대한안이비인후피부과학회지 제17권 제1호(2004년 4월)
The Journal of Oriental Medical Ophthalmology & Otolaryngology & Dermatology 2004;17(1):163-171

Lonicera japonica Inhibits the Production of NO through the Suppression of NF-κB Activity in LPS-stimulated Mouse Peritoneal Macrophages

Young-hee Kim[1] · Han-do Kim[2]

Korea NanoBioTechnology Center, Pusan National University, Busan, 609-735, Korea.[1]

Department of Molecular Biology, College of Natural Sciences, Pusan National University, Busan, 609-735, Korea[2]

The flowers of *Lonicera japonica* Thunb. (Caprifoliaceae) has been used as anti-inflammatory drug in the folk medicine recipe and been proved its anti-inflammatory effect in the oriental medicine. However, the action mechanism of *Lonicera japonica* that exhibits anti-inflammatory effects has not been determined. Since nitric oxide (NO) is one of the major inflammatory parameter, we studied the effect of aqueous extracts of *Lonicera japonica* (AELJ) on NO production in lipopolysaccharide (LPS)-stimulated mouse peritoneal macrophages. NO and inducible NO synthase (iNOS) level were significantly reduced in LPS-stimulated macrophages by AELJ compared to those without. Electrophoretic mobility shift assay (EMSA) indicated that AELJ blocked the activation of nuclear factor kappa B (NF-κB), which was considered to be a potential transcription factor for the iNOS expression. AELJ also blocked the phosphorylation and degradation of inhibitor of kappa B-alpha (IκB-α). Furthermore, IκB kinase alpha (IKKα), which is known to phosphorylate serine residues of IκB directly, is inhibited by AELJ *in vivo* and *in vitro*. These results suggest that AELJ could exert its anti-inflammatory actions by suppressing the synthesis of NO through inhibition of NF-κB activity.

Key words : *Lonicera japonica* Thunb., Nitric oxide (NO); Inducible nitric oxide synthase (iNOS); Nuclear Factor kappa B (NF-κB); Inhibitor kappa B (IκB); IκB kinase (IKK)

INTRODUCTION

NO has been known to be an important regulatory molecule in diverse physiological functions such as vasodilation, neural communication, and also toxic for bacteria and tumor cells[1, 2]. However a large quantity of NO induces an inflammatory response to inhibit the growth of invading microorganisms and tumor cells. This strong inflammatory response to foreign cells

Corresponding author : YoungHee Kim, Ph.D., Korea NanoBio-Technology Center, Pusan National University, Busan, 609-735, Korea,
(Tel : 82-51-510-2526, Fax : 82-51-513-9258,
E-mail : yheekim@pusan.ac.kr)

could also cause further damage for the neighboring cells and tissues of the host[3]. Therefore, the reduction of the harmful effects is seemed to be important in inflammation therapy.

NO is produced from conversion of L-arginine to citrulline *in vivo* by three distinct isoforms of NO synthase (NOS): neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II), and endothelial NOS (eNOS or NOS III)[4]. While nNOS and eNOS are constitutively expressed and regulated at post-translational level by Ca^{2+}-calmodulin, the activity of iNOS is regulated at the transcriptional level by mediators such as IL-2, IFN-α and inflammatory stimuli including bacterial lipopolysaccharide (LPS)[5, 6].

The transcriptional activator protein NFκB plays a critical role in iNOS gene expression[7]. NFκB is a heterodimeric transcription factor and controls a number of genes that are important for immunity and inflammation. In its unstimulated form, NFκB is present in the cytosol bound to the inhibitory protein I kappa B (IκB). In response to cell stimulation, IκB becomes phosphorylated and recognized by a specific E3 ubiquitin ligase complex and then degradated by the 26S proteasome. The free NFκB from IκB, which are spared from degradation, translocates to the nucleus to activate gene transcription[8, 9].

Recent studies have identified an IκB kinase (IKK), which consists of two catalytic subunits, IKKγ and IKKγ, and regulatory subunits, IKKγ[10]. IKK is induced by inflammatory signals and able to phosphorylate two conserved N-terminal serine residues of IκBα and IκBβ in RAW 264.7 murine macrophages, HeLa human epithelial cells, and U937 human histiocytic lymphoma cells[11, 12].

The flowers of *Lonicera japonica* Thunb. (Caprifoliaceae) has been used to remove fever from blood, to nourish yin, to quench fire, and to counteract toxicity traditionally in the oriental medicine recipe[13]. Since these effects are regarded totally as anti-inflammatory functions we hypothesize that this drug is correlated with the function of NO production, one of key parameters of inflammation. Therefore, in this study, we examined the effects of the aqueous extracts of *Lonicera japonica* (AELJ) on NO production from LPS-stimulated mouse peritoneal macrophages and investigated possible mechanisms of the effects of the medicine.

MATERIALS AND METHODS

Preparation of extract

The flowers of *Lonicera japonica* were purchased from a local herb store, Kwang Myoung Dang (Busan, Korea) in February 1999. The roots were identified and authenticated by Professor W. S. Ko. The dry roots (200 g) were extracted with distilled water at 100°C for 2 hr. The extract was filtered through 0.45 ㎛ filter and the filterate was freeze-dried (yield, 6 g) and kept at 4 °C. The dried filterate was dissolved in phosphate buffered saline (PBS) and filtered through 0.22 ㎛ filter before use.

Macrophage culture

C57BL/6 mice purchased from Dae Han Animal Center (DHAC, Korea) were used between 8 to 12 weeks of age (25-30 g). TG-elicited macrophages were harvested 3 days after i.p. injection of 2.5 ml TG into mice and isolated as reported previously[14]. Peritoneal lavage was performed by using 8 ml HBSS. Cells were then suspended in RPMI 1640, which was

supplemented with 10% FBS, and incubated at 37 °C in an atmosphere of 5% CO_2 for 5 hr. Nonadherent cells were removed by suction, and then freshly prepared complete media were added.

Measurement of nitrite concentration

NO synthesis in cell cultures was measured by a microplate assay method. After cells plated in 24 well for 24 hr, 100 ㎕ each cultured medium was mixed with the same volume of the Griess reagent (1% sulfanilamide /0.1% N- (1-naphthyl)-ethylenediamine dihydrochloride /2.5% H3PO4). Nitrite concentration was determined by measuring the absorbance at 540 nm with a Vmax microplate reader (Molecular Devices).

Cytotoxicity assay

The cytotoxicity of AELJ was assessed using the MTT assay in the remaining cells after Griess reaction. To measure viability, 0.5 mg/ml of MTT solution was added to each well. After incubation for 2 h at 37 °C and 5% CO_2, the supernatant was removed and the formed formazan crystals in viable cells were measured at 540 nm with Vmax microplate reader (Molecular Devices).

Western blot analysis

The cells were washed with PBS three times and scraped off and lysed with lysis buffer [1% Triton X-100, 1% Deoxycholate, 0.1% NaN_3]. Protein concentration of lysates was determined and equal amounts of protein (25 ㎍) were separated electrophoretically using 10% SDS-PAGE, and then the gel was transferred to 0.45 ㎛ polyvinylidene fluoride (PVDF). The blot was incubated with anti-iNOS, IkB-α, p-IκB-α or IKKα antibody at room temperature and secondary antibody, and then was detected by the enhanced chemiluminescence detection system according to the recommended procedure (ECL, Amersham).

Preparation of nuclear extract

Nuclear extracts were prepared as described[15] with some modifications. Briefly, cells were incubated in 100 mm dishes and scraped off. Then cells washed with PBS three times, resuspended in 500 ㎕ of ice-cold buffer A [10 mM HEPES-KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF)] and allowed on ice for 15 min. Then cell extract was added Nonidet P-40 (NP-40), incubated on ice for 5 min and centrifuged at 12000 g for 30 s at 4 °C. After removal of the supernatant, containing cytosolic proteins, nuclear proteins were extracted by addition of 100 ㎕ of buffer B [20 mM HEPES, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF, protease inhibitor cocktail] for 30 min at 4 °C with occasional vortexing. After centrifugation at 13,000 g for 5 min 4 °C, supernatants were collected and stored at - 70 °C for use as nuclear extract.

Eletrophoretic Mobility Shift Assay (EMSA)

Gel shift assay of nuclear extracts was performed according to the manufacture's instructions (Promega, Madison, WI) with some modifications. In brief, the probe consisted of a double-stranded oligonucleotide containing the consensus binding sequence for NF-κB (5'-AGTTGAGGGGACTTTCCCAGGC-3', Promega, Madison, WI) was end-labeled with (-^{32}P-ATP(3000Ci /mmol at 10μCi/ml) using T4 polynucleotide kinase at 37 °C for 1 hour and purified in G-25 spin column

(BM, Indianapolis, IN). Nuclear extracts (5 (g) were incubated with gel shift binding buffer [10 mM HEPES, pH 7.9, 50 mM KCl, 0.2 mM EDTA, 2.5 mM DTT, 10% glycerol, 0.05% NP-40, 0.25 mg/ml poly dI/poly dC, protease inhibitor cocktail] for 10 min at room temperature and then the mixture was incubated with ^{32}P-labeled probe for 20 min at room temperature. The incubation mixture was loaded onto 6% nondenaturating gel (30:1 acrylamide:bis- acrylamide) and run in 0.25(Tris/borate/EDTA buffer. Gels were dried and exposed to X-ray film at -70 °C.

IKK Assay

IKK was assayed as performed by Yomaoka *et al.*[16], with some modification. Whole cell extracts were lysed with lysis buffer [10% glycerol, 1% Triton X-100, 1 mM EGTA, 5 mM EDTA, 1 mM Sodium pyrophosphate, 20 mM Tris-HCl (Ph 7.9), 10 mM β -glycerophosphate, 137 mM NaCl, 1 mM PMSF, 10 mM NaF, 1 mM sodium orthivanadate, protease inhibitor cocktail] for 15 min at 4°C. The cell lysates were clarified by centrifugation at 12000 g for 10 min at 4 °C. Equal amounts of total cellular protein (500 μg) were immunoprecipitated with IKKα specific antibody in TNT buffer [20 mM NaCl, 20 mM Tris-HCl (pH7.5), 1% Triton X-100, 300 μM sodium orthovanadate, 2 mM PMSF, protease inhibitor cocktail] for 2 h. The IKKα-antibody complex was precipitated with protein A/G sepharose beads for 1 h at 4 °C, washed three times with TNT buffer, and finally washed with kinase buffer [20 mM HEPES, 10 mM $MgCl_2$, 50 mM NaCl, 20 μM β-glycerophosphate, 300 uM sodium orthovanadate, 1 mM NaF, 2 mM DTT, 500 mM PMSF, protease inhibitor cocktail]. The kinase assay was carried out in kinase buffer containing 5 (Ci [(-^{32}p] ATP and GST-IκBα fusion protein 500 ng as substrate and incubated for 30 min at 30 °C. Each sample was mixed with Laemmli's loading buffer, heated for 10 min 100 °C and subjected to 10% SDS-PAGE. The gels were dried, visualized by autoradiography.

RESULTS

Suppression of NO production and iNOS expression by AELJ in LPS-stimulated mouse peritoneal macrophages

To determine the effect of AELJ on NO generation in LPS-stimulated mouse peritoneal macrophages, Greiss Method was employed. TG-elicited mouse peritoneal macrophages were pre-incubated in 24-well tissue culture plates (2(10 cells/well) with AESB 1 hr and stimulated with 1 μg/ml LPS for 24 hr. LPS alone increased the production of nitrite about 8-fold over basal levels. This induction in nitrite generation by LPS was inhibited by AELJ in dose dependent manner (Fig. 1A). We next investigated whether AELJ could affect iNOS protein levels in LPS-stimulated mouse peritoneal macrophages. Western blot analysis indicated that the level of iNOS was gradually decreased with increasing concentration of AELJ (Fig. 1B). This result strongly suggests that the inhibitory effect of AELJ on NO release is caused by the gene expression level of iNOS. Cell viability was not affected by AELJ 0.25~5 mg/ml as determined with MTT assay, so inhibition of NO synthesis was not due to cytotoxicity of AELJ (Fig. 1C).

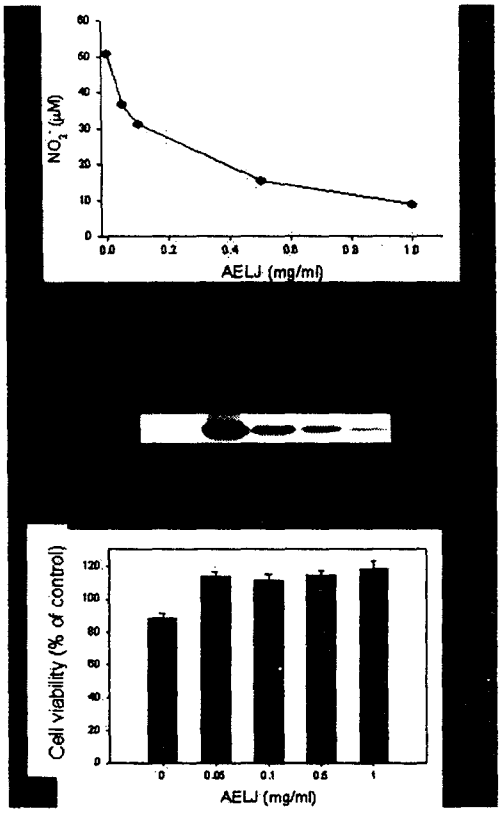

Fig. 1. Effect of AELJ on NO production and iNOS expression in LPS-stimulated macrophages. TG-elicited mouse peritoneal macrophages were incubated with various concentrations of AELJ for 1 h and stimulated with 1 μg/ml LPS for 24 h at 37 oC. (A) At the end of incubation, the culture medium was collected for Griess reaction. (B) Whole cell extracts were separated by SDS-PAGE and analyzed by western blotting. (C) MTT assay was performed in the remaining cells after Griess reaction. Results were presented as the means (S.E. of four individual experiments performed in duplicate.

Suppression of LPS-induced NF-κB activation by AELJ in mouse peritoneal macrophages

In mouse peritoneal macrophage, NFκB is transcritional factor that is activated in response to stimulation by LPS and also probably controls transcriptional initiation of iNOS gene and thus the inhibitory effect of AELJ on NO production might be based on the trabscriptional level of iNOS in the participation of NFκB. To assess whether AELJ suppresses NFκB activation, EMSA was performed. TG-elicited mouse peritoneal macrophages were incubated with AELJ for 1 hr and stimulated with 1 μg/ml LPS for 30 minutes. EMSA using a consensus NFκB oligonucleotide showed a low level of binding affinity in unstimulated macrophages; however, it was largely increased by LPS. This increased NFκB binding affinity was inhibited markedly by AELJ in dose dependent manner (Fig. 2).

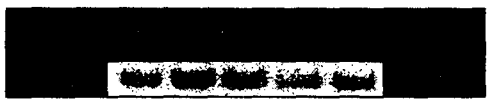

Fig. 2. Inhibition of LPS-stimulated NF-κB activity by AELJ. TG-elicited mouse peritoneal macrophages were incubated with various concentrations of AELJ for 1 h and stimulated with 1 μg/ml LPS for 30 min at 37 oC. Nuclear proteins were extracted and assayed for NF-κB DNA binding affinity by EMSA.

Inhibition of LPS-induced phosphorylation and degradation of IκBα

Functional NFκB dimmers are bound in the cytoplasm to one or more inhibitory proteins, identified as IκB. The activation of NFκB involves the signal induced degradation of IκB protein. The details of this process are not fully known, but it involves the hyper-phosphorylation of IκB by an multisubunit protein kinase (IKK), the subsequent attachment of ubiquitin to hyper-phosphorylated IκB, and finally degradation of ubiquitinated IκB by the cytoplasmic

proteasome complex. To determine whether the action of AELJ was due to its inhibitory effect on IκBα degradation, the level of IκBα protein with AELJ pre-treatment was examined by western blotting. We examined that IκBα was degradated transiently (about 15 min) by LPS, whereas AELJ (0.5 mg/ml) suppressed degradation of IκBα (Fig. 3A). Science IIκ Bα is degradated rapidly after phosphorylated, the cells were incubated with LPS and MG132, the proteasome inhibitor, for detection of the level of phospho-IκBα. Western blot analysis of cell extracts with antibody specific for phospho-IκBα showed that phosphorylation of IκBα caused by LPS was reduced by treatment with 1 mg/ml of AELJ (Fig. 3B).

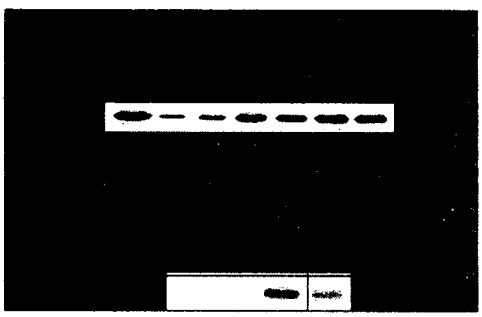

Fig. 3. Effect of AELJ on LPS-stimulated phosphorylation and degradation of IκBα in mouse peritoneal macrophages. (A) TG-elicited mouse peritoneal macrophages were incubated with AELJ as described in Fig. 1 and stimulated with or without LPS (1 ㎍/ml) for indicated period at 37 oC. Cells were harvested and the level of IκBα was determined by Western blot. (B) Cells were incubated with AELJ as described above and stimulated with or without LPS (1 ㎍/ml) plus MG132 (10 μM) for 15 min 37 oC. Cells were harvested and the level of phospho-Ik Bα was determined by Western blot.

Inhibition of LPS-induced IKKα activity

In many mammal cells, IκB phosphorylation is due to rapid activation of multisubunit protein kinase, IKK. To directly measured IKK activity in cells, IKKα was immunoprecipitated and assayed using recombinant GST-IκBα(1-317) as a substrate. In this study, we demonstrated that IKKα activity is increased significantly in LPS-induced mouse peritoneal macrophages. After stimulation with LPS, GST-IκBα fusion protein was phosphorylated strongly, indicating stimulation of IKKα activity. This induction of IKK activity is suppressed by AELJ in dose dependent manner (Fig. 4A). Western blot analysis showed that the level of IKKα protein was not changed by incubation with AELJ. The inhibitory effect of AELJ on LPS- induced IKKα activity was also confirmed *in vitro* kinase assay (Fig. 4B).

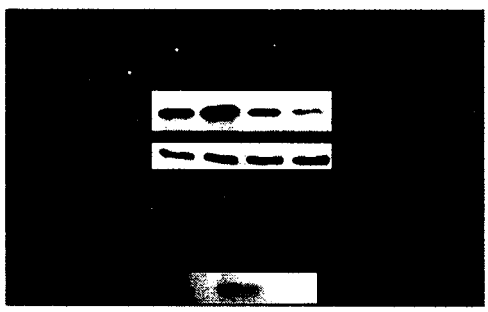

Fig. 4. Inhibition of LPS-stimulated IKKα activity by AELJ. (A) Mouse peritoneal macrophages were incubated with AELJ for 1 h, and then stimulated with 1 ㎍ /ml LPS for 10 min at 37oC. Whole cell extracts were immunoprecipitated with anti-IKKα antibody. The precipitates were incubated with GST-IκBα (1-317) and [γ-32P] ATP, resolved by SDS-PAGE and analyzed by autoradiography (upper panel). Western blotting for IKKα was performed as a loading control (lower panel). (B) In vitro kinase assay was performed. IKKα-antibody complexes from protein extracts of LPS stimulated cell were incubated with 1 mg/ml AELJ for 10 min at 37oC and then were incubated with GST-IκBα (1-317) and [γ-32P] ATP, resolved by SDS-PAGE and analyzed by autoradiography.

DISCUSSION

Mammals are in contact with Gram-negative bacteria and their LPS[17]. Low dose of LPS are thought to be beneficial for the host, e.g. in causing immunostimulation and enhancing resistance to infections and malignancies. On the other hand, the presence of large amounts of LPS can lead to dramatic pathophysiological reactions such as fever, leukopenia, tachycardia, hypotension, disseminated intravascular coagulation, and multiorgan failure[3, 18]. It has been established that iNOS produces large amount of NO several hours after exposure to LPS in macrophage[19]. In this study, AELJ significantly inhibited LPS induced NO production and iNOS expression in mouse peritoneal macrophages without appreciable cytotoxic effects. These results suggest that AELJ could do potent anti-inflammatory action via inhibition of NO release by affecting the iNOS expression level.

The expression of murine macrophage iNOS is regulated at the transcriptional level. NFκB is activated in response to the stimulation by LPS, and its activation is essential step in inducing iNOS gene expression in macrophage[20]. In nonstimulated cells, NF κB dimers are maintained in the cytoplasm through interaction with inhibitory proteins, the IkB. However, under LPS exposure, NFκB is activated by phosphorylation and subsequent degradation of IkB in RAW264.7 mouse macrophage[21, 22]. Our study showed that NFκB was positively reglated by LPS, and AELJ cotreatment significantly inhibited NFκB activity mouse peritoneal macrophages. AELJ also suppressed LPS-stimulated phosphorylation and degradation of IκB α.

Considerable efforts were to identify the stimulus-responsive serine kinase(s) responsible for IκB phosphorylation. These efforts bore fruit in 1996 with the biochemical identification and eventual purification of IKK complex[12, 23, 24]. IKK was defined through its ability to catalyze the phosphorylation of the N-terminal regulatoty serines on IκBαand IκBβas well as its rapid activation in response to cell stimulation by LPS in several cell lines including THP-1 human monocytic cells and RAW264.7 cells[25, 26]. It is not demonstrated that whether IKK is activated in LPS-stimulated mouse peritoneal macrophage yet. In this study, we demonstrated that IKKα was activated by LPS and its activity was significantly suppressed by AELJ in mouse peritoneal macrophages. This result suggests that the reduction of IKKα activity by AELJ can be mediated by direct effect on the IKKα or on events upstream from IKKα in the signal transduction pathway.

In summary, these results suggest that AELJ could inhibit LPS-stimulated NO production expression of iNOS gene and this biological effect may involve the inhibition of NFκB through negative regulation of IKK pathway. Further experiments will explore the isolation and characterization of the active chemical constitutes of AELJ in the inhibition of NO production.

ACKNOWLEDGEMENT

This work was supported by the grant No. R03-2003-000-10067-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

REFERENCES

1. Moncada, S., Palmer, R. M., Higgs, E. A. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109-42, 1991.
2. Marletta, M. A., Yoon, P. S., Iyengar, R., Leaf, C.D., Wishnok, J. S. Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. Biochemistry 27, 8706-11, 1988.
3. MacMicking, J., Xie, Q. W. and Nathan, C. Nitric oxide and macrophage function. Annu. Rev. Immunol. 15, 323-350, 1997.
4. Xie, W. Q., Kashiwabara, Y., and Nathan, C. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. J. Biol. Chem. 269, 4705-4708, 1994.
5. Duval, D. L., Miller, D. R., Collier, J., and Billings, R. E. Caracterization of hepatic nitric oxide synthase: Identification as the cytokine-inducible form primarily regulated by oxidents. Mol. Parmacol. 50, 277-284, 1996.
6. Yuan, T., Vogel, H. T., Sutherland, C., and Walsh, M. P. Characterization of the Ca2+ -dependent and - independent interactions between calmodulin and its binding domain of inducible nitric oxide synthase. FEBS lett. 431, 210-214, 1998.
7. May, M. J., and Ghosh, S. Signal transduction through NF-κB. Immunol Today 19, 80-88, 1998.
8. Spencer, E., Jiang, J., Chen, Z. J. Singal-induced ubiquitination of IB by the F-box protein Slimb/-TrCP. Genes Dev. 13, 284-294, 1999.
9. Winston, J. T., Strack, P., Beer-Romero, P., Chu, C. Y., Elledge, S. J., Harper, J. W. The SCF -TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IB and -catenin and stimulates IB ubiquitination in vitro. Genes Dev. 13, 270-283, 1999.
10. Rothwarf, D.M. and Karin, M. The NF-κB activation Pathway: A Paradigm in Information Transfer from Membrane to Nucleus. Sci STKE. 1999(5):RE1, 1999.
11. Chen, Z. J., Parent, L., Maniatis, T. Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity. Cell 84, 853-62, 1996.
12. DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E., Karin, M. A cytokine-responsive IκB kinase that activates the transcription factor NF-κ B. Nature 388, 548-54, 1997.
13. Choi, Y. J. Utility and cultivation of medicinal herb. O-sung express, 1994.
14. Kim, Y. H., Ko, W. S., Ha, M.S., Lee, C. H., Choi, B. T., Kang, H. S., Kim, H. D. The production of nitric oxide and TNF-α in peritoneal macrophages is inhibited by Dichroa febrifuga Lour. J. Ethnopharm. 69, 35-43, 2000.
15. Andrews, N. C., Faller, D. V., A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res 19, 2499 1991.
16. Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., and Kay, R. J. Complementation cloning of NEMO, a component of the IκB kinsae complex essential for NF-κB activation. Cell 93, 1231-1240, 1998.
17. Schletter, J., H. Heine, A. J. Ulmer, and E. T. Rietschel. Molecular mechanisms of endotoxin activity. Arch. Microbiology 164, 383-389, 1995
18. Lin, Y. L., Lin, J. K. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. Mol.

Pharmacol. 52, 465-72, 1997.

19. Wang, W. W., Jenkinson, C. P., Griscavage, J. M., Kern, R. M., Arabolos, N. S., Byrns, R. E., Cederbaum, S. D., Ignarro, L. J. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem. Biophys. Res. Commun. 210, 1009-16, 1995.
20. Kim, Y. M., Lee, B. S., Yi, K. Y., and Paik, S. G. Upstream NF-κB site is required for the maximal expression of mouse inducible nitric oxide synthase gene in interferon-g plus lipopolysaccharide-induced RAW264.7 macrophage. Biochem. Biophys. Res. Commun. 236, 655-660, 1997.
21. Baldwin, A. S. Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu. Rev. Immunol. 14, 649-83, 1996.
22. Tebo, J. M., Chaoqun, W., Ohmori, Y., Hamilton, T. A. Murine inhibitory protein-kappa B alpha negatively regulates kappa B-dependent transcription in lipopolysaccharide-stimulated RAW 264.7 macrophages. J. Immunol. 153, 4713-20, 1994.
23. Karin, M., Delhase, M. The IkB kinase (IKK) and NFkB: key elements of proinflammatory signalling Seminars in Immunology 12, 85-98, 2000.
24. Mercurio, F., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J., Young, D. B., Barbosa, M., Mann, M., Manning, A., Rao, A. IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. Science 278, 860-6, 1997.
25. O'Connell, M. A., Bennett, B. L., Mercurio, F., Manning, A. M., Mackman, N. Role of IKK1 and IKK2 in lipopolysaccharide signaling in human monocytic cells. J. Biol. Chem. 273, 30410-4, 1998.
26. Jeon, K. I., Jeong, J. Y., Jue, D. M. Thiol-reactive metal compounds inhibit NF-kappa B activation by blocking I kappa B kinase. J. Immunol. 164, 5981-9, 2000.