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Original Article

The Inhibitory Effect of Lycii Fructus on LPS-stimulated NF-κB Activation and iNOS Expression in RAW 264.7 Macrophages

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Objective : Anti-inflammatory effects of the extract of Lycii Fructus on lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophage cells were investigated.

Method : In order to assess the cytotoxic effect of Lycii Fructus on the raw 264.7 macrophages 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay was performed. Reverse transcription-polymerase chain reaction(RT-PCR) analysis of the mRNA levels of tumor necrosis factor-α(TNF-α) and inducible nitric oxide synthase(iNOS) was performed in order to provide an estimate of the relative level of expression of these genes. The protein level of the inhibitor of nuclear factor-κB(IκB) and nuclear factor-κB(NF-κB) activity was investigated by Western blot assay. NO production was investigated by NO detection.

Result : Lycii Fructus suppressed NO production by inhibiting the LPS-induced expressions of iNOS and TNF-α mRNA and iNOS protein in RAW 264.7 macrophage cells. Also, Lycii Fructus suppressed activation of NF-κB in the nucleus.

Conclusion : These results show that the extract of Lycii Fructus has anti-inflammatory effect probably by suppressing iNOS expressions through the down-regulation of NF-κB binding activity.

Key Words : Lycii Fructus, lipopolysaccharide, nitric oxide, inducible nitric oxide synthase, NF-κB

Introduction

Lycium chinense Miller, a plant of the Solanaceae family, distributed in northeast Asia, have been used as a tonic in Traditional Oriental Medicine. The fruit of this plant known as Lycii Fructus is used to exhibit hypotensive, hypoglycemic, antipyretic activities, and anti-stress activities[1,2]. Lycii Fructus has been reported to exhibit various biological activities, including nourishing the blood, improving eye brightness and tonyfying the kidney and liver in many Asian countries[3,4]. The main active component of Lycii Fructus is known as a carotenoid, polysaccharide and betaine. It has been reported to posses immunomodulatory, anti-tumor, anti-fungal and hepatoprotective activities[5-7].

Lipopolysaccharide(LPS) stimulates the production of inflammatory mediators such as nitric oxide(NO), tumor necrosis factor-α(TNF-α), interleukins, prostaglandin E_2(PGE_2), and leukotrienes in microglia cells[8-11]. Microglia is the macrophage-like cells of the central nervous system, and they generally considered as imm-

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unologically quiescent under normal conditions. Exaggerated pain is induced by the activation of astrocytes and microglia. Astrocytes and microglia are activated by pain-inducing neurotransmitters including substance P, glutamate and fractalkine[12,13]. These neurotransmitters are known to excite the pain-responsive neurons, resulting in the production of reactive oxygen species(ROS), nitric oxide(NO), prostaglandins (PGs) and growth factors[14].

NO is synthesized from L-arginine by nitric oxide synthase(NOS). According to physical and chemical properties, NOS is divided into neuronal NOS(nNOS), inducible NOS(iNOS), and endothelial NOS(eNOS). Especially, nNOS and eNOS are considered as consititutive NOS because they exist in normal state, whereas iNOS expressed by special stimulants such as LPS and cytokines is regarded as inducible NOS[15].

TNF-α, inflammatory cytokine, was produced by the various cells including macrophages, lymphocytes, neutrophils and mast cells. TNF-α is known to be a key mediator for the induction of apoptosis and development of humoral immune response. However, high level of TNF-α activity is closely associated with inducing tissue injury and potentiating septic shock[16-18]. TNF-α may have a homeostatic effect in limiting the extent of an inflammatory response as well as acting as an anti-malarial agent and functioning in intra-membranous bone repair[19,20].

Nuclear factor kappa B(NF-κB) plays a critical role in the expression of many genes involved in immune and inflammatory response[21-23]. iNOS expression is related with the upregulation of NF-κB[24], the site that was identified in the promoter region of iNOS gene[25]. NF-κB is constitutively localized in the cytosol as a homodimer or a heterodimer, and is associated with inhibitory IκB protein(IκB). Also, activation of NF-κB is induced by activation of IκB kinase(IKK), which phosphorylates IκB, leading to its degradation and translocation of NF-κB to the nucleus[26].

Hepatoprotective effect of Lycii Fructus has been studied and reported, however, the mechanism of the anti-inflammatory action of Lycii Fructus has not been clarified yet.

In the present study, the effect of Lycii Fructus on the LPS-stimulated expression of NF-κB, TNF-α and iNOS was investigated. In order to assess the cytotoxic effect of Lycii Fructus on the raw 264.7 macrophages 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay was performed. Reverse transcription-polymerase chain reaction(RT-PCR) analysis of the mRNA levels of TNF-α and iNOS was performed in order to provide an estimate of the relative level of expression of these genes. The protein level of the inhibitor of nuclear factor-κB(IκB) and NF-κB activity was investigated by Western blot assay. NO production was investigated by NO detection.

Materials and methods

1. Cell culture

Raw 264.7 macrophages were cultured in Dulbecco's modified Eagle's Medium(DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum(FBS; Gibco BRL) at 37°C in 5% CO_2 -95% O_2 in a humidified cell incubator. The cells were plated onto culture dishes at a density of 2×10^4 cells/cm^2 24 h prior to drug treatments.

2. Reagents

Lipopolysacharride(LPS), pyrrolidine dithiocarbamate(PDTC), were purchased from Sigma Chemical Co(St. Louis, MO, USA).

3. Preparation of the extract of Lycii Fructus

To obtain the extract of Lycii Fructus, 50 g of Lycii Fructus was added to distilled water and extraction was performed by heating at 80°C for 2 h, concentrating with rotary evaporator(Eyela, Tokyo, Japan), and lyophilizing by a drying machine(Ilsin, Kyungkido, Korea) for 24 h. The resulting powder, weighting 19.51 g(yield of 39.02%) was diluted to the concentrations needed with autoclaved distilled water and filtered through a 0.22 ㎛ syringe filter before use.

4. MTT cytotoxicity assay

Raw 264.7 macrophages were grown in a final volume of 100 ㎕ culture medium per well in 96-well plates. In order to determine the cytotoxicity of Lycii Fructus, the cells were treated with Lycii Fructus at concentrations of 1, 10, 100, 1,000 and 10,000 ㎍/㎖ for 24 h. The cells in the control group were left untreated. After adding 10 μl of the MTT labeling reagent containing 5 ㎎/㎖ 3-(4,5-dimethylthiazol -2yl)-2,5-diphenyltetrazolium bromide in phosphate-buffered saline to each well, the plates were incubated for 2 h. Solubilization solution 100 μl containing 10% sodium dodecyl sulfate in 0.01 M hydrochloric acid was added to each well, and the cells were incubated for another 12 h. The absorbance was then measured with a microtiter plate reader(Bio-Tek, Winooski, VT, USA) at a test wavelength of 595 nm with a reference wavelength of 690 nm. The optical density(O.D.) was calculated as the difference between the absorbance at the reference wavelength and that observed at the test wavelength. Percent viability was calculated as(O.D. of drug-treated sample/control O.D.) × 100.

5. RNA Isolation and RT-PCR

To identify the expression of TNF-α and iNOS mRNA, RT-PCR was performed. The total RNA was isolated from raw 264.7 macrophages using RNAzol^{TM}B(TEL-TEST, Friendswood, TX, U.S.A.). 2 ㎍ of RNA and 2 ㎕ of random hexamers (Promega, Madison, WI, USA.) were added together, and the mixture was heated at 65°C for 15 min. 1 ㎕ of AMV reverse transcriptase (Promega), 5 ㎕ of 2.5 mM dNTP(Promega), 1 ㎕ of RNasin(Promega), and 8 ㎕ of 5 × AMV RT buffer(Promega) were then added to the mixture, and the final volume was brought up to 40 ㎕ volume with diethylpyrocarbonate (DEPC) -treated water. The reaction mixture was then incubated at 42°C for 2 h.

PCR amplification was performed in a reaction volume of 40 ㎕ containing 1 ㎕ of the appropriate cDNA, 0.5 ㎕ of each set of primers at a concentration of 10 pM, 4 ㎕ of 10 × RT buffer, 1 ㎕ of 2.5 mM dNTP, and 0.2 ㎕ of Taq DNA polymerase(Takara, Shiga, Japan).

For mouse TNF-α, the primer sequences were 5'-TCATACCAGGGTTTGAGCTCAG-'(a 22-mer sense oligonucleotide) and 5'-TCCCC-AAAGGGATGAGAAGTT-3'(a 21-mer anti-sense oligonucleotide). For mouse iNOS, the primer sequences were 5'-CAAGAGTTTGACCAGA-GGACC-3'(a 21-mer sense oligonucleotide) and 5'-TGGAACCACTCGTACTTGGGA-3'(a 21-mer anti-sense oligonucleotide). For cyclophilin, the internal control used in the study, the primer sequences were 5'-ACCCCACCGTGT-TCTTCGAC-3'(a 20-mer sense oligonucleotide)

and 5'-CATTTGCCATGGACAAGATG-3'(a 20-mer anti-sense oligonucleotide). The expected size of the PCR product was 615 bp for TNF-α, 180 bp for iNOS, and 650 bp for cyclophilin.

For TNF-α, the PCR procedures were carried out using a PTC-0150 MiniCycler(Bio-Rad, Hercules, CA, USA) under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 amplification cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec, with an additional extension step at the end of the procedure at 72°C for 10 min. For iNOS, the PCR procedures were under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 amplification cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 45 sec, with an additional extension step at the end of the procedure at 72°C for 10 min. For cyclophilin, under the following conditions: initial denaturation at 94°C for 5 min, followed by 25 amplification cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec, with an additional extension step at the end of the procedure at 72°C for 10 min. The final amount of RT-PCR product for each of the mRNA species was calculated densitometrically using Imaging-Pro®Plus(Media Cyberbetics Inc., Silver Spring, MD, USA).

6. Preparation of whole cell extract

The cells were lysed in a ice-cold whole cell lysate buffer containing 50 mM HEPES(pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM magnesium chloride hexahydrate, 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-N, N'-tetraacetic acid(EGTA), 1 mM phenylmethylsulfonyl fluoride(PMSF), 2 ㎍/㎖ leupeptin, 1 ㎍/㎖ pepstatin, 1 mM sodium ortho vanadate, and 100 mM sodium floride, and then the mixture was incubated on ice for 30 min. The cells were centrifuged at 14,000 × g for 15 min at 4°C.

7. Preparation of nuclear and cytosolic extracts

The cells were collected and suspended in hypotonic buffer(10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.2 mM PMSF, 0.5 mM DTT, 10 ㎍/㎖ aprotinin) and incubated on ice for 10 min. They were lysed by the addition of 0.1% Nonidet P-40 and vigorous vortexing for 10 sec. The cells were centrifuged at 4,000 × g for 5 min at 4°C. The supernatants containing protein were collected. The pellets acquired from cytosolic protein extraction were resuspended in high salt buffer (20 mM HEPES, pH 7.9, 25% glycerol, 400 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 1 mM NaF, 1 mM sodium vanadate). The cells were centrifuged at 14,000 × g for 5 min at 4°C.

8. Western blot assay

Whole protein extract was used to analyze the expressions of iNOS proteins. Cytosolic extract was used for the detection of IκB-α protein expression, while nuclear extract was used for the NF-κB(p65) protein expression. The protein concentrations were measured using a Bio-Rad colorimetric protein assay kit(Bio-Rad, Hercules, CA, USA). Protein 40 ㎍ was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane(Schleicher & Schuell GmbH, Dassel, Germany). Rabbit iNOS antibody(1:500; Santa Cruz Biot-

ech), rabbit NF-κB(p65) antibody(1:500; Santa Cruz Biotech) and rabbit IκB-α antibody(1:500; Santa Cruz Biotech) were used as the primary antibody. Horseradish peroxidase-conjugated anti-goat antibody(1:4000; Santa Cruz Biotech) was used to probe for iNOS, NF-κB(p65) and IκB-α was used as the secondary antibody. Band detection was performed using the enhanced chemiluminescence(ECL) detection system(Santa Cruz Biotech).

9. Determination of NO nitric oxide production

In order to determine the effect of Lycii Fructus on NO production, the amount of nitrite in the supernatant was measured using a commercially available NO detection kit(iNt- RON, INC., Seoul, Korea). After collection of 100 ㎕ of supernatant, 50 ㎕ of N1 buffer was added to each well, and the plate was incubated at room temperature for 10 min. N2 buffer was then added, and the plate was incubated at room temperature for 10 min. The absorbance of the content of each well was measured at a wavelength of 540 nm. The nitrite concentration was calculated from a nitrite standard curve.

10. Statistical analysis

The results are presented as the mean ± standard error of the mean(SEM). The data were analyzed by one-way ANOVA followed by Duncan's post-hoc test using SPSS(ver 10.0). The differences were considered statistically significant at $P < 0.05$.

Results

1. Cell viability of Lycii Fructus on raw 264.7 macrophage cell

In order to assess the cytotoxic effect of the Lycii Fructus on the raw 264.7 macrophages, the cells were cultured with Lycii Fructus at final concentrations of 1, 10, 100, 1,000 and 10,000 ㎍/㎖ for 24 h, and MTT assays were then carried out. The experiments were repeated four times. The cells cultured in Lycii Fructus-free media were used as the no-treated group. The viability of cells incubated with Lycii Fructus at concentrations of 1, 10, 100, 1,000 and 10,000 ㎍/㎖ for 24 h was 95.89±1.37, 94.96 ±0.98, 97.80±0.80, 95.93±1.43 and 65.09±0.97% of the control value, respectively (Fig. 1).

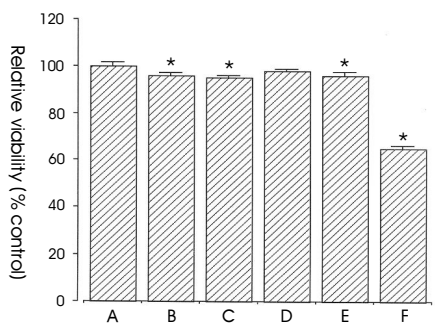


Fig. 1. Cell viability of Lycii Fructus on raw 264.7 macrophage cell. A, No-treated group ; B, 1 ㎍/㎖ Lycii Fructus treated group ; C, 10 ㎍/㎖ Lycii Fructus treated group for 24 h; D, 100 ㎍/㎖ Lycii Fructus treated group ; E, 1,000 ㎍/㎖ Lycii Fructus treated group ; F, 10,000 ㎍/㎖ Lycii Fructus treated group. The results are presented as the mean ± SEM. * $F < 0.05$ compared to the group A.

The present results showed that Lycii Fructus exerted no cytotoxicity until it was at a concentration of 1,000, however, 10,000 ㎍/㎖ of Lycii Fructus reduced cell viability. Then, we used 100 and 1,000 ㎍/㎖ of the Lycii Fructus for the next experiments.

2. Effect of Lycii Fructus on mRNA expression of TNF-α and iNOS

RT-PCR analysis of the mRNA levels of TNF-α and iNOS was performed in order to provide an estimate of the relative level of expression of these genes. In this study, the mRNA level of TNF-α and iNOS in the control cells was set as 1.00. Cyclophilin mRNA was used as the internal control.

The level of TNF-α mRNA following a treatment with 1 ㎍/㎖ LPS for 24 h was markedly increased to 4.43±0.74. The level of TNF-α mRNA was decreased to 3.64±0.77, 2.18±0.22 and 1.43±0.11 in cells pre-treated for 1 h with Lycii Fructus extract at 100, 1,000 ㎍/㎖ and 10μM PDTC, following 1 ㎍/㎖ LPS treatment for 24 h(Fig. 2).

The level of iNOS mRNA following a treatment with 1 ㎍/㎖ LPS for 24 h was markedly increased to 4.96±0.61. The levels of iNOS mRNA were decreased to 4.54±0.51, 3.19±0.43 and 2.60±0.41 in cells pre-treated for 1 h with Lycii Fructus extract at 100, 1,000 ㎍/㎖ and 10μM PDTC, following 1 ㎍/㎖ LPS treatment for 24 h(Fig. 2).

The present results show that LPS enhanced TNF-α and iNOS mRNA expression in raw 264.7 macrophages and pre-treatment with Lycii Fructus suppressed LPS-induced TNF-α and iNOS mRNA expression.

3. Effect of Lycii Fructus on the expression of iNOS protein

Analysis of the expressions of iNOS protein was performed in order to provide an estimate of the relative level of expressions of these proteins. In this study, the expressions of iNOS

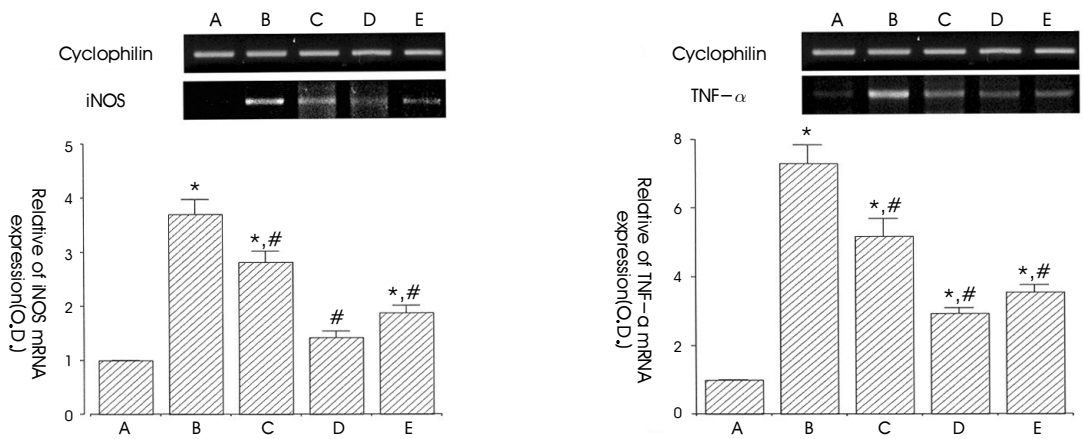


Fig. 2. Results of RT-PCR analysis of the mRNAs Level of TNF-α and iNOS. A, No-treated group ; B, LPS-treated group ; C, LPS- and 100 ㎍/㎖ Lycii Fructus-treated group ; D, LPS and 1,000 ㎍/㎖ Lycii Fructus-treated group; E, LPS and 10μM PDTC-treated group. * $P < 0.05$ compared to the group A. # $P < 0.05$ compared to the group B.

protein in the control cells were set as 1.00. Cyclophilin mRNA was used as the internal control.

The expression of iNOS protein following a treatment with 1 ㎍/㎖ LPS for 24 h was 2.34 ±0.13. When the cells were pre-treated for 1 h with Lycii Fructus at 100, 1,000 ㎍/㎖, and 10 μM PDTC following 1 ㎍/㎖ LPS treatment for 24 h, the expressions of iNOS protein was 1.93±0.61, 1.28±0.03 and 1.44±0.07, respectively (Fig. 3).

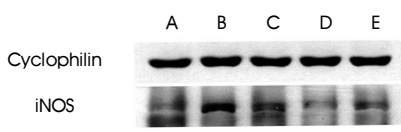
The present results showed that LPS enhanced the expression of iNOS protein in raw 264.7 macrophages and that Lycii Fructus and PDTC suppressed LPS-induced expression of iNOS protein.

4. Effect of Lycii Fructus on the expression of NF-κB(p65) protein in the nuclear fraction and IκB protein in the cytosolic fraction

The effect of Lycii Fructus on the expression of NF-κB protein in the nuclear fraction following LPS treatment for 20 min was investigated. The expression of NF-κB(p65) protein in the nuclear fraction in the control cells was set as 1.00. Actin was used as the internal. The expression of NF-κB(p65) protein in the nuclear fraction was markedly increased to 2.29±0.11 following the treatment with 1 ㎍/㎖ LPS for 20 min. The expression of NF-κB(p65) protein in the nuclear fraction was decreased to 1.67±0.07, 1.22±0.01 and 1.39± 0.08 in the cells pre-treated for 1 h with Lycii Fructus at 100, 1,000 ㎍/㎖ and 10μM PDTC, respectively, following 1 ㎍/㎖ LPS treatment for 20 min (Fig. 4).

The expression of IκB-α protein in the cytosolic fraction in the control cells was set as 1.00. The expression of IκB-α protein in the nuclear fraction was markedly increased to 0.46±0.07 following the treatment with 1 ㎍/㎖ LPS for 20 min. The expression of IκB-α protein in the cytosolic fraction was increased to 0.58±0.07, 0.61±0.06 and 0.67±0.01 in the cells pre-treated for 1 h with Lycii Fructus at 100, 1,000 ㎍/㎖ and 10 μM PDTC, respectively, following 1 ㎍/㎖ LPS treatment for 20 min(Fig. 4).

The present results showed that treatment



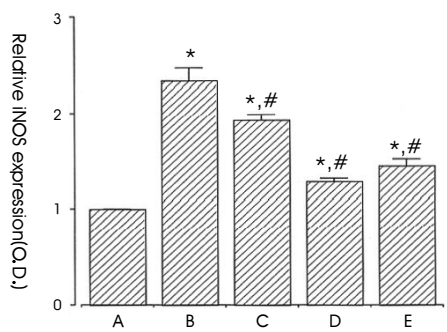


Fig. 3. Results of Western blot assay of the protein levels of iNOS. A, No-treated group; B, LPS-treated group; C, LPS- and 100 ㎍/㎖ Lycii Fructus-treated group; D, LPS- and 1,000 ㎍/㎖ Lycii Fructus-treated group; E, LPS- and 10μM PDTC-treated group. * $P < 0.05$ compared to the group A. # $P < 0.05$ compared to the group B.

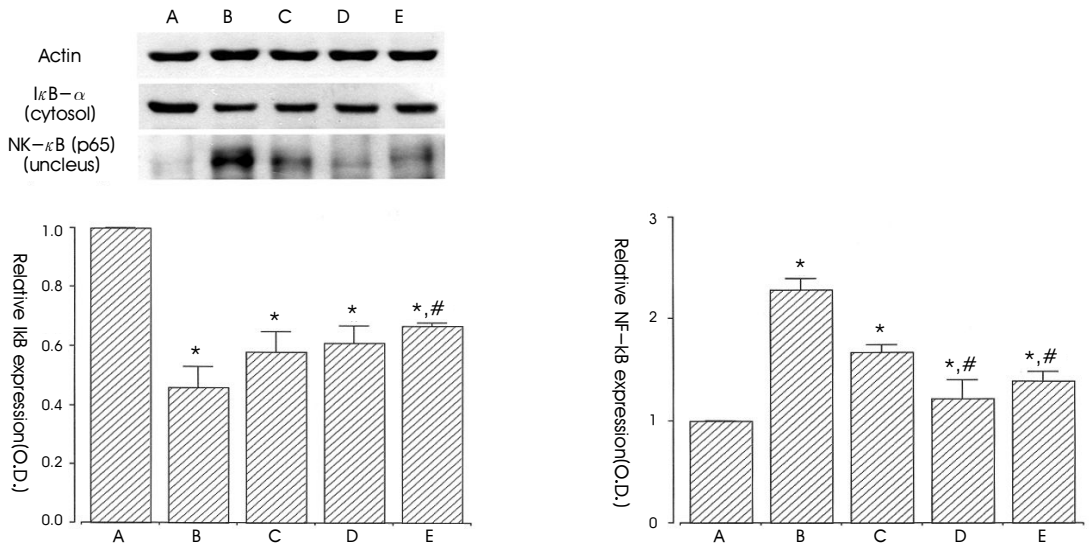


Fig. 4. Results of Western blot assay of NF-κB(p65) and degradation of IκB-α. A, No-treated group; B, LPS-treated group; C, LPS- and 100 ㎍/㎖ Lycii Fructus-treated group; D, LPS- and 1,000 ㎍/㎖ Lycii Fructus-treated group; E, LPS- and 10 μM PDTC-treated group. The results are presented as the mean ± SEM. * $P < 0.05$ compared to the group A. # $P < 0.05$ compared to the group B.

with Lycii Fructus decreased LPS-induced the expression of NF-κB(p65) protein in the nuclear fraction, while increased LPS-induced decreasing of IκB-α protein in the cytosolic fraction.

5. Effect of Lycii Fructus on NO production

NO was detected and analyzed by enzyme-linked immunosorbent assay(ELISA). The experiments were repeated four times. From the NO

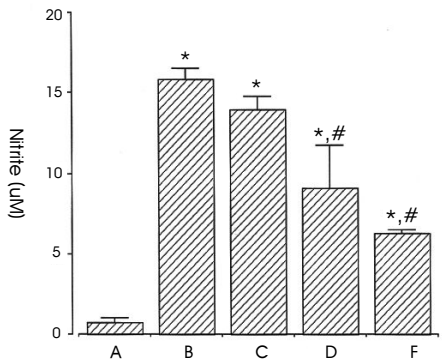


Fig. 5. Measurement of NO production in raw 264.7 macrophages. A, No-treated group; B, LPS-treated group; C, LPS- and 100 ㎍/㎖ Lycii Fructus-treated group; D, LPS- and 1,000 ㎍/㎖ Lycii Fructus-treated group; E, LPS- and 10 μM PDTC. The results are presented as the mean ± SEM. * $P < 0.05$ compared to the group A. # $P < 0.05$ compared to the group B.

detection assay, the amount of nitrite was increased from 0.76±0.25 to 15.81±0.70 μM after 24 h of exposure to LPS. NO production was decreased to 13.92±0.81, 9.10±2.62 and 6.29±1.15 μM by pre-reatment for 1 h with Lycii Fructus at 100, 1,000 ㎍/㎖, and 10μM PDTC, following 1 ㎍/㎖ LPS treatment for 24 h(Fig. 5).

The present results showed that LPS enhanced NO production in raw 264.7 macrophages and Lycii Fructus, and PDTC suppressed LPS-induced NO production.

Discussion

Inflammation is a response of the organism to injury related to physical or chemical noxious stimuli or microbiological substances, which is involved in multiple pathologies such as arthritis, asthma, multiple sclerosis, colitis, inflammatory bowel disease and atherosclerosis[27]. Nowadays, there are many efforts to research drug that has less side effect and more anti-inflammatory functions. It has been found that interleukin (IL)-1β and TNF-α, which is regulated by NF-κB, are concerned in many inflammatory response and immune disease[28,29]. Activated microglia generates inflammatory cytokines, such as TNF-α and IL-1β, NF-κB, and NO that probably cause or exacerbate neuronal degeneration[30-35].

NO is known to regulate inflammatory and immune responses in several ways. iNOS, absolutely produces large amounts of NO, and excessive NO production induces pathologic states[36]. Actually NO causes inflammatory damages, genetic alterations and autoimmune diseases[37-39]. In addition, some studies have shown a correlation between the release of NO by microglia and the progression of neurodegeneration[8,40]. In the present results, LPS treatment enhanced iNOS expression, and it increased the synthesis of NO in raw 264.7 macrophages.

NF-κB is believed to play an important role in the regulation of inflammatory response. NF-κB activation was associated with the phosphorylation and degradation of IκB-α and the nuclear translocation of p65[41,42]. Excessive proinflammatory cytokine and NO production through NF-κB activation plays in inflammatory diseases[43]. Activation of NF-κB is also critical for the induction of iNOS gene expression in LPS-stimulated macrophages[25,44]. The activation of NF-κB can induce the transcription of cytokines such as TNF-α, IL-1β, and IL-6 genes[45]. The NF-κB signaling pathway plays an important role in regulating inflammation through transcription of COX, iNOS and cytokine genes[46]. Baeuerle[47] reported that the expressions of iNOS in raw 264.7 macrophages are dependent on NF-κB activation. In the present results, LPS treatment enhanced the NF-κB and TNF-α expression in raw 264.7 macrophages, while decreased the IκB expression.

Lycii Fructus is known to contain betaine, carotene, nicotinic acid, zeaxanthin and a cerebroside[48]. Among these, betaine was reported to be the biologically active component. Betaine is known to suppress inflammation via NF-κB pathway. It has been reported that betaine suppresses certain proinflammatory signaling factors via NF-κB pathway[49]. In addition, It has been reported that Lycii Fructus showed inhibitory effect on edema in models of inflammation in rats[50].

In these results, we have found that Lycii Fructus, suppressed NO and TNF-α production, iNOS expression by LPS via down-regulation of NF-κB activation in raw 264.7 macrophages.

Also, LPS-induced NO production was decreased by PDTC, which is a NF-κB inhibitor. PDTC also suppressed LPS-induced expressions of NF-κB and TNF-α and decreased IκB-α expression in raw 264.7 macrophages.

Here in this study, we have shown that Lycii Fructus inhibited LPS-induced NO and TNF-α production. Moreover, these observation suggest that Lycii Fructus may involve the inhibition of inflammation through regulation of the NF-κB pathway. Thus, Lycii Fructus appears to be a potential therapeutic agent for treating LPS-induced inflammation.

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