Arch Pharm Res Vol 27, No 9, 923-929, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Stylopine from *Chelidonium majus* Inhibits LPS-Induced Inflammatory Mediators in RAW 264.7 Cells

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(Received July 30, 2004)

Stylopine is a major component of the leaf of *Chelidonium majus* L. (Papaveraceae), which has been used for the removal of warts, papillomas and condylomas, as well as the treatment of liver disease, in oriental countries. Stylopine *per se* had no cytotoxic effect in unstimulated RAW 264.7 cells, but concentration-dependently reduced nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor-a (TNF-α) and interleukin-1β (IL-1β), and the IL-6 production and cyclooxygenase-2 (COX-2) activity caused by the LPS stimulation. The levels of inducible nitric oxide synthase (iNOS) and COX-2 protein expressions were markedly suppressed by stylopine in a concentration dependent manner. These results suggest that stylopine suppress the NO and PGE_2 production in macrophages by inhibiting the iNOS and COX-2 expressions. These biological activities of stylopine may contribute to the anti-inflammatory activity of *Chelidonium majus.*

Key words: *Chelidonium majus*, Stylopine, Nitric oxide, Tumor necrosis factor-α, Interleukin-1β, Interleukin-6

INTRODUCTION

Nitric oxide (NO), prostaglandin E_2 (PGE_2) and the cytokines, such as interleukin-1 beta (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), are well known for their involvement in the development of inflammation (Lee *et al.*, 1992; Moncada *et al.*, 1991; Sautebin, 2000; Wheeler and Bernard, 1999). Macrophages play significant roles in inflammatory diseases by producing these inflammatory mediators. Following exposure to immune stimulants, including bacterial toxins, such as lipopolysaccharide (LPS) and lipoteichoic acid, the production of these mediators from macrophages has been found in many inflammatory tissue along with the increased expressions of their mRNAs (Penglist *et al.*, 2000; Yamashita *et al.*, 2000). Although NO and pro-inflammatory cytokines are involved in the host defense mechanism, their overproduction contributes to the pathogenesis of several diseases, such as sepsis, rheumatoid arthritis, atherosclerosis, pulmonary fibrosis and chronic hepatitis (Coker and Laurent, 1998; Isomaki and Punnonen, 1997). Thus, the inhibition of the production of these inflammatory mediators may prevent or suppress various inflammatory diseases.

Chelidonium majus L. (Papaveraceae) (*C. majus*) is a plant of great interest for its usage as an herbal medicine for various diseases in Chinese and European countries. The crude extracts from various parts of the plant, such as the roots, shoots and leaves, have been reported to contain several alkaloids, such as stylopine, sanguinarine, chelidonine, chelerythrine, berberine and coptisisine (Colombo and Bosisio, 1996). Both the crude extracts of *C. majus* and purified compounds from these extracts have been reported to exhibit interesting anti-viral, anti-inflammatory, anti-tumor and anti-microbial properties both *in vitro* and *in vivo* (Colombo and Bosisio, 1996;

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Lenfeld *et al.*, 1981; Biswas and Khuda-Bukhsh, 2002). Stylopine is a major component of various phyto-medicinal plants, including *C. majus*, which has been reported to exhibit allosteric modulation of the GABAA receptor, detoxification of xenobiotics and anti-inflammatory properties (Haberlein *et al.*, 1996; Sauto *et al.*, 2002; Ikezawa *et al.*, 2003). However, the effects of stylopine from *C. majus* on LPS-stimulated NO, PGE_2 and pro-inflammatory cytokine production remaine to be defined. Therefore, the effect of stylopine from *C. majus* on the production of NO, PGE_2, and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), and the expressions of inducible nitric oxide synthase (iNOS) and COX-2 were investigated in RAW 264.7 macrophages activated by LPS.

MATERIALS AND METHODS

Chemicals and reagents

The Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and antibiotics were purchased from GIBCO BRL (Grand Island, NY). The Rabbit anti-iNOS, rabbit anti-COX-1 and COX-2 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The LPS (phenol extracted *Salmonella entritidis*), Tween 20, bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium dodecyl sulfate (SDS) and N^G-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemical Co. (ST Louis, MO). The TNF-α, IL-1β, IL-6, and PGE_2 immunoassay kits (Quantikine™) were purchased from R &D System (Minneapolis, MN, USA). Ninety-six well tissue culture plates and other tissue culture reagents were purchased from Life Technologies (Gaithersburg, MD). All reagents were tested for their LPS content using a colorimetric Limulus amoebocyte lysate assay (detection limit, 10 pg/mL; Whitaker Bioproducts, Walkersville, MD).

Plant material

The leaves of *Chelidonium majus* L. (Papaveraceae) were purchased from the herbal medicine co-operative association of Jeonbuk Province, Korea, in October 2003. A voucher specimen (no. LGF777) was deposited at the Herbarium of the College of Oriental Medicine, Wonkwang University (Korea).

Extraction and isolation

The dried and powered root (2.0 kg) of *C. majus* was extracted three times with MeOH (6 L) for 7 days at room temperature. The combined MeOH extract was concentrated under reduced pressure to yield dark brown syrup (72 g). The MeOH extract (70 g) was suspended in H_2O (1 L) and sequentially partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH. A portion of the CH_2Cl_2 soluble fraction (14 g) was subjected to chromatography on a silica gel column (400 g) using 100% *n*-hexane, *n*-hexane-EtOAc (50:1, 25: 1, 12:1, 5:1, 1:1), then 100% EtOAc to obtain 6 fractions (Fr. SM-31; 41 mg, 400 mL, Fr. SM-32; 686 mg, 800 mL, Fr. SM-33; 2250 mg, 800 mL, Fr. SM-34; 890 mg, 800 mL, Fr. SM-35; 231 mg, 800 mL, Fr. SM-36; 760 mg). A portion (150 mg) of the active fraction (Fr. SM-33) was subjected to recycling-preparative HPLC ($CHCl_3$, flow rate; 3.5 mL/min) to yield a compound (91 mg, tr = 26.5 min). The compound was identified as stylopine (Fig. 1) by the comparison of its spectral data (MS, 1D NMR, and 2D NMR) with those of the alkaloid reported in the literature (Suau *et al.*, 2002). ^{1}H-NMR (500 Mz, Pyridine-d_5): 2.56 (2H, *m*, H-5 and H-6), 2.96 (1H, *dd*, J = 15.6, 16 Hz, H-5), 3.07 (1H, *m*, H-6), 3.34, 3.57 (1H, *dddd*, J = 2.7, 3.65, 11.9, 15.6 Hz, H-13), 3.55 (2H, *dd*, J = 11.9, 15.6 Hz, H-8), 4.18 (1H, *d*, J = 15.1, H-14), 5.93, 6.00 (2H, *dd*, J = 0.9, 1.35 Hz, $-OCH_2O-$), 5.98, 5.99 (2H, *dd*, J = 1.4, 1.4 Hz, $-OCH_2O-$), 6.64 (1H, *s*, ArH), 6.72 (2H, *d*, J = 7.8 Hz, ArH), 6.83 (2H, *d*, J = 7.75 Hz, ArH), 6.93 (1H, *s*, ArH), ^{13}C-NMR (125 MHz, Pyridine-d_5): 29.4 (C-5), 36.5 (C-13), 51.2 (C-6), 52.9 (C-8), 59.9 (C-14), 101.2 (C-A), 101.4 (C-B), 106.1 (C-1), 106.9 (C-11), 108.6 (C-4), 117.0 (C-8'), 121.3 (C-12), 127.9 (C-4'), 128.9 (C-12'), 130.8 (C-14'), 143.6 (C-10), 145.4 (C-2), 146.4 (C-3), 146.6 (C-9).

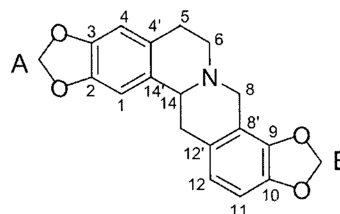

Fig. 1. Chemical structure of stylopine isolated from *C. majus*

Cell culture

The murine macrophage RAW 264.7 cell line, obtained from American Type Culture Collection (ATCC, TIB 71, Maryland, USA), was maintained in 1×10^6 cells/mL cultures in DMEM supplemented with 10% heat inactivated FBS, penicillin G (100 IU/mL), streptomycin (100 μg/mL) and L-glutamine (2 mM), and incubated at 37°C in a humidified 5% CO_2 and 95 % air atmosphere. On the following day, the medium was replaced with fresh DMEM, and the cells then stimulated with LPS (1 μg/mL), in the presence or absence of stylopine from *Chelidonium majus,* for the indicated periods. The stylopine was dissolved in DMSO and diluted with the medium to the final concentration.

MTT assay for cell viability

The cells were plated at a density of 2.5×10^5 cells/mL into a 96 well plate, containing 100 μL of DMEM medium supplemented with 10% FBS, and incubated overnight. Following 24 h of incubation after seeding, 100 μL of new media or stylopine (1-20 mg/mL) solution was added, and the plates incubated for a further 24 h. The cells were washed once before adding 50 μL FBS-free medium containing 50 μg/mL MTT. After incubation for 4 h at 37°C, the medium was discarded and the formazan blue, that had formed in the cells, was dissolved in 50 μL DMSO. The optical density at 570 nm was determined with a microplate reader, with that of the formazan formed in the control (untreated) cells taken as 100% viability.

Nitrite assay

The accumulated nitrite, an oxidation product of NO, was measured in the culture medium by the Griess reaction. Briefly, 100 μL of cell culture medium were mixed with 100 μL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydorchloride/2.5% phosphoric acid) and incubated at room temperature for 10 min, and then the absorbance at 540 nm measured in a microplate reader, with fresh culture medium used as the blank. The nitrite levels in the samples were calculated from a standard nitrite curve freshly prepared in culture medium.

PGE_2, TNF-α, IL-1β and IL-6 assay

The cells (1×10^6/mL) were pre-incubated for 2 h with stylopine, and further cultured for 6 h or 18 h with LPS (1 μg/mL) in 24-well plates. The supernatants were removed at the allotted times and the levels of PGE_2, TNF-α, IL-1β and IL-6 quantified using their respective immunoassay kits, according to the manufactureís protocols.

Western blot

Cellular proteins were extracted from the control and stylopine-treated RAW 264.7 cells. The washed cell pellets were resuspended in cold lysis buffer (10 mM Tris-base, 5 mM EDTA, 50 mM NaCl, 1% triton X-100, 5 mM phenylmethylsulfonyl fluoride, 2 mM sodium orthovanadate, 10 μg/mL leupeptin and 25 μg/mL aprotinin) and incubated for 30 min at 4°C. The nuclei and cell debris were removed by microcentrifugation followed by quick freezing of the supernatants. 30 μg of the cellular proteins from the treated and untreated cell extracts were electroblotted onto nitrocellulose membranes following their separation by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The immunoblots were incubated overnight with blocking solution (5% skim milk) at 4°C, followed by incubation for 4 h with appropriate dilutions of the primary antibodies (against rabbit anti-iNOS, rabbit anti-COX-1, and rabbit anti-COX-2). The blots were washed twice with PBS, incubated with a 1:5000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG for 1 h at room temperature. The blots were washed a further three times in Tween 20/Tris-buffered saline (TTBS), developed with 10 mL of a 1:1 solution of the ECL detection system for 1 min, dried quickly, and exposed to a film for 2-20 min. The protein concentration was determined using the Bio-Rad protein assay reagent, according to the manufactureís instruction.

Data analysis

All values are expressed as the mean±S.D. of three independent determinations. All experiments were performed at least three times, with three or more independent observations each time. An analysis of variance (ANOVA) and Student's *t*-tests were used for the statistical analysis.

RESULTS

Effects of stylopine on NO production and iNOS expression in LPS-stimulated RAW 264.7 Cells

The effects of stylopine on the NO production and iNOS protein expression were examined in RAW 264.7 cells activated with LPS. First, the stylopine at the treated concentrations in the present study was confirmed to have no effects on the NO production and iNOS protein expression in non-stimulated RAW 264.7 cells (data not shown). However, stylopine markedly inhibited the NO production in LPS (1 μg/mL) treated RAW 264.7 cells. To further evaluate whether the reduction of the NO production by stylopine was correlated with iNOS, the expression of iNOS protein was examined by Western blotting analysis. From the reduction of NO production, as shown in Fig. 2A, stylopine consistently inhibited the expression of iNOS protein in RAW 264.7 cells stimulated with LPS, in a concentration dependent manner (Fig. 2B). The NO inhibitor, L-NAME (10 μM), as a positive control, also inhibited the production of NO in activated RAW 264.7 cells (Fig. 2A). These results clearly demonstrated that stylopine produced a concentration-dependent inhibition of NO production and iNOS expression in response to LPS, without any cytotoxicity.

Effects of stylopine on PGE_2 secretion in LPS-stimulated RAW 264.7 Cells

The effects of stylopine on the production of PGE_2 were examined in RAW 264.7 cells activated with LPS. As shown in Fig. 3, LPS alone secreted large amounts of PGE_2 in RAW 264.7 cells. Treatment with various concentrations of stylopine inhibited LPS-induced PGE_2 secretion, in a dose dependent manner (Fig. 3).

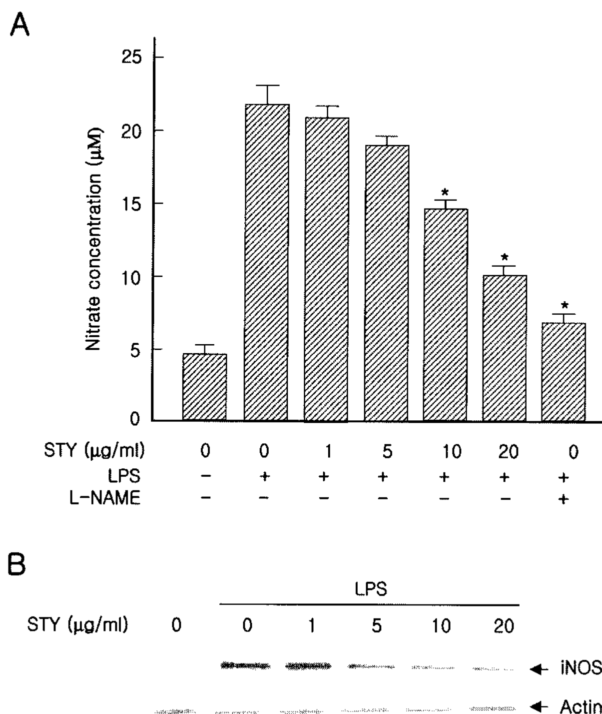

Fig. 2. The effects of stylopine on NO production (A) and iNOS protein expression (B) in RAW 264.7 cells. Cells (2.5×10^5/mL) were incubated for 24 h (for NO assay) or 18 h (for iNOS Western blot) with medium, supplemented with either LPS (1 μg/mL) or LPS plus stylopine (1-20 μg/mL). The NO concentration was determined by Griess reagent, as described in Materials and Methods. Each column represents the mean ±S.D. of three independent experiments. *P<0.01 indicates a significant difference from the LPS treated control group. Western immunoblot analysis was carried out for the determination of intracellular iNOS protein, as described in Materials and Methods.

Effects of stylopine on COX-2 activity and expression in LPS-stimulated RAW 264.7 Cells

To investigate the mechanism of the inhibition of PGE_2 secretion by stylopine, the activity of COX-2 and expression of COX-2 protein in the RAW 264.7 cells stimulated with LPS were examined. As shown in Fig. 4A, LPS alone induced high level of COX-2 production in the RAW 264.7 cells stimulated with LPS. However, the treatment with stylopine at different concentrations inhibited the LPS-induced COX-2 activity in a dose-dependant manner. In the range of doses used, stylopine showed a dose-dependent decrease in the LPS-induced COX-1 and COX-2 protein expressions (Fig. 4B). The activity and protein expression of COX-2 were inhibited, but those of COX-1 were not suppressed at the same stylopine dose. These results clearly demonstrated that stylopine produced a dose-dependent inhibition of PGE_2 production by inhibiting the COX activity and protein expression in RAW 264.7 cells stimulated with LPS.

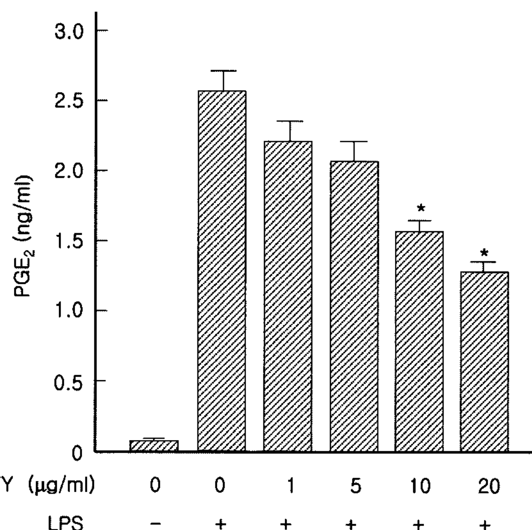

Fig. 3. The effects of stylopine on PGE_2 production in RAW 264.7 cells. Cells (1×10^6/mL) were incubated with or without LPS (1 μg/mL) for 18 h, in the presence or absence of stylopine at the indicated concentrations. PGE_2 production in the culture medium was determined as described in Materials and Methods. Each column represents the mean±S.D. of three independent experiments. *P<0.01 indicates a significant difference from the LPS treated control group.

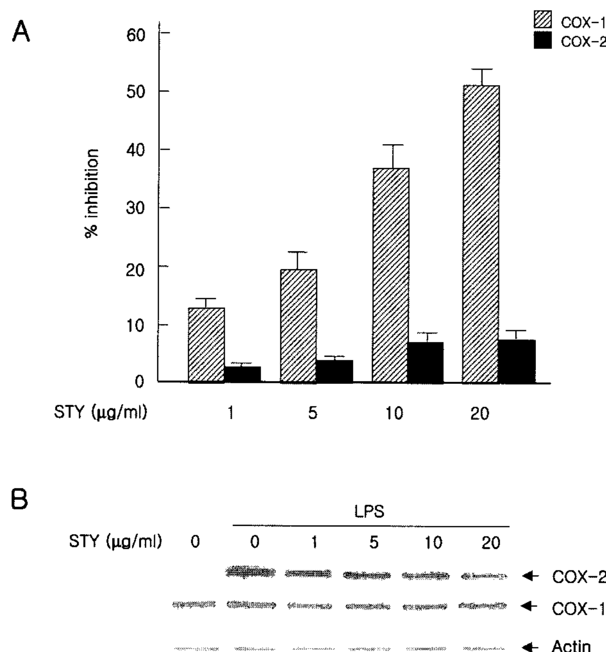

Fig. 4. The effects of stylopine on the COX activity (A) and COX protein expression (B) in RAW 264.7 cells. Cells (1×10^6/mL) were incubated with or without LPS (1 μg/mL) for 18 h, in the presence or absence of stylopine at the indicated concentrations. COX activity in the culture medium was determined as described in Materials and Methods. Each column represents the mean±S.D. of three independent experiments. Western immunoblot analysis was carried out as described in Materials and Methods.

Effects of stylopine on TNF-α, IL-1β, and IL-6 in LPS-stimulated RAW 264.7 Cells

To determine whether stylopine modulates the production of pro-inflammatory cytokines, the productions of TNF-α, IL-1β and IL-6 were examined using ELISA methods in the stimulated RAW 264.7 macrophage soup. Stylopine alone had no significant effect on the TNF-α, IL-1β and IL-6 secretions in the unstimulated RAW 264.7 cells (data not shown). The treatment with different concentrations of stylopine resulted in a dose-dependent inhibition of the LPS-induced TNF-α, IL-1β, and IL-6 secretions (Fig. 5). These observed effects were not due to the cytotoxicity of stylopine, as catapolside showed no impairment of the cell viability in dose ranges used in this study, compared with the cells treated with LPS alone (Fig. 5D).

DISCUSSION

In order to validate the use of stylopine from *C. majus* as an anti-inflammatory drug in traditional herbal medicine, the effects of stylopine on the productions of NO, PGE_2, COX-2, TNF-α, IL-1β, and IL-6 in LPS-stimulated RAW 264.7 cells were investigated. It was shown that stylopine inhibited the production of the major macrophage-derived inflammatory mediators, in dose-dependent manners.

NO and PGE_2, which are produced by iNOS and COX-2, respectively, have been implicated as important mediators

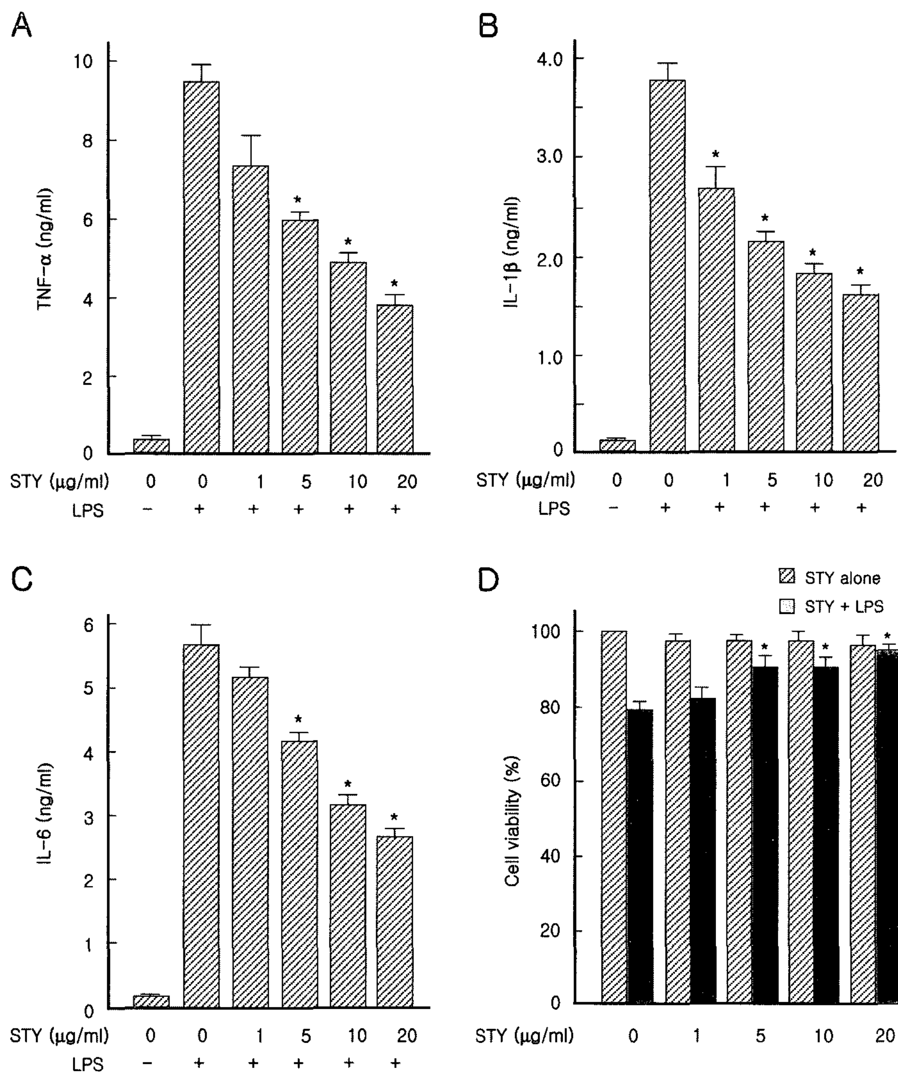

Fig. 5. The effects of stylopine on TNF-α (A), IL-1b (B) and IL-6 (C) in RAW 264.7 cells. Cells (1×10^6/mL) were incubated with or without LPS (1 μg/mL) TNF-α and IL-6 for 6 h or IL-1β for 12 h, in the presence or absence of stylopine at the indicated concentrations. The productions of TNF-α, IL-1β or IL-6 in the culture medium were determined as described in Materials and Methods. The cell viability (D) was measured after incubation for 24 h. Each column represents the mean±S.D. of three independent experiments. *P<0.01 indicates a significant difference from the LPS treated control group.

under endotoxemia and inflammatory conditions (Ahmad *et al.*, 2002). It has been demonstrated that NO plays a important role as an immune regulator, neurotransmitter and vasodilator in a variety of tissues at physiological concentrations (Moncada *et al.*, 1991). The high levels of NO produced by iNOS, however, have been defined as a cytotoxic molecule in inflammation and endotoxemia (Kroncke *et al.*, 1997). PGE_2, like NO, is a pleiotropic mediator produced at inflammatory sites by COX-2, which gives rise to pain, swelling and stiffness (Dinarello, 1999). Thus, the potential inhibitors of iNOS and COX-2 have been considered as potential anti-inflammatory drugs. Here, it has been demonstrated that stylopine significantly inhibited the protein expression of iNOS and COX-2, resulting in the suppression of NO and PGE_2 productions in LPS-stimulated RAW 264.7 cells (Figs. 2-4).

A body of evidence indicates that pro-inflammatory cytokines, such as IL-1, TNF-α, and IL-6, have been shown to control inflammation both *in vitro* and *in vivo* (Dinarello, 1999; Feldmann *et al.*, 1996; Mannel and Echtenacher, 2000; Straub *et al.*, 2000). These cytokines appear to be interlinked in a cascade, being produced serially by cells during an inflammatory response. Cumulative evidence indicates that an abnormality in the productions or functions of TNF-α, IL-1β and IL-6 play essential roles in many inflammatory lesions (De Nardin, 2001). Exposure to LPS causes inflammatory liver damage and septic shock due to the production of the high levels of these cytokines (Mojena *et al.*, 2001). The inhibition of cytokine production or function serves as a key mechanism in the control of inflammation (Shapira *et al.*, 1996). The present study showed that stylopine suppressed the production of the major macrophage-derived pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in LPS-stimulated RAW 264.7 cells.

The development of potent inhibitors of NO, PGE_2, and pro-inflammatory cytokines for potential therapeutic use would be valuable. According to the literature, the therapeutic effects of many Chinese herbs are attributable to the alkaloids, flavonoids and ilidoids (Recio *et al.*, 1994). Indeed, these compound-producing plants have often been used as anti-inflammatory medicines (Hu *et al.*, 1992). The papaveraceous plant, *C. majus,* is rich in specific alkaloid and flavonoid components. The chemistry and the biogenesis of these plant alkaloids have been the subject of many publications. This plant displays a variety of biological properties, such as antiviral, anti-microbial, anti-tumoral and anti-inflammatory effects (Colombo and Bosisio, 1996). Therefore, some are important in medicine and others can be considered as promising in this respect. Stylopine is a major component isolated from the leaf of *C. majus*, which has been used for the removal of warts, papillomas and condylomas and in the treatment of liver disease in Oriental countries (Colombo and Bosisio, 1996). Stylopine was found in this study to suppress inflammatory mediators and pro-inflammatory cytokines in LPS-stimulated RAW 264.7 cells.

Conversely, nuclear factor-κB (NF-κB) response elements have been demonstrated to be essential for the expressions of iNOS, COX-2 and the pro-inflammatory cytokine gene involved in the inflammatory process by providing NO, PGE_2 and pro-inflammatory cytokines, respectively (Barnes and Karin, 1997; Kotake *et al.*, 1998; Roshak *et al.*, 1996). Therefore, further investigation will be required to confirm whether stylopine suppresses NF-κB activation and the components leading to its activation.

In conclusion, stylopine from *C. majus* can suppress the productions NO, PGE_2, TNF-α, IL-1β and IL-6 and the expression COX-2 in LPS-stimulated RAW 264.7 cells. This anti-inflammatory effect occurs by down-regulation of iNOS and COX-2. Thus, these findings provide evidence that the anti-inflammatory activity of this plant is mainly due to stylopine, which may have potential as a medicine.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Small and Medium Business Administration of Korea in 2003 and partially by Wonkwang University in 2002.

REFERENCES

Ahmad, N., Chen, L. C., Gordon, M. A., Laskin, J. D., and Laskin, D. L., Related Articles, Links regulation of cycolooxygenase-2 by nitric oxide in activated hepatic macrophages during acute endotoxemia. *J. Leukoc. Biol.*, 71, 1005-1011 (2002).

Barnes, P. J. and Karin, M., Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.*, 336, 1066-1071 (1997).

Biswas, S. J. and Khuda-Bukhsh, A. R., Effect of a homeopathic drug, Chelidonium, in amelioration of p-DAB induced hepatocarcinogenesis in mice. *BMC Complement Altern. Med.*, 10; 2(1): 4. (2002).

Coker, P. K. and Laurent, G. J., Pulmonary fibrosis: cytokines in the balance. *Eur. Resp. J.*, 11, 1218 (1998).

Colombo, M. L. and Bosisio, E., Pharmacological activities of *Chelidonium majus* L. *Pharmacol. Res.*, 33, 127-134 (1996).

De Nardin, E., The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. *Ann. Periodontol.*, 6, 30-40 (2001).

Dinarello, C. A., Cytokines as endogenous pyrogens. *J. Infect. Dis.*, 179, Suppl 2, S294-304 (1999).

Feldmann, M., Brennan, F. M., and Maini, R. N., Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.*, 14, 397-440 (1996).

Haberlein, H., Tschiersch, K. P., Boonen, G.., and Hiller, K. O. *Chelidonium majus* L.: components with *in vitro* affinity for the FABAA receptor. *Planta Med.*, 62, 227- 231 (1996).

Hu, S., Cai. W., Ye, J., Qian, Z., and Sun. Z., Influence of medicinal herbs on phagocytosis by bovine neutrophils. *Zentralbl. Veterinarmed A.*, 39, 593-599 (1992).

Ikezawa, N., Tanaka, M., Nagayoshi, M., Shinkyo, R., Sakaki, T., Inouye, K., and Sato, F., *J. Biol. Chem.*, 278, 38557-38565 (2003)

Isomaki, P. and Punnonen, J., Pro- and anti-inflammatory cytokines inrheumatoid arthritis. *Ann. Med.*, 29(6), 499-507 (1997).

Kotake, Y., Sang, H., Miyajima, T., and Wallis, G.. L., Inhibition of NF-kappaB, iNOS mRNA, COX-2 mRNA, and COX catalytic activity by phenyl-*N-tert*-butylnitrone (PBN). *Biochim. Biophys. Acta*, 1448, 77-84 (1998).

Kroncke, K. D., Fehsel, K., and Kolb-Bachofen. V., Nitric oxide: cytotoxicity versus cytoprotectionóhow, why, when, and where? *Nitric Oxide*, 1, 107-120 (1997).

Lee, S H., Soyoola, E., Chanmugam, P., Hart, S., Sun, W., Zhong, H., Liou, S., Simmons, D., and Hwang, D., Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. *J. Biol. Chem.*, 267, 25934-25938 (1992).

Lenfeld, J., Kroutil, M., Marsalek, E., Slavik, J., Preininger, V., and Simanek, V., Anti-inflammatory activity of quaternary benzophenanthridine alkaloids from *Chelidonium majus. Planta Med.*, 43, 161-165 (1981).

Mannel, D. N. and Echtenacher, B., TNF in the inflammatory response. *Chem. Immunol.*, 74, 141-161 (2000).

Mojena, M., Hortelano, S., Castrillo, A., Diaz-Guerra, M. J., Garcia-Barchino, M. J., Saez, G. T., and Bosca, L., Protection by nitric oxide against liver inflammatory injury in animals carrying a nitric oxide synthase-2 transgene. *FASEB J.*, 15, 583-585 (2001).

Moncada, S., Palmer, R. M., and Higgs, E. A., Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, 43, 109-142. (1991).

Penglis, P. S., Cleland, L. G., Demasi, M., Caughey, G. E., and James, M. J., Differential regulation of prostaglandin E2 and thromboxane A2 production in human monocytes: implications for the use of cyclooxygenase inhibitors. *J. Immunol.*, 165, 1605-1611. (2000).

Recio, M. C., Giner, R. M., Manez, S., and Rios, J. L., Structural considerations on the iridoids as anti-inflammatory agents. *Planta Med.*, 60, 232-234 (1994).

Roshak, A. K., Jackson, J. R., McGough, K., Chabot-Fletcher, M., Mochan, E., and Marshall, L. A., Manipulation of distinct NFkappaB proteins alters interleukin-1beta-induced human rheumatoid synovial fibroblast prostaglandin E2 formation. *J. Biol. Chem.*, 271, 31496-31501 (1996)

Satou, T., Koga, M., Matsuhashi, R., Koike, K., Tada, I., and Nikaido, T., Characterisation of the 33kDa piroplasm surface antigen of Theileria orientalis/ sergenti/ buffeliisolates from West Java. *Indonesia Veterinary Parasitology.*, 104, 117-131 (2002).

Sautebin L., Prostaglandins and nitric oxide as molecular targets for anti-inflammatory therapy. *Fitoterapia*, 71, S48-S57 (2000).

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L., and Isakson, P., Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc. Natl. Acad. Sci. U.S.A.*, 91, 12013-12017 (1994).

Shapira, L., Soskolne, W. A., Houri, Y., Barak, V., Halabi, A., and Stabholz, A., Protection against endotoxic shock and lipopolysaccharide-induced local inflammation by tetra cyclin: correlation with inhibition of cytokine secretion. *Infect. Immun.*, 64, 825-828 (1996).

Straub, R. H., Linde, H. J., Mannel, D. N., Scholmerich, J., and Falk, W., A bacteria-induced switch of sympathetic effector mechanisms augments local inhibition of TNF-alpha IL-6 secretion in the spleen. *FASEB J.*, 14, 1380-1388 (2000)

Suau R., Cabezudo B., Rico R., and Najera F., Direct determination of alkaloid contents in Fumaria species by GC-MS. *Phytochem. Anal.*, 13, 363-367 (2002).

Wheeler, A. P. and Bernard, G..R., Treating patients with sever sepsis. *N. Engl. J. Med.*, 340, 207-214 (1999).

Yamashita, T., Kawashima, S., Ohashi, Y., Ozaki, M., Ueyama, T., Ishida, T., Inoue, N., Hirata, K., Akita, H., and Yokoyama, M., Resistance to endotoxin shock in transgenic mice overex endothelial nitric oxide synthase. *Circulation*, 101, 931-937 (2000).