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 lipopolysaccharide (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 (CEF) 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 PGE2 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 wortmannin 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

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 PGE2, 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 wortmannin 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.

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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-hydroxyccinnamate (MH), a methyl ester of hydroxyccinnamic acid, lacks only a hydroxyl group in the catechol of caffeic acid methyl ester. Methyl p-hydroxyccinnamate 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-hydroxyccinnamate possesses anti-adipogenic activity (Lee et al., 2013c). However, the underlying mechanism by which methyl p-hydroxyccinnamate exerts its biological activities has not been clearly demonstrated. Methyl p-hydroxyccinnamate has been reported to be present in various medicinal plants including Clauessa harmandiana (Maneerat et al., 2013), Plumeria obtuse (Saleem et al., 2011), and Idesia polycarpa (Lee et al., 2013c). Methyl p-hydroxyccinnamate, 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-hydroxyccinnamate 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-hydroxyccinnamate was isolated and identified from the stem of Sorghum bicolor (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, Grand Island, NY, USA) at 37°C, 5% CO₂. In all experiments, cells were incubated in the presence of the indicated concentration of methyl p-hydroxyccinnamate 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 5×10⁵ cells per well and incubated with methyl p-hydroxyccinnamate 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% CO₂. 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.

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-hydroxyccinnamate-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-hydroxyccinnamate 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 Hybond 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-hydroxyccinnamate 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 double (**) arks represent statistical significance in p<0.05 and p<0.01, respectively.

RESULTS

Methyl p-hydroxyccinnamate suppresses NO and PGE₂ production in LPS-stimulated RAW264.7 macrophage cells

Excessive production of pro-inflammatory mediators such as NO and PGE₂ is a characteristic feature of inflammatory conditions of macrophages (Ock et al., 2009; Lee et al., 2012). Therefore, suppressive effects of methyl p-hydroxyccinnamate on NO and PGE₂ production in LPS-stimulated RAW264.7 macrophage cells were examined. Cells were incubated with indicated concentrations of methyl p-hydroxyccinnamate for 1 hr.

![Fig. 1. Chemical structure of methyl p-hydroxyccinnamate (MH).](www.biomolther.org)
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 PGE₂ 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 PGE₂ 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 PGE₂ 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 degradation of IκB

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.
Vo et al.  Anti-Inflammatory Effects of Methyl p-Hydroxycinnamate

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 PGE2 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-
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 ρ-hydroxycinnamate significantly attenuated LPS-induced IκB degradation. In addition, methyl ρ-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 ρ-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 ρ-hydroxycinnamate. Furthermore, blockade of Akt with wortmannin, a PI3K inhibitor, significantly attenuated methyl ρ-hydroxycinnamate-induced Akt phosphorylation, suggesting that Akt signaling pathway plays a key role in methyl ρ-hydroxycinnamate-induced Akt phosphorylation. However, inhibition of PI3K/Akt pathway with wortmannin could not completely recover methyl ρ-hydroxycinnamate-induced suppression of iNOS and COX-2 expression, suggesting that although Akt might play a major role in

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 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.

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.
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 PGE2 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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REFERENCES

Guha, M. and Mackman, N. (2001) LPS induction of gene expression in human monocytes. Cell. Signal. 13, 85-94.

Ha, Y. M., Ham, S. A., Kim, Y. M., Lee, Y. S., Kim, H. J., Seo, H. G., Lee, J. H., Park, M. K. and Chang, K. C. (2011) β-adrenergic receptor-mediated HO-1 induction, via PI3K and p38 MAPK, by isoproterenol in RAW264.7 cells leads to inhibition of HMGB1 release in LPS-activated RAW264.7 cells and increases in survival rate of CLP-induced septic mice. Biochem. Pharmacol. 82, 769-777.

Hooper, S. N., Jurgens, T., Chandler, R. F. and Stevens, M. F. G. (1984) Methyl p-coumarate: a cytotoxic constituent from Comptonia peregrina. Phytochemistry 23, 2096-2097.

Itharat, A. and Hiransai, P. (2012) Dioscoreanone suppresses LPS-induced nitric oxide production and inflammatory cytokine expression in RAW264.7 macrophages by NF-kappaB and ERK1/2 signaling transduction. J. Cell. Biochem. 113, 3427-3435.

Jiang, W. L., Tian, J. W., Fu, F. H., Zhu, H. B. and Hou, J. (2010) Neuroprotective efficacy and therapeutic window of Forsythoside B: in a rat model of cerebral ischemia and reperfusion injury. Eur. J. Pharmacol. 640, 75-81.

Jung, W. K., Lee, D. Y., Park, C., Choi, Y. H., Choi, I., Park, S. G., Seo, S. K., Lee, S. W., Yea, S. S., Ahn, S. C., Lee, C. M., Park, W. S., Ko, J. H. and Choi, I. W. (2010) Cistolastol is anti-inflammatory in BV2 microglial cells by inactivating nuclear factor-kappaB and...
Kubo, I., Nihei, K. and Tsujimoto, K. (2004) Methyl p-coumarate, a
http://dx.doi.org/10.4062/biomolther.2013.095
Biomol Ther 22(1), 10-16 (2014)
Lee, J. W., Bae, C. J., Choi, Y. J., Kim, S. I., Kim, N. H., Lee, H. J.,
Kwon, Y. S. and Kim, C. M. (2003) Antioxidant constituents from the
melanin formation inhibitor in B16 mouse melanoma cells. Bioorg.
Med. Chem. 12, 5349-5354.
Kuprash, D. V., Udalova, I. A., Turetskaya, R. L., Rice, N. R. and Nedo-
spasov, S. A. (1995) Conserved kappa B element located down-
stream of the tumor necrosis factor alpha gene: distinct NF-kappa B
binding pattern and enhancer activity in LPS activated murine
macrophages. Oncogene 11, 97-106.
Kwon, Y. S. and Kim, C. M. (2003) Antioxidant constituents from the
stem of Sorghum bicolor. Arch. Pharm. Res. 26, 535-539.
Lapchak, P. A. (2007) The phenylpropanoid mucronin chlorogenic acid
improves clinical rating scores in rabbits following multiple in-
farct ischemic strokes: synergism with tissue plasminogen activa-
tor. Exp. Neurol. 205, 407-413.
Lee, J. W., Bae, C. J., Choi, Y. J., Kim, S. I., Kim, N. H., Lee, H. J.,
Kim, S. S., Kwon, Y. S. and Chun, W. (2012) 3,4,5-Trimethoxy-
nyloxyacetamide compound KT-15087 in microglia cells. Biol. Pharm.
Biotechnol. Biochem. 76, 1122-1127.
Kubo, I., Nihei, K. and Tsujiimoto, K. (2004) Methyl p-coumarate, a
melanin formation inhibitor in B16 mouse melanoma cells. Bioorg.
Med. Chem. 12, 5349-5354.
Kuprash, D. V., Udalova, I. A., Turetskaya, R. L., Rice, N. R. and Nedo-
spasov, S. A. (1995) Conserved kappa B element located down-
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Kwon, Y. S. and Kim, C. M. (2003) Antioxidant constituents from the
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Lapchak, P. A. (2007) The phenylpropanoid mucronin chlorogenic acid
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tor. Exp. Neurol. 205, 407-413.
Lee, J. W., Bae, C. J., Choi, Y. J., Kim, S. I., Kim, N. H., Lee, H. J.,
Kim, S. S., Kwon, Y. S. and Chun, W. (2012) 3,4,5-Trimethoxy-
nyloxyacetamide compound KT-15087 in microglia cells. Biol. Pharm.
Biotechnol. Biochem. 76, 1122-1127.
Kubo, I., Nihei, K. and Tsujiimoto, K. (2004) Methyl p-coumarate, a
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Med. Chem. 12, 5349-5354.
Kuprash, D. V., Udalova, I. A., Turetskaya, R. L., Rice, N. R. and Nedo-
spasov, S. A. (1995) Conserved kappa B element located down-
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macrophages. Oncogene 11, 97-106.
Kwon, Y. S. and Kim, C. M. (2003) Antioxidant constituents from the
stem of Sorghum bicolor. Arch. Pharm. Res. 26, 535-539.
Lapchak, P. A. (2007) The phenylpropanoid mucronin chlorogenic acid
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farct ischemic strokes: synergism with tissue plasminogen activa-
tor. Exp. Neurol. 205, 407-413.
Lee, J. W., Bae, C. J., Choi, Y. J., Kim, S. I., Kim, N. H., Lee, H. J.,
Kim, S. S., Kwon, Y. S. and Chun, W. (2012) 3,4,5-Trimethoxy-
nyloxyacetamide compound KT-15087 in microglia cells. Biol. Pharm.
Biotechnol. Biochem. 76, 1122-1127.
Kubo, I., Nihei, K. and Tsujiimoto, K. (2004) Methyl p-coumarate, a
melanin formation inhibitor in B16 mouse melanoma cells. Bioorg.
Med. Chem. 12, 5349-5354.
Kuprash, D. V., Udalova, I. A., Turetskaya, R. L., Rice, N. R. and Nedo-
spasov, S. A. (1995) Conserved kappa B element located down-
stream of the tumor necrosis factor alpha gene: distinct NF-kappa B
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Kwon, Y. S. and Kim, C. M. (2003) Antioxidant constituents from the
stem of Sorghum bicolor. Arch. Pharm. Res. 26, 535-539.
Lapchak, P. A. (2007) The phenylpropanoid mucronin chlorogenic acid
improves clinical rating scores in rabbits following multiple in-
farct ischemic strokes: synergism with tissue plasminogen activa-
tor. Exp. Neurol. 205, 407-413.
Lee, J. W., Bae, C. J., Choi, Y. J., Kim, S. I., Kim, N. H., Lee, H. J.,
Kim, S. S., Kwon, Y. S. and Chun, W. (2012) 3,4,5-Trimethoxy-
nyloxyacetamide compound KT-15087 in microglia cells. Biol. Pharm.
Biotechnol. Biochem. 76, 1122-1127.