Natural Product Sciences
15(2) : 83-89 (2009)

Anti-inflammatory Effect of MeOH Extracts of the Stem of *Polygonum multiflorum* in LPS-stimulated Mouse Peritoneal Macrophages

Dong Seok Cha[1] and Hoon Jeon[1,2]*

[1]*College of Pharmacy, Woosuk University, Chonbuk 565-701, Korea*
[2]*Center for Healthcare Technology Development, Woosuk University, Chonbuk 565-701, Korea*

Abstract – Polygoni multiflori Ramulus (PM), the stem of *Polygonum multiflorum* Thunb. has been widely used as a traditional medicine for the treatment of lots of diseases. In macrophages, nitric oxide is released as an inflammatory mediator and has been proposed to be an important modulator of many pathophysiological conditions in inflammation. In the present study, it was investigated that the inhibitory effects on NO and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and the mechanism of down-regulation of immune response by 85% methanol extracts of PM in mouse (C57BL/6) peritoneal macrophages. Extracts of PM (0.1, 1 mg/ml) suppressed NO production and showed inhibition of pro-inflammatory cytokines like TNF-α, IL-6 and it attenuated iNOS and COX-2 expression via down-regulation of NF-κB activation. The present results indicate that the 85% methanol extracts of PM has an inhibitory effect on the production of NO through down-regulation of iNOS expression in LPS stimulated mouse peritoneal macrophages and therefore may be beneficial in diseases which related to macrophage-mediated inflammatory disorders.
Keywords – Nitric Oxide, NF-κB, iNOS, COX-2, Polygoni multiflori Ramulus

Introduction

Inflammation is characterized by redness, heat, swelling, pain and dysfunction of the organs. It is first response of the immune system to infection or irritation and may be referred to as the innate cascade including various cells and cytokines. The inflammatory response is out of proportion to the external irritation. Thus, the result can be more damage to the body than the agent itself would have produced. Many types of autoimmune diseases and allergies such as asthma, rheumatoid arthritis and multiple sclerosis are example of excessive inflammatory responses (Rakel *et al*., 2005).

Macrophages play a central role in host defense and maintenance as a major immune cell in inflammation, since they are concerned in not only natural immunity but specific acquired immunity. Lipopolysaccharide (LPS) is a component of the outer cell membrane of gram-negative bacteria. It is an endotoxin, which induces septic shock and stimulates the production of inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukins, prostanoids and leukotrienes (Chen *et al*., 2005; Erridge *et al*., 2002; Hewett *et al*., 1993). The stimulation of macrophages with LPS also induces expression of the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Cao *et al*., 2006).

Expression of an iNOS, which closely related with up-regulation of nuclear factor kappa B (NF-κB), result in catalyze the abundant production of NO from L-arginine and molecular oxygen (Jeon *et al*., 2006). High levels of NO have been described in a variety of patho-physiological processes including various forms of circulatory shock (Szabo *et al*., 1995), inflammation (MacMicking *et al*., 1997) and carcinogenesis (Ohshima *et al*., 1994). NO up-regulates the release of inflammatory mediators by mouse macrophages (Marcinkiewicz *et al*., 1995).

It is widely acknowledged that a number of pro-inflammatory cytokines such as TNF-α and IL-6 play an important role in the regulation of immune response (Lee *et al*., 2005). TNF-α and IL-6, which macrophages release simultaneously by pathogenic germs or toxicants, lead to secondary immune response such as proliferation of T and B cells and killing of microorganisms (Shacter *et al*., 1993; Haslberger *et al*., 1992). It is well known that

*Author for correspondence
Tel: +82-63-290-1576; E-mail: hoonj6343@hanmail.net

NO, synthesized by iNOS, is released from macrophage intimately correlated with the pathophysiology in inflammation and lots of diseases (Kim *et al*., 2005; Thiemermann *et al*., 1990) and increased expression of iNOS and its catalytic activity has been observed in several human tissues and in chemically-induced animal tumors and also in inflammatory disorders (Goldstein *et al*., 1998; Wilson *et al*., 1998; Ambs *et al*., 1998).

Another enzyme that plays a important role in mediating inflammation is COX-2. The inducible isoform, COX-2, is induced by LPS, certain serum factors, growth factors, cytokines and is a predominant cyclooxygenase at a site of inflammation (Thiemermann *et al*., 1990; Shacter *et al*., 1993). Expression of the inducible enzymes such as iNOS, COX-2 and cytokines in macrophages is regulated mainly at the transcriptional level, particularly by NF-κB and it plays a key role in inflammatory responses, cell growth, cell adhesion and apoptosis (Baeuerle *et al*., 1996; Verma *et al*., 1995). NF-κB exists in the cytoplasm of unstimulated cells as a latent form and is bound to IκB, the inhibitory protein (Karin *et al*., 2000). LPS stimulate NF-κB activation by phosphorylation and degradation of IκB-α and then NF-κB is translocated to the nucleus (Sanchez-Perez *et al*., 2002). It binds to DNA at the κB site in the promoter region of target genes and activates gene expression (Chen *et al*., 1995). In mammalian cells, regulation of iNOS expression is predominantly governed by the ubiquitously expressed NF-κB which is required for the inducible expression of genes associated with inflammatory responses (Kim *et al*., 2005). Therefore, inhibition of signal transduction proteins in the pathways leading to activation of NF-κB is now widely recognized as a reasonable strategy to inflammatory disease. Since NO production, related enzymes, pro-inflammatory cytokines might cause inflammatory damage, many studies about inflammation focused to find materials which selective modulate these inflammatory mediators from traditional plant-derived medicines (Lee *et al*., 2005).

The dried stem of *Polygonum multiflorum* Thunb. (Polygonaceae) has been widely used crude drugs as an oriental medicine, with beneficial effects in numerous diseases, including insomnia, hyperhidrosis in Korea and China. The major types of chemical constituents, stilbenes and anthraquinones, were analyzed from *Polygonum multiflorum*. The root of *P. multiflorum* have been found to possess antioxidant (Chiu *et al*., 2002), myocardial protective (Yim *et al*., 1998), neuroprotective (Wang *et al*., 2006; Li *et al*., 2005), anti-aging (Xiao *et al*., 1993), estrogenic (Kang *et al*., 2006; Zhang *et al*., 2005), cognitive enhancing (Um *et al*., 2006; Chan *et al*., 2003), antimutagenic activity (Zhang *et al*., 1999), arthritic pain and skin infections (Li *et al*., 2003). It also has been demonstrated that 2,3,5,4'-tetrahydroxystilbene-2-O-beta-D-glucoside, an active component of *P. multiflorum* have protective effects on experimental colitis (Wang *et al.,* 2008) and inhibitory effects on experimental inflammation and cyclooxygenase 2 acitivity (Zhang *et al.,* 2007). However, the anti-inflammatory mechanism of the stem of *P. multiflorum* are not clearly understood.

In the present study, the inhibitory effect of the MeOH extracts of Polygoni multiflori Ramulus (PM) on NO and cytokines such as TNF-α, IL-6 was investigated in LPS stimulated mouse peritoneal macrophage cell. For clear the evident mechanism of NO suppression, the author assessed the effect of PM on expression level of iNOS, COX-2 and NF-κB.

Experimental

Preparation of the stem of PM – The plant materials were purchased from Wansanyakupsa (Jeonju, South Korea) in October 2005. A voucher specimen (WME005) has been deposited at the Department of Oriental Pharmacy, College of Pharmacy, Woosuk University. An extract was obtained twice from the dried sample (250 g) with 6,000 ml of 85% MeOH under ultrasonification for 2 h. It was evaporated and lyophilized to yield an MeOH extract of PM (Yield : 4.185%), which was then stored at –20 °C until use.

Peritoneal macrophage culture – TG-elicited macrophages were harvested 3 ~ 4 days after i.p. injection of 2.5 ml TG to the mice and isolated. Using 8 ml of HBSS containing 10 U/ml heparin, peritoneal lavage was performed. Then, the cells were distributed in DMEM, which was supplemented with 10% heat-inactivated FBS, in 4-well tissue culture plates (3×10^5 cells/well) incubated for 3 h at 37 °C in an atmosphere of 5% CO_2, washed three times with HBSS to remove non-adherent cells and equilibrated with DMEM that contained 10% FBS before treatment.

3-(3,4-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay – Cell respiration, an indicator of cell viability, was performed by the mitochondrial-dependent reduction of 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan, as described by Mosmann (Mosmann *et al*., 1983). The extent of the reduction of MTT to formazan within cells was quantified by measuring the optical density (OD) at 540 nm using an automated microplate reader (GENios,

Tecan, Austria).

Assay of nitrite concentration – Peritoneal macrophages (3×10^5 cells/well) were pretreated with 0.01, 0.1 and 1 mg/ml of PM for 30 min. The cells were then stimulated with rIFN-γ (20 U/ml). After 6 h, the cells were finally treated with LPS (10 μg/ml) for 48 h. NO synthesis in cell cultures was measured by a microplate assay method. To measure nitrite, 100 μl aliquots were removed from conditioned medium and incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphtyl)-ethylenediamine dihydrochloride, 2.5% H_3PO_4) at room temperature for 10 min. The absorbance at 540 nm was determined by an automatic microplate reader. NO_2^- was determined by using sodium nitrite as a standard. The cell-free medium alone contained 5 to 9 M of NO_2^-. This value was determined in each experiment and subtracted from the value obtained from the medium with peritoneal macrophages.

Assay of cytokine release – Peritoneal macrophages (3×10^5 cells/well) were treated with various with concentrations of PM. The cells were then stimulated rIFN-γ (20 U/ml) plus LPS (10 μg/ml) and incubated for 24 h. TNF-α and IL-6 in supernatants from the cells (3×10^5 cells/ml, culture medium DMEM with 10% FBS were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) according to manufacturer's protocol. Absorption of the avidin-horseradish peroxidase color reaction was measured at 405 nm and compared with serial dilutions of mouse TNF-α and IL-6 recombinant as a standard.

Preparation of nuclear extracts – Nuclear extracts were prepared essentially according to described previously (Baek *et al*., 2002). Briefly, the cells were allowed to swell by adding lysis buffer (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol and 0.5 mM phenylmethylsulfonylfluoride). Pellets containing crude nuclei were resuspended in extraction buffer (20 mM HEPES pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride) and incubated for 30 min on ice. The samples were centrifuged at 12,000 rpm for 10 min to obtain the supernatant containing nuclear extracts. Extracts were stored at −70 °C until use.

Western blot analysis – Whole cell lysates were made by boiling peritoneal macrophages in sample buffer (62.5 mM Tris-HCl pH 6.8, 2% sodium dodecyl sulfate (SDS), 20% glycerol and 10% 2-mercaptoethanol). Proteins in the cell lysates were then separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. The membrane was then blocked with 5% skim milk in PBS-tween 20 (Sigma, SL, USA) for 2 h at room temperature and then incubated with anti-iNOS (SantaCruz, CA, USA), COX-2 (Cayman, MI, USA) and NF-κB antibodies (SantaCruz, CA, USA). After washing in with phosphate buffered saline (PBS) containing 0.05% tween 20 three times, the blot was incubated with secondary antibody (anti-rabbit, anti-mouse) for 1 h and the antibody specific proteins were visualized by the enhanced chemiluminesence detection system according to the recommended procedure (Amersham Corp. Newark, NJ, Germany).

Statistical analysis – All measurement are expressed as the mean ± S.D. of independent experiments. Data between groups were analyzed by a paired Student's *t*-test and *P*-values less than 0.001 were considered significant.

Results and Discussion

Macrophages play a crucial role in eliciting the response cascade in the acute phase of inflammatory processes and after being stimulated, produce many kinds of inflammatory mediators including NO, chemokine, cytokines, iNOS and COX-2 for the primary protection of the host (Cao *et al*., 2006). However, excessive production of such NO, inducible enzymes and cytokines could cause inflammatory damage to the host reversely. Therefore, variation in levels of NO, cytokines, iNOS and COX-2 can be thought of as a marker of immunomodulation.

NO is a free radical produced from L-arginine by NOS and has diverse physiological roles and also contributes to the immune defense against viruses, bacteria and other parasites (Seo *et al*., 2001). But, over production of NO is recognized to play a central role in the pathogenesis of inflammation and result in septic shock, neurologic disorders, rheumatoid arthritis and autoimmune diseases (Thiemermann *et al*., 1990). Therefore, the modulation of NO production is one of the major contributing factors on inflammatory-mediated diseases. To determine the effect of PM on the production of NO in mouse peritoneal macrophages, nitrite accumulation was measured by the Griess reaction. The amount of NO in rIFN-γ and LPS treated cells was increased about 30 folds (86.5 ± 2.27 μM) compare with unstimulated cells. When PM was pre-treated in primed cell, PM significantly inhibits NO production dose dependently and over 80% ($P < 0.001$) inhibition of NO production was shown at the concentration of 1 mg/ml (Fig. 2). No significant effect on cell viability was observed at a test concentration up to 1 mg/ml PM (Fig. 1). Thus, the inhibitory effect of NO by PM was not due to a toxic action on the cells.

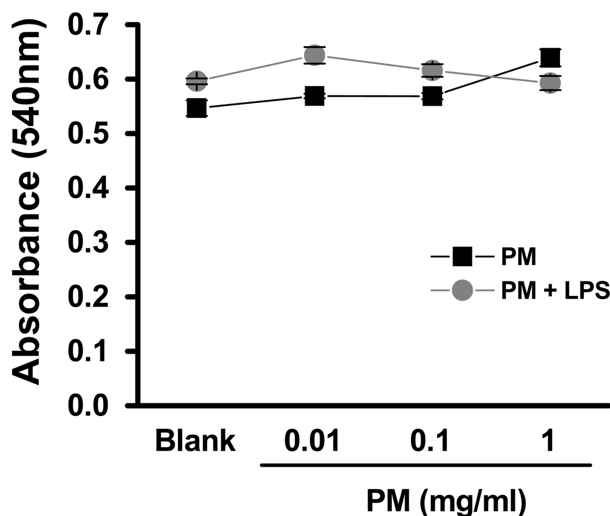

Fig. 1. Effects of PM on the viability in rIFN-γ/LPS stimulated peritoneal macrophages. Following pretreatment with various concentrations of PM for 30 min, the peritoneal macrophages (3 × 10^5 cells/well) were primed for 6 h with rIFN-γ (20 U/ml). The peritoneal macrophages were then stimulated with LPS (10 μg/ml) for 24 h. Cell viability was evaluated by MTT colorimetric assay as described in the method. The results are expressed as means ± S.D. of three independent experiments duplicate in each run.

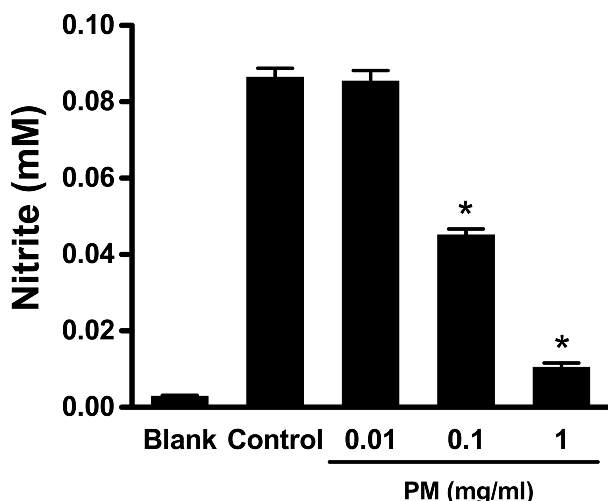

Fig. 2. Dose-dependent effects of PM on NO inhibition in rIFN-γ and LPS-stimulated peritoneal macrophages. Following pretreatment with various concentrations of PM for 30 min, the peritoneal macrophages (3 × 10^5 cells/well) were primed for 6 h with rIFN-γ (20 U/ml). The peritoneal macrophages were then stimulated with LPS (10 μg/ml). After 48 h of culture, NO release was measured by the Griess method. NO (nitrite) released into the medium is presented as the mean ± S.D. of three independent experiments duplicate in each run; *$P < 0.001$ compared to rIFN-γ + LPS

The macrophage-derived mediators, TNF-α and IL-6 are considered to play a important role in inflammatory and immune response, based on their occurrence at inflammatory sites and their ability to induce many hallmarks in the inflammatory response (Park *et al*., 2002). The pro-inflammatory cytokine, TNF-α regulates systemic responses to microbial infection or tissue injury (Evans *et al*., 1995). The involvement of TNF-α as a pathogenic factor has been documented in several inflammatory diseases, including arthritic diseases, inflammatory bowel diseases, type 1 diabetes mellitus, multiple sclerosis and Guillain-Barre syndrome (O'Shea *et al*., 2002). In patients with rheumatoid arthritis, juvenile idiopathic arthritis and ankylosing spondylitis, for example, neutralizing anti-TNF antibodies and soluble TNF-α receptors are powerful means of controlling disease activity (Gorman *et al*., 2002). IL-6, a macrophage-or monocyte-related cytokine (Sradnyk *et al*., 1997) plays an important role in the development of plasma cells and the induction of acute phase response and it has also been demonstrated to induce neurite outgrowth in cell culture system. The secretion of IL-6 has been found to play a central role in the regulation of defense mechanism, haematopoiesis and particularly important in the production of acute phase proteins (Park *et al*., 1999). We examined the inhibitory effect of PM on LPS-stimulated TNF-α and IL-6 production. Mouse peritoneal macrophages secreted low levels of TNF-α and IL-6 after 24 h incubation with medium alone. The basal level of TNF-α and IL-6 was little increased when incubated PM only. Upon rIFN-γ (20 U/ml) plus LPS (10 μg/ml) treatment for 24 h, TNF-α and IL-6 drastically increased in these cells and pre-treatment of cells with various concentration of PM (0.01, 0.1, 1 mg/ml) for 30 min significantly inhibited TNF-α and IL-6 induction in mouse peritoneal macrophages (Fig. 3).

iNOS and COX-2 are up-regulated in response to inflammatory and pro-inflammatory mediators and their products can influence many aspects of the inflammatory cascade. The level of iNOS play a crucial role of excess production of NO in activated macrophages. Therefore, suppression of NO production via inhibition of iNOS expression levels might be an attractive therapeutic target for the treatment of numerous pathological conditions, including inflammation. COX, another key enzyme in inflammation, is the rate-limiting enzyme that catalyzes the formation of prostaglandins (PGs) from arachidonic acid. Levels of PGs increase early in the course of the inflammation (Wallace *et al*., 1999). COX-2 is rapidly stimulated by tumor promoters, growth factors, cytokines and pro-inflammatory molecules (Minghetti *et al*., 1998) and responsible for the production of the high levels of PGs in several pathological conditions such as inflammation. Since COX-2 is induced by stimulation in

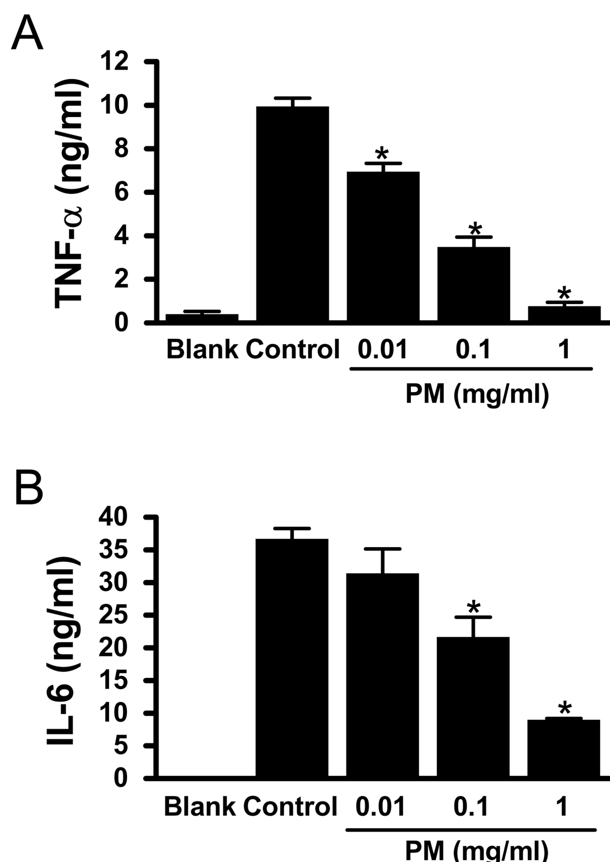

Fig. 3. Effects of PM on the rIFN-γ and LPS-stimulated TNF-α and IL-6 production in peritoneal macrophages. Following pretreatment with various concentrations of PM for 30 min, the peritoneal macrophages (3×10^5 cells/well) were primed for 6 h with rIFN-γ (20 U/ml). The peritoneal macrophages were then stimulated with LPS (10 μg/ml). The amount of TNF-α (A) and IL-6 (B) secretion was measured by ELISA method after 24 h incubation. Data represent mean ± S.D. of three independent experiments duplicate in each run; $^*P < 0.001$ compared to rIFN-γ + LPS.

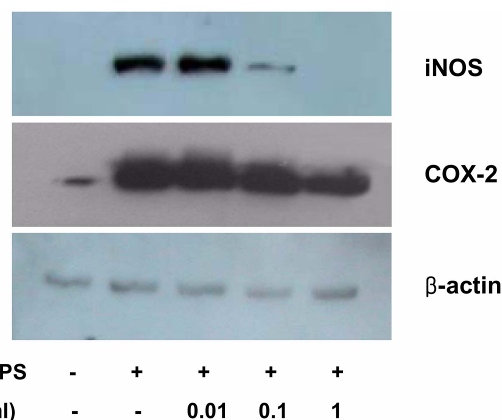

Fig. 4. Effects of PM on the expression of iNOS and COX-2 by rIFN-γ/LPS activated peritoneal macrophages. Peritoneal macrophages (5×10^6 cells/well) were pretreated with PM for 30 min and then stimulated for 6 h with rIFN-γ (20 U/ml). The peritoneal macrophages were then stimulated with LPS (10 μg/ml) for 24 h. The protein extracts were prepared; samples were analyzed for iNOS and COX-2 expression by western blotting as described in the method.

inflammatory cells, inhibitors of COX-2 induction might be candidates for the new type of nonsteroidal anti-inflammatory drugs (NSAIDs). In order to investigate the mechanism of action of PM on the inhibition of NO production, the effect of the PM on iNOS and COX-2 expression was identified by western blotting. As shown in Fig. 4, the expression of iNOS and COX-2 protein were markedly increased after rIFN-γ (20 U/ml) plus LPS (10 μg/ml) challenge for 24 h. This enhanced expression of iNOS and COX-2 protein was significantly reduced by PM in a dose-dependent manner (Fig. 4).

The expression of iNOS and COX-2 is largely regulated by transcriptional activation (Park *et al*., 2006). NF-κB is a protein that is essential for the transcription of genes that encode a number of pro-inflammatory molecules which participate in the acute inflammatory response, including iNOS, COX-2, TNF-α and IL-6 (Muller *et al*., 1993). In unstimulated cells, it present in the cytoplasm through interaction with the inhibitory protein, IκB. However, in active state, Following the induction of NF-κB by appropriate extracellular stimulation such as LPS, TNF-α, or tissue plasminogen activator, it translocates to the nucleus with phosphorylation, ubiquitation and degradation of IκBα and also acts upon the pro-inflammatory gene promoter to activate transcription (Park *et al*., 2006). As shown in Fig. 5, the activation of NF-κB was markedly increased after rIFN-γ (20 U/ml) plus LPS (10 μg/ml) challenge for 10 min. This increased expression of NF-κB was significantly reduced by PM (Fig. 5) and In summary, the production of NO, TNF-α and IL-6 takes an important part of the immune response to many inflammatory stimulti. Nonetheless, excessive overproduction of these mediators is implicated in acute and chronic inflammatory diseases including septic shock, hemorrhagic shock, multiple selerosis, rheumatoid arthritis, ulcerative colitis and atherosclerosis (Bertolini *et al*., 2001). The present results demonstrate that the stem of PM inhibits over production of NO in mouse peritoneal macrophages stimulated with LPS. This inhibitory effect was consistent with its down-regulation effect on the expression of iNOS in murine macrophages. Activation of NF-κB is thought play a key role in the LPS-stimulated expression of iNOS. PM also suppressed expression of COX-2 and TNF-α, IL-6 release through decreased NF-κB transcriptional factor and DNA binding activities in a concentration-dependent manner. The findings from this

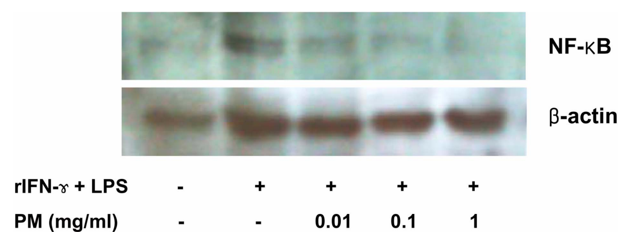

Fig. 5. Effects of PM on the activation of NF-κB by LPS-stimulated peritoneal macrophages. Peritoneal macrophages (5 × 10^6 cells/well) were pretreated with PM for 30 min and then stimulated for 10 min with LPS (10 µg/ml). The nuclear extracts were prepared; samples were analyzed by western blotting as described in the method.

study provide scientific supporting evidence for the use of clinical practice of PM in the treatment of inflammation. In conclusion, these results establish that PM has potent anti-inflammatory effects and may hold great promise for use in macrophage-mediated inflammatory diseases.

Acknowledgement

This work was supported by the Research Grant from Woosuk University.

References

Ambs, S., Merriam, W.G., Bennett, W.P., Felley-Bosco, E., Ogunfusika, M.O., Oser, S.M., Klein, S., Shields, P.G., Billiar, T.R., and Harris, C.C., Frequent nitric oxide synthase-2 expression in human colon adenomas: implication for tumor angiogenesis and colon cancer progression. *Cancer Res*. **58**, 334-341 (1998).

Baek, W.K., Park, J.W., Lim, J.H., Suh, S.I., Suh, M.H., Gabrielson, E., and Kwon, T.K., Molecular cloning and characterization of the human budding uninhibited by benomyl (BUB3) promoter. *Gene* **295**, 117-123 (2002).

Baeuerle, P.A. and Baltimore, D., NF-kappa B: ten years after. *Cell* **87**, 13-20 (1996).

Bertolini, A., Ottani, A., and Sandrini, M., Dual acting anti-inflammatory drugs: a reappraisal. *Pharmacol. Res*. **44**, 437-450 (2001).

Cao, X.Y., Dong, M., Shen, J.Z., Wu, B.B., Wu, P.M., Du, X.D., Wang, Z., Qi, Y.T., and Li, B.Y., Tilmicosin and tylosin have anti-inflammatory properties via modulation of COX-2 and iNOS gene expression and production of cytokines in LPS-induced macrophages and monocytes. *Int. J. Antimicrob. Agents* **27**, 431-438 (2006).

Chan, Y.C., Wang, M.F., and Chang, H.C., *Polygonum multiflorum* extracts improve cognitive performance in senescence accelerated mice. *Am. J. Chin. Med*. **31**, 171-179 (2003).

Chen, F., Sun, S.C., Kuh, D.C., Gaydos, L.J., and Demers, L.M., Essential role of NF-kappa B activation in silica-induced inflammatory mediator production in macrophages. *Biochem. Biophys. Res. Commun*. **214**, 985-992 (1995).

Chen, Y.J., Hsu, K.W., Tssai, J.N., Hung, C.H., Kuo, T.C., and Chen, Y.L., Involvement of protein kinase C in the inhibition of lipopoly-saccharide-induced nitiric oxide production by thapsigargin in RAW 264.7 macrophages. *Int. J. Biochem. Cell. Biol*. **37**, 2574-2585 (2005).

Chiu, P.Y., Mak, D.H., Poon, M.K., and Ko, K.M., In vivo antioxidant action of a lignan-enriched extract of Schisandra fruit and an anthraquinone-containing extract of Polygonum root in comparison with schisandrin B and emodin. *Planta. Med*. **68**, 951-956 (2002).

Erridge, C., Bennett-Guerrero, E., and Poxton, I.R., Structure and function of lipopolysaccharides. *Microbes. Infect*. **4**, 837-851 (2002).

Evans, C.H., Nitric oxide: what role does it play in inflammation and tissue destruction? *Agents Actions Supplement* **47**, 107-116 (1995).

Goldstein, S.R., Yang, G.Y., Chen, X., Curtis, S.K., and Yang, C.S., Studies of iron deposits, inducible nitric oxide synthase and nitrotyrosine in a rat model for esophageal adenocarcinoma. *Carcinogenesis* **19**, 1445-1449 (1998).

Gorman, J.D., Sack, K.E., and Davis, J.C., Treatment of ankylosing spondylitis by inhibition of tumor necrosis factor alpha. *New Engl. J. Med*. **346**, 1349-1356 (2002).

Haslberger, A., Romanin, C., and Koerber, R., Membrane potential modulates release of tumor necrosis factor in lipopolysaccharide-stimulated mouse macrophages. *Mol. Biol. Cell*. **3**, 451-460 (1992).

Hewett, J.A., and Roth, R.A., Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides. *Pharmacol. Rev*. **45**, 382-411 (1993).

Jeon, Y.J., Li, M.H., Lee, K.Y., Kim, J.S., You, J.H., Lee, S.K., Sohn, H.M., Choi, S.J., Koh, J.W., and Chang, I.Y., KIOM-79 inhibits LPS-induced iNOS gene expression by blocking NF-κB/Rel and p38 kinase activation in murine macrophages. *J. Ethnopharmacol*. **108**, 38-45 (2006).

Kang, S.C., Lee, C.M., Choi, H., Lee, J.H., Oh, J.S., Kwak, J.H., and Zee, O.P., Evaluation of oriental medicinal herbs for estrogenic and antiproliferative activities. *Phytother. Res*. **20**, 1017-1019 (2006).

Karin, M. and Ben-Neriah, Y., Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu. Rev. Immunol*. **18**, 621-663 (2000).

Kim, K.S., Cho, H.S., Lee, S.D., Kim, K.H., Cho, J.Y., Chung, K.H., Lee, Y.C., Moon, S.K., and Kim, C.H., Inhibitory effect of *Buthus martensi* Karsch extracts on interleukin-1 beta-induced expression of nitric oxide (NO) synthase and production of NO in human chondrocytes and LPS-induced NO and prostaglandin E_2 production in mouse peritoneal macrophages. *Toxicol. In Vitro* **19**, 757-769 (2005).

Kim, Y.H., Woo, K.J., Lim, J.H., Kim, S., Lee, T.J., Jung, E.M., Lee, J.M., Park, J.W., and Kwon, T.K., 8-Hydroxyquinoline inhibits iNOS expression and nitric oxide production by down-regulating LPS-induced activity of NF-κB and C/EBPβ in Raw 264.7 cells. *Biochem. Biophys. Res. Commun*. **329**, 591-597 (2005).

Lee, Y.S., Han, O.K., Park, C.W., Yang, C.H., Jeon, T.W., Yoo, W.K., Kim, S.H., and Kim, H.J., Pro-inflammatory cytokine gene expression and nitric oxide regulation of aqueous extracted Astragali radix in RAW 264.7 macrophage cells. *J. Ethnopharmacol*. **100**, 289-294 (2005).

Li, R.W., David, L.G., Myers, S.P., and Leach, D.N., Anti-inflammatory activity of Chinese medicinal vine plants. *J. Ethnopharmacol*. **85**, 61-67 (2003).

Li, X., Matsumoto, K., Murakami, Y., Tezuka, Y., Wu, Y., and Kadota, S., Neuroprotective effects of *Polygonum multiflorum* on nigrostriatal dopaminergic degeneration induced by paraquat and maneb in mice. *Pharmacol. Biochem. Behav*. **82**, 345-352 (2005).

Ohshima, H. and Bartsch, H., Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat. Res*. **305**, 253-264 (1994).

O'Shea, J.J. and Ma A, Lipsky., Cytokines and autoimmunity. *Nature Reviews Immunology* **2**, 37-45 (2002).

MacMicking, J., Xie, Q.W., and Nathan, C., Nitric oxide and macrophage function. *Annu. Rev. Immunol*. **15**, 323-350 (1997).

Marcinkiewicz, J., Grabowska, A., and Chain, B., Nitric oxide up-regulates the release of inflammatory mediators by mouse macrophages. *Eur. J. Immunol*. **25**, 947-951 (1995).

Minghetti, L. and Levi, G., Microglia as effector cells in brain damage and repair: focus on prostanoids and nitric oxide. *Prog. Neurobiol*. **54**, 99-125 (1998).

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65: 55-63.

Muller, J.M., Ziegler-Heitbrock, H.W., and Baeuerle, P.A., Nuclear factor kappa B, a mediator of lipopolysaccharide effects. *Immunobiology* **187**, 233-256 (1993).

Park, J.E., Kang, Y.J., Park, M.K., Lee, Y.S., Kim, H.J., Seo, H.G., Lee, J.H., Shin, J.S., Lee, H.W., Ahn, S.K., and Chang, K.C., Enantiomers of higenamine inhibit LPS-induced iNOS in a macrophage cell line and improve the survival of mice with experimental endotoxemia. *Int. Immunopharmacol*. **6**, 226-233 (2006).

Park, S.J., Lee, S.C., Hong, S.H., and Kim, H.M., Degradation of IkappaBalpha in activated RAW264.7 cells is blocked by the phosphatidylinositol 3-kinase inhibitor LY294002. *Cell. Biol. Toxicol*. **18**, 121-130 (2002).

Park, S.J., Jung, J.A., Lee, J.S., and Kim, H.M., The phosphatidylinositol 3-kinase inhibitor, LY294002, blocks TNF-α and IL-6 production in activated RAW-264.7 cells. *Bulletin of Life Science and Biotechnology* **6**, 3-17 (1999).

Park, Y.M., Won, J.H., Yun, K.J., Ryu, J.H., Han, Y.N., Choi, S.K., and Lee, K.T., Preventive effect of *Ginkgo biloba* extract (GBB) on the lipopolysaccharide-induced expressions of inducible nitric oxide synthase and cyclooxygenase-2 via suppression of nuclear factor-kappaB in RAW 264.7 cells. *Biol. Pharm. Bull.* **29**, 985-990 (2006).

Rakel, D.P. and Rindfleisch, A., Inflammation: nutritional, botanical, and mind-body influences. *South Med. J.* **98**, 303-310 (2005).

Sanchez-Perez, I., Benitah, S.A., Martinez-Gomariz, M., Lacal, J.C., and Perona, R., Cell stress and MEKK1-mediated c-Jun activation modulate NF-kappaB activity and cell viability. *Mol. Biol. Cell* **13**, 2933-2945 (2002).

Seitz, M., Loetscher, P., Dewald, B., Towbin, H., Rordorf, C., Gallati, H., and Gerber, N.J., Interleukin 1 (IL-1) receptor antagonist, soluble tumor necrosis factor receptors, IL-1 beta, and IL-8 markers of remission in rheumatoid arthritis during treatment with methotrexate. *J. Rheumatol*. **23**, 1512-1516 (1996).

Seo, W.G., Pae, H.O., Oh, G.S., Chai, K.Y., Kwon, T.O., Yun, Y.G., Kim, N.Y., and Chung, H.T., Inhibitory effects of methanol extract of *Cyperus rotundus* rhizomes on nitric oxide and superoxide productions by murine macrophage cell line, RAW 264.7 cells. *J. Ethnopharmacol* **76**, 59-64 (2001).

Shacter, E., arzadon, G.K., and Williams, J.A., Stimulation of interleukin-6 and prostaglandin E_2 secretion from peritoneal macrophages by polymers of albumin. *Blood* **82**, 2853-2864 (1993).

Sradnyk, A.W., Gillan, T.L., and Anderson, R., Respiratory syncytial virus triggers synthesis of IL-6 in BALB/c mouse alveolar macrophages in the absence of virus replication. *Cell Immunol*. **176**, 122-126 (1997).

Szabo, C., Alterations in nitric oxide production in various forms of circulatory shock. *New Horiz*. **3**, 2-32 (1995).

Thiemermann, C. and Vane, J., Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. *Eur. J. Pharmacol*. **182**, 591-595 (1990).

Um, M.Y., Choi, W.H, Aan, J.Y., Kim, S.R., and Ha, T.Y., Protective effect of *Polygonum multiflorum* Thunb on amyloid beta-peptide 25-35 induced cognitive deficits in mice. *J. Ethnopharmacol* **104**, 144-148 (2006).

Verma, I.M., Stevenson, J.K., Schwarz, E.M., Van Antwerp, D., and Miyamoto, S., Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes Dev.* **9**, 2723-2735 (1995).

Wallace, J.L., Distribution and expression of cyclooxygenase (COX) isoenzymes, their physiological roles, and the categorization of nonsteroidal anti-inflammatory drugs (NSAIDs). *Am. J. Med*. **13**, 107,11S-16S; discussion 16S-17S (1999).

Wang, W., Cao, C.Y., Wang, D.Q., and Zhao, D.Z., Effect of prepared *Polygonum multiflorum* on striatum extracellular acetylcholine and choline in rats of intracerebral perfusion with sodium azide. *Zhongguo Zhong Yao Za Zhi* **31**, 751-753 (2006).

Wang, X., Zhao, L., Han, T., Chen, S., and Wang, J., Protective effects of 2,3,5,4'-tetrahydroxystilbene-2-O-beta-D-glucoside, an active component of *Polygonum multiflorum* Thunb, on experimental colitis in mice. *Eur. J. Pharmacol.* **578**, 339-348 (2008).

Wilson, K.T., Fu, S., Ramanujam, K.S., and Meltzer, S.J., Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. *Cancer Res*. **58**, 2929-2934 (1998).

Xiao, P.G., Xing, S.T., and Wang, L.W., Immunological aspects of Chinese medicinal plants as antiageing drugs. *J. Ethnopharmacol*. **38**, 167-175 (1993).

Yim, T.K., Wu, W.K., Mak, D.H., and Ko, K.M., Myocardial protective effect of an anthraquinone-containing extract of *Polygonum multiflorum* ex vivo. *Planta. Med.* **64**, 607-611 (1998).

Zhang, C.Z., Wang, S.X., Zhang, Y., Chen, J.P., and Liang, X.M., In vitro estrogenic activities of Chinese medicinal plants traditionally used for the management of menopausal symptoms. *J. Ethnopharmacol*. **98**, 295-300 (2005).

Zhang, H., Jeong, B.S, and Ma, T.H., Antimutagenic property of an herbal medicine, *Polygonum multiftorum* Thunb. detected by the Tradescantia micronucleus assay. *J. Environ. Pathol. Toxicol. Oncol*. **18**, 127-130 (1999).

Zhang, Y.Z., Shen, J.F., Xu, J.Y., Xiao, J.H., and Wang, J.L., Inhibitory effects of 2,3,5,4'-tetrahydroxystilbene-2-O-beta-D-glucoside on experimental inflammation and cyclooxygenase 2 activity. *J. Asian Nat. Prod. Res*. **9**, 355-363 (2007).

Received April 19, 2009
Revised June 11, 2009
Accepted June 11, 2009