Antioxidant effects of glycyrol isolated from Glycyrrhiza uralensis in LPS-stimulated RAW264.7 macrophages

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Abstract

The anti-inflammatory effects of glycyrol, a benzofuran coumarin isolated from Glycyrrhiza Radix, were studied. Glycyrol of 5, 25 and 50 µM dose-dependently inhibited nitric oxide (NO) production by down-regulating inducible nitric oxide synthase (iNOS), and alleviated cyclooxygenase-2 (COX-2) expression in LPS-stimulated RAW264.7 macrophages, in both the mRNA and the protein. Furthermore, glycyrol dose-dependently decreased the mRNA of the pro-inflammatory cytokines IL-1β and IL-6. LPS-induced NF-κB activation was prevented in RAW264.7 macrophages by inhibition of IκBα phosphorylation. In addition, administration of glycyrol (30 and 100 mg/kg, i.p) reduced the thickness of carrageenan-induced mouse-paw edema swelling. Taken together, our results indicate that glycyrol is an important anti-inflammatory constituent of Glycyrrhiza Radix, and that its anti-inflammatory effect is attributed to the inhibition of IκBα phosphorylation.

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1. Introduction

Inflammation is the response of vascularized living tissue to local injury. Various factors, such as microbial infections, chemicals, and immunologic reactions, can cause inflammation. The function of inflammation is to enclose injury, destroy invading microorganisms and inactivate toxins, and to restore the tissue or organ for recovery [1,2]. The process

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of inflammation and repair, however, can potentially be harmful, because most chemical inflammatory mediators also potentially have insidious effects causing noxious hypersensitivity reactions and, with chronic inflammation, progressive organ damage [3,4].

Macrophages are considered to play essential roles in inflammation. When activated by endotoxin, macrophages produce inflammatory cytokines, which in turn activate other macrophages and other nearby cells to promote e.g. iNOS gene induction [5]. Although NO is indispensable to physiological cellular activities, uncontrolled overproduction of NO by iNOS results in a catastrophic breakdown of important physiological functions [6]. NO is also reported to modulate the activity of prostaglandin endoperoxide H synthase 2 (cyclooxygenase-2) in a concentration-dependent manner [7].

Cyclooxygenase (COX), an enzyme also known as prostaglandin (PG) H synthase (EC 1.14.99.1), converts arachidonic acid to prostaglandins, which play a crucial role as mediators of inflammatory responses [8]. COX-2, unlike the other isoenzymes COX-1 and -3, is inducible in certain cells in response to inflammatory stimuli such as endotoxin, cytokines, growth factors, mitogens, and tumor promoters [8,9]. The adverse effects of COX-2 have been evidenced in various pathogeneses of chronic inflammatory diseases, and its selective antagonists have been favorably reported in diverse experiments and clinical treatments [10,11].

Nitric oxide (NO) is a short-living free radical that is produced from L-arginine by catalytic reaction of NO synthases (NOSs) within mammalian immune, cardiovascular, and neural systems, where it functions as a signaling or cytotoxic molecule [12]. Among the three isomer NOSs reported to date, endothelial NOS (eNOS, NOS I) and neuronal NOS (nNOS, NOS III) are constitutive NOSs, whereas inducible NOS iNOS, NOS II can be induced by immunostimulatory cytokines and microbial products in various cell types, including endothelium, smooth muscle cells, hepatocytes, monocytes, mast cells, and macrophages [13].

The transcription factor nuclear factor kappa B (NF-κB) plays a fundamental role in the inflammatory and acute response [14]. NF-κB subunits are normally inactive, being bound to a protein called I-κBα. Signaling through cytokines such as TNF-α and IL-1 cause dissociation of NF-κB from I-κBα. The transcription factor translocates to the nucleus and binds to the DNA regulating the transcription of various inflammatory mediators, such as iNOS or COX-2 [15-17].

**Glycyrrhiza uralensis** (Leguminosae) has long been used throughout the world as a sweetener and in folk medicine because of its antioxidative, anti-inflammatory, antibacterial, antiangiogenic and antiallergenic properties. Its main components are considered to be the triterpene saponins glycyrrhizin and glycyrrhetic acid [18-20].

As part of our project to screen medicinal plants for anti-inflammatory compounds, glycyrol, a coumarin derivative, was isolated from the total extract of Glycyrrhizae Radix. In the present study, we explored its anti-inflammatory activity and mechanisms in LPS-stimulated RAW264.7 macrophages and investigated the *in vivo* anti-inflammatory effect of glycyrol in a mouse model of acute inflammation.

### 2. Materials and Methods

#### 2.1. Plant material

The roots of *G. uralensis* were purchased at a local market, Daegu, South Korea and identified by Prof. Je-Hyun Lee, College of Oriental Medicine, Dongguk University, Gyeongju, South Korea. A voucher specimen has been deposited at the College of Pharmacy, Yeungnam University, South Korea.

#### 2.2. Isolation of glycyrol

The roots of *G. uralensis* (10 kg) were extracted with methanol (50 L) at room temperature. The methanol extract was evaporated under reduced pressure to obtain a residue (2.6 kg), which was dissolved in water (3.5 L) and partitioned with methylene chloride (3.5 L X 3). The methylene chloride soluble fraction (230 g) was chromatographed on silica gel (6.2 kg), eluted with n-hexane, with the polarity gradually increased with ethyl acetate (100:0, 98:2, 95:5, 90:10, 85:15, 80:20, 5 L for each gradient) to give sixteen fractions (fr. 1-16). Fr. 4 (3200 mL, n-hexane-ethyl acetate, 98:2) and fr. 8 (1200 mL, n-hexane-ethyl acetate, 90:10) were combined and crystallized from cold methanol to yield crude glycycrol (450 mg, purity 90%), followed by the purification using the preparative HPLC (25 mm i.d. x 350 mm, Shim-pack Prep-ODS column; Shimadzu, Japan) eluting with MeOH–H2O (70:30 (v/v), 7 mL/min) to afford the pure compound (30 mg, purity 99%). The chemical structure of glycycrol was identified by comparison of spectral data (MS, 1H- and 13C-NMR) in the literature [21] and is shown in Fig. 1.

#### 2.3. Mice, chemicals and reagents

Male ICR mice (Samtako, Osan, Korea) weighing 25–30 g were used in this study. All animal studies were carried out in a pathogen-free barrier zone of Seoul National University Animal Laboratory, according to procedures outlined in the Guide for the Care and Use of Laboratory Animals (Seoul National University, Korea). All animals were acclimated for at least one week, caged ten per group or fewer, and fed a diet of animal chow and water ad lib. The animals were housed at 23±0.5 °C and 10% humidity in a 12 h light–dark cycle. Unless otherwise indicated, all the chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO). The antibodies against iNOS, COX-2, phosphor-I-κBα, I-κBα, and β-actin were obtained from Santa Cruz Biotechnology, USA.
2.4. Cell culture

RAW 264.7 murine macrophages were obtained from the American Type Culture Collection (Manassas, USA). These cells were maintained at sub-confluence in a 95% air and 5% CO2 humidified atmosphere at 37 °C. The medium used as the routine subculture was DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL). The RAW264.7 cells harboring pNF-κB-secretory alkaline phosphatase (SEAP)-NPT reporter construct [22] were cultured under the same conditions except that the media was supplemented with 500 μg/mL geneticin.

2.5. Cell viability

The concentration of glycyrol affecting cell viability was evaluated using a Cell Counting Kit (CCK-8) purchased from Dojindo Laboratories (Tokyo, Japan), as previously described [22]. Briefly, RAW264.7 cells were plated at a density of 1×10⁴ per well in a 96-well plate, and incubated at 37 °C for 24 h. The cells were treated with various concentrations of glycyrol or vehicle alone, and incubated at 37 °C for an additional 24 h. After incubation, 10 μL of CCK8 solution was added to each well and incubated under the same conditions for another 3 h. Water-soluble tetrazolium salt (WST8-[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] in CCK8 produces a water-soluble formazan dye upon reduction, and the resulting color was assayed at 450 nm using a microplate reader (Emax, Molecular Devices, Sunnyvale, CA, USA).

2.6. Nitric oxide determination

Nitrite concentration in the medium was measured as an indicator of nitric oxide production according to the Griess reaction method, as previously described [22]. Briefly, 1×10⁵ RAW264.7 cells were plated in 24-well plates, incubated for 24 h and pre-treated with the indicated concentrations of glycyrol or vehicle for another 2 h, then challenged with LPS (1 μg/mL) for an additional 18 h. Equal volumes of cultured medium and Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in distilled water) were mixed, the absorbance at 540 nm was determined with a microplate reader (Molecular Devices, Emax, Sunnyvale, CA, USA), and the absorption coefficient was calibrated using a sodium nitrite solution standard. For this experiment, 2-amino-5, 6-dihydro-6-mercaptopurine (MUP) was used as a positive control.

2.7. NF-κB SEAP reporter gene assay

To determine the inhibitory activity of glycyrol in LPS-challenged RAW264.7 macrophages, NF-κB-dependent reporter gene transcription was analyzed by secretory alkaline phosphatase (SEAP) assay, as previously described with some modifications [23]. In brief, 1×10⁶ RAW264.7 macrophages transfected with pNF-κB-SEAP-NPT encoding four copies of NF-κB sequence and SEAP gene as a reporter were pre-incubated with different concentrations of glycyrol for 2 h and challenged with LPS (1 μg/mL) for additional 16 h. Aliquots of the cell-free culture medium were heated at 65 °C for 5 min and then given to an assay buffer (2 M diethanolamine, 1 mM MgCl₂, 500 μM 4-methylumbelliferyl phosphate) in the dark at 37 °C for 1 h. The fluorescence from the products of the SEAP/MUP was measured using a 96-well microplate fluorometer (Molecular Devices, Gemini XS, Sunnyvale, CA, USA) according to the excitation at 360 nm and the emission at 449 nm.

2.8. Semi-quantitative reverse transcription (RT)-PCR

To perform a semi-quantitative RT-PCR, 1 μg of isolated total RNA (easy-BLUE™, iNtRON Biotech, Korea) was reverse-transcriptased to cDNAs, employing a RT PreMix Kit (iNtRON Biotech, Korea) according to the manufacturer’s instructions. The amount and purity of the RNA preparation was confirmed both by measuring the absorbance ratio at 260/280 nm and the 28S/18S intensity ratio in agarose gel electrophoresis. Two microliters of cDNA products were directly used in the PCR. The primers and the amplification conditions were the same as previously described [24] with modification of 30–35 cycles for amplification. The amplified cDNA products were separated in 2% agarose gel electrophoresis and stained with ethidium bromide. The gels were viewed using Doc-It LS Image Analysis software (UVP Inc, CA, USA) and quantified using UN-SCAN-IT™ software (Silk Scientific Corp, USA). The PCR products were normalized to the amount of GAPDH for each band.

2.9. Western immunoblot analysis

RAW264.7 macrophages were pre-treated with the indicated concentrations of glycyrol or vehicle for 2 h and stimulated with LPS (1 μg/mL) for 5 min (phosphor-1-βx), 15 min (1-βx) and 18 h (COX-2 and iNOS). All of the primary and secondary antibodies were purchased from Santa Cruz Biotechnology (USA). Twenty micrograms of total proteins for iNOS, 1-βx, and phosphor-1-βx, and 1 μg for COX-2, were separated on an SDS-PAGE, 8% (iNOS) and 10% (COX-2, phosphor-1-βx, 1-βx and β-actin), respectively. After electrophoresis, the proteins were electro-transferred to nitrocellulose membranes (Whatman GmbH, Germany), blocked with 5% non-fat milk in TBS-T buffer, blotted to each primary antibody (1:1000) and its corresponding secondary antibody (1:5000) according to the manufacturer’s instructions, and detected with WEST-SAVE Up™ luminol-based ECL reagent (LabFrontier, Korea). The target bands were quantified using UN-SCAN-IT™ software (Silk Scientific Corp, USA).

2.10. Electrophoretic mobility shift assay (EMSA)

To investigate the inhibitory effect on NF-κB DNA binding we performed an electrophoretic mobility shift assay (EMSA), as previously described [25]. Briefly, nuclear extracts prepared from LPS-treated cells were incubated with [32P]-end-labeled 22-mer double-stranded NF-κB consensus oligonucleotide (Promega, USA; sequence: 5'-AGT TGA GGC GAC TTT CCC AGG C-3') for 30 min at room temperature, and the DNA–protein complexes were separated from the free oligonucleotides on 6% native polyacrylamide gels. The signals
obtained from the dried gel were quantitated with an FLA-3000 apparatus (Fuji) using the BAS reader version 3.14 and Aida Version 3.22 softwares (Amersham Biosciences, USA). The binding conditions were optimized by Zable et al. [26].

2.11. Carrageenan-induced paw edema test in mice

To test its inhibitory effects on acute inflammation in an animal model, paw edema was induced by subcutaneous injection of 0.05 mL of carrageenan (1%) into the right hind paw, as previously described [27]. Control animals received identical treatment but with the vehicle, which, in this study, was 10% Tween 80 (10 mL/kg, i.p. Sigma, USA) in saline. Thirty minutes prior to carrageenan administration, the animals received an i.p. injection of either (saline or dexamethasone 50 mg/kg) or glycyrol (30 or 100 mg/kg), and the paw thickness was measured using a dial thickness gauge (No. 2046F, Mitutoyo, Japan) before and every hour after edema induction for 5 h. The percent increase of paw thickness was calculated based on the volume difference between the two paws with/without carrageenan injection.

2.12. Data analysis

The results, unless otherwise stated, were expressed as means ± standard deviation (S.D.) from three different experiments. A one-way analysis of variance (ANOVA) followed by Dunnett’s t-test was applied to assess the statistical significance of the differences among the study groups (SPSS version 10.0, Chicago, USA). A value of $P<0.05$ was chosen as the criterion of statistical significance.

3. Results

3.1. Cell viability

The cytotoxic effects of glycyrol are shown in Fig. 2. No cytotoxic effect was observed up to 50 µM.

![Figure 2](image_url) Effects of glycyrol on cell viability in RAW264.7 macrophages using a Cell Counting Kit. RAW264.7 cells were cultured with the indicated concentrations of glycyrol or vehicle alone at 37 °C in a 96-well plate for 24 h. Cell viability was evaluated as described in "Materials and Methods," and expressed as a percentage of the vehicle control. Values are expressed as mean±S.D. of three individual experiments. CON stands for vehicle control.

Figure 3  Dose-dependent inhibition of nitric oxide production in LPS-challenged RAW264.7 macrophages. The Griess reagent assay was carried out to measure nitrite produced as described in "Materials and Methods". The data were obtained from three independent experiments and expressed as mean±S.D. (*** $P<0.001$ indicates a significant difference from the LPS-challenged group. ###$P<0.001$ indicates a significant difference from the unstimulated control group. Con, control; LPS, LPS-treated alone; AMT, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine.

![Figure 3](image_url)

3.2. Glycyrol inhibits nitrite production in RAW264.7 macrophages

The amount of produced NO was determined by the amount of nitrite, a stable metabolite of NO. During incubation time of 18 h, RAW264.7 macrophages produced 3.04±0.13 µM nitrite in the resting state. After LPS (1 µg/mL) stimulation, NO production increased dramatically to 33.32±0.049 µM after 18 h. Glycyrol inhibited nitrite production 20 h after LPS stimulation in a dose-dependent manner corresponding to 29.1% at 20 µM and 87.1% inhibition at 50 µM (Fig. 3). The positive iNOS inhibitor, AMT, significantly inhibited LPS-induced NO production (Fig. 3).

![Figure 4](image_url) Dose-dependent suppression of LPS-induced and NF-kB dependent alkaline phosphatase (SEAP) expression by glycyrol in transfectant RAW264.7 macrophages. Data were derived from three independent experiments and expressed as mean±SD. (*** $P<0.001$ indicates a significant difference from the LPS-challenged group. ###$P<0.001$ indicates a significant difference from the unstimulated control group. Con, control; LPS, LPS-treated alone; TPCK, N-p-tosyl-L-phenylalanyl chloromethyl ketone; RFU, relative fluorescence unit.

![Figure 4](image_url)
3.3. Glycyrol inhibits NF-κB transcriptional activity in LPS-stimulated RAW264.7 macrophages

To determine the inhibitory effect of glycyrol on NF-κB transcriptional activity, pNF-κB-SEAP-NPT-construct-transfected RAW264.7 macrophages were employed. Upon exposure to LPS alone, SEAP expression was increased approximately 3-to-4-fold over the basal level, confirming successful NF-κB dependent transcription in the cell system. Glycyrol inhibited LPS-induced SEAP expression in a dose-dependent manner, to 52.4% at 30 µM and to 84.9% at 50 µM. TPCK was used as a control inhibiting LPS-induced SEAP expression to 77.2% at 20 µM (Fig. 4).

3.4. Glycyrol suppresses COX-2, iNOS, IL-1β and IL-6 mRNA expression in LPS-stimulated RAW264.7 macrophages

COX-2 and iNOS are important mediators in inflammation regulated by the transcription factor NF-κB. To understand whether glycyrol can inhibit LPS-induced activation of iNOS and COX-2 mRNA, a semi-quantitative RT-PCR was performed. The expression of iNOS and COX-2 mRNA drastically increased upon LPS stimulation at 6 h and decreased after glycyrol incubation in a dose-dependent manner, corresponding to 36.4% inhibition at 5 µM and 60.4% inhibition at 50 µM for COX-2 (Fig. 5A), and 26.1% inhibition at 25 µM and 65.7% inhibition at 50 µM for iNOS (Fig. 5B). Additionally, mRNA
expression of TNF-α, IL-1β and IL-6 in the LPS-induced RAW264.7 macrophages was determined by semi-quantitative RT-PCR analysis. The total lysates of the proteins were subjected to Western immuno blot analysis, as described in "Materials and Methods". The values of relative ratio (%) are expressed as the mean±S.D. of three individual experiments. Both the COX-2 (A) and iNOS (B) signals were normalized to the β-actin signal. *P<0.05, **P<0.01 and ***P<0.001 indicate significant differences from the LPS-treated group. **P<0.001 indicates a significant difference from the unstimulated control group.

3.5. Glycyrol inhibits iNOS and COX-2 protein expression in LPS-stimulated RAW264.7 macrophages

In order to confirm that the concentration-dependent NO decrease is due to its influence on iNOS, the inhibitory effect of glycyrol on iNOS was measured by Western immunoblot analysis. In addition, the inhibitory activity of glycyrol on COX-2 was also confirmed by means of Western blot analysis. The COX-2 and iNOS proteins were fully induced upon LPS stimulation for 18 h. Glycyrol decreased LPS-induced synthesis of iNOS as well as COX-2 protein in a dose-dependent manner (Fig. 6). For COX-2, glycyrol showed inhibitory activity corresponding to 19.1% at 5 µM, 39.2% at 25 µM and 55.3% at 50 µM (Fig. 6A). The selective NF-κB inhibitor, TPCK showed an inhibitory activity of 48.5% at 20 µM (Fig. 6A). Additionally, glycyrol showed an inhibitory effect of 36.6% at 50 µM on inhibition of iNOS expression (Fig. 6B). The positive control TPCK inhibited LPS-induced iNOS expression in RAW264.7 macrophages by 58.6% at 20 µM (Fig. 6B).

3.6. Glycyrol inhibits LPS-induced phosphorylation and degradation of I-κBα

To determine the inhibitory activity of glycyrol on I-κBα, I-κBα phosphorylation and degradation were examined by immunoblot analyses. Glycyrol exhibited a dose-dependent inhibitory activity of TNF-α, IL-1β and IL-6 in the LPS-induced RAW264.7 macrophages was determined by semi-quantitative RT-PCR analysis. The representative level of IL-1β and IL-6 is shown in Figs. 5C and D. Among the pro-inflammatory cytokines tested, glycyrol inhibited IL-1β mRNA expression by 53.9% at 50 µM. TPCK showed 68.7% inhibition at 20 µM (Fig. 5C). In the case of IL-6, glycyrol suppressed IL-6 mRNA expression by 24.43% at 5 µM and 24.32% at 50 µM. TPCK inhibited 44.06% at 20 µM (Fig. 5D), while glycyrol showed no inhibitory activity on TNF-α mRNA expression (Fig. 5E). Furthermore, glycyrol dose-dependently increased TNF-α mRNA expression (Fig. 5F).

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on phosphorylation and consequently on degradation of IκBα in LPS-stimulated RAW264.7 macrophages, corresponding to 34.7% at 25 µM and 88.3% at 50 µM for phosphorylation, and 31.1% at 25 µM and 56.6% at 50 µM for degradation. Furthermore, the positive control TPCK showed strong inhibitions of IκBα phosphorylation and degradation, 89.1% and 96.9%, respectively, at 20 µM (Fig. 7A and B).

3.7. Glycyrol has no inhibitory effect on NF-κB nuclear translocation but induces IκBα production

To examine whether the observed inhibition of IκBα degradation also results in a reduced NF-κB translocation into the nucleus an EMSA assay was performed after preparation of nuclear extracts. Surprisingly, glycyrol showed no reduction of NF-κB DNA binding (Fig. 8). Parthenolide, a sesquiterpene lactone, showed a potent inhibition of NF-κB DNA binding at 20 µM (Fig. 8A). However, glycyrol dose-dependently increased the production of IκBα (Fig. 8B).

3.8. Glycyrol alleviates carrageenan-induced paw edema in mice

In order to determine the anti-inflammatory activity of glycyrol in vivo in acute-phase inflammation, a carrageenan-induced paw-swelling experiment was carried out. Glycyrol, at two concentrations (30 and 100 mg/kg, i.p.), showed a dose-dependent inhibitory activity in carrageenan-induced paw swelling from 1–5 h after carrageenan injection: Specifically, a 100 mg/kg concentration of glycyrol reduced carrageenan-induced paw swelling after injection, by 22.4% at 1 h, 26.9% at 2 h, 43.1% at 3 h, 37.1% at 4 h and 28.4% at 5 h (Table 1). The positive control dexamethasone (50 mg/kg) effected a continuous decrease in paw swelling compared with the vehicle control group, which received an equal volume of vehicle only (10% Tween80), corresponding to 39.3% at 3 h, 46.4% at 4 h and 53.3% at 5 h (Table 1). Glycyrol (100 mg/kg) showed a notably better inhibitory activity in the early phase of paw swelling (22.4% at 1 h and 26.9% at 2 h) than did dexamethasone (50 mg/kg; 4.3% at 1 h and 8.7% at 2 h). Nevertheless, glycyrol’s inhibitory activity decreased after 4 h, in consequence of its relatively short half-life (Table 1).

### Table 1 Effect of glycyrol on carrageenan-induced paw edema in mice

<table>
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<th>Control</th>
<th>Dexamethasone</th>
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<td>25.4±3.4*</td>
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</tr>
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The percent increase of paw thickness is presented as means±S.D. of 9 mice per group. *P<0.05, **P<0.01 and ***P<0.001 indicate significant differences from the vehicle control group.

4. Discussion

For many years, G. uralensis has been used to treat various disorders including allergies, hepatitis and other inflammatory diseases [19,28]. The main medicinal ingredients of G. uralensis are triterpene saponins, glycyrrhizin and glycyrrhetinic acid. Glycyrrhizin has been found to significantly and dose-dependently decrease neutrophil-generated –O2, –H2O2 and –OH’, These reactive oxygen species (ROS) are considered to be the most potent inflammatory mediators at the site of inflammation [18]. Moreover, glycyrrhetinic acid (ED50, 200 mg kg⁻¹) shows an inhibitory effect on carrageenan-induced rat paw edema and anti-allergic activity [28]. Whereas the anti-inflammatory activity of glycyrol has not been well known, glycyrol has been reported to have antibacterial activity against upper-airway respiratory tract bacteria such as Streptococcus pyogenes, Haemophilus influenzae and Moraxella catarrhalis [21,29].

In order to investigate the anti-inflammatory activity of glycyrol in LPS-stimulated RAW264.7 macrophages, several important inflammation mediators were examined. Glycyrol showed dose-dependent inhibitory effects on NO production, iNOS mRNA and protein expression. In addition, glycyrol dose-dependently down-regulated LPS-induced COX-2 production, both on mRNA and protein level. Furthermore, glycyrol was tested at different times against LPS stimulations (Supplementary data Fig. 1), and showed dose-dependent inhibitory effects on NO, iNOS and COX-2 production, regardless of pre- or post-administration. Additionally, glycyrol showed the pronounced inhibitory effect on IL-1β mRNA expression in the LPS-stimulated RAW264.7 macrophages. However, glycyrol was revealed to have no inhibitory effect on the mRNA of TNF-α, due to its competitive dose-dependent TNF-α producing activity.
With these in vitro results, we explored the anti-inflammatory effect of glycyrol in a rodent model. Reportedly, carrageenan-induced paw edema is a highly sensitive tool to evaluate the efficacy of acute inflammation [27,30]. Decreased carrageenan-induced paw swelling by reduced levels of IL-8, IL-1β and TNF-α, NO via iNOS inhibition, and PGE2 via COX-2 inhibition, have all been documented [31,32]. In the present study, glycyrol showed dose-dependent inhibitory activity in carrageenan-induced paw edema.

To investigate the inhibitory activity on NF-κB action, glycyrol was tested in various experiments. From the NF-κB-SEAP reporter gene assay, glycyrol showed inhibitory activity on NF-κB transcription in a dose-dependent manner. Moreover, glycyrol was revealed to inhibit IκBα phosphorylation and degradation in a concentration-dependent manner, but did not impair NF-κB DNA binding. Instead, glycyrol was revealed to have an IκBα producing activity. From these results, we speculate that glycyrol might act like glucocorticoids. Glucocorticoids target the NF-κB pathway at different sites, inducing IκBα expression. Moreover, glucocorticoids reduce phosphorylation at p65 and influence p300, by which the transactivating activity is reduced, but still bind to the DNA [33–35]. Although further investigations are still required to clarify the detailed mechanism, we conclude that glycyrol isolated from G. uralensis is a potential compound candidate for inflammatory diseases.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.intimp.2008.06.008.

References


