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Methyl *p*-Hydroxycinnamate Suppresses Lipopolysaccharide-Induced Inflammatory Responses through Akt Phosphorylation in RAW264.7 Cells

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Abstract

Derivatives of caffeic acid have been reported to possess diverse pharmacological properties such as anti-inflammatory, anti-tumor, and neuroprotective effects. However, the biological activity of methyl *p*-hydroxycinnamate, an ester derivative of caffeic acid, has not been clearly demonstrated. This study aimed to elucidate the anti-inflammatory mechanism of methyl *p*-hydroxycinnamate in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. Methyl *p*-hydroxycinnamate significantly inhibited LPS-induced excessive production of pro-inflammatory mediators such as nitric oxide (NO) and PGE_2 and the protein expression of iNOS and COX-2. Methyl *p*-hydroxycinnamate also suppressed LPS-induced overproduction of pro-inflammatory cytokines such as IL-1β and TNF-α. In addition, methyl *p*-hydroxycinnamate significantly suppressed LPS-induced degradation of IκB, which retains NF-κB in the cytoplasm, consequently inhibiting the transcription of pro-inflammatory genes by NF-κB in the nucleus. Methyl *p*-hydroxycinnamate exhibited significantly increased Akt phosphorylation in a concentration-dependent manner. Furthermore, inhibition of Akt signaling pathway with wortmaninn abolished methyl *p*-hydroxycinnamate-induced Akt phosphorylation. Taken together, the present study clearly demonstrates that methyl *p*-hydroxycinnamate exhibits anti-inflammatory activity through the activation of Akt signaling pathway in LPS-stimulated RAW264.7 macrophage cells.

Key Words: Methyl *p*-hydroxycinnamate, RAW 264.7 cells, Lipopolysaccharide, iNOS, COX-2, NF-κB

INTRODUCTION

Although macrophages play an essential role in host defense against a variety of infections (Rehman *et al*., 2012), aberrant activation of macrophages has also been reported to play pathogenic roles in inflammatory disorders such as rheumatoid arthritis, atherosclerosis, and sepsis (Kim *et al*., 2012). In those conditions, pathogenic macrophages produce excessive amount of various pro-inflammatory mediators and cytokines through NF-κB-mediated transcription, which eventually leads to the aggravation of the conditions (Itharat and Hiransai, 2012). The most common cause of macrophage activation is an exposure to lipopolysacharide (LPS), which is a component of the outer membrane of Gram-negative bacteria (Rietschel and Brade, 1992). It has been widely reported that LPS-induced activation of macrophages results in a wide range of responses, including secretion of pro-inflammatory mediators, expression of adhesion molecules and coagulation factors, phagocytosis, and cytoskeletal rearrangement (Sweet and Hume, 1996). Therefore, attenuation of aberrant macrophage activation may have important therapeutic potential for the treatment of inflammation-related disorders.

Hydroxycinnamic acids have been reported to possess a wide variety of biological properties such as anti-inflammatory, anti-tumor, and neuroprotective activities (Nagasaka *et al*., 2007; Steinbrecher *et al*., 2008; Kim, 2010). Recently, it has been reported that 3,4,5-trihydroxycinnamic acid, a derivative of hydroxycinnamic acid, significantly attenuates LPS-induced inflammatory responses in microglial cells (Lee *et al*., 2012; Lee *et al*., 2013a). In addition, ester derivatives of hydroxycinnamic acids have been also reported to exhibit various biological activities (Lee *et al*., 2011). Caffeic acid phenylethyl ester

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(CAPE) has been reported to inhibit cytokine-mediated NF-κB activation in murine macrophage cells (Lee *et al*., 2010). Methyl *p*-hydroxycinnamate (MH), a methyl ester of hydroxycinnamic acid, lacks only a hydroxyl group in the catechol of caffeic acid methyl ester. Methyl *p*-hydroxycinnamate has been reported to possess a variety of biological activities such as anti-tumor (Hooper *et al*., 1984), anti-oxidant (Kwon and Kim, 2003), and depigmenting (Kubo *et al*., 2004) activities. Recently, it has been also reported that methyl *p*-hydroxycinnamate possesses anti-adipogenic activity (Lee *et al*., 2013c). However, the underlying mechanism by which methyl *p*-hydroxycinnamate exerts its biological activities has not been clearly demonstrated. Methyl *p*-hydroxycinnamate has been reported to be present in various medicinal plants including *Clausena harmandiana* (Maneerat *et al*., 2013), *Plumeria obtuse* (Saleem *et al*., 2011), and *Idesia polycarpa* (Lee *et al*., 2013c). Methyl *p*-hydroxycinnamate, used in the present study, was isolated from the stem of *Sorghum bicolor* (Kwon and Kim, 2003).

The present study was carried out to examine the anti-inflammatory activity of methyl *p*-hydroxycinnamate and its underlying mechanism in LPS-stimulated RAW264.7 macrophage cells in order to provide a novel pharmacological agent that could inhibit abnormally activated macrophages in inflammatory conditions.

MATERIALS AND METHODS

Reagents and cell culture

Bacterial lipopolysaccharide (LPS) from *Escherichia coli* serotype 055:B5 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methyl *p*-hydroxycinnamate was isolated and identified from the stem of *Sorghum bicholor* (Kwon and Kim, 2003) (Fig. 1). The compound was dissolved in dimethyl sulfoxide (DMSO) and added to the cell culture at the desired concentrations. The macrophage RAW264.7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) containing 5% heat-inactivated fetal bovine serum and penicillin-streptomycin (Gibco BRL) at 37°C, 5% CO_2. In all experiments, cells were incubated in the presence of the indicated concentration of methyl *p*-hydroxycinnamate before the addition of LPS (200 ng/ml).

Cell viability

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The RAW264.7 cells were seeded at 5×10^5 cells per well and incubated with methyl *p*-hydroxycinnamate at various concentrations for 24 hr at 37°C. After incubation, MTT (0.5 mg/ml in PBS) was added to each well, and the cells were incubated for 3 hr at 37°C and 5% CO_2. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO). Absorbance was determined at 540 nm. The results were expressed as a percentage of surviving cells over control cells.

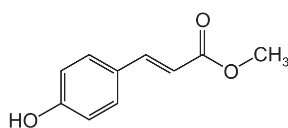

Fig. 1. Chemical structure of methyl *p*-hydroxycinnamate (MH).

Nitrite quantification assay

The production of NO was estimated by measuring the amount of nitrite, a stable metabolite of NO, using the Griess reagent as described (Lee *et al*., 2012). After methyl *p*-hydroxycinnamate-pretreated RAW264.7 macrophage cells were stimulated with LPS in 12-well plates for 24 hr, 100 μl of the cell supernatant was mixed with an equal volume of Griess reagent. Light absorbance was read at 540 nm. The results were expressed as a percentage of released NO from LPS-stimulated RAW264.7 cells. To prepare a standard curve, sodium nitrite was used to prepare a standard curve.

Western blot analysis

The RAW264.7 macrophage cells were pretreated with methyl *p*-hydroxycinnamate for 1 hr and then stimulated with LPS. Cells were washed with PBS and lysed in PRO-PREP lysis buffer (iNtRON Biotechnology, Seongnam, Korea). Equal amounts of protein were separated on 10% SDS-polyacrylamide gel. Proteins were transferred to Hypond PVDF membrane (Amersham Biosciences, Piscataway, NJ, USA) and blocked in 5% skim milk in TBST for 1 hr at room temperature. Specific antibodies against iNOS, COX-2, extracellular signal-regulated kinase (ERK), phosphorylated (p)-ERK, p38, p-p38, c-Jun N-terminal kinase Akt, p-Akt (1:1,000; Cell Signaling Technology), IκB-α (1:1,000; Santa Cruz Biotechnology Inc), and β-actin (1:2,500; Sigma) were diluted in 5% skim milk. After thoroughly washing with TBST, horseradish peroxidase-conjugated secondary antibodies were applied. The blots were developed by the enhanced chemiluminescence detection (Amersham Biosciences).

ELISA assay for cytokines

The RAW264.7 macrophage cells were treated with methyl *p*-hydroxycinnamate in the absence or presence of LPS. After 24 hr incubation, TNF-α and IL-1β levels in culture media were quantified using monoclonal anti-TNF-α or IL-1β antibodies according to the manufacturer's instruction (R&D Systems).

Statistical analysis

All values shown in the figures are expressed as the mean ± SD obtained from at least three independent experiments. Statistical significance was analyzed by two-tailed Student's *t*-test. Data with values of $p<0.05$ were considered as statistically significant. Single (* and #) and double (** and ##) arks represent statistical significance in $p<0.05$ and $p<0.01$, respectively.

RESULTS

Methyl *p*-hydroxycinnamate suppresses NO and PGE_2 production in LPS-stimulated RAW264.7 macrophage cells

Excessive production of pro-inflammatory mediators such as NO and PGE_2 is a characteristic feature of inflammatory conditions of macrophages (Ock *et al*., 2009; Lee *et al*., 2012). Therefore, suppressive effects of methyl *p*-hydroxycinnamate on NO and PGE_2 production in LPS-stimulated RAW264.7 macrophage cells were examined. Cells were incubated with indicated concentrations of methyl *p*-hydroxycinnamate for 1

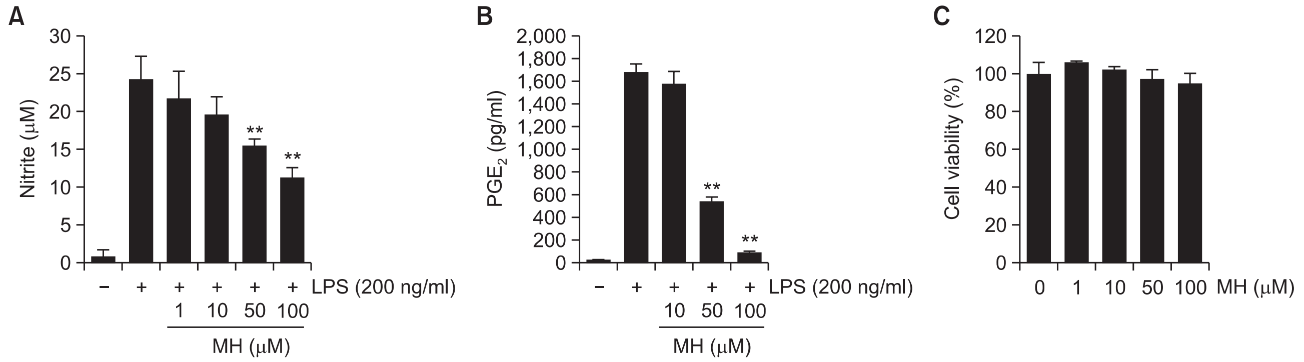

Fig. 2. MH attenuated LPS-induced NO and PGE_2 production in RAW264.7 macrophage cells. RAW264.7 cells were pretreated with various indicated concentrations of MH for 1 hr, then incubated with LPS (200 ng/ml) for 24 hrs. The concentrations of NO (A) and PGE_2 (B) in the supernatants were measured by Griess and ELISA assay as described in the methods. MH exhibited a significant suppression of LPS-induced NO (A) and PGE_2 (B) secretion in a concentration-dependent manner. No noticeable cell death was observed at MH concentrations used in the present study (C). The data are expressed as mean ± S.D. for three or more independent experiments. **Statistically significant differences compared with LPS treatments alone, $p<0.01$.

hr prior to LPS treatment and, for NO measurement, Griess reaction was used as an index for NO synthesis. Methyl *p*-hydroxycinnamate significantly inhibited NO production in LPS-stimulated RAW264.7 cells (Fig. 2A) and PGE_2 production in a concentration-dependent manner (Fig. 2B). In addition, methyl *p*-hydroxycinnamate showed no noticeable cytotoxicity in concentration ranges (Fig. 2C).

Methyl *p*-hydroxycinnamate inhibits LPS-induced protein expression of iNOS and COX-2

As methyl *p*-hydroxycinnamate inhibited LPS-induced NO and PGE_2 production in RAW264.7 cells (Fig. 2), protein expression levels of iNOS and COX-2 were examined using Western blot analysis. LPS resulted in markedly increased expression of iNOS and COX-2 proteins and methyl *p*-hydroxycinnamate significantly suppressed protein levels in a concentration-dependent manner (Fig. 3), demonstrating that suppressed production of NO and PGE_2 is due to decreased expression of their responsible proteins, iNOS and COX-2, respectively, with methyl *p*-hydroxycinnamate treatment.

Methyl *p*-hydroxycinnamate attenuates LPS-induced release of pro-inflammatory cytokines such as IL-1β and TNF-α

To examine the effects of methyl *p*-hydroxycinnamate on the extracellular secretion of pro-inflammatory cytokines such as IL-1β and TNF-α, secretion of these cytokines was examined using ELISA assay in LPS-stimulated RAW264.7 cells. LPS treatment resulted in excessive extracellular release of IL-1β and TNF-α in RWA 264.7 cells. Methyl *p*-hydroxycinnamate significantly attenuated LPS-induced extracellular release of IL-1β and TNF-α (Fig. 4).

Methyl *p*-hydroxycinnamate attenuates LPS-induced degradation of IKB

NF-κB has been widely considered to be a predominant transcription factor of multiple pro-inflammatory genes. IκB inhibits the nuclear translocation of NF-κB by sequestering NF-κB in the cytoplasm given the fact that cytosolic NF-κB has to translocate to the nucleus for transcription of pro-inflammatory genes (Zheng *et al*., 2008b; Ock *et al*., 2009). In the present study, whether methyl *p*-hydroxycinnamate inhibits LPS-induced degradation of cytosolic IκB was examined. LPS treatment resulted in evident degradation of IκB and methyl *p*-hydroxycinnamate attenuated LPS-induced degradation of IκB in a concentration-dependent manner (Fig. 5), suggesting that methyl *p*-hydroxycinnamate presumably suppress LPS-induced NF-κB activity in RAW264.7 cells.

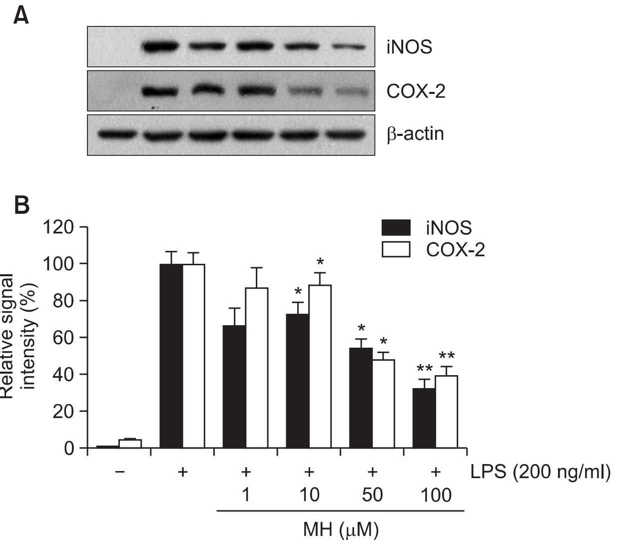

Fig. 3. MH suppressed LPS-induced iNOS and COX-2 expressions in RAW264.7 macrophage cells. (A) RAW264.7 cells were pretreated with various indicated concentrations of MH for 1 hr, then incubated with LPS (200 ng/ml) for 24 hrs. The cell lysates were prepared and subjected to Western blotting analysis by using antibodies specific for iNOS and COX-2 as described in the methods. (B) The relative protein levels were quantified by scanning densitometry and normalized to β-actin. The data indicated that MH significantly decreased the over-expression of iNOS and COX-2 induced by LPS in a concentration-dependent manner. The images shown are representatives of three independent experiments that showed consistent results and the relative protein values are expressed as mean ± S.D. for three experiments. *$p<0.05$ and **$p<0.01$ indicate statistically significant differences from treatments with LPS alone.

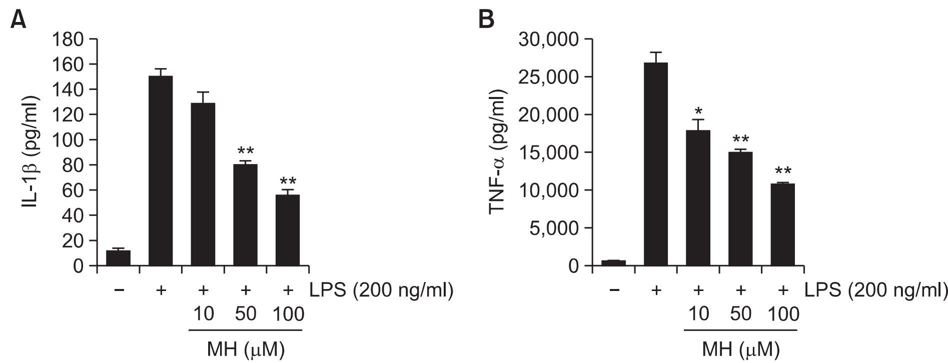

Fig. 4. MH inhibited the release of IL-1β and TNF-α cytokines stimulated by LPS in RAW264.7 macrophage cells. RAW264.7 cells were pretreated with indicated concentrations of MH for 1 hr, then incubated with LPS (200 ng/ml) for 24 hrs. The concentrations of IL-1β (A) and TNF-α (B) in collected cell culture media were measured by ELISA assay as described in the methods. MH meaningfully reduced LPS-stimulated both IL-1β and TNF-α cytokines in a concentration-dependent manner. The values are expressed as mean ± S.D. for three independent experiments. **p*<0.05 and ***p*<0.01 indicate statistically significant differences from treatments with LPS alone.

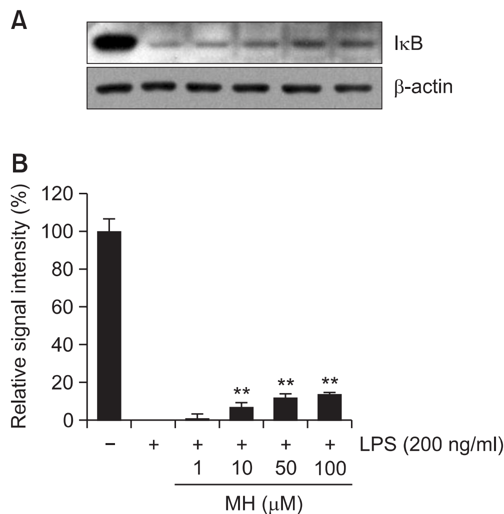

Fig. 5. MH slightly prevented IκBα degradations caused by LPS in RAW264.7 macrophage cells. (A) RAW264.7 cells were pretreated with various indicated concentrations of MH for 1 hr, then exposed to LPS (200 ng/ml) for 15 mins. The cell lysates were prepared and subjected to Western blotting analysis by using antibodies specific for IκBα as described in the methods. (B) The relative protein levels were quantified by scanning densitometry and normalized to β-actin. The data showed that MH significantly prevented LPS-induced IκBα degradations, albeit not strong enough to completely recover. The images shown are representatives of three independent experiments that showed consistent results and the relative protein values are expressed as mean ± S.D. for three experiments. *Statistically significant differences compared with LPS treatments alone, *p*<0.05.

Akt signaling pathway mediates the anti-inflammatory activity of methyl *p*-hydroxycinnamate

To examine the underlying signaling pathway by which methyl *p*-hydroxycinnamate exerts its anti-inflammatory activity, possible involvement of multiple kinase signaling pathways was examined. The effect of methyl *p*-hydroxycinnamate on LPS-stimulated phosphorylation of MAP (JNK, ERK, and p38) kinases and Akt in RAW264.7 macrophage cells was measured. Cells were pretreated with methyl *p*-hydroxycinnamate at various concentrations and then were treated with LPS (200 ng/ml) for 30 min. No significant changes were observed in the phosphorylation levels of MAP kinases (Fig. 6). However, methyl *p*-hydroxycinnamate significantly resulted in the increased phosphorylation of Akt in the LPS-stimulated RAW264.7 cells (Fig. 7). Furthermore, inhibition of Akt signaling pathway with wortmannin significantly abolished the methyl *p*-hydroxycinnamate-induced Akt phosphorylation (Fig. 8A), suggesting that activation of Akt signaling mediate the anti-inflammatory action of methyl *p*-hydroxycinnamate. However, inhibition of PI3K/Akt pathway with wortmannin did not completely recover methyl *p*-hydroxycinnamate-induced suppression of iNOS and COX-2 expressions (Fig. 8B), suggesting that other signaling pathways rather than Akt/PI3K might be also involved in the anti-inflammatory property of methyl *p*-hydroxycinnamate.

DISCUSSION

The present study clearly demonstrated that methyl *p*-hydroxycinnamate possesses anti-inflammatory properties through the activation of Akt signaling pathway in LPS-stimulated RAW264.7 macrophage cells. Methyl *p*-hydroxycinnamate significantly attenuated LPS-induced secretion of NO and PGE_2 and expression of iNOS and COX-2. Methyl *p*-hydroxycinnamate also suppressed the secretion of pro-inflammatory cytokines such as IL-1β and TNF-α, and significantly attenuated LPS-induced IκB degradation.

Methyl *p*-hydroxycinnamate belongs to a family of phenylpropanoids, which are a group of plant-derived secondary metabolites that are biosynthesized from phenylalanine to coumaric acid and other acids including caffeic acid, ferulic acid, and trihydroxycinnamic acid. Hydroxycinnamic acids and their ester derivatives have been reported to possess a variety of pharmacological activities including antitumor, antiviral, anti-inflammatory, immunosuppressive, and neuroprotective activities (Nagasaka *et al*., 2007; Kim, 2010; Lee *et al*., 2011). Recently, we observed that 3,4,5-trihydroxycinnamic acid (THC), a derivative of hydroxycinnamic acids, significantly attenuated LPS-induced inflammatory responses through the activation of a cytoprotective transcription signal-

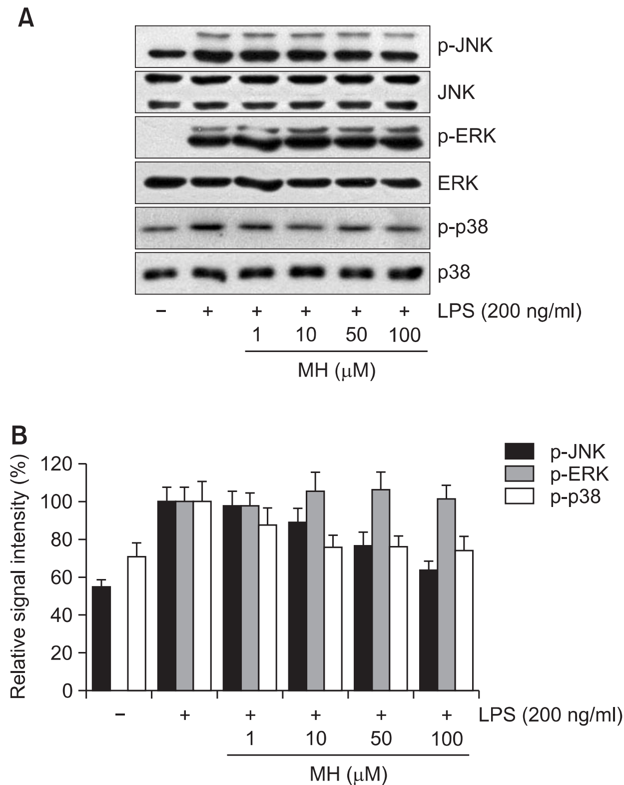

Fig. 6. MH did not affect MAPKs signaling pathways in RAW264.7 macrophage cells. (A) RAW264.7 cells were pretreated with various indicated concentrations of MH for 1 hr, then exposed to LPS (200 ng/ml) for 30 mins. The cell lysates were prepared and subjected to Western blotting analysis by using antibodies specific for total and phosphorylated forms (shown as p-) of JNK, ERK and p38. (B) The relative protein levels of p-JNK, p-ERK and p-p38 were quantified by scanning densitometry and normalized to total JNK, ERK and p38, respectively. The images shown are representatives of three independent experiments that showed consistent results and the relative protein values are expressed as mean ± S.D. for three experiments. The data presented no effect of MH on MAPKs.

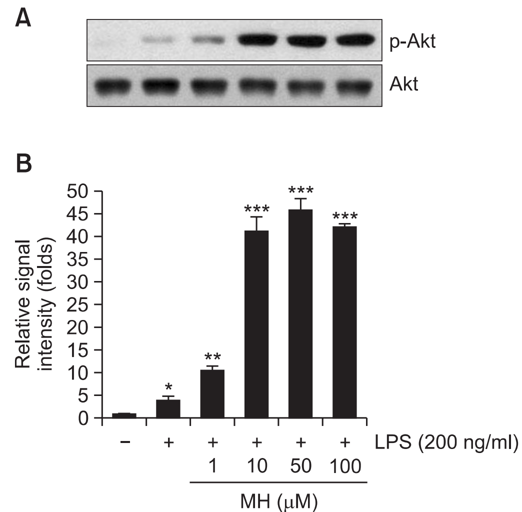

Fig. 7. MH significantly increased Akt phosphorylation in RAW264.7 macrophage cells. (A) RAW264.7 cells were pretreated with various indicated concentrations of MH for 1 hr, then exposed to LPS (200 ng/ml) for another 1 hr. The cell lysates were prepared and subjected to Western blotting analysis by using antibodies specific for total and phosphorylated forms of Akt. (B) The relative protein levels of p-Akt were quantified by scanning densitometry and normalized to total Akt. The data exhibited that MH strongly increased Akt activation in a concentration-dependent manner. The images shown are representatives of three independent experiments that showed consistent results and the relative protein values are expressed as mean ± S.D. for three experiments. *$p<0.05$, **$p<0.01$, ***$p<0.001$ indicate statistically significant differences from treatments with control alone.

ing pathway of Nrf2 (Lee *et al*., 2013a). Ester derivatives of phenylpropanoids have been reported to exhibit a wide range of biological activities including antioxidant, anti-inflammatory, and anti-apoptotic properties (Lapchak, 2007; Wei *et al*., 2008; Jiang *et al*., 2010). Previously, we demonstrated that 1-docosanoyl cafferate (DC), a high molecular fatty alcohol ester of caffeic acid, exhibited a significant anti-inflammatory activity in LPS-stimulated murine immortalized microglial cells (Lee *et al*., 2011). In the present study, methyl *p*-hydroxycinnamate, a methyl ester of hydroxycinnamic acid, showed significant anti-inflammatory activity in LPS-stimulated macrophage cells.

Macrophages play essential roles in the host defense against bacterial infection (Rehman *et al*., 2012). However, abnormally activated macrophages also play a key role in inflammation-related disorders including sepsis by producing a wide variety of pro-inflammatory mediators and cytokines (Rietschel and Brade, 1992). Macrophages have been reported to undertake LPS-induced gene transcription when LPS binds to its membrane receptor, TLR4, which leads to the phosphorylation of multiple kinases, which in turn activates various transcription factors including NF-κB and AP-1 families (O'Connell *et al*., 1998; Guha and Mackman, 2001). We previously observed that LPS causes increased expression of pro-inflammatory mediators and the increased degradation IκB (Vo *et al*., 2012; Lee *et al*., 2013b). LPS exhibited noticeable degradation of IκB in the present study. Methyl *p*-hydroxycinnamate significantly attenuated LPS-induced IκB degradation. In addition, methyl *p*-hydroxycinnamate suppressed LPS-induced extracellular secretion of IL-1β and TNF-α. Although IL-1β and TNF-α and NF-κB have been reported to play an essential role in inflammatory response, other cytokines and transcription factors might also play a certain role. Therefore, further studies are necessary to clearly elucidate the effect of methyl *p*-hydroxycinnamate on wide range of cytokines and transcription factors in LPS-mediated inflammatory conditions.

Akt signaling pathway has been reported to exert a significant anti-inflammatory effect through the regulation of pro-inflammatory mediators through the suppression of NF-κB-mediated transcription (Ha *et al*., 2011). The present data showed that phosphorylation of Akt was significantly increased with methyl *p*-hydroxycinnamate. Furthermore, blockade of Akt with wortmannin, a PI3K inhibitor, significantly attenuated methyl *p*-hydroxycinnamate-induced Akt phosphorylation, suggesting that Akt signaling pathway plays a key role in methyl *p*-hydroxycinnamate-induced Akt phosphorylation. However, inhibition of PI3K/Akt pathway with wortmannin could not completely recover methyl *p*-hydroxycinnamate-induced suppression of iNOS and COX-2 expression, suggesting that although Akt might play a major role in

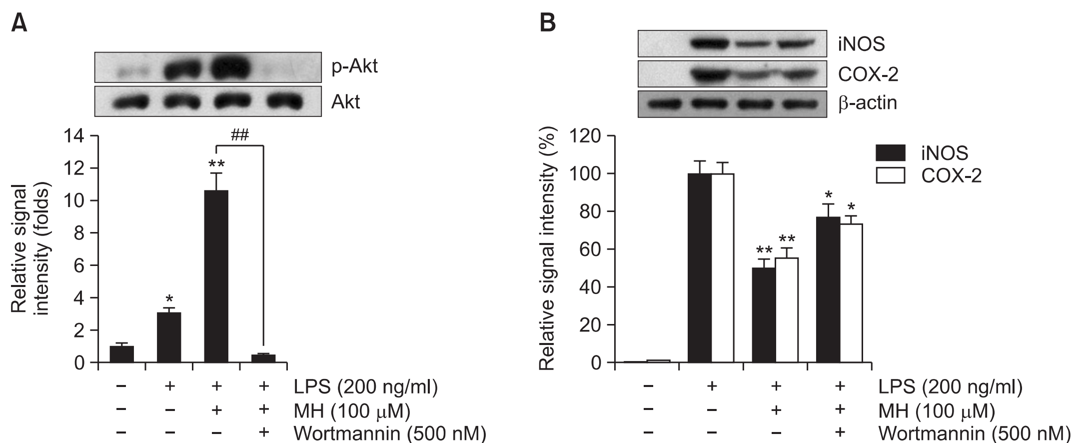

Fig. 8. PI3K/Akt was required for MH-mediated suppression of LPS-induced iNOS and COX-2 expressions in RAW264.7 macrophage cells. RAW264.7 cells were pretreated with MH (100 μM) for 1 hr in the presence or absence of wortmannin, then exposed to LPS (200 ng/ml) for 1 hr. The cell lysates were prepared and subjected to Western blotting analysis by using antibodies specific for total and phosphorylated forms of Akt, which showed that wortmannin completely abolished MH-mediated Akt activation (A). The relative protein levels of iNOS and COX-2 were also examined. MH-mediated suppression of LPS-induced iNOS and COX-2 expressions were significantly attenuated with wortmannin but not completely (B). The images shown are representatives of three independent experiments that showed consistent results and the relative protein values are expressed as mean ± S.D. for three experiments. *$p<0.05$, **$p<0.01$ indicate statistically significant differences from treatments with control alone. ##$p<0.01$ represents statistical significance between two indicated groups.

methyl *p*-hydroxycinnamate-mediated anti-inflammatory actions, other signaling pathway might be also involved in the anti-inflammatory property of methyl *p*-hydroxycinnamate. Although MAPK signaling pathways have been reported to play certain roles in anti-inflammatory mechanism (Jung *et al*., 2010), no noticeable changes were not observed in the present study, suggesting that the anti-inflammatory activity of methyl *p*-hydroxycinnamate presumably be through the activation of Akt, but not MAPK, signaling pathway in the present cell culture model. However, further studies are necessary to delineate the exact mechanism by which methyl *p*-hydroxycinnamate activates Akt signaling pathway.

NF-κB is a major transcription factor responsible for the expression of a variety of pro-inflammatory genes such as mediators such as iNOS, COX-2, cytokines (Siebenlist *et al*., 1994; Kuprash *et al*., 1995). Therefore, the aberrant activation of NF-κB has been associated with various pathological conditions including cancers and autoimmune diseases (Karin *et al*., 2001; Li and Verma, 2002). LPS has been reported to cause the nuclear translocation of p65 subunit of NF-κB through increased degradation of IκB (Moon *et al*., 2007; Zheng *et al*., 2008a). The present data showed that methyl *p*-hydroxycinnamate significantly attenuated LPS-induced IκB degradation, which presumably prevents subsequent nuclear translocation of p65 in LPS-induced RAW264.7 macrophage cells. We previously observed that inhibition of IκB degradation eventually retains p65 subunit of NF-κB in cytoplasm (Lee *et al*., 2013b). However, further studies are necessary to delineate the exact mechanism by which methyl *p*-hydroxycinnamate-mediated Akt activation leads to the attenuation of IκB degradation.

In conclusion, the data clearly demonstrate that methyl *p*-hydroxycinnamate possesses anti-inflammatory activity such as suppression of NO and PGE_2 production and cytokine secretion through the inhibition of nuclear translocation of NF-κB in LPS-stimulated RAW264.7 macrophage cells. The anti-inflammatory activity of methyl *p*-hydroxycinnamate presumably considered to be through the activation of Akt signaling pathway. However, further studies are necessary to delineate the exact mechanism by which methyl *p*-hydroxycinnamate activates Akt signaling pathway. The present study strongly suggests that methyl *p*-hydroxycinnamate might be a valuable therapeutic adjuvant in the treatment of inflammation-related pathogenic conditions such as rheumatoid arthritis, atherosclerosis, and sepsis.

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