Coptis chinensis Extract Inhibits the Production of Inflammatory Mediators and Delayed Type Hypersensitivity in Mice

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Background: *Coptis chinensis* rhizome has been used as a medicinal herb in traditional Oriental medicine. We investigated the effects of *Coptis chinensis* extract on inflammatory mediators and delayed type hypersensitivity in mice. **Methods:** The inhibitory effect of ethanolic extract of Coptis chinensis (CCE) on cell proliferation was evaluated using MTS assay. The lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and the Con A-activated mouse splenocytes were cultured with various concentrations of CCE. Total nitric oxide (NO) production was determined by Griess reaction. The amounts of secreted prostaglandine E2 (PGE_2), interleukin (IL)-2 and IFN-γ were measured by ELISA. To investigate the *in vivo* anti-inflammatory effect of CCE, oxazolone-induced delayed type hypersensitivity (DTH) model was used. **Results:** The CCE at 100 μg/ml significantly blocked the LPS-induced production of pro-inflammatory mediators (NO and PGE) in RAW264.7 macrophages. Also, it significantly inhibited cell proliferation and cytokine (IL-2 and IFN-γ) production in splenocytes. Furthermore, when splenocytes from CCE fed mice (200 mg/kg for 2 weeks) were activated with Con A, cell proliferation and cytokine production were significantly inhibited. In addition, CCE decreased *in vivo* inflammation in oxazolone-induced DTH model mice. **Conclusion:** We suggest that *Coptis chinensis* can be used as an anti-inflammatory drug by exerting an inhibitory effect in inflammatory mediator- and cell-mediated inflammation.
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INTRODUCTION

An inflammatory reaction is characterized by a series of pathophysiological processes such as edema, tissue damage and cellular proliferation, that results from injury, infection or autoimmunity. With this reaction, activated macrophages release various kinds of inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 (1). While these inflammatory mediators are required for tissue repair and protection (2,3), their overproduction can lead to an unnecessary immune reaction and inflammation, even in normal tissues (4,5). In particular, it was reported that NO primarily contributes to vasodilatation and tissue damage and that PGE_2 mediates the pain and febrile reaction (6). Therefore, effective anti-inflammatory drugs are expected to inhibit the production of inflammatory mediators and the proliferation of inflammatory cells. Anti-inflammatory drugs are used to treat chronic inflammatory diseases, including rheumatoid arthritis, with the goal of reducing tissue destruction by controlling excessive inflammation and reducing pain. However, the long-term administration of non-steroidal anti-inflammatory drugs (NSAIDs) may result in serious adverse side effects such as gastrointestinal ulcer bleeding, renal dysfunction and the elevation of blood pressure (7).

The use of NSAIDs has been recently reported to increase the risk of vascular thrombosis and myocardial infarction (8). Thus, we still need to develop newer anti-inflammatory drugs

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with less adverse effects.

There is an increasing interest in the effects of natural substances that have been used in complementary and alternative medicine. The rhizome of *Coptis chinensis* Franch (Ranunculaceae) has been widely used as a tonic remedy for liver and heart disorders for years in traditional Oriental medicine (9). According to recent reports, this medicinal herb possesses a broad spectrum of pharmacological effects including anti-bacterial (10), anti-cancer (11), and anti-inflammatory effects (12). We are interested in identifying and developing clinically useful and safe products from herbal medicines. In the present study, we sought to investigate the anti-inflammatory effects of the ethanolic extract from *Coptis chinensis* and its inhibitory effects on delayed type hypersensitivity (DTH) in extract-fed mice.

MATERIALS AND METHODS

Reagents

Concanavalin A (Con A), lipopolysaccharide (LPS), carboxymethyl cellulose (CMC), dimethylsulfoxide (DMSO), mercaptoethanol, dexamethasone, olive oil, 4-ethoxymethylene-2-phenyloxazolone, and Greiss regent (1% sulfanilamide and 0.1% N-(napthyl)ethylenediamine dihydrochloride in 2.5% H_3PO_4) were procured from Sigma. ELISA kits for interleukin-2 and interferon-γ and the immunoassay kit for PGE_2 were purchased from R&D Systems (Minneapolis, MN, USA). The cell culture media RPMI 1640 and DMEM (Gibco, Invitrogen Ltd, Paisley, UK), antibiotic-antimycotic solution (Gibco) and fetal bovine serum (CAMBREX, Walkersville, MD, USA) were used for the cell culture.

Preparation of *Coptis chinensis* extract (CCE)

The air-dried rhizomes of *Coptis chinensis* Franch were obtained from Kyung-Hee Oriental Medical Hospital, Korea and powdered. This powder (100 g) was then extracted with 50% ethanol (500 ml) for 16 h at room temperature. The combined alcoholic extract was filtered through filter paper and concentrated in a rotary evaporator at 40°C to yield 23.3 g of *Coptis chinensis* ethanol extract. The ethanol extract obtained at the end of this process was then solved in DMSO for the *in vitro* experiment or in 0.5% CMC for the *in vivo* experiment of feeding mice. The extract from the *Coptis chinensis* rhizome with 50% ethanol is hereafter referred to as CCE. The voucher specimen of the plants used in the present study was kept in our lab herbaricum for future reference.

Animals

Female BALB/c mice, 5~6 weeks old, weighing 16~18 g were obtained from Orient Co. Ltd. (Gapyoung, Korea). All animals were maintained in plastic cages at 21~24°C with free access to pellet food and water and were kept on a 12 h light/dark cycle. This study was in compliance with the current ethical regulations on animal research.

RAW264.7 macrophages culture

Murine macrophages (RAW264.7) were cultured with DMEM media containing 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were stimulated with LPS (1 μg/ml) in the presence of CCE for 24 h and were cultured for 72 h at a concentration of 2×10^4 cells/well/200 μl of media on 96-well plates for the NO assay, or for 24 h at a concentration of 1×10^6 cells/well/1ml of media on 24-well plates for the PGE_2 assay.

Splenocytes preparation

Splenocytes were prepared by disrupting the spleen between glass slides in complete RPMI 1640 medium. After 10 min of centrifugation at 1,500 rpm to separate the cells from the debris (13), the cells were washed in RPMI medium, which was followed by the lysis of erythrocytes in lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM Na_2EDTA) at 4°C for 10 min. The splenocytes were cultured in complete RPMI 1640 medium containing 50 μM of mercaptoethanol.

Measurement of NO and PGE_2 content

To assay the total production of NO, 100 μL of each culture supernatant were incubated at room temperature for 10 min with 100 μL of Griess reagent (1% sulfanilamide, 0.2% N-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% H_3PO_4). The OD values of the samples were read at 570 nm. A standard curve using $NaNO_2$ was then used to calculate the NO_2^- concentration. The enzyme immunoassay kit was used to measure the production of PGE_2 in the culture supernatant according to the manufacturer's instructions.

Western blot analysis of iNOS and COX-2

RAW264.7 macrophages were adjusted to 1×10^6 cells/well in a 6-well plate. The cells were treated with 0~100 μg/mL of CCE and stimulated by LPS (1 μg/mL) for 24 hours. After washing the cells with PBS, whole cell lysates were prepared using the PRO-PREPTM protein extraction solution (iNtRON Biotechnology, INC.). Equal amounts of total protein (10 μg/

ane) were separated by 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophresis (SDS-PAGE) and transferred to a Hybond ECL nitrocellulose membrane (Amersham Pharmacia). The membrane was blocked with 5% skim milk for 1 h at room temperature. After washing, the blot was incubated with specific anti-iNOS and anti-COX-2 antibodies for 30 min and the antibody specific proteins were visualized by the enhanced chemiluminescence detection system (ECL-plus; Amersham Pharmacia Biotech., Braunschweig, Germany).

Measurement of cell proliferation by MTS assay

The inhibitory effect of the plant extract on cell proliferation was evaluated using an MTS assay kit from Promega (Madison, WI, USA). The cells were cultured in a 200 μl volume in accordance with the manufacture's protocol. The freshly prepared MTS/PMS solution (20 μl) was added to each well of the 96-well plate. The plate was incubated for 3 hr in a humidified, 5% CO_2 atmosphere. The incubation was terminated by the addition of 10% sodium dodesyl sulfate. The optical density at 490 nm was measured by an Emax microplate reader (Molecular Devices, Sunnyvale, CA).

Induction of in vivo cell-mediated inflammatory response

The mice were sensitized via the epicutaneous application of 25 μl of a mixture of acetone and olive oil (4 : 1) containing 2% 4-ethoxymethylene-2-phenyloxazolone on the shaved abdomen and thorax skin as previously described (14). One, three, and five days following the sensitization of the mice, 200 μl of CCE (400 mg/kg) or dexamethasone (1 mg/kg) dissolved in 0.5% carboxymethyl cellulose (CMC) were orally administered to the mice from each group (n=6) three times. We used dexamethasone (Sigma) as a reference, positive control. The negative control group received 200 μl of 0.5% CMC. After 6 days, all of the mice were challenged by the application of 10 μl of 0.5% oxazolone dissolved in the mixture of acetone and olive oil on both sides of the right ear. The inhibitory effect of the DTH reaction by CCE was analyzed by comparing it with that of the 0.5% CMC-fed mice. The intensity of the DTH reaction was expressed by subtracting left ear thickness from right ear thickness at 24 h after challenging with 0.5% oxazolone.

High Performance Liquid Chromatography (HPLC) analysis

HPLC analysis was performed using a Waters model(Waters Delta 600 system, Waters Corp, Milford, USA) and the column was YMC Hydrosphere C18 (250 mm×4.6 mm, i.d., S-5 μm, The temperature of column was 40°C and the injection volume was 20 μl. Detection was done at UV 345 nm using Waters 287 detector. The extract was eluted in HPLC using a binary gradient at a flow rate of 1.0 mL/min. and the mobile phase was 0.1% phosphoric acid in water-100% acetonitrile (60 : 40). Berberine and palmatine were quantified in the extract. The linearity of the HPLC method was investigated for berberine in the range 62.7～627.5 μg/mL and palmatine in the range 10.5～105.2 μg/mL at five concentration levels, using the successive dilutions of stock solutions berberine 627.5 μg/mL and palmatine 105.2 μg/mL, respectively. Calibration plots with correlation coefficient were obtained by reporting peak areas as a function of analyte concentration.

Statistical analysis

The results were expressed as the means±SD. Statistical significance was determined by Dunnet's test using SigmaStat version 3.0. p values below 0.05 were considered significant.

RESULTS

Effect of CCE on cell viability

To rule out the toxic effect of CCE, we tested its effect on the viability of RAW264.7 macrophages by 0.025% trypan blue dye exclusion. The exposure of cells to CCE at 1～1,000 μg/ml for 72 h showed no significant adverse effect on the cell viability versus the untreated controls, although the cell viability at 1,000 μg/ml was reduced to 80% of that of the untreated controls (data not shown).

Effect of CCE on NO and PGE_2 production in RAW264.7 macrophages

In an effort to investigate the effect of CCE, we first examined whether CCE inhibits the production of NO and PGE_2 in RAW264.7 macrophages stimulated with LPS (1 μg/ml). CCE at 1～100 μg/ml significantly inhibited LPS-induced NO (Fig. 1A) and PGE_2 production (Fig. 1B), however, at 1 μg/ml its inhibition of NO was substantial but statistically insignificant.

Effect of CCE on iNOS and COX-2 expression in RAW264.7 macrophages

When CCE was added to RAW264.7 cells activated with LPS (1 μg/ml), the expression of the iNOS protein was significantly inhibited compared with that of the control. The ex-

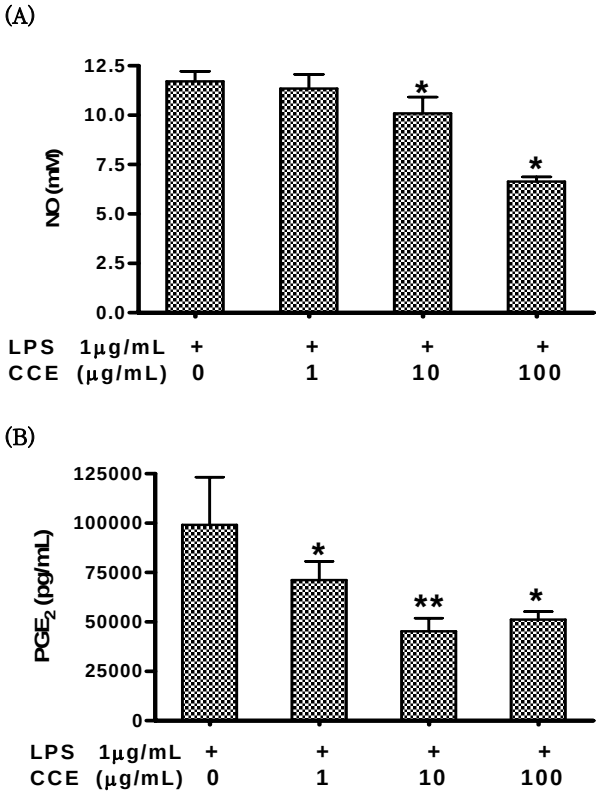

Figure 1. Effects of *Coptis chinensis* extract (CCE) on NO (A) and PGE_2 (B) formation in RAW264.7 macrophages. RAW264.7 macrophages were adjusted to 2×10^4 cells/well in a 96-well plate. The cells were stimulated with LPS (1 μg/ml) in the presence of CCE for 24 h. The culture supernatants were used to measure the NO and PGE_2 levels. Values are the means of five wells in triplicate experiments and are expressed as the mean$\pm$S.D. (*$p<0.05$, **$p<0.01$ vs. cells treated with DMSO as a control).

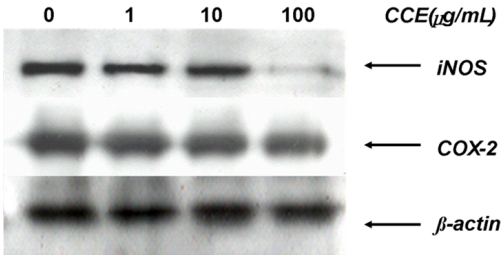

Figure 2. Inhibitory effect of CCE on the expression of the iNOS and COX-2 proteins in RAW264.7 macrophages. RAW264.7 macrophages were adjusted to 1×10^6 cells/well in a 6-well plate. The cells were treated with 0~100 μg/mL of CCE and stimulated by LPS (1 μg/mL) for 24 hours. Equal amounts of total protein (10 μg/lane) were subjected to 12.5% SDS PAGE, and the expression levels of the iNOS and COX-2 proteins was determined by Western blotting with specific anti-iNOS and anti-COX-2 antibodies. Actin was used as an internal control.

pression of COX-2 did not show an important change despite its subtle suppression (Fig. 2).

Effect of CCE on lymphocyte proliferation and cytokine production

The effect of CCE on lymphocyte proliferation was in-

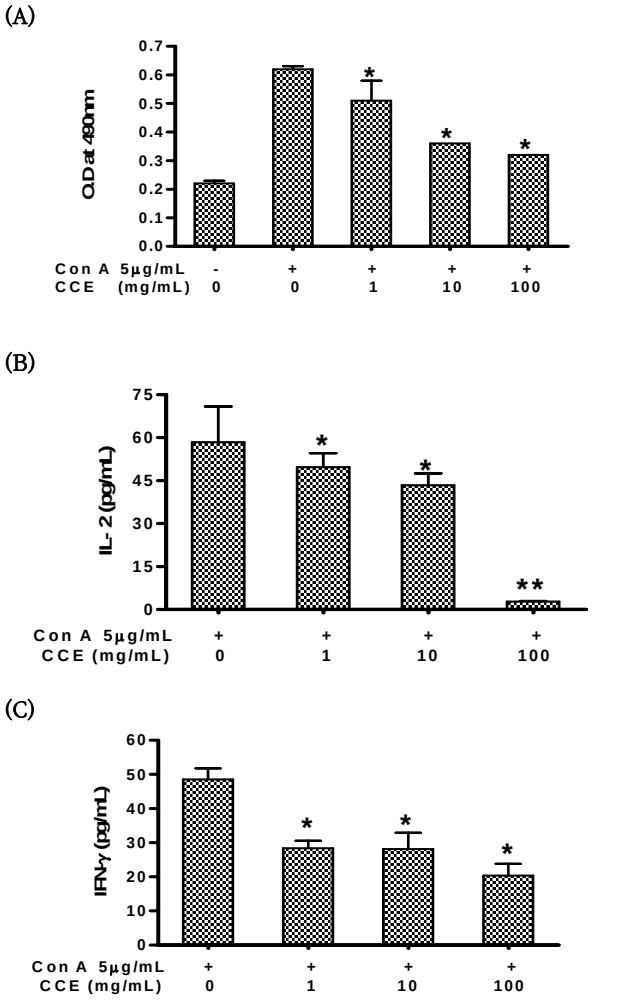

Figure 3. The *in vitro* inhibitory effect of *Coptis chinensis* extract (CCE) on the cell proliferation and cytokine production. Splenocytes were adjusted to 2×10^5 cells/well/200 μL of media in a 96-well plate. The cells were treated with 0, 1, 10, and 100 μg/mL of CCE dissolved in DMSO 30 min before stimulation with Con A (5 μg/ml). The inhibition of cell proliferation was assayed by MTS after 72 hr incubation (A). The culture supernatants were collected at 24 h and analyzed for cytokines by ELISA (B and C). Values are the mean of five wells from one of the three representative experiments and are expressed as the mean$\pm$SD (*$p<0.05$, **$p<0.01$ versus cell treated with DMSO as a control).

vestigated. Splenocytes were stimulated with Con A at 5 μg/ml for 72 h in the presence of CCE at concentrations of 1-100 μg/ml. CCE suppressed the proliferation of Con A-stimulated splenocytes in a dose-dependent manner (Fig. 3A). CCE inhibited the proliferation of splenocytes by about 51.6% at a concentration of 100 μg/ml in comparison to that of the vehicle-treated groups. The inhibition of splenocyte proliferation might be related to the reduction of the pro-inflammatory cytokines required for T cell proliferation. Thus, IL-2 and IFN-γ were analyzed from the culture supernatants. CCE (100 μg/ml) caused a 95.2% and 58.0% reduction in the production of IL-2 and IFN-γ, respectively (Fig. 3B and 3C).

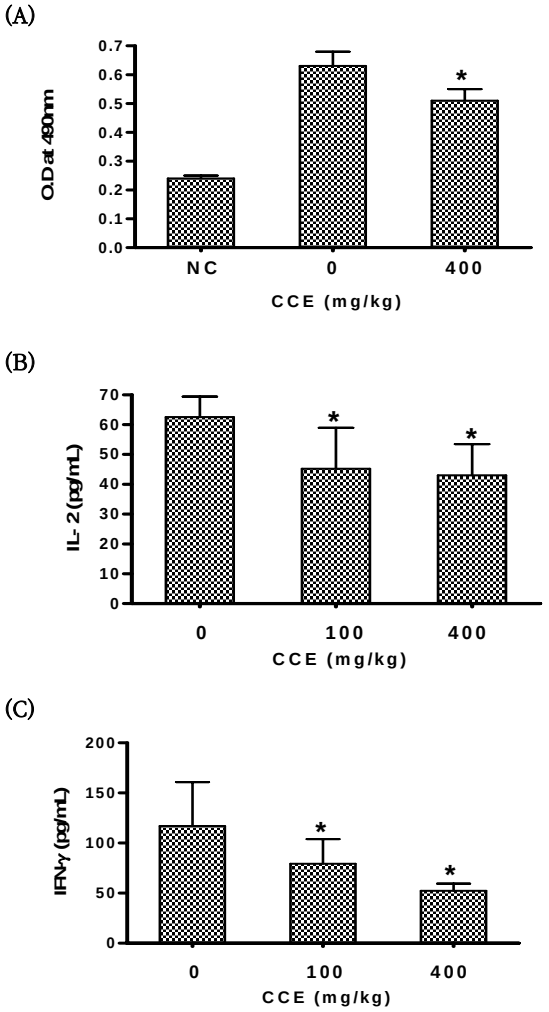

Figure 4. The anti-proliferative and anti-inflammatory effect of CCE *in vivo*. Splenocytes from CCE-fed mice were isolated and adjusted to 2×10^5 cells/well/200 μL of RPMI media in a 96-well plate, and stimulated with Con A for 24 h or 72 h in the absence of CCE. Cell proliferation was measured by MTS assays at 72 h of incubation (A). The productions of IL-2 (B) and IFN-γ (C) in the culture supernatants after 24 h of incubation were measured by ELISA. The values are expressed as the means $\pm$ SD for six mice. The data are representative of two separate experiments (*$p<0.05$ vs. cells treated with 0.5% CMC, NC: non-stimulated cells).

The anti-inflammatory effects of CCE *in vivo*

To further investigate the in vivo effect of CCE, six mice from each group were orally administrated CCE (100, 400 mg/kg) dissolved in 200 μl of a 0.5% CMC solution or 200 μl of a 0.5% CMC solution for the control group for 2 weeks. The splenocytes were isolated and stimulated by Con A at a 5-μg/ml concentration for 24 or 72 h without CCE. The cell proliferation at 72 h was significantly inhibited in the CCE-treated group in comparison to that of the control group (Fig. 4A). At 24 h of culture, the cell supernatant from the splenocyte of CCE-fed mice showed a reduction of the amount of IL-2 (31.3%) and IFN-γ (55.2%), compared to the control group, respectively (Fig. 4B and 4C).

Effect of CCE on delayed type hypersensitivity (DTH)

We also investigated whether CCE exhibits inhibitory effects on cell-mediated inflammation *in vivo* using a DTH mouse model. DTH mice induced by oxazolone were orally administrated CCE (400 mg/kg/200 μl of 0.5% CMC), dexamethasone (1 mg/kg/200 μl of 0.5% CMC) and 200 μl of 0.5% CMC as

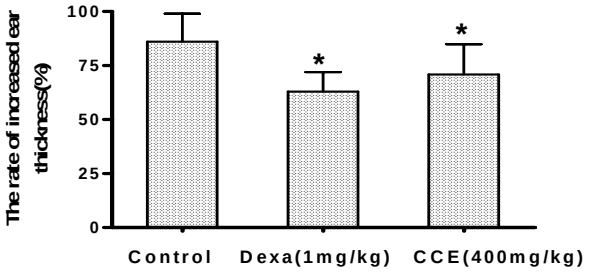

Figure 5. Anti-inflammatory effect of CCE on delayed type hypersensitivity (DTH) reactions to topical oxazolone in mouse skin. DTH was induced in BALB/c mice by sensitizing the skin with 25 μl of 2% oxazolone dissolved in the mixture of acetone and olive oil (4 : 1). At one, three and five days after sensitization, the mice were orally fed CCE (400 mg/kg) or dexamethasone (1 mg/kg) dissolved in 200 μl of a 0.5% CMC solution. The control mice received a 0.5% CMC solution (200 μl) orally. One day after the last feeding of CCE, both sides of the right ear of mice from each group were challenged with 10 μl of 0.5% oxazolone solution. After 24 h, the right and left ear thicknesses were measured in order to analyze the anti-inflammatory effect of CCE (A). The right ear thickness was compared with that of left ear. Six mice were used for each group. The data show one of two separate experiments. Each value represents the mean $\pm$ SD (n=6).

a control group. The ear thickness of each mouse of these three groups was compared in order to analyze the inhibitory effect of CCE. The ear thickness of the immunized right ear in the control group was increased to 86% in comparison to that of the non-immunized left ear. The ear thickness in the CCE- and the dexamethasone-fed group was increased to a lesser extent up to 70.8% and 62.9%, respectively (Fig. 5).

HPLC analysis of CCE

HPLC chromatogram of the extract showed a number of peaks with the peaks at RT 43.0 min of berberine and RT 39.2 min of palmatine (Fig. 6). Berberine and palmatine contents were found to be 22.5% and 5.7% w/w respectively from dry starting material. HPLC analysis showed that berberine was the main components of the extract of *Coptis* rhizome.

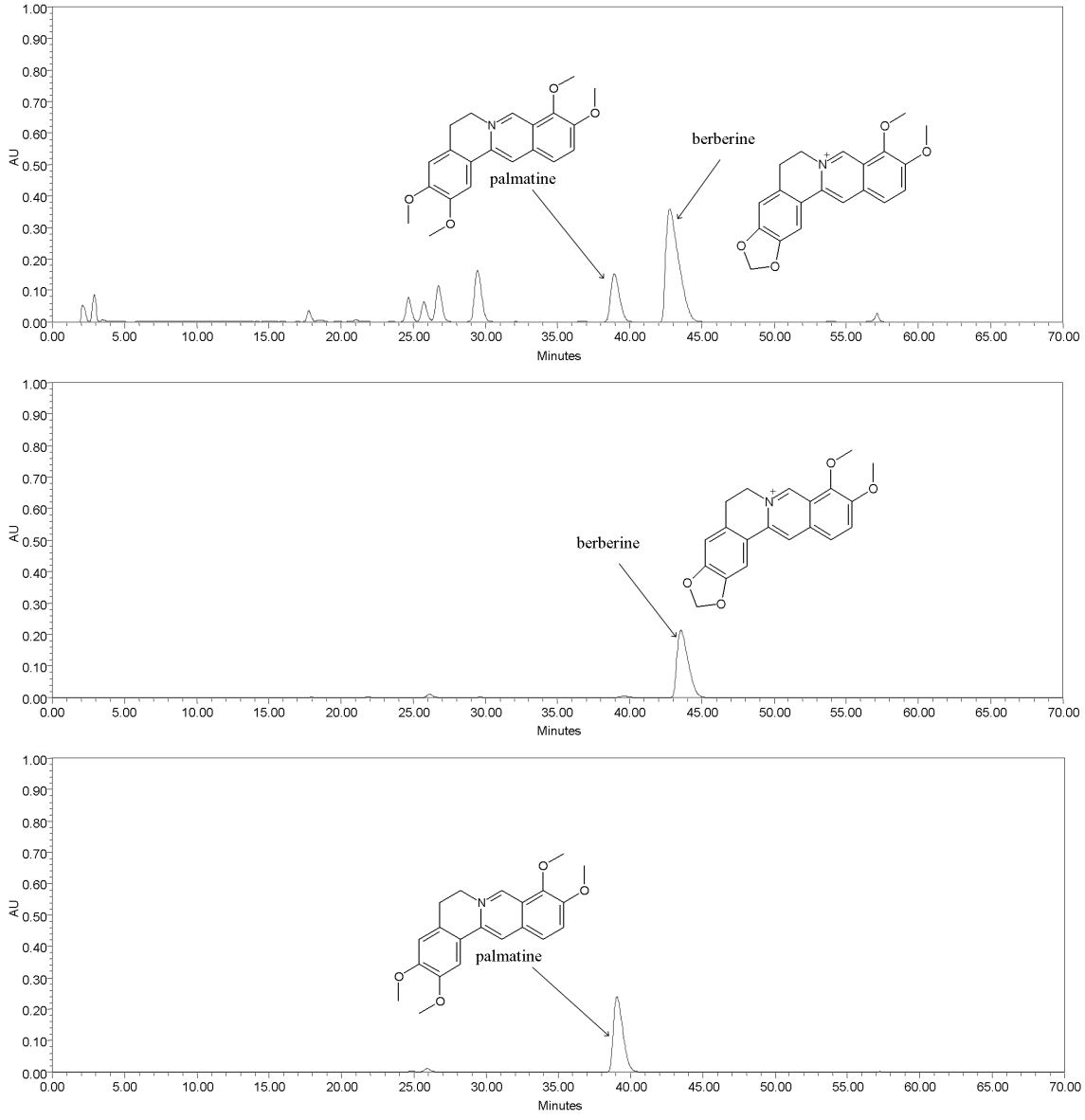

Figure 6. HPLC Chromatogram of *Coptis* rhizome. HPLC analysis showed that berberine and palmatine were the main component of the extract of *Coptis* rhizome.

DISCUSSION

This study showed the inhibitory effect of CCE on the production of pro-inflammatory mediators (NO and PGE_2) and inflammatory cytokines (IL-2, IFN-γ) in activated murine macrophages and splenocytes, respectively. In addition, we further demonstrated its immunosuppressive effect on oxazolone-induced DTH.

Among the inflammatory mediators, NO reacts with the superoxide anion (O_2^-) to produce other toxic free radicals, such as peroxynitfite ($ONOO^-$), and these exert undesirable effects on the majority of organ systems. NO is generated by NO synthetase (NOS) (15). Its overproduction is mediated by iNOS detected in activated macrophages (16). PGE_2, a type of prostaglandin (PG) produced by cyclo-oxygenase (COX), plays a role in the development of erythema, edema, and fever. COX exists in at least two isoforms. COX-1 is constitutively expressed, whereas COX-2 is expressed only in response to pro-inflammatory stimuli and plays an important role in the inflammatory reaction (17). Therefore, effective anti-inflammatory drugs are required to selectively suppress the expression of iNOS and COX-2 as well as the generation of NO and PGE_2. In this study, we demonstrated the ability of CCE to inhibit the production of NO and COX-2 in RAW264.7 macrophages. Furthermore, CCE seems to be able to reduce the expression of the iNOS and COX-2 proteins.

CCE added *in vitro* significantly inhibited the cell proliferation and release of the inflammatory cytokines IL-2 and IFN-γ in Con A-activated mouse splenocytes in a dose-dependent manner. IL-2 is required for T cell proliferation (18). Thus, the inhibition of IL-2 production might be involved in the decreased proliferation of lymphocytes from the spleen. IFN-γ, secreted by Th1 cells, induces the differentiation of naïve CD4+ T cells to Th1 cells and suppresses the proliferation of Th2 cells (19). Th1 and Th2 cells mediate cellular and humoral immunity, respectively (20). The inhibition of IFN-γ production by CCE suggests the possible immunosuppression in cell-mediated immunity.

Similarly, the lymphocyte proliferation and the cytokine production (IL-2 and IFN-γ) were significantly inhibited in splenocytes from the CCE-fed mice. These results indicate that CCE might induce a regulatory immune system *in vivo* (21), such as regulatory T cells or directly affect lymphocyte proliferation.

A model of cell-mediated inflammation, oxazolone-induced DTH, was used to investigate the immunosuppressive effect of CCE, and a significant difference in ear thickness was observed between the CCE-fed mice and the negative control (0.5% CMC)-fed mice. DTH is an immune reaction that is known to be T cell, macrophage and granulocyte-dependent (22). The *in vivo* inhibitory effects on DTH indirectly suggest that the *in vitro* inhibitory effect of CCE was not due to cell cytotoxicity, but rather to an immunomodulatory effect.

In the present study, we identified that berberine was the primary component of CCE through the HPLC analysis (data not shown). Moreover, it was recently shown that berberine, an isoquinoline derivative of alkaloid, has many pharmacological properties including anti-bacterial, anti-cancer, and anti-inflammatory effects (9-11). Therefore, the anti-inflammatory activity observed may be attributed to the berberine component.

Berberine containing herbal extracts have been used for many centuries as a tonic remedy for liver and cardiac diseases. According to recent reports, berberine displays a wide range of biological actions, including a potent antibiotic effect against bacteria, viruses and fungii (10), an anti-cancer effect by inducing DNA topoisomerase poisoning and cellular apotosis in HeLa and leukemia cells (11), and an anti-inflammatory effect. Berberine exhibited the inhibition of phorbol ester-induced inflammation (23), COX-2 expression (24), and the LPS-induced production of COX-2, TNF-α and iNOS in alveolar macrophages (25).

It is remarkable that the total alkaloids from the rhizoma of *Coptis chinensis* were capable of reducing ulcer formation in experimental models (26) and inhibiting the *in vitro* proliferation of vascular smooth muscle cells, which is an important mechanism of atherosclerosis (27). These findings suggest that berberine may overcome existing complications associated with NSAIDs, such as gastrointestinal ulceration and cardiovascular morbidity. Thus, berberine can be a potential candidate to be developed as a newer anti-inflammatory drug suitable for long-term use.

Anti-inflammatory drugs are frequently used to improve the symptoms and inhibit tissue damage in chronic inflammatory diseases, such as rheumatoid arthritis. Corticosteroid, one of the most potent anti-inflammatory drugs, is well known for its rapid action. However, the long-term administration of corticosteroids is undesirable because it is associated with a number of serious side effects such as infection, osteoporosis, hypertension, Cushing syndrome and so on. NSAIDs, which are commonly prescribed drugs, have a high probability of gastrointestinal problems as previously mentioned. COX-2 se-

lective inhibitors, which were developed in an effort to reduce these complications, were recently reported to increase the risk of cardiovascular events. Accordingly, the development of new anti-inflammatory drugs for the safe treatment of chronic inflammatory diseases is highly desirable.

In our study, the ethanolic extract of *Coptis chinensis* showed an anti-inflammatory effect via the inhibition of the expression of pro-inflammatory mediators and an immunosuppressive effect in oxazolone-induced DTH mice. These results suggest that *Coptis chinensis* may be useful in treating chronic inflammatory diseases with immunologic mechanisms such as rheumatoid arthiritis. However, further studies investigating the organ toxicity at therapeutic concentrations, detailed pharmacokinetics, and individual studies of berberine, will be needed to validate the value of *Coptis chinensis* as an anti-inflammatory drug.

REFERENCES

1. Wu MJ, Wang L, Ding HY, Weng CY, Yen JH. Glossogyne tenuifolia acts to inhibit inflammatory mediator production in a macrophage cell line by downregulating LPS-induced NF-Kb. J Biomed Sci 11:186-199, 2004
2. Dinarello CA. Interleukin-1, interleukin-1 receptors and interlekin-1 receptor antagonist. Int Rev Immunol 16:457-499, 1998
3. Nathan C. Natural resistance and nitric oxide. Cell 82: 873-876, 1995
4. Fenton MJ, Golenbock DT. LPS-binding proteins and receptors. J Leukoc Biol 64:25-32, 1998
5. Glauser MP. The inflammatory cytokines. New developments in the pathophysiology and treatment of septic shock. Drugs 52(suppl):9-17, 1996
6. Duan H, Zhang Y, Xu J, Qiao J, Suo Z, Hu G, Mu X. Effect of anemonin on NO, ET-1 and ICAM-1 production in rat intestinal microvascular endothelial cells. Journal of Ethnopharmacology 104:362-366, 2006
7. Lefkowith JB. Cyclooxygenase-2 specificity and its clinical implications. American Journal of Medicine 106:43S-50S, 1999
8. Solomon SD. Cyclooxygenase-2 inhibitors and cardiovascular risk. Curr Opin Cardiol 21:613-617, 2006
9. Ikram M. A review on the chemical and pharmacological aspects of genus Berberis. Planta Med 28:353-358, 1975
10. Schmeller T, Latz-Bruning B, Wink M. Biochemical activities of berberine, palmaltine and sanguinarine mediating chemical defense against microorganisms and herbivores. Phytochemistry 44:257-266, 1997
11. Kettmann V, Kosfalova D, Jantova S, Cernakova M, Drimal J. *In vitro* cytotoxicity of berberine against HeLa and L1210 cancer cell lines. Pharmazie 59:548-551, 2004
12. Kuo CL, Chi CW, Liu TY. The anti-inflammatory potential of berberine *in vitro* and *in vivo*. Cancer Lett 20:127-137, 2004
13. Pathak N, Khandelwal S. Oxidative stress and apoptotic changes in murine splenocytes exposed to cadmium. Toxicology 220:26-36, 2006
14. Blaylock BL, Newsom KK, Holladay SD, Shipp BK, Bartow TA, Mehendale HM. Topical exposure to chlordane reduces the contact hypersensitivity response to oxazolone in BALB/c mice. Toxicology Letters 81:205-211, 1995
15. Mayer B, Hemmens B. Biosynthesis and action of nitric oxide in mammalian cells. Trends Biochem Sci 22:477-481, 1997
16. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J 6:3051-3064, 1992
17. Akarasereenont P, Bakhle YS, Thiemermann C, Vane JR. Cytokine-mediated induction of cyclo-oxygenase 2 by activation of tyrosine kinase in bovine endothelial cells stimulated by bacterial lipopolysaccharide. Br J Pharmacol 115:401-408, 1994
18. Rothenberg EV, Diamond RA, Pepper KA, Yang JA. IL-2 gene inducibility in T cells before T cell receptor expression. Changes in signaling pathways and gene expression requirements during intrathymic maturation. The Journal of Immunology 144:1614-1624, 1990
19. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100:655-669, 2000
20. Mullen AC, High FA, Hutchins AS. Role of T-bet in commitment of Th1 cells before IL-12-depedent selection. Science 292:1907-1910, 2001
21. Schwartz RH. Natural regulatory T cells and self-tolerance. Nature Immunology 6:327-330, 2005
22. Verdrngh M, Jonsson IM, Holmdahl R, Tarkowski A. Genistein as an anti-inflammatory agent. Inflammation Research 52:341-346, 2003
23. Yasukawa K, Takido M, Ikekawa T, Shimada F, Takeuchi M, Nakagawa S. Relative inhibitory activity of berberine-type alkaloids against 12-O-tetra decanoylphorbol-13-acetate-induced inflammation in mice. Chem Pharm Bull 39:1462-1465, 1991
24. Fukuda K, Hibiya Y, Mutoh M, Koshiji M, Akao S, Fujiwara H. Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells. J Ethnopharmacol 66:227-233, 1999
25. Lee DU, Kang YJ, Park MK, Lee YS, Seo HG, Kim TS, Kim CH, Chang KC. Effects of 13-alkylsubstituted berberine alkaloids on the expression of COX-II, TNF-a, iNOS, and IL-12 production in LPS stimulated macrophages. Life Sci 73:1401-1412, 2003
26. Li B, Shang JC, Zhou QX. Study of total alkaloids from Rhizoma *Coptis Chinensis* on experimental gastric ulcers. Chin J Integr Med 11:217-221, 2005
27. Liang KW, Ting CT, Yin SC, Chen YT, Lin SJ, Liao JK, Hsu SL. Berberine suppresses MEK/ERK-dependent Egr-1 signaling pathway and inhibits vascular smooth muscle cell regrowth after *in vitro* mechanical injury. Biochem Pharmacol 71:806-817, 2006