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Anti-inflammatory and Analgesic Actions of Aqueous Extract of Amygdalin from *Armeniacae semen* and Manufactured Amygdalin in Mouse BV2 Microglial Cells

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ABSTRACT

Microglia are responsible for the brain inflammation and activated by stimulants such as injury and lipopolysaccharide. Amygdalin is abundant in *Armeniacae semen*. *Armeniacae semen* is the seed of *Prunus armeniaca L. var. ansu Maxim* which is classified into Rosaceae. *Armeniacae semen*. *Armeniacae semen* has been used for the treatment of pain and inflammatory diseases. We prepared the aqueous extract of the amygdalin from *Armeniacae semen*. In this study, we compared the effects of the manufactured amygdalin and amygdalin extracted from *Armeniacae semen* on the lipopolysaccharide (LPS)-stimulated expressions of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) in the mouse BV2 microglial cells. For this study, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, reverse transcription-polymerase chain reaction (RT-PCR), prostaglandin E_2 (PGE_2) immunoassay, and nitric oxide (NO) detection were performed. The present results showed that the amygdalin extract from *Armeniacae semen* suppressed PGE_2 synthesis and NO production by inhibiting the LPS-stimulated enhancement of COX-2 and iNOS mRNA expressions, while the manufactured amygdalin did not significantly reduce PGE_2 synthesis and NO production. Here in this study, we showed that the aqueous extract of amygdalin from *Armeniacae semen* exerts anti-inflammatory and analgesic effects, however, the manufactured amygdalin dose not have such effects.

Key words: *Armeniacae semen,* lipopolysaccharide, cyclooxygenase-2, prostaglandin E_2, inducible nitric oxide synthase (iNOS)

INTRODUCTION

Amygdalin is abundant in *Armeniacae semen*, the seeds of *Prunus armeniaca L. var. ansu Maxim* which is classified into Rosaceae. *Armeniacae semen* has traditionally been used for the treatment of asthma, bronchitis, emphysema, constipation, nausea, leprosy, and leucoderma (Pak et al., 1999; Hwang et al., 2003). Amygdalin is also known as vitamain B_{17}, which was used to treat cancers, named as laetrile (Fukuta et al., 2003). It was also reported that amygdalin is effective for relieving

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pain of the cancer patients (Ellison et al., 1978).

Brain inflammation has been implicated in the pathogenesis of brain infection, trauma and several neurodegenerative disorders including Alzheimer's disease and Parkinson's disease. Anti-inflammatory drugs are known to reduce the risk and progression of Alzheimer's disease and the neuronal damage in animal models of Parkinson's disease (McGeer et al., 1988; Rogers et al., 1988; Kyrkanides et al., 2002).

Microglia, the major inflammatory cells in the brain, are responsible for the brain inflammation and activated by stimulants such as injury and lipopolysaccharide (LPS) (González-Scarano and Baltuch, 1999; Vegeto et al., 2001). LPS initiates a number of major cellular responses that play vital roles in the pathogenesis of inflammatory responses including activation of inflammatory cells and the production of cytokines and other mediators. Among these, prostaglandin E_2 (PGE_2) is a key inflammatory mediator that is synthesized from arachidonic acid *via* a cyclooxygenase (COX)-dependent pathway. There are two isoforms of COX: COX-1 and COX-2. While COX-1 is a constitutively expressed form in normal physiologic functions, COX-2 is expressed only in response to inflammatory signals such as cytokines and the bacterial endotoxin like LPS. COX-2 produces a large amount of PGE_2, and this induces inflammation (Mitchell et al., 1995; Fiebich et al., 2000).

Nitric oxide (NO) is endogenously generated from $_L$-arginine by NO synthase (NOS) and plays important role in the regulation of many physiological processes. Several isoforms of NOS exist, and these isoforms fall into three major classes: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Of these, iNOS is an important enzyme involved in the regulation of inflammation (Yui et al., 1991). In addition, it was reported that LPS up-regulates iNOS expression in macrophages (Korhonen et al., 2002) and microglial cells (Vegeto et al., 2001).

We prepared the aqueous extract of the amygdalin from *Armeniacae semen*. In the present study, we compared the effects of the manufactured amygdalin and amygdalin extracted from *Armeniacae semen* on the LPS-stimulated expressions of COX-1, COX-2, and iNOS in the mouse BV2 microglial cells. For this study, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, reverse transcription-polymerase chain reaction (RT-PCR), PGE_2 immunoassay, and NO detection were performed.

MATERIALS AND METHODS

Cell culture

Mouse BV2 microglial cells 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 in culture dishes at a density of $2\sim3\times10^4$ cells/cm^2 24 h prior to amygdalin treatments.

Preparation of amygdalin

The manufactured amygdalin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The aqueous extract of amygdalin was prepared from *Armeniacae semen*. *Armeniacae semen* used in this experiment was obtained from the Kyungdong market (Seoul, Korea). After immersing in 0.1% citric acid for 1 min, *Armeniacae semen* was rinsed and dried in room temperature for 24 h. Then, it was pulverized by crusher (Hanil, Seoul, Korea) and the fine power was sifted from the course particles by a mesh screen with a pore diameter of 2 mm. In order to obtain the aqueous extract of amygdalin from *Armeniacae semen*, the fine powder was subsequently heat-extracted by distilled water, pressure-filtered, and concentrated with a rotary evaporator (Eyela, Tokyo, Japan). The resulting 34.48 g powder (yield of 6.88%) was obtained from 500 g of *Armeniacae semen* through lyophilization by a drying machine (Ilsin, Kyungkido, Korea) for 24 h.

MTT cytotoxicity assay

The cells were grown in final volume of 100 μl culture medium per well in 96-well plates. In order to determine the cytotoxicity of amygdalins, the cells were treated with the manufactured amygdalin and the aqueous extract of amygdalin from *Armeniacae semen* at concentrations of 0.1 μg/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml, 1,000 μg/ml for 24 h.

The cells in the control group were left untreated. After adding 10 μl of the MTT labeling reagent containing 5 mg/ml 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide in phosphate-buffered saline to each well, the plates were incubated for 4 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.

RNA isolation and RT-PCR

To identify expressions of COX-1, COX-2, and iNOS mRNA, RT-PCR was performed. Total RNA was isolated from BV2 cells using RNAzol^{TM}B (TEL-TEST, Friendswood, TX, USA). Two μg of RNA and 2 μl of random hexamers (Promega, Madison, WI, USA) were added together, and the mixture was heated at 65°C for 10 min. One μl of AMV reverse transcriptase (Promega), 5 μl of 10 mM dNTP (Promega), 1 μl of RNasin (Promega), and 5 μl of 10×AMV RT buffer (Promega) were then added to the mixture, and the final volume was brought up to 50 μl with diethylpyrocarbonate (DEPC)-treated water. The reaction mixture was then incubated at 42°C for 1 h.

PCR amplification was performed in a reaction volume of 40 μl containing 1 μl of the appropriate cDNA, 1 μl of each set of primers at a concentration of 10 pM, 4 μl of 10×RT buffer, 1 μl of 2.5 mM dNTP, and 2 units of Taq DNA polymerase (Takara, Shiga, Japan). For mouse COX-1, the primer sequences were 5'-AGTGCGGTCCAACCTTATCC-3' (a 20-mer sense oligonucleotide) and 5'-CCGCAGGTGATACTGTCGTT-3' (a 20-mer anti-sense oligonucleotide). For mouse COX-2, the primer sequences were 5'-TGCATGTGGCTGTGGATGTCATCAA-3' (a 25-mer sense oligonucleotide) and 5'-CACTAAGACAGACCCGTCATCTCCA-3' (a 25-mer anti-sense oligonucleotide). For mouse iNOS, the primer sequences were 5'-GTGTTCCACCAGGAGATGTTG-3' (a 21-mer sense oligonucleotide) and 5'-CTCCTGCCCACTGAGTTCGTC-3' (a 21-mer anti-sense oligonucleotide). For cyclophilin, the internal control used in the study, the primer sequences were 5'-ACCCCACCGTGTTCTTCGAC-3' (a 20-mer sense oligonucleotide) and 5'-CATTTGCCATGGACAAGATG-3' (a 20-mer anti-sense oligonucleotide). The expected sizes of the PCR products were 381 bp for COX-1, 583 bp for COX-2, 500 bp for iNOS, and 299 bp for cyclophilin.

For COX-1, COX-2, and iNOS, the PCR procedure was 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 45 sec, annealing at 60°C for 45 sec, and extension at 72°C for 60 sec, with an additional extension step at the end of the procedure at 72°C for 10 min. For cyclophilin, the PCR procedure was 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 55°C, 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 Molecular Analyst^{TM} version 1.4.1 (Bio-Rad, Hercules, CA, USA).

Measurement of prostaglandin E_2 synthesis

Assessment of PGE_2 synthesis was performed using a commercially available PGE_2 competitive enzyme immunoassay kit (Amersham Pharmacia Biotech. Inc., Piscataway, NJ, USA). Supernatant 100 μl from culture medium and standards were put into different wells on the goat anti-mouse IgG-coated microtiter plate provided in the kit. Mouse anti-PGE_2 antibody and peroxidase-conjugated PGE_2 were added to each well, and the plate was incubated at room temperature with shaking for 1 h. The wells were drained and washed, and 3,3',5,5'-tetramethylbenzidine/hydrogen peroxide solution was added. The plate was incubated at room temperature with shaking, and the reaction was stopped after 30 min through the addition of H_2SO_4. The absorbance of the content of each well was then measured at 450 nm.

Determination of nitric oxide production

In order to determine the effect of amygdalin on NO production, the amount of nitrite in the supernatant was measured as an indicator of NO production based on the Griess reaction. After collection of 100 μl of cell culture medium, 50 μl of 1% sulfanilamide was added to each well, and the plate was incubated at room temperature for 10 min. 0.1% naphtylethylenediamine containing 5% phosphoric acid 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 540 nm. The nitrite concentration was calculated from a nitrite standard curve generating by mixing 0 to 200 μM sodium nitrite solutions with Griess reagent. Standard curve was typically linear between 0 and 200 μM of sodium nitrite.

Statistical analysis

The results are presented as the mean±standard error of the mean (S.E.M.). The data were analyzed by one-way ANOVA followed by Duncan's post-hoc test using SPSS 12.0. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of amygdalin on viability of BV2 cells

In order to assess the cytotoxic effect of the manufactured amygdalin and the aqueous extract of amygdalin from *Armeniacae semen* on the mouse BV2 microglial cells, BV2 cells were cultured with two types of amygdalin at final concentrations of 0.1 μg/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml, and 1,000 μg/ml for 24 h, and MTT assay was then carried out.

The viability of cells incubated with the manufactured amygdalin at concentrations of 0.1 μg/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml, and 1,000 μg/ml for 24 h was 91.61±2.27%, 94.32±2.24%, 92.20±2.99%, 90.23±4.00%, and 90.83±2.56% of the control value. The viability of cells incubated with the aqueous extract of amygdalin from *Armeniacae semen* at concentrations of 0.1 μg/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml, and 1,000 μg/ml for 24 h was 94.53±2.46%, 91.43±3.51%, 91.04±3.00%, 92.84±3.04%, and 92.58±3.08% of the control value, respectively. The results show that both types of amygdalin exerted no significant cytotoxicity until it reached at a concentration of 1,000 μg/ml (Fig. 1).

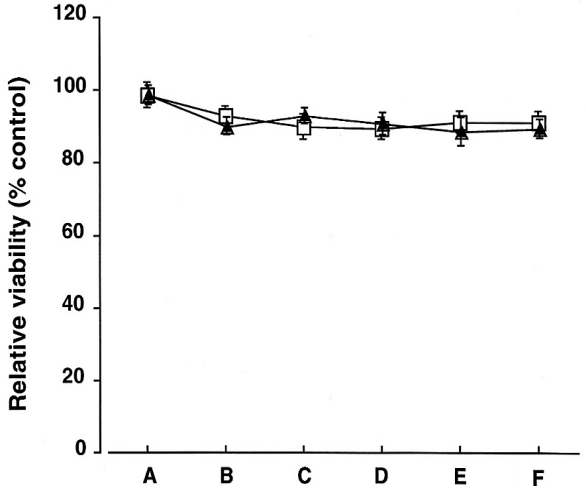

Fig. 1. Cytotoxicity effect of amygdalin. (A) Control cells; (B) 0.1 μg/ml amygdalin-treated cells; (C) 1 μg/ml amygdalin-treated cells; (D) 10 μg/ml amygdalin-treated cells; (E) 100 μg/ml amygdalin-treated cells; (F) 1 mg/ml amygdalin-treated cells. (▲) manufactured amygdalin, (□) aqueous extract of amygdalin from *Armeniacae semen*.

Effect of amygdalin on the mRNA expressions of COX-1, COX-2, and iNOS

RT-PCR analysis of the mRNA levels of COX-1, COX-2, and iNOS was performed in order to provide an estimate of the relative level of expressions of these genes. In the present study, the mRNA levels of COX-1, COX-2, and iNOS in the control cells were set as 1.00. The level of COX-1 mRNA following a treatment with 2 μg/ml LPS for 6 h was 1.06±0.13. The level of COX-1 mRNA in the cells treated with the manufactured amygdalin at concentrations of 10 μg/ml and 100 μg/ml was 0.99±0.12 and 1.01±0.20, respectively. The level of COX-1 mRNA in the cells treated with the aqueous extract of amygdalin from *Armeniacae semen* at concentrations of 10 μg/ml, 100 μg/ml, and 100 μM acetylsalicylic acid (ASA) was 0.87±0.02, 0.65± 0.06, and 0.51±0.01, respectively (Fig. 2).

The level of COX-2 mRNA was remarkably increased to 2.40±0.08 following a treatment with 2 μg/ml LPS for 6 h. The level of COX-2 mRNA in the cells treated with the manufactured amygdalin at concentrations of 10 μg/ml and 100 μg/ml was 2.35±0.09 and 2.26±0.16, respectively. The level of COX-2 mRNA in the cells treated with the aqueous

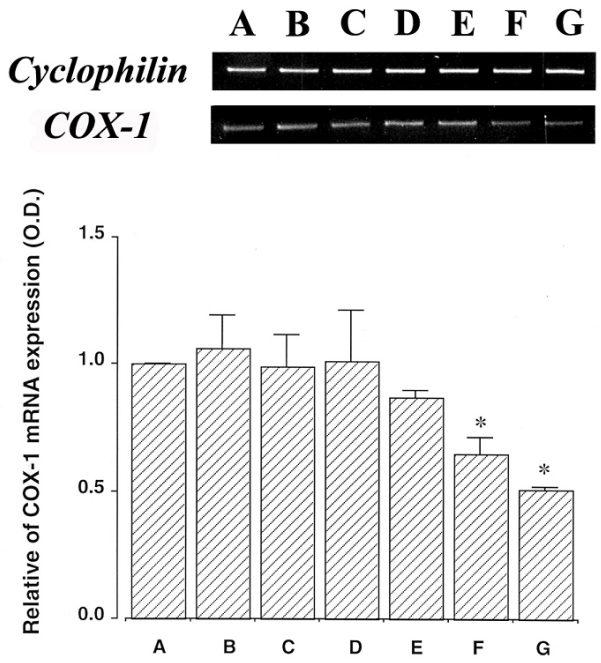

Fig. 2. Effect of amygdalin on the mRNA expression of cyclooxygenase-1 (COX-1). *p < 0.05 compared to the control. (A) Control group; (B) 2 μg/ml lipopolysaccharide (LPS)-treated group; (C) 2 μg/ml LPS and 10 μg/ml manufactured amygdalin- treated group; (D) 2 μg/ml LPS and 100 μg/ml manufactured amygdalin-treated group; (E) 2 μg/ml LPS and 10 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (F) 2 μg/ml LPS and 100 μg/ml aqueous extract of amygdalin from Armeniacae semen-treated group; (G) 2 μg/ml LPS and 500 μM acetylsalic acid (ASA)-treated group.

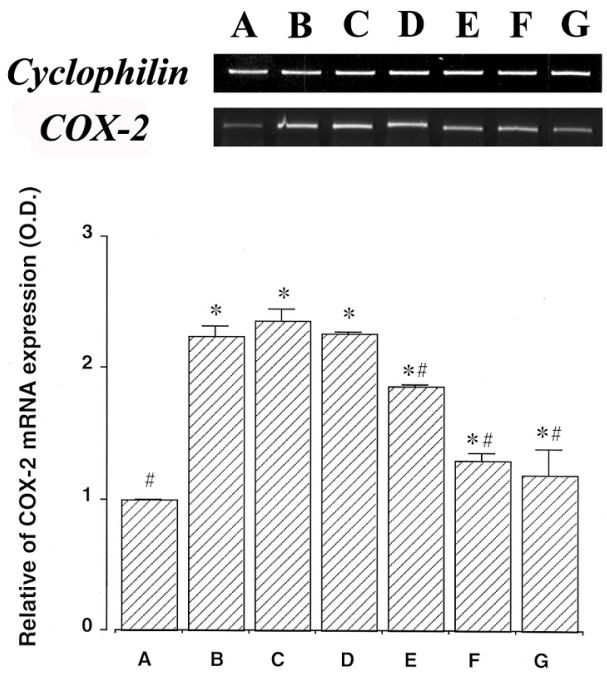

Fig. 3. Effect of amygdalin on the mRNA expression of cyclooxygenase-2 (COX-2). *p < 0.05 compared to the control. # p < 0.05 compared to the lipopolysaccharide (LPS)-treated group. (A) Control group; (B) 2 μg/ml lipopolysaccharide (LPS)-treated group; (C) 2 μg/ml LPS and 10 μg/ml manufactured amygdalin- treated group; (D) 2 μg/ml LPS and 100 μg/ml manufactured amygdalin-treated gruop; (E) 2 μg/ml LPS and 10 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (F) 2 μg/ml LPS and 100 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (G) 2 μg/ml LPS and 500 μM acetylsalic acid (ASA)-treated group.

extract of amygdalin from *Armeniacae semen* at concentrations of 10 μg/ml, 100 μg/ml, and 100 μM acetylsalicylic acid (ASA) was 1.87±0.01, 1.30±0.05, and 1.19±0.20, respectively (Fig. 3).

The level of iNOS mRNA following a treatment with 2 μg/ml LPS for 6 h was conspicuously increased to 3.13±0.63. The level of iNOS mRNA in the cells treated with the manufactured amygdalin at concentrations of 10 μg/ml and 100 μg/ml was 3.51±0.84 and 3.31±0.78, respectively. The level of iNOS mRNA in the cells treated with the aqueous extract of amygdalin from *Armeniacae semen* at concentrations of 10 μg/ml, 100 μg/ml, and 100 μM acetylsalicylic acid (ASA) was 2.64±0.39, 1.73±0.26, and 1.51±0.13, respectively (Fig. 4).

The present results show that LPS enhanced the COX-2 and iNOS mRNA expressions in the BV2 cells, however, LPS did not exert significant effect on the COX-1 mRNA expression. It was also shown that two types of amygdalin acted differently on these gene expressions. The manufactured amygdalin at concentration of 10 μg/ml and 100 μg/ml did not significantly decrease the COX-2 mRNA and the iNOS mRNA expressions. On the other hand, the aqueous extract of amygdalin from *Armeniacae semen* at concentration of 10 μg/ml and 100 μg/ml suppressed the LPS-induced COX-2 and iNOS mRNA expressions.

Effect of amygdalin on PGE_2 synthesis

From the results of the PGE_2 immunoassay, the amount of PGE_2 from the culture medium was increased from 148.63±6.56 pg/well to 311.99±4.62 pg/well after 24 h of exposure to LPS. The PGE_2 synthesis was 272.01±26.94 pg/well and 303.60±2.08 pg/well by the treatment with the manufactured amygdalin at concentrations of 10 μg/ml and 100 μg/ml. Although the manufactured

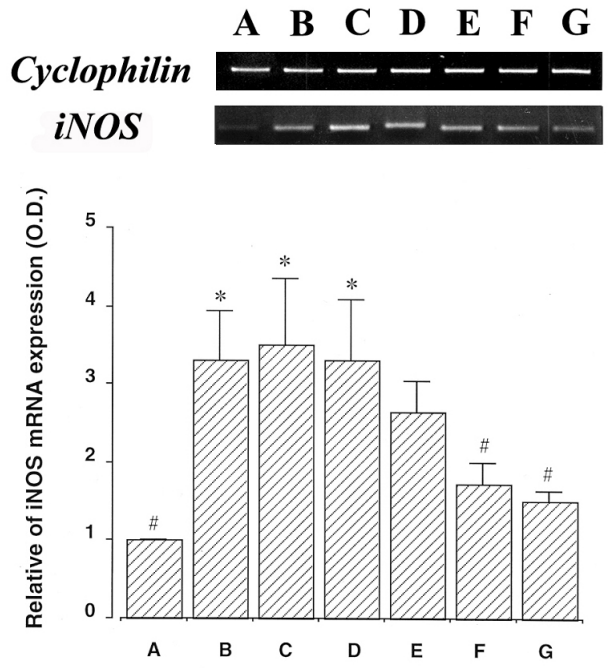

Fig. 4. Effect of amygdalin on the mRNA expression of inducible nitric oxide synthase (iNOS). *$p < 0.05$ compared to the control. #$p < 0.05$ compared to the lipopolysaccharide (LPS)-treated group. (A) Control group; (B) 2 μg/ml lipopolysaccharide (LPS)-treated group; (C) 2 μg/ml LPS and 10 μg/ml manufactured amygdalin- treated group; (D) 2 μg/ml LPS and 100 μg/ml manufactured amygdalin-treated gruop; (E) 2 μg/ml LPS and 10 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (F) 2 μg/ml LPS and 100 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (G) 2 μg/ml LPS and 500 μM acetylsalic acid (ASA)-treated group.

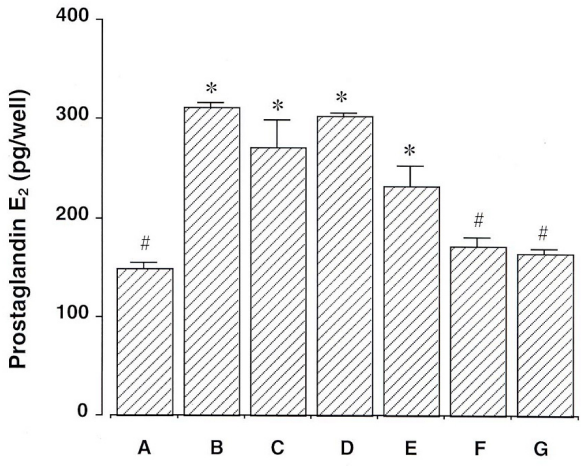

Fig. 5. Measurement of prostaglandin E_2 (PGE_2) synthesis in the BV2 microglial cells. *$p < 0.05$ compared to the control. #$p<0.05$ compared to the LPS-treated group. (A) Control group; (B) 2 μg/ml lipopolysaccharide (LPS)-treated group; (C) 2 μg/ml LPS and 10 μg/ml manufactured amygdalin-treated group; (D) 2 μg/ml LPS and 100 μg/ml manufactured amygdalin-treated gruop; (E) 2 μg/ml LPS and 10 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (F) 2 μg/ml LPS and 100 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (G) 2 μg/ml LPS and 500 μM acetylsalic acid (ASA)-treated group.

amygdalin at these concentrations slightly reduced PGE_2 synthesis, the reduction was not statistically significant. On the other hand, the PGE_2 synthesis was decreased to 232.66±20.40 pg/well, 172.23± 8.32 pg/well, and 164.47±4.34 pg/well by the treatment with the aqueous extract of amygdalin from *Armeniacae semen* at concentrations of 10 μg/ml, 100 μg/ml, and 100 μM ASA, respectively (Fig. 5).

The present results show that LPS enhanced the PGE_2 synthesis in the BV2 cells and that only the aqueous extract of amygdalin from *Armeniacae semen* suppressed the LPS-induced PGE_2 synthesis.

Effect of amygdalin on the NO production

From the NO detection assay, the amount of nitrite was increased from 4.04±0.22 μM to 8.83± 0.43 μM after 24 h of exposure to the LPS. The NO production was 9.52±0.09 μM and 8.34±0.64 μM by the treatment with the manufactured amygdalin at concentrations of 10 μg/ml and 100 μg/ml. NO production was not reduced significantly by the manufactured amygdalin. On the other hand, NO production was decreased to 7.68±0.44 μM, 6.14± 0.09 μM, and 5.63±0.05 μM by the treatment with the aqueous extract of amygdalin from *Armeniacae semen* at concentrations of 10 μg/ml, 100 μg/ml, and 100 μM ASA, respectively (Fig. 6).

The present results show that LPS enhanced the NO production in BV2 cells and that only the aqueous extract of amygdalin from *Armeniacae semen* suppressed the LPS-induced NO production.

DISCUSSION

Amygdalin is one of many nitrilosides, which are natural cyanide-containing substances abundant in the seeds of prunasin family plant such as apricots, almonds, peaches, apples, and other rosaceous plants. Among the prunasins, amygdalin is plentiful in *Armeniacae semen*, the seeds of *Prunus armeniaca L. var. ansu Maxim* which is classified into Rosaceae. *Prunus armeniaca L.* is known to have antidiarrhoeic, antipyretic, antiemetic, and

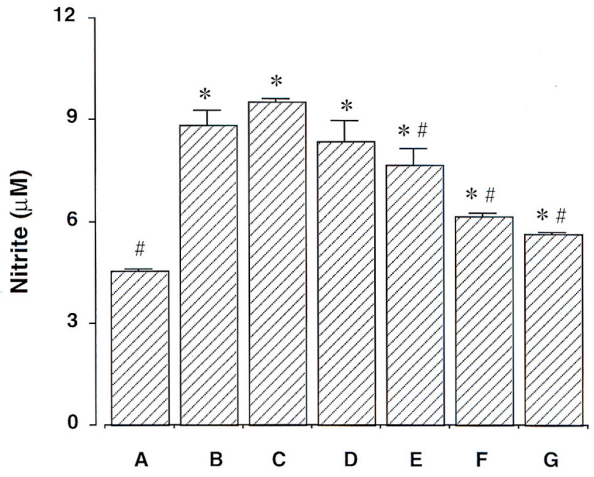

Fig. 6. Measurement of nitric oxide (NO) production in the BV2 microglial cells. *$p<0.05$ compared to the control. #$p<0.05$ compared to the LPS-treated group. (A) Control group; (B) 2 μg/ml lipopolysaccharide (LPS)-treated group; (C) 2 μg/ml LPS and 10 μg/ml manufactured amygdalin-treated group; (D) 2 μg/ml LPS and 100 μg/ml manufactured amygdalin-treated gruop; (E) 2 μg/ ml LPS and 10 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (F) 2 μg/ml LPS and 100 μg/ml aqueous extract of amygdalin from *Armeniacae semen*-treated group; (G) 2 μg/ml LPS and 500 μM acetylsalic acid (ASA)-treated group.

anthelmintic effects. In addition, anti-inflammatory and analgesic activities of this herb have been reported (Prasad, 1999). *Armeniacae semen* has been traditionally used as ingredient of prescriptions for the relieving of pain and inflammation. Practically, Xiao Qing Long Tang including *Armeniacae semen* has been used for the treatment of bronchitis, asthma with fever, allergic rhinitis, and cold symptoms. Sang Ju Yin including *Armeniacae semen*, prescribed for the treatment of febrile diseases, also has the effect on preventing viral infections (Nagai et al., 2004; Poon et al., 2006). Amygdalin, a major ingredient of *Armeniacae semen*, has been used to treat cancers and relieve pain of the cancer patients (Ellison et al., 1978; Fukuta et al., 2003). In addition, it has been reported that amygdalin has anti-inflammatory activity in murine marcrophage raw 264.7 cells (Shin et al., 2003).

Here in this study, we investigated whether amygdalin has anti-inflammatory and analgesic effects and which type of amygdalin between the manufactured amygdalin and the aqueous extract of amygdalin from *Armeniacae semen* has more potent effectiveness on inflammation induced by LPS in mouse BV2 microglia cells.

Microglia is macrophage-like cells of the central nervous system (CNS), and generally considered as immunologically quiescent under normal conditions. However, astrocytes and microglia are activated by pain-inducing neurotransmitters including substance P, glutamate, and fractalkine (Watkins and Maier, 2000). In addition, many studies have reported that LPS stimulates the production of inflammatory mediators such as NO, tumor necrosis factor-α (TNF-α), interleukins, PGE_2, and leukotrienes in microglia cells (Chao et al., 1992; Lee et al., 1992; Kubes and McCafferty, 2000; Vegeto et al., 2001). Activated microglia enhance iNOS and COX-2 expressions which are involved in the processes of inflammation and carcinogenesis. Especially, COX-2 and iNOS produce pro-inflammatory mediators such as PGE_2 and NO (Schmidt and Walter, 1994; Simon, 1999; Shin et al., 2003). Particularly, PGE_2 can perform pro-inflammatory and immuno-depressive action by inducing hyperthermia, increasing vascular permeability, and reducing T-lymphocyte proliferation and major histocompatibility complex (MHC) class II antigen expression (Snyder et al., 1982; Fretland, 1992; Rothwell, 1992; Meinl et al., 1994). Actually, it was reported that PGE_2 is implicated in the pathogenesis of acute and chronic inflammatory states (Hinz et al., 2000). PGE_2 is a major metabolite of the COX-2, and COX-2 is induced by cytokines, mitogens, and endotoxins in inflammatory cells (Mitchell et al., 1995; Dubois et al., 1998; Smith and Langenbach., 2001). Elevation of COX-2 activity is closely associated with the occurrence of cancers, arthritis, several types of neurodegenerative disorders, and especially inflammation. Crofford et al. (2000) and Shao et al. (2000) reported that specific COX-2 inhibitors attenuate the symptoms of inflammation.

In the present results, LPS treatment enhanced the expressions of COX-2 and production of PGE_2 in the mouse BV2 microglia cells, whereas the aqueous extract of amygdalin from *Armeniacae semen* was shown to inhibit LPS-stimulated enhancement of COX-2 enzyme activity and PGE_2 production. However, the manufactured amygdalin did not significantly suppress COX-2 enzyme activity and PGE_2 production.

COX activity and subsequent production of PGE_2 are closely related to the generation of NO radical. Salvemini et al. (1993) reported that NO modulates the activity of COX-2 in a cGMP-independent manner and plays a critical role in the release of PGE_2 by direct activation of COX-2. NO produced by the constitutive isoform of NOS is a key regulator of homeostasis and an important mediator of inflammation in animal models (Vane et al., 1994). Especially, generation of NO by iNOS plays an important role in inflammation, host-defense responses, and tissue repair (Nathan and Xie, 1994). After exposure to endogenous and exogenous stimulators such as LPS and viral infections, iNOS is induced quantitatively in various cells, and triggers autoimmune diseases and several deleterious cellular responses inducing inflammation, sepsis, stroke, genetic alterations (Stuehr et al., 1991; Yui et al., 1991; Kleemann et al., 1993; McCartney-Francis et al., 1993; Nakashima et al., 2003). In addition, some studies have shown a correlation between the release of NO by microglia and the progression of neurodegeneration (Boje and Arora, 1992; Chao et al., 1992). Therefore, inhibition on the iNOS expression has been suggested as another possible mechanism of non-steroidal anti-inflammatory drugs (Amin et al., 1995).

In the present results, LPS treatment enhanced the expressions of iNOS and NO production in the mouse BV2 microglia cells, whereas the aqueous extract of amygdalin from *Armeniacae semen* was shown to inhibit LPS-stimulated enhancement of iNOS expression and NO production. However, the manufactured amygdalin did not significantly suppress iNOS expression and NO production.

Here in this study, we have shown that the only aqueous extract of amygdalin from *Armeniacae semen* exerts anti-inflammatory and analgesic effects by suppressing of COX-2 and iNOS expressions, resulting in inhibition of PGE_2 and NO synthesis.

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