

Inhibition of Prostaglandin and Nitric Oxide Production in Lipopolysaccharide-treated RAW 264.7 Cells by Tanshinones from the Roots of *Salvia miltiorrhiza* Bunge

Su Jin Jeon, Kun Ho Son, Yeong Shik Kim¹, Yong Hwan Choi², and Hyun Pyo Kim²

Dept. Food and Nutrition, Andong National University, Andong 760-749, Korea, ¹Natural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 110-460, Korea, and ²College of Pharmacy, Kangwon National University, Chuncheon 200-701, Korea

(Received January 28, 2008; Revised February 24, 2008; Accepted April 21, 2008)

This study examined the effects of tanshinone derivatives (tanshinone I, cryptotanshinone, 15,16-dihydrotanshinone I) on prostaglandin (PG) and nitric oxide (NO) metabolism in an attempt to establish their anti-inflammatory mechanisms and to present a scientific rationale for the use of *Salvia miltiorrhiza* (danshen) in inflammatory conditions. From lipopolysaccharide-treated RAW 264.7 cells, cyclooxygenase-2 (COX-2)-mediated PGE₂ production was inhibited by tanshinone I, cryptotanshinone and 15,16-dihydrotanshinone I, while only cryptotanshinone and 15,16-dihydrotanshinone I inhibited inducible NO synthase (iNOS)-mediated NO synthesis at 1-50 μM. Particularly, cryptotanshinone was found to be a down-regulator of proinflammatory molecule expression, including COX-2 and iNOS. The electrophoretic mobility shift assay showed that cryptotanshinone and 15,16-dihydrotanshinone I also inhibited the activation of the transcription factors, such as nuclear transcription factor-κB and activator protein-1. Moreover, cryptotanshinone exhibited *in vivo* anti-inflammatory activity against carrageenan-induced paw edema in rats. Overall, these results provide additional scientific rationale for the anti-inflammatory use of danshen in Chinese medicine. Especially, cryptotanshinone and 15,16-dihydrotanshinone I are important constituents.

Key words: *Salvia miltiorrhiza* Bunge, Tanshinone, Cryptotanshinone, 15,16-Dihydrotanshinone, Cyclooxygenase, Inducible nitric oxide synthase, Anti-inflammation

INTRODUCTION

Among the chemical mediators of inflammation, prostaglandins (PG) and nitric oxide (NO) are important for provoking and maintaining an inflammatory condition. In particular, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressed by certain inflammatory signals are the enzymes responsible for producing large amounts of proinflammatory PGs and NO, respectively (Gallin and Snyder, 1999). Therefore, an interference of these enzymatic pathways may have an anti-inflammatory effect. In this respect, many candidates for anti-inflammatory agents need to be evaluated for their effects on the PG and NO metabolism.

The roots of *Salvia miltiorrhiza* Bunge Labiatae (danshen) and a related species, *Salvia officinalis*, have been used in folk medicine for treating several inflammatory diseases including rheumatism (Duke and Ayensu, 1985; Kintzios, 2000; Bae, 2000). As its major constituents, tanshinones were previously demonstrated to possess anti-inflammatory and antiallergic activities. For example, tanshinones inhibited mast cell degranulation (Ryu *et al.*, 1999; Choi and Kim, 2004), suggesting their anti-allergic action. In immune cells, tanshinones inhibited IL-12 and IFN-γ production (Kang *et al.*, 2000). Our group showed that tanshinone I has *in vivo* anti-inflammatory activity and inhibits PGE₂ production from lipopolysaccharide (LPS)-treated RAW 264.7 cells, but it did not affect COX-2. Instead, this compound was suggested to be a phospholipase A₂ inhibitor (Kim *et al.*, 2002). It was also reported that tanshinone IIA inhibits iNOS expression from the activated RAW 264.7 cells (Jang *et al.*, 2003). Choi *et al.* (2004) examined the effects of four tanshinones on NO production from LPS-

Correspondence to: Hyun Pyo Kim, College of Pharmacy, Kangwon National University, Chuncheon, Korea
Tel: 82-33-2506915, Fax: 82-33-2558721
E-mail: hpkim@kangwon.ac.kr

treated RAW 264.7 cells. They claimed that tanshinone IIA, cryptotanshinone and 15,16-dihydrotanshinone I reduced the level of NO production. Among the derivatives, cryptotanshinone was demonstrated to reduce iNOS expression. They have also reported that tanshinone IIA inhibits the nuclear translocation of nuclear transcription factor- κ B (NF- κ B) and cryptotanshinone inhibits the activation of extracellular signal-regulated kinase (ERK). In addition, cryptotanshinone was reported to inhibit COX-2 activity in arachidonic acid-stimulated insect sf-9 cells without affecting its expression (Jin *et al.*, 2006). However, these previous reports are far from complete. The effects of tanshinones on the signal transduction pathways are not completely understood. Furthermore, the effects of 15,16-dihydrotanshinone I on COX-2 have not been described. Therefore, this study examined the effects of tanshinones on PG and NO metabolism to determine their anti-inflammatory cellular mechanisms and to provide scientific rationale of the use of danshen in inflammatory condition.

MATERIALS AND METHODS

Chemicals

N-[2-cyclohexyloxy-4-nitrophenyl]methane sulfonamide (NS-398) was obtained from Biomol (Plymouth Meeting, PA). 2-Amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT) was purchased from Tocris Cookson Ltd. (UK). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), LPS (*Escherichia coli* 0127:B8) and λ -carrageenan were purchased from Sigma Chem. (St. Louis, MO). DMEM and other cell culture reagents including fetal bovine serum (FBS) were products of Gibco BRL (Grand Island, NY). Protein assay kit was purchased from Bio-Rad Lab. (Hercules, CA).

Isolation of tanshinones

The dried roots of *Salvia miltiorrhiza* (17.5 Kg) were percolated with methanol three times and the extract was concentrated in vacuo. The dried residue (2.4 Kg) was suspended in H₂O and partitioned successively with hexane, CH₂Cl₂, EtOAc and *n*-butanol, to give hexane (48.8 g), CH₂Cl₂ (27.9 g), EtOAc (212.2 g), and *n*-butanol (149.1 g) soluble fractions, respectively. A portion of the hexane fraction and CH₂Cl₂ fraction (76.7 g) were subjected to a silica gel column chromatography, eluted with a stepwise gradient of hexane:CH₂Cl₂ to yield eleven fractions (HC1~HC11) on their polarities. Fraction HC5 was recrystallized with methanol to give tanshinone I (Fig. 1). Fractions HC8~10 (2 g) were rechromatographed on a silica gel column, eluted with hexane:CH₂Cl₂ (4:6~6:4) to give three subfractions (HC8.1~HC8.6). HC8.3 and H8.5 were recrystallized with methanol enabled the isolation of pure cryptotanshinone and 15,16-dihydrotanshinone I (Fig.

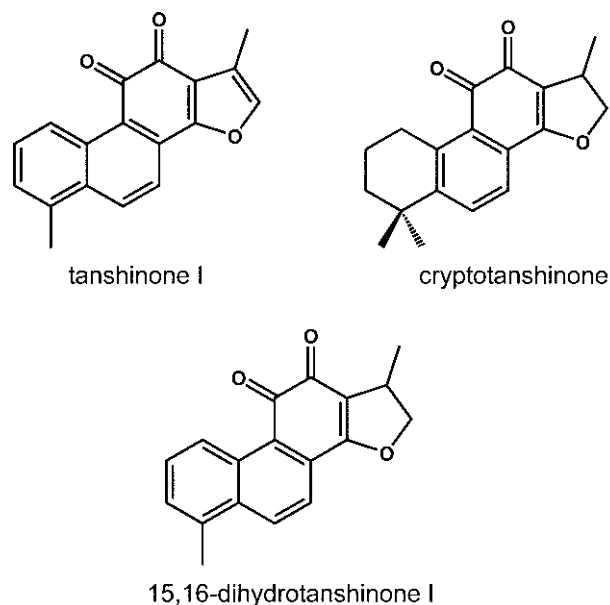


Fig. 1. Chemical structures of tanshinones used in this study

1), respectively. The structure of tanshinone I, cryptotanshinone, and 15,16-dihydrotanshinone I were verified by comparison of the NMR data with those of the reported in literature (Kang *et al.*, 1997; Ryu *et al.*, 1997). Test compounds dissolved in DMSO were diluted with serum-free DMEM into appropriate concentrations. Final concentration of DMSO in the culture medium was adjusted to 0.1% (v/v).

RAW 264.7 cell culture and measurement of NO and PGE₂ concentrations

RAW 264.7 cells obtained from American Type Culture Collection (ATCC, Rockville, MD) were cultured in DMEM supplemented with 10% FBS and 1% antibiotics under 5% CO₂ at 37°C based on the previously described procedures (Chi *et al.*, 2001). Briefly, cells were plated in 96-well plates (2 × 10⁵ cells/well). After pre-incubation for 2 h, the test compounds and LPS (1 mg/mL) were added and the cells were incubated further for 24 h. Cell viability was assessed with MTT assay as described previously (Mossman, 1983). From the culture medium, NO and PGE₂ concentrations were measured. For determination of NO concentration, the stable conversion product of NO, nitrite (NO₂⁻), was measured using Griess reagent [1:1 mixture (v/v) of 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% H₃PO₄]. Medium (100 μL) and Griess reagent (100 μL) were mixed in the 96-well plates and left for 10 min. Optical density was checked at 550 nm. PGE₂ concentration in the medium was measured using ELISA kit for PGE₂ (Cayman Chem. Co., Ann Arbor, MI) according to the manufacturer's recommendation.

Western blot of COX-2 and iNOS

For measuring the protein level of COX-2 and iNOS, Western blotting technique was used (Chi *et al.*, 2001). RAW 264.7 cells were cultured in 6-well plates (5×10^6 cells/well) in the presence or absence of LPS ($1 \mu\text{g/mL}$) with/without the test compounds for 20 h. The cells were washed, harvested, and homogenized. And the supernatant was obtained by centrifugation at 15,000 g for 30 min. Using Tris-glycine gel (4-15%, Novex Lab., San Diego, CA), electrophoresis was carried out and the bands were blotted to PVDF membranes. COX-2 antibody (160106, Cayman Chem.) and iNOS antibody (610332, Transduction Lab., Franklin Lakes, NJ) were incubated and bands were visualized by the treatment of secondary antibody and DAB reagent (Vector Lab., Burlingame, CA).

Electrophoretic mobility shift assay (EMSA)

Test samples were pre-incubated in RAW 264.7 cells without LPS for 2 h. LPS was added and the cells were incubated further for 30 min. Nuclear extracts from RAW 264.7 macrophages were prepared as previously described (Kim *et al.*, 1995). EMSA was analyzed by gel shift assay system (Promega, Madison, WI). Briefly, NF- κ B and activator protein-1 (AP-1) consensus oligo nucleotides (Promega, Madison, WI) were phosphorylated by T4 polynucleotide kinase with $10 \mu\text{Ci}$ of [γ - ^{32}P] ATP ($3,000 \text{ Ci/mmol}$) at 37°C for 10 minutes. Unincorporated oligonucleotides were removed by Microspin G-25 column (Amersham, UK). Nuclear extract containing $5 \mu\text{g}$ protein was incubated with [^{32}P]-labeled NF- κ B and AP-1 consensus oligonucleotides in gel shift binding buffer at room temperature for 20 minutes. The incubation mixture was subjected to electrophoresis on a 4% polyacrylamide gel in $0.5 \times \text{TBE}$ buffer at 350 V. The gel was dried and exposed to X-ray film overnight at -70°C .

λ -Carageenan (CGN)-induced paw edema in rats

In order to examine *in vivo* anti-inflammatory activity, rat CGN-induced paw edema assay was used with slight modification of Winter *et al.* (1962). Specific-pathogen free male SD rats (150-200 g) were purchased from Orient Bio (Seoul, Korea) and acclimatized in animal facility with lab. chow and water *ad libitum* at least for 7 days prior to experiment. Cryptotanshinone or reference compound dissolved in DMSO (0.1 mL/rat) was administered intraperitoneally. One hour later, 1% CGN (w/v) dissolved in pyrogen-free sterile saline solution (0.1 mL/paw) was injected to right hind paw and, after 5 h, paw volume was measured using plethysmometer (Ugo Basil, Italy). The paw volume increased from the initial non-treated paw volume was regarded as edema.

Statistical analysis

Experimental values were represented as arithmetic mean \pm SD. One-way ANOVA followed by Dunnet's test was used to determine the statistical significance.

RESULTS

COX-2 and iNOS were induced from LPS-treated RAW 264.7 cells to produce high amount of PGE_2 and NO, respectively. In a typical experiment, PGE_2 concentration increased to $43.8 \pm 0.2 \text{ nM}$ from the basal level of $3.1 \pm 0.1 \text{ nM}$. NO concentration also increased to $35.1 \pm 0.2 \mu\text{M}$ from the basal level of $0.4 \pm 0.1 \mu\text{M}$ after 24 h incubation with LPS ($1 \mu\text{g/mL}$). Under this condition, the cells were treated concomitantly with the test compounds and LPS, and incubated for 24 h. As shown in Fig. 2a, tanshinones

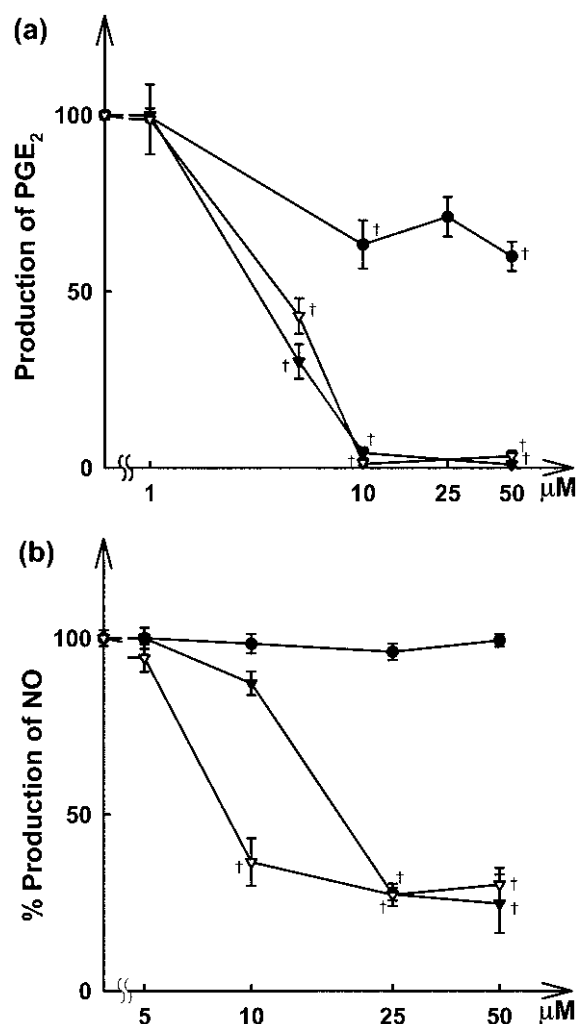


Fig. 2. Effects of tanshinones on COX-2-mediated PGE_2 and iNOS-mediated NO production from LPS-treated RAW 264.7 cells. (a) Effects on PGE_2 production. (b) Effects on NO production. Tanshinone I (●), cryptotanshinone (▼), 15,16-dihydrotanshinone I (▽), †: $P < 0.01$, significantly different from the LPS-treated control group ($n = 3$).

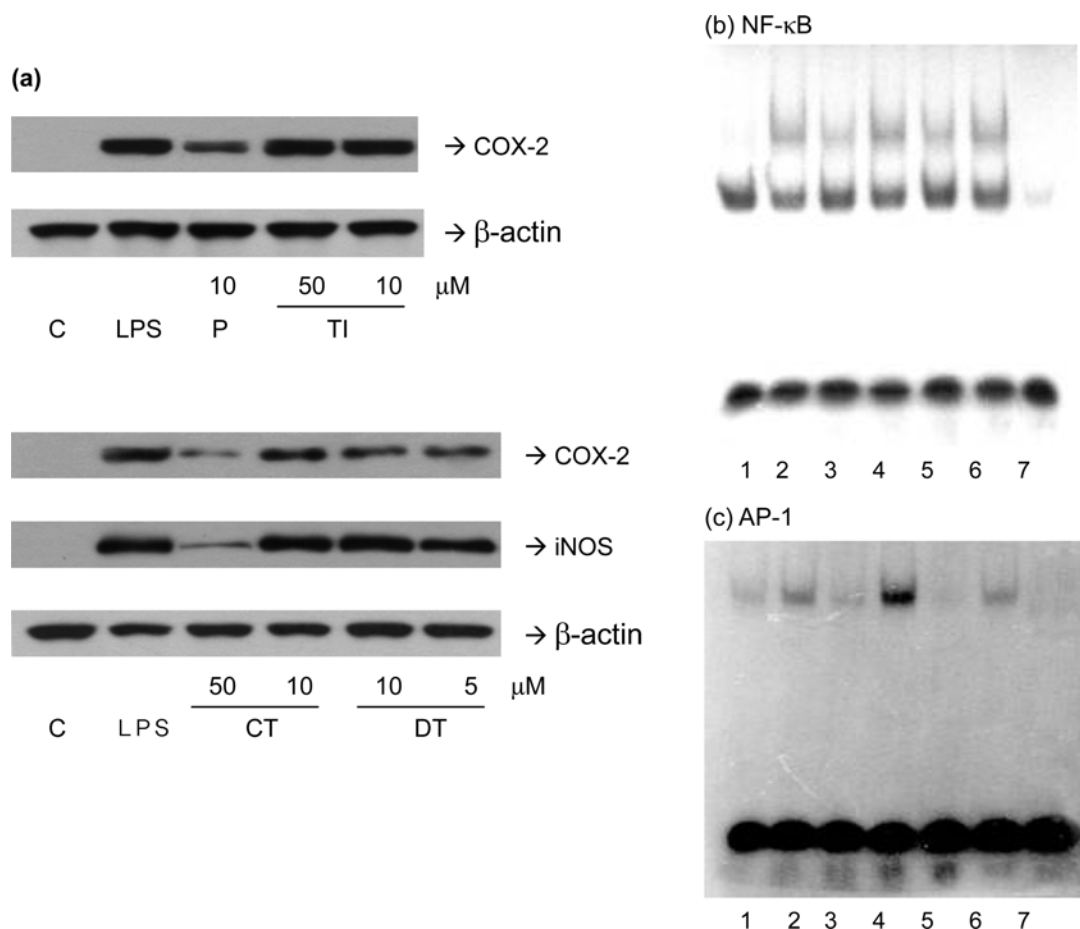


Fig. 3. Effects of tanshinones on COX-2 and iNOS expression and transcription factor activation from LPS-treated RAW 264.7 cells. (a) Western blotting analysis of COX-2 and iNOS expression. Control (C), prednisolone (P), tanshinone I (TI), cryptotanshinone (CT), 15,16-dihydrotanshinone I (DT), (b) EMSA of NF- κ B activation. (c) EMSA of AP-1 activation. Lane 1: control, lane 2: LPS-treated control, lane 3: LPS + CT (50 μ M), lane 4: LPS + CT (10 μ M), lane 5: LPS + DT (50 μ M), lane 6: LPS + DT (10 μ M), lane 7: LPS + specific competitor.

more or less inhibited COX-2-mediated PGE₂ production. The IC₅₀ values for tanshinone I, cryptotanshinone, and 15,16-dihydrotanshinone I were found to be > 50.0, 3.9, and 4.5 μ M, respectively, with cryptotanshinone being the most potent. Against iNOS-mediated NO production, cryptotanshinone and 15,16-dihydrotanshinone I showed strong inhibition with the IC₅₀ values of 19.3 and 8.8 μ M, respectively, while tanshinone I did not significantly inhibit NO production at the concentrations up to 50 μ M (Fig. 2b). As expected, the reference compounds, NS-398 (COX-2 inhibitor) and AMT (iNOS inhibitor) potently inhibited PGE₂ and NO production (> 90% inhibition) at 1 μ M, respectively.

The expression of COX-2 and iNOS was examined to determine the cellular mechanism for the inhibition of PGE₂ and NO production. As shown in Fig. 3a, cryptotanshinone clearly down-regulated COX-2 and iNOS expression at 50 μ M, while 15,16-dihydrotanshinone I inhibited COX-2 expression at 5-10 μ M without down-regula-

tion of iNOS. As expected, tanshinone I did not affect COX-2 expression. The cytotoxic effect of these compounds on RAW 264.7 cells was examined using MTT assay. None of the test compounds affected the cell viability under the experimental conditions except for 15,16-dihydrotanshinone I (data not shown). 15,16-Dihydrotanshinone I at concentrations 25 μ M showed some cytotoxicity against RAW 264.7 cells in the 6-well plate culture after 20 h incubation for Western blotting analysis. Therefore, the concentrations of 5 and 10 μ M 15,16-dihydrotanshinone I were used to examine the expression level. Under this condition, iNOS down-regulating property of 15,16-dihydrotanshinone I could not be observed. EMSA analysis showed that cryptotanshinone and 15,16-dihydrotanshinone I inhibited NF- κ B and AP-1 activation at 50 μ M (Fig. 3b and 3c), suggesting that these tanshinones inhibit both COX-2-mediated PGE₂ and iNOS-mediated NO production, at least in part, by inhibiting the activation of these transcription factors leading to down-regulation of the

Table I. Inhibition of λ -carrageenan (CGN)-induced paw edema in rats by cryptotanshinone

Compounds	Dose (mg/kg)	Increased paw volume (mL)	% inhibition
CGN	-	1.58 \pm 0.19	-
Indomethacin	20	0.96 \pm 0.27*	39.1
Cryptotanshinone	20	1.20 \pm 0.18*	24.2

All compounds were intraperitoneally administered.

*: $P < 0.05$, Significantly different from the CGN-treated control group ($n = 5$).

expression of certain inducible proinflammatory enzymes, such as COX-2 and iNOS. From these results, it was suggested that 15,16-saturated bond in the chemical structures of tanshinones is important for down-regulating the expression of proinflammatory molecules.

In addition, cryptotanshinone showed *in vivo* anti-inflammatory activity against CGN-induced paw edema in rats by intraperitoneal injection (Table I).

DISCUSSION

The present study clearly demonstrates that all the tanshinone derivatives tested had anti-inflammatory activity with some different cellular action mechanisms depending on their chemical structures. Particularly, cryptotanshinone down-regulated the expression of proinflammatory molecules including COX-2 and iNOS. This compound and 15,16-dihydrotanshinone I also inhibited the activation of the transcription factors, such as NF- κ B and AP-1. Since the previous investigations have revealed that inhibition of NF- κ B and AP-1 activation was closely related with COX-2 and iNOS down-regulation in LPS-treated RAW 264.7 cells (Kim *et al.*, 1995; Suh *et al.*, 1998; Hsu *et al.*, 2001), these two compounds may inhibit PGE₂ and NO production by COX-2 and iNOS down-regulation through the inhibition of these transcription factors. The ability of cryptotanshinone and 15,16-dihydrotanshinone I to inhibit NO production is well coincided with the experimental results reported by Choi *et al.* (2004). On the other hand, tanshinone I did not affect NO production. Tanshinone I only inhibited COX-2-mediated PGE₂ production without COX-2 down-regulating capacity. Tanshinone I may behave as a phospholipase A₂ inhibitor as previously suggested (Kim *et al.*, 2002).

There is some controversy regarding the experimental results reported by Jin *et al.* (2006) and the present results. Jin *et al.* (2006) demonstrated that cryptotanshinone inhibited COX-2-mediated PGE₂ production, and claimed that cryptotanshinone did not affect COX-2 expression. However, in the present study, cryptotanshinone clearly down-regulated COX-2 and iNOS expression. Currently,

the precise reason for this discrepancy is not known. It is speculated that these controversial results may be due, at least in part, to the different cells and stimulants used. Jin *et al.* (2006) used human U937 promonocytes stimulated with LPS plus phorbolmyristate acetate (PMA). U937 cells normally express COX-1, but they can be induced to express COX-2 by several agents, such as PMA. PMA activates protein kinase C (PKC) directly. Therefore, cryptotanshinone can affect mitogen-activated protein kinase pathway involved in LPS signaling, as reported previously (Suh *et al.*, 2006), but may not affect PKC signaling pathway.

Danshen is used frequently in Chinese medicine as an anti-inflammatory and antiallergic agent. As major constituents, tanshinone derivatives were isolated and some of their anti-inflammatory activities were previously described (Kim *et al.*, 2002; Jang *et al.*, 2003; Choi *et al.*, 2004; Jin *et al.*, 2006; Lee *et al.*, 2006). From this investigation, cryptotanshinone was found to be a down-regulator of proinflammatory molecule expression, including COX-2 and iNOS. 15,16-dihydrotanshinone I was proved to down-regulate COX-2 induction at noncytotoxic concentrations. The electrophoretic mobility shift assay showed that cryptotanshinone and 15,16-dihydrotanshinone I also inhibited the activation of the transcription factors, such as nuclear transcription factor- κ B and activator protein-1. The present study may provide additional scientific rationale for the anti-inflammatory use of danshen in Chinese medicine. Especially, cryptotanshinone and 15,16-dihydrotanshinone I are important constituents.

ACKNOWLEDGEMENTS

This study was financially supported by the research fund of Studies on the identification of the Efficacy of Biologically Active Components from Oriental Herbal Medicines from Korean Food and Drug Administration (2006) and post-BK21 project.

REFERENCES

- Bae, K., In Bae, K. (Ed.). The medicinal plants of Korea, Kyo-Hak Pub. Co., Seoul, pp. 444, (2000).
- Chi, Y. S., Cheon, B. S., and Kim, H. P., Effect of wogonin, a plant flavone from *Scutellaria radix*, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cells. *Biochem. Pharmacol.*, 61, 1195-1203 (2001).
- Choi, H. -S., Cho, D. -I., Choi, H. -K., Im, S. Y., Ryu, S. -Y., and Kim, K. -M., Molecular mechanisms of inhibitory activity of tanshinones on lipopolysaccharide-induced nitric oxide generation in RAW 264.7 cells. *Arch. Pharm. Res.*, 27, 1233-1237 (2004).

- Choi, H. S. and Kim, K. M., Tanshinones inhibit mast cell degranulation by interfering with IgE receptor-mediated tyrosine phosphorylation of PLC γ 2 and MAPK. *Planta Med.*, 70, 178-180 (2004).
- Duke, J. A. and Ayensu, E. S., In Duke, J. A. and Ayensu, E. S. (Eds.). Medicinal plants of China. Reference Publications, Inc., Algonquin, Michigan, Vol. 2, pp. 381, (1985).
- Gallin, J. I. and Snyderman, R., Overview, In Gallin, J. I. and Snyderman, R. (Eds.). Inflammation: Basic principles and clinical correlates 3rd ed. Lippincott Williams & Wilkins, Philadelphia, pp. 1-4, (1999).
- Hsu, Y. -W., Chi, K. -H., Huang, W. -C., and Lin, W. -W., Ceramide inhibits lipopolysaccharide-mediated nitric oxide synthase and cyclooxygenase-2 induction in macrophages: Effects on protein kinases and transcription factors. *J. Immunol.*, 166, 5388-5397 (2001).
- Jang, S. I., Jeong, S. I., Kim, K. J., Kim, H. J., Yu, H. H., Park, R., Kim, H. M., and You, Y. O., Tanshinone IIA from *Salvia miltiorrhiza* inhibits inducible nitric oxide synthase expression and production of TNF- α , IL-1 β and IL-6 in activated RAW 264.7 cells. *Planta Med.*, 69, 1057-1059 (2003).
- Jin, D. Z., Yin, L. L., Ji, X. Q., and Zhu, X. Z., Cryptotanshinone inhibits cyclooxygenase-2 enzyme activity but not its expression. *Eur. J. Pharmacol.*, 549, 166-172 (2006).
- Kang, B. Y., Chung, S. W., Kim, S. H., Ryu, S. Y., and Kim, T. S., Inhibition of interleukin-12 and interferon- γ production in immune cells by tanshinones from *Salvia miltiorrhiza*. *Immunopharmacol.*, 49, 355-361 (2000).
- Kang, H. S., Chung, H. Y., Jung, J. H., Kang, S. S., and Choi, J. S., Antioxidant effect of *Salvia miltiorrhiza*. *Arch. Pharm. Res.*, 20, 496-500 (1997).
- Kim, H., Lee, H. S., Chang, K. T., Ko, T. H., Baek, K. J., and Kwon, N. S., Chloromethyl ketones block induction of nitric oxide synthase in murine macrophages by preventing activation of nuclear factor- κ B. *J. Immunol.*, 154, 4741-4748 (1995).
- Kim, S. Y., Moon, T. C., Chang, H. W., Son, K. H., Kang, S. S., and Kim, H. P., Effects of tanshinone I isolated from *Salvia miltiorrhiza* on arachidonic acid metabolism and in vivo inflammatory responses. *Phytotherapy Res.*, 16, 616-620 (2002).
- Dweck, A. C., The folklore and cosmetic use of various *Salvia* species, In Kintzios, S. E. (Ed.). SAGE The genus *Salvia*, Overseas Publishers Association, New York, pp. 9-18, (2000).
- Lee, P., Hur, J., Lee, J., Kim, J., Jeong, J., Kang, I., Kim, S. Y., and Kim, H., 15,16-Dihydro-tanshinone I suppresses the activation of BV-2 cell, a murine microglia cell line, by lipopolysaccharide. *Neurochem. Int.*, 48, 60-66 (2006).
- Mosmann, T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assays. *J. Immunol. Methods*, 65, 55-63 (1983).
- Ryu, S. Y., No, Z., Kim, S. H., and Ahn, J. W., Two novel abietane diterpenes from *Salvia miltiorrhiza*. *Planta Med.*, 63, 44-46 (1997).
- Ryu, S. Y., Oak, M. H., and Kim, K. M., Inhibition of mast cell degranulation by tanshinones from the roots of *Salvia miltiorrhiza*. *Planta Med.*, 65, 654-655 (1999).
- Suh, N., Honda, T., Finlay, H. J., Barchowsky, A., Williams, C., Benoit, N. E., Xie, Q., Nathan, C., Gribble, G. W., and Sporn, M. B., Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. *Cancer Res.*, 58, 712-723 (1998).
- Suh, S. J., Jin, U. H., Choi, H. J., Chang, H. W., Son, J. K., Lee, S. H., Jeon, S. J., Son, K. H., Chang, Y. C., Lee, Y. C., and Kim, C. H., Cryptotanshinone from *Salvia miltiorrhiza* Bunge has an inhibitory effect on TNF- α -induced matrix metalloproteinase-9 production and HASMC migration via down-regulated NF- κ B and AP-1. *Biochem. Pharmacol.*, 72, 1680-1689 (2006).
- Winter, C. A., Risley, E. A., and Nuss, G. W., Carrageenan-induced edema in the hindpaw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, 111, 544-547 (1962).