Park, Y. D., Choi, D. S., Kim, D. S., Kang, S. Y., Seo, K. I. and Jeong, I. Y. (2010) Isoegomaketone inhibits lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophages through the heme oxygenase-1 induction and inhibition of the interferon-β-STAT-1 pathway. *J. Agric. Food Chem.* **58**, 860-867.

Kaspar, J. W., Niture, S. K. and Jaiswal, A. K. (2009) Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic. Biol. Med.* **47**, 1304-1309.

Khan, M. S., Priyadarshini, M. and Bano, B. (2009) Preventive effect of curcumin and quercetin against nitric oxide mediated modification of goat lung cystatin. *J. Agric. Food Chem.* **57**, 6055-6059.

Kim, J. S. and Jobin, C. (2005) The flavonoid luteolin prevents lipopolysaccharide-induced NF-κB signaling and gene expression by blocking IκB kinase activity in intestinal epithelial cells and bone-marrow derived dendritic cells. *Immunology* **115**, 375-387.

Kim, M. J., Kadayat, T., Kim, D. E., Lee, E. S. and Park, P. H. (2014) TI-I-174, a synthetic chalcone derivative, suppresses nitric oxide production in murine macrophages via heme oxygenase-1 induction and inhibition of AP-1. *Biomol. Ther. (Seoul)* **22**, 390-399.

Kwon, S. J., Lee, J. H., Moon, K. D., Jeong, I. Y., Ahn, D. U., Lee, M. K. and Seo, K. I. (2014) Induction of apoptosis by isoegomaketone from Perillafrutescens L. in B16 melanoma cells is mediated through ROS generation and mitochondrial-dependent, -independent pathway. *Food Chem. Toxicol.* **65**, 97-104.

Lee, J. H., Cho, H. D., Jeong, I. Y., Lee, M. K. and Seo, K. I. (2014) Sensitization of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant primary prostate cancer cells by isoegomaketone form *Perillafrutescens*. *J. Nat. Prod.* **77**, 2438-2443.

Lee, S. E., Jeong, S. I., Kim, G. D., Yang, H., Park, C. S., Jin, Y. H. and Park, Y. S. (2011) Upregulation of heme oxygenase-1 as an adaptive mechanism for protection against crotonaldehyde in human umbilical vein endothelial cells. *Toxicol. Lett.* **201**, 240-248.

Lee, S. E., Yang, H., Jeong, S. I., Jin, Y. H., Park, C. S. and Park, Y. S. (2012) Induction of heme oxygenase-1 inhibits cell death in crotonaldehyde-stimulated HepG2 cells via the PKC-δ-p38-Nrf2 pathway. *PLoS ONE* **7**, e41676.

Liu, X. M., Peyton, K. J., Shebib, A. R., Wang, H. and Durante, W. (2011) Compound C stimulates heme oxygenase-1 gene expression via the Nrf2-ARE pathway to preserve human endotherial cell survival. *Biochem. Pharmacol.* **82**, 371-379.

McNally, S. J., Harrison, E. M., Ross, J. A., Garden, O. J. and Wiqmore, S. J. (2007) Curcumin induces hemeoxygenase 1 through generation of reactive oxygen species, p38 activation and phosphatase inhibition. *Int. J. Mol. Med*. **19**, 165-172.

Nishizawa, A., Hoda, G. and Tabata, M. (1989) Determination of final steps in biosynthesis of essential oil components in *Perillafrutescens*. *Planta Med*. **55**, 251-253.

Otterbein, L. E. and Choi, A. M. (2000) Hemeoxygenase: colors of defense against cellular stress. *Am. J. Physiol. Lung Cell Mol. Physi-*

ol. **279**, L1029-L1037
Park, P. H., Kim, H. S., Jin, X. Y., Jin, F., Hur, J., Ko, G. and Sohn, D. H. (2009a) KB-34, a newly synthesized chalcone derivative, inhibits lipopolysaccharide-stimulated nitric oxide production in RAW264.7 macrophages via heme oxygenase-1 induction and blockade of activator protein-1. *Eur. J. Pharmacol*. **606**, 215-224.
Park, Y. D., Kang, M. A., Lee, H. J., Jin, C. H., Choi, D. S., Kim, D. S., Kang, S. Y., Byun, M. W. and Jeong, I. Y. (2009b) Inhibition of an inducible nitric oxide synthase expression by a hexane extract from *Perilla frutescens* cv. Chookyoupjaso mutant induced by mutagenesis with gamma-ray. *Bangsaseon San Eob Haghoeji* **3**, 13-18.
Rojo, A. I., Salina, M., Salazar, M., Takahashi, S., Suske, G., Calvo, V., de Sagarra, M. R. and Cuadrado, A. (2006) Regulation of heme oxygenase-1 gene expression through the phosphatidylinositol 3-kinase/PKC-ζ pathway and Sp1. *Free Radic. Biol. Med.* **41**, 247-261.
Ryter, S. W., Alam, J. and Choi, A. M. (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic application. *Physiol. Rev*. **86**, 583-650.
Shih, R. H., Cheng, S. E., Hsiao, L. D., Kou, Y. R. and Yang, C. M. (2011) Cigarette smoke extract upregulates heme oxygenase-1 via PKC/NADPH oxidase/ROS/PDGFR/PI3K/Akt pathway in mouse brain endothelial cells. *J. Neuroinflammation* **8**, 104
Thapa, D., Meng, P., Bedolla, R. G., Reddick, R. L., Kumar, A. P. and Ghosh, R. (2014) NQO1 suppresses NF-κB-p300 interaction to regulate inflammatory mediators associated with prostate tumorigenesis. *Cancer Res.* **74**, 5644-5655.
True, A. L., Olive, M., Boehm, M., San, H., Westrick, R. J., Raghavachari, N., Xu, X., Lynn, E. G., Sack, M. N., Munson, P. J., Gladwin, M. T. and Nabel, E. G. (2007) Heme oxygenase-1 deficiency accelerates formation of arterial thrombosis through oxidative damage to the endothelium, which is rescued by inhaled carbon monoxide. *Circ. Res*. **101**, 893-901.
Tsoyi, K., Kim, H. J., Shin, J. S., Kim, D. H., Cho, H. J., Lee, S. S., Ahn, S. K., Yun-Choi, H. S., Lee, J. H., Seo, H. G. and Chang, K. C. (2008) HO-1 and JAK-2/STAT-1 signals are involved in preferential inhibition of iNOS over COX-2 gene expression by newly synthesized tetrahydroisoquinoline alkaloid, CKD712, in cells activated with lipopolysaccharide. *Cell. Signal.* **20**, 1839-1847.
Turdi, S., Han, X., Huff, A. F., Roe, N. D., Hu, N., Gao, F. and Ren, J. (2012) Cardiac-specific overexpression of catalase attenuates lipopolysaccharide-induced myocardial contractile dysfunction: Role of autophagy. *Free Radic. Biol. Med*. **53**, 1327-1338.
Ueda, H. and Yamazaki, M. (1997) Inhibition of tumor necrosis factor-α production by orally administering a perilla leaf extract. *Biosci. Biotechnol. Biochem.* **61**, 1292-1295.
Yamada, N., Yamaya, M., Okinaga, S., Nakyama, K., Sekizawa, K., Shibahara, S. and Sasaki, H. (2000) Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am. J. Hum. Genet.* **66**, 187-195.
Zhang, M., An, C., Gao, Y., Leak, R. K., Chen, J. and Zhang, F. (2013) Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Prog. Neurobiol.* **100**, 30-47.
Zhang, X., Wang, G., Gurley, E. C. and Zhou, H. (2014) Flavonoid apigenin inhibits lipopolysaccharide-induced inflammatory response through multiple mechanism in macrophages. *PLoS ONE* **9**, e107072.