

Anti-allergic Activity of an Ethanol Extract from *Salviae miltiorrhiza*

Ju Hye Yang, Kun Ho Son¹, Jong Keun Son, and Hyeun Wook Chang

College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea and ¹Department of Food and Nutrition, Andong National University, Andong 760-749, Korea

(Received October 1, 2008/Revised November 18, 2008/Accepted November 18, 2008)

As part of an ongoing investigation aimed at the discovery of novel bioactive medicinal herbs with anti-inflammatory properties, the effects of an ethanolic extract from the parts of *Salviae miltiorrhiza* Bunge (ESM) were evaluated using *in vitro* and *in vivo* animal model analysis. ESM inhibited cyclooxygenase-2 (COX-2) and COX-1-dependent phases of prostaglandin D₂ (PGD₂) generation in bone marrow-derived mast cells (BMMC) in a concentration-dependent manner with IC₅₀ values of 3.96 µg/mL and 21.54 µg/mL, respectively. Furthermore, ESM inhibited leukotriene C₄ (LTC₄) production with an IC₅₀ value of 2.6 µg/mL. These results clearly demonstrated the dual COX-2 selective/5-lipoxygenase inhibitory activity that ESM possessed. ESM strongly inhibited a degranulation reaction in a dose dependent manner within a BMMC system, with an IC₅₀ value of 22.4 µg/mL. Additionally, ESM was tested in a rat passive cutaneous anaphylaxis (PCA) reaction assay by oral administration (25 to 100 mg/kg). ESM dose-dependently inhibited the PCA reaction, which was activated by anti-dinitrophenyl (DNP) IgE. These results suggested that ESM might be beneficial in regulating various allergic reactions.

Key words: *Salviae miltiorrhiza*, Allergy, Bone marrow derived mast cells, Passive cutaneous anaphylaxis (PCA) reaction

INTRODUCTION

Mast cells are one of the most important cells during the allergic diseases. When activated, mast cells release a number of biologically active molecules through various processes. Histamine, serotonin, proteoglycans, and serine proteases are released through exocytosis, lipid mediators such as eicosanoids are released through the activation of the cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) pathways and various cytokines are synthesized *de novo* (Stevens et al., 1989; Yamaguchi et al., 1999; Murakami et al., 2001). Prostaglandins (PGs) elicit a variety of important biological responses. Among these properties are the ability to induce pain, fever, and the symptoms associated with inflammatory responses. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the pain and inflammatory swelling by blocking PGs synthesis at the COX stage. However, most NSAIDs which have been used clinically inhibit the production of PGs which are associated with the inflammatory processes as well as

the maintenance of normal physiological processes. The main limitation of NSAIDs are the side-effects associated with it including gastrointestinal ulcerogenic activity and kidney dysfunction, which limits the therapeutic value of their safe and long-term usage (Vane et al., 1971; Whittle et al., 1980).

The enzyme responsible for PGs synthesis exists as two isoforms, COX-1 (constitutive isoform) and COX-2 (inducible form) (O'Banion et al., 1992). Arachidonic acid can also be converted to cysteinyl leukotrienes (LTs) by the action of 5-LOX. The cysteinyl LTs are potent mediators of allergy and inflammation by modulation of the smooth muscle tone and vascular permeability (Kanaoka et al., 2006; Tokita et al., 2006). In addition, they play an important role in asthma and other respiratory diseases (Lewis et al., 1990; McMillan et al., 1992; Henderson, 1994). Thus, the development of dual inhibitors that can simultaneously inhibit both the COX-2 and 5-LOX reactions, might enhance their individual anti-inflammatory effects and reduce the undesirable side-effects that are generally associated with NSAIDs. For this purpose, about 300 plant extracts were evaluated for possible inhibitory activity on PGD₂ and LTC₄ production from mouse bone marrow-derived mast cells (BMMC) stimulated with *c-kit* ligand (KL)/IL-10/lipopolysaccharide (LPS) *in vitro* (Moon

Correspondence to: Hyeun Wook Chang, College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Korea
Tel: 82-53-810-2811; Fax: 82-53-812-9459
E-mail: hwchang@yu.ac.kr

et al., 1998).

Salvia miltiorrhiza (Labiatae), an eminent herbal plant, has been widely used in traditional Chinese medicine for the treatment of heart, liver diseases and vascular diseases such as hypertension (Wu et al., 1998; Chen et al., 2001; Chen et al., 2001b; Hung et al., 2001; Ji et al., 2003; Lay et al., 2003). Several active components such as tanshinones, D(+)-3,4-dihydroxyphenol lactic acid, protocatechuic aldehyde, salvianolic acids (A, B, C, D, E, F), and rosmarinic acid (Li 1997; Wang et al., 1991) have been isolated and identified. Tanshinones, which are contained within the non-polar extracts of *S. miltiorrhiza*, are known to inhibit platelet-aggregation and protect the myocardium against ischemia-induced derangement. The phenolic compounds present in the crude extract of *S. miltiorrhiza* are effective in protecting liver microsomes, hepatocytes, and erythrocytes against oxidative damage (Liu et al., 2001; Li et al., 2002). It was also reported that *S. miltiorrhiza* improved cognitive impairment in a broad range of animal models of memory deficiency (Hsieh et al., 2000). Previously we reported that that *S. miltiorrhiza* inhibited matrix metalloproteinase-9 activity and migration of tumor necrosis factor- α (TNF- α)-induced human aortic smooth muscle cells (Jin et al., 2006) and that tanshinone I, isolated from *S. miltiorrhiza* showed *in vivo* anti-inflammatory activity in rat carrageenan-induced paw oedema and adjuvant-induced arthritis (Kim et al., 2002). Although the underlying mechanisms for the anti-inflammatory properties of *S. miltiorrhiza* have not yet been completely elucidated, the evidence that has already been presented suggests that SM may have some effects on allergic reactions.

The present study was therefore conducted to investigate the possible anti-allergic effects of the ethanolic extracts of *S. miltiorrhiza* (ESM) on passive cutaneous anaphylaxis (PCA) reaction induced by anti-dinitrophenyl (DNP) IgE in rats. This was determined by testing the effects of ESM on the arachidonic acid metabolizing enzymes and degranulation reactions from cytokine stimulated mouse bone marrow-derived mast cells (BMMC) and using an *in vivo* animal model.

MATERIALS AND METHODS

Materials

Dried leaves of *S. miltiorrhiza* were collected from the herbal garden at the College of Pharmacy, Yeungnam University and positively identified by professor Ki-Hwan Bae (College of Pharmacy, Chungnam National University, Daejeon, Korea). A voucher specimen has been deposited at the herbarium of the College of Pharmacy, Yeungnam University (YNU 6027). The dried aerial parts of *S. miltiorrhiza* (1 kg) were cut into small pieces and extract-

ed repeatedly with 70% ethanol. The solution was filtered and evaporated *in vacuo* to yield a powdered extract (100.3 g).

Animals

Male Sprague-Dawley (SD) rats (180-200 g) obtained from Daehan Biolink, Eumsung (Korea) were acclimatized in the animal facility under conditions of 20°-22°C, 40%-60% relative humidity and 12 h/12 h (light/day) cycle for at least for 7 days.

Preparation and activation of BMMC

Bone marrow cells from male Balb/cJ mice were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics and 10% fetal calf serum) and 50% WEHI-3 cell-conditioned medium as a source of IL-3. After 3 weeks, > 98% of the cells were confirmed to be BMMC using a previously described procedure (Murakami et al., 1999).

Determination of prostaglandin D₂ (PGD₂)

In order to measure the inhibitory activity of ESM on COX-2, BMMC suspended at a cell density of 5×10^5 cells/mL in enriched medium were pre-incubated with aspirin (10 μ g/mL) for 2 h in order to irreversibly inactivate any pre-existing COX-1. After washing, BMMC were activated with *c-kit* ligand (KL, 100 ng/mL), IL-10 (100 U/mL), and LPS (100 ng/mL) at 37°C for 8 hours in the presence or absence of ESM that was previously dissolved in dimethylsulfoxide (DMSO). All reactions were stopped by centrifugation at 120 g at 4°C for 5 minutes. The supernatant and cell pellet were immediately frozen in liquid N₂ and stored at -80°C for further analysis. Concentrations of PGD₂ in the supernatant were measured using a PGD₂ assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Under these assay conditions, the COX-1 and COX-2-dependent phases of the PGD₂ generation reached approximately 7.6 and 7.8 ng/10⁶ cells, respectively. All data reported are representative of the arithmetic mean of triplicate determinations.

Analysis of LTC₄

BMMC suspended in enriched medium at a cell density of 1×10^6 cells/mL were pre-treated with ESM for 15 minutes at 37°C and stimulated with KL (100 ng/mL). After 20 minutes of stimulation, the supernatants were isolated for further analysis. LTC₄ was determined using an enzyme immunoassay kit (EIA) (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Under the conditions employed, LTC₄ reached approximately 8.9 ng/10⁶ cells. All data reported are re-

presentative of the arithmetic mean of triplicate determinations.

Passive Cutaneous Anaphylaxis (PCA) analysis

Male SD rats weighing 180-200 g (Daehan Biolink, Eumsung, Korea) were used in the PCA test. The animals were housed in cages at least 1 week prior to the experiments. Briefly, 100 μ L/site of monoclonal anti-DNP mouse IgE (1:1,000 dilution; Seikagaku-kougyo, Japan) was injected intradermally into the shaved back of the rat. After 48 h, PCA was evoked by an intravenous injection of the antigen (Ag; 1 mg of DNP-BSA (Sigma Chemical Co.) in PBS containing 1 mL of 1% Evans blue (1:4). ESM (25-100 mg/kg body weight, *p.o*) and dexamethasone (1-10 mg/kg, *p.o*) were administered 1 h before the Ag challenge. After 30 minutes, the skin was removed and evans blue was leaked into the skin, which was then extracted and quantified, as described previously (Katayama et al., 1978). ESM did not show any toxicity such as decrease in body-weight or morphological changes of the spleen and liver at doses up to 2 g/kg.

Statistical analysis

All the values were represented as arithmetic mean $\pm$ SD. One-way ANOVA was used to determine the statistical significance.

RESULTS AND DISCUSSION

S. miltiorrhiza Bunge, commonly known as Danshen, has long been used as a traditional Korean medicine for angina pectoris and the treatment of cardiovascular and inflammatory diseases (Chang et al., 1986, Koo et al., 2004). Several active ingredients have been identified that are effective on the inflammatory animal model (Kim et al., 2002). However, the underlying mechanisms for its anti-allergic reaction have not been sufficiently elucidated.

Previously, it has been reported that BMMC showed biphasic PGD₂ synthetic responses over time, as well as COX-1-dependent immediate and COX-2-dependent delayed responses (Murakami et al., 1994). The immediate PGD₂ generation occurring within 2 hours was associated with the coupling of COX-1. The delayed PGD₂ generation, which occurred after several hours of culturing (2-10 hours), was associated with the *de novo* induction and function of COX-2 after stimulation with particular combinations of cytokines and LPS (Murakami et al., 1994). This cell model appeared to be suitable for analyzing the effects of 5-LOX inhibitors since the immediate LTC₄ generation, elicited by the IgE-dependent or cytokine-initiated stimulus, occurred in BMMC through 5-LOX (Murakami et al., 1995). The BMMC system is therefore widely reported to be useful in the screening processes of

selective COX-2 or 5-LOX and COX-2/5-LOX dual inhibitors from various sources (Moon et al., 1999; Lee et al., 2004; Son et al., 2005; Son et al., 2006).

When BMMC were activated with the combination of KL, IL-10, and LPS in the presence or absence of ESM, the COX-2-dependent phase of PGD₂ generation was inhibited in a dose-dependent manner with an IC₅₀ value of approximately 3.96 μ g/mL, while that of the COX-1-dependent phase of PGD₂ generation was about 21.54 μ g/mL (Fig. 1A & B). The relative value for selectivity was about 5 fold (IC₅₀ for COX-1/IC₅₀ for COX-2).

Arachidonic acid can also be converted into LTC₄ by the action of 5-lipoxygenase (5-LOX) in BMMC (Murakami et al., 1995). The inhibition of 5-LOX has been suggested as the ideal treatment for various allergic diseases and asthma (Singh et al., 1994). The inhibitory activity of ESM on the generation of LTC₄ in BMMC was thus analyzed in the present study. Fig. 2 shows that BMMC stimulated with KL for 15 minutes produced 9.0 ng/mL of LTC₄. Pre-incubation of BMMC with ESM resulted in a dose-dependent suppression of this LTC₄ biosynthesis with an IC₅₀ value of 2.6 μ g/mL (Fig. 2).

Mast cells have long been implicated in the pathology

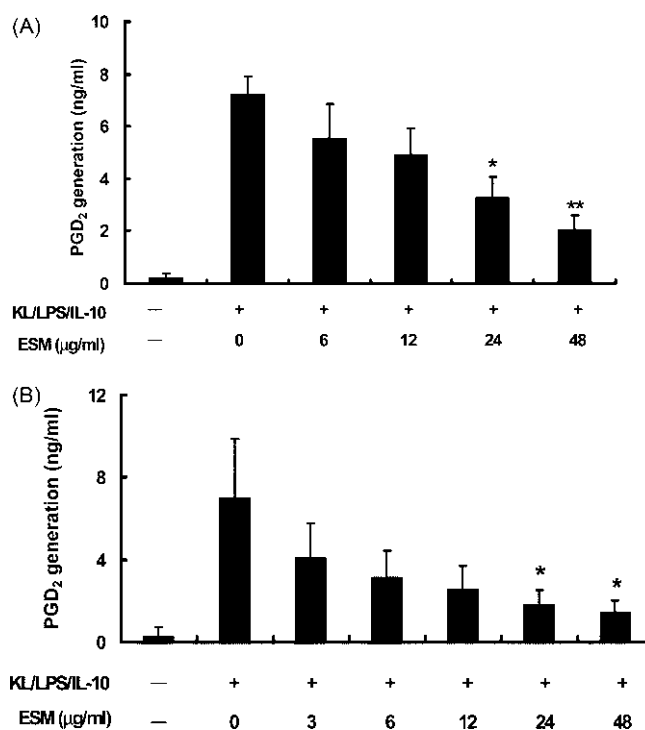


Fig. 1. Effect of ESM on the generation of COX-1 dependent PGD₂ (A) and COX-2 dependent PGD₂ (B) generation. BMMC were incubated with KL (100 ng/mL), IL-10 (100 U/mL) and LPS (100 ng/mL) at 37°C for 1 h or 8 h in the presence or absence of the indicated concentration of ESM. PGD₂ released into the supernatant was quantified using the EIA kit.

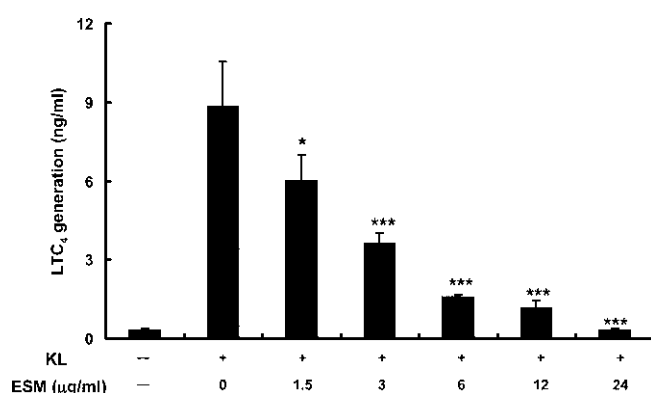


Fig. 2. Effect of ESM on the generation of LTC₄. BMMC were pre-incubated for 30 minutes with the indicated concentrations of ESM and then stimulated with 100 ng/mL of KL for 15 minutes. LTC₄ released into the supernatant was quantified using the EIA kit.

and mortality of anaphylaxis and other allergic disorders by virtue of their ability to be activated through FcεRI bound antigen-specific IgE and their concentration at surfaces that interface with the external environment (Marone et al., 1999; Katz 2002). Mast cells may also be activated by various cytokines through each cytokine receptor (Okayama et al., 1995). Activation through any of these receptors leads to the release of a number of biologically active molecules through the process of exocytosis. These molecules include histamine, serotonin, proteoglycans and neutral proteases, which are stored in cytoplasmic granules. Histamine is one of the most important chemical mediators in the pathologic allergic reaction (Yamaguchi et al., 1999). When mast cells are activated by various stimuli, the release of histamine bears a close parallel to that of β-hexosaminidase (β-Hex), which is a biological marker of the degranulation reactions. Therefore, the inhibitory activity of ESM on the degranulation reaction in BMMC was examined. As shown in Fig. 3, ESM caused a dose-dependent inhibition of β-Hex release with an IC₅₀ value of 22.4 µg/mL.

In order to test the anti-allergic activity of ESM *in vivo*, the effect of ESM on IgE-induced PCA was examined using a rat model. When 50 mg/kg of ESM was orally injected an hour prior to the antigen (Ag) challenge, the PCA reaction was suppressed by approximately 36.4% and the inhibition was observed in a dose-dependent manner (Fig 4). Under the same conditions, dexamethasone (1 mg/kg), which is a steroidal anti-inflammatory drug, inhibited the PCA reaction by 72.7%. This result clearly showed that ESM exhibited a potent anti-allergic activity, but that its inhibitory activity was weaker than that of dexamethasone. The weak inhibitory effect of oral administration of ESM may be attributed to its poor bio-availability. The precise mechanism for this result is not

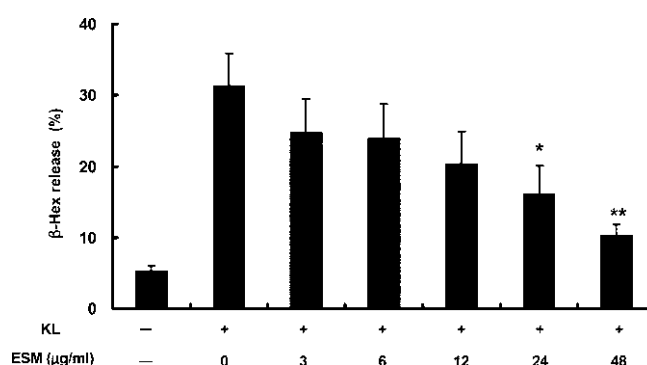


Fig. 3. Effect of ESM on the release of β-hexosaminidase (β-Hex). BMMC were pre-incubated for 30 minutes with the indicated concentrations of ESM and then stimulated with 100 ng/mL of KL for 15 minutes. β-Hex released into the supernatant cell lysate was measured using a procedure that has been detailed within the materials and methods section. All data presented was the arithmetic mean of triplicate determinations.

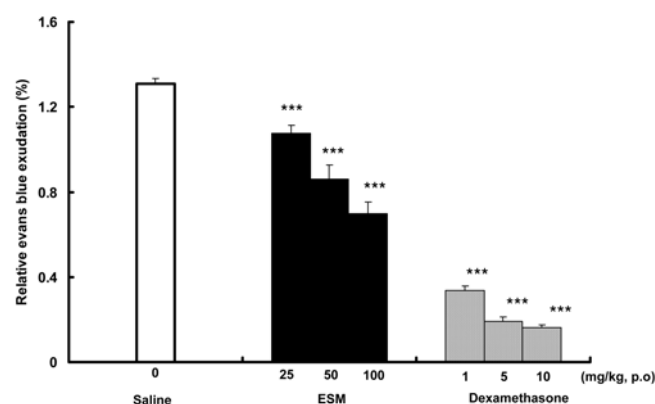


Fig. 4. Inhibition of IgE induced PCA reaction by ESM. The PCA reaction was induced by IgE as described in the materials and methods section. (A) ESM (25-100 mg/kg) was orally administered 30 minutes before the antigen challenge. Dexamethasone was used as the positive control. Data are presented as arithmetic mean ± SD (n=6). ***: P < 0.001 was considered to be significantly different from the control (saline) by one way ANOVA test.

understood but it is believed the *in vivo* anti-allergic activity of ESM on the PCA reaction is related to the inhibition of the both PGD₂ and LTC₄ generation as well as the inhibition of degranulation reaction in mast cells. Future studies should be aimed at clarifying the active compounds and further elucidating the anti-allergic mechanism of ESM.

Analysis of the chemical composition of ESM by HPLC showed ESM contains one major compound, salvianolic acid B, as well as several minor compounds such as 15,16-dihydrotanshinone, tanshinones, and cryptotanshinone (data not shown). Many groups reported *in vitro* anti-

inflammatory and anti-allergic effect of cryptotanshinone, 15,16-dihydrotanshinone (Ryu et al., 1999; Jin et al., 2006) Salvianolic acid B (Chen et al., 2006), tanshinones (Choi et al., 2004; Choi et al., 2004; Jeon et al., 2008), and rosmarinic acid (Osakabe et al., 2004). From those reports together with our *in vitro* and *in vivo* results, it is suggest that all theses compounds might contribute to the potential anti-allergic components of ESM.

The results from this study also provide an additional rationale for the use of *S. miltiorrhiza* as a Korean traditional herb in the treatment of allergy-related disorders. The apparent lack of significant toxicity of this compound after oral administration in rats indicated that it could be used safely in humans within moderate doses.

ACKNOWLEDGMENTS

This work was supported by a Yeungnam University Research Grant in 2008.

REFERENCES

- Chang, H. M. and But, P. P., Pharmacology and Applications of Chinese Material Medica, *World Scientific Publication, Singapore* (1986).
- Chen, Y. H., Lin, S. J., Ku, H. H., Shiao, M. S., Lin, F. Y., Chen, J. W., and Chen, Y. L., Salvianolic acid B attenuates VCAM-1 and ICAM-1 expression in TNF-alpha-treated human aortic endothelial cells. *J. Cell. Biochem.*, 82, 512-21 (2001b).
- Chen, Y. L., Yang, S. P., Shiao, M. S., Chen, J. W., and Lin, S. J., *Salvia miltiorrhiza* inhibits intimal hyperplasia and monocyte chemotactic protein-1 expression after balloon injury in cholesterol-fed rabbits. *J. Cell. Biochem.*, 83, 484-93(2001).
- Chen, Y. L., Hu, C. S., Lin, Y. H., Sheu, L. M., Ku, H. H., Shiao, M. S., Chen, J. W., and Lin, S. L., Salvianolic acid B attenuates cyclooxygenase-2 expression in vitro in LPS-treated human aortic smooth muscle cells and in vivo in the apolipoprotein-E-deficient mouse aorta. *J. Cell. Biochem.*, 98, 618-631 (2006).
- Choi, H. S., Choi, D. I., Choi, H. K., Im, S. Y., Ryu, S. Y., and Kim, K. M., Molecular mechanisms of inhibitory activities 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 PLCgamma2 and MAPK. *Planta Med.*, 70,178-80 (2004)
- Henderson, W. R. Jr., The role of leukotrienes in inflammation. *Ann. Intern. Med.*, 121, 684-697 (1994).
- Hsieh, M. T., Peng, W. H., Wu, C. R., and Wang, W. H., The ameliorating effects of the cognitive-enhancing Chinese herbs on scopolamine-induced amnesia in rats. *Phytother. Res.*, 14, 375-377 (2000).
- Hung, H. H., Chen, Y. L., Lin, S. J., Yang, S. P., Shih, C. C., Shiao, M. S., and Chang, C.H., A salvianolic acid B-rich fraction of *Salvia miltiorrhiza* induces neointimal cell apoptosis in rabbit angioplasty model. *Histol. Histopathol.*, 16, 175-183 (2001).
- Ji, X., Tan, B. K., Zhu, Y. C., Linz, W., and Zhu, Y. Z., Comparison of cardioprotective effects using ramipril and DanShen for the treatment of acute myocardial infarction in rats. *Life Sci.*, 73, 1413-1426 (2003).
- Jin, D. Z., Yin, L. L., Ji, X. Q., and Zhu, X. Z., Cryptotanshinone inhibits cyclooxygenase-2 enzyme activity. *Fur. J. Phatmacol.*, 549, 166-172 (2006).
- Jin, U. H., Kang, S. K., Suh, S. J., Hong, S. Y., Park, S. D., Kim, D. W., Chang, H. W., Son, J. K., Lee, S. H., Son, K. H., and Kim, C. H., Inhibitory effect of *Salvia miltiorrhiza* BGE on matrix metalloproteinase-9 activity and migration of TNF-alpha-induced human aortic smooth muscle cells. *Vascul. Pharmacol.*, 44, 345-353 (2006).
- Jeon, S. J., Son, K. H., Kim, Y. S., Choi, Y. H., and Kim, H. P., Inhibition of prostaglandin and nitric oxide production in lipopolysaccharide-treated RAW 264.7 cells by tanshinones from the roots of *Salvia miltiorrhiza bunge*. *Arch. Pharm. Res.*, 31, 758-763 (2008).
- Kanaoka, Y. and Boyce, J. A., Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. *J. Immunol.*, 173, 1503-1510 (2004).
- Katayama, S., Shionoya, H., and Ohtake, S., A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Microbiol. Immunol.*, 22, 89-101 (1978).
- Katz, H. R., Inhibition of anaphylactic inflammation by the gp49B1 receptor on mast cells. *Mol. Immunol.*, 38, 1301-1305 (2002).
- 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 *Savia miltiorrhiza* Bunge on the arachidonic acid metabolism and *in vivo* inflammatory responses. *Phytother. Res.*, 16, 616-620 (2002).
- Koo, B. S., Kwon, T. S., and Kim, C. H., *Salviae miltiorrhizae* radix inhibits superoxide generation by activated rat microglia and mimics the action of amphetamine on in vitro rat striatal dopamine release. *Neurochem. Res.*, 29, 1837-1845 (2004).
- Lay, I. S., Chiu, J. H., Shiao, M. S., Lui, W. Y., and Wu, C. W., Crude extract of *Salvia miltiorrhiza* and salvianolic acid B enhance in vitro angiogenesis in murine SVR endothelial cell line. *Planta Med.*, 69, 26-32 (2003).
- Lee, S. H., Son, M. J., Ju, H. K., Lin, C. X., Moon, T. C.,

- Choi, H. G., Son, J. K., and Chang, H. W., Dual inhibition of cyclooxygenases-2 and 5-lipoxygenase by deoxypodophyllotoxin (anthricin) in mouse bone marrow-derived mast cells. *Biol. Pharm. Bull.*, 27, 786-788 (2004).
- Lewis, R. A., Austen, K. F., and Soberman, R. J., Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N. Engl. J. Med.*, 323, 645-655 (1990).
- Li, H. Y., Li, Y., Yan, C. H., Li, L. N., and Chen, X. G., Inhibition of tumor growth by S-3-1, a synthetic intermediate of salvianolic acid A. *J. Asian Nat. Prod. Res.*, 4, 271-280 (2002).
- Li, L. N., Water soluble active components of *Salvia miltiorrhiza* and related plants. *J. Chin. Pharm. Sci.*, 6, 57-64 (1997).
- Liu, P., Hu, Y., Liu, C., Liu, C., and Zhu, D., Effects of salvianolic acid A (SA-A) on liver injury: SA-A action on hepatic peroxidation. *Liver*, 21, 384-390 (2001).
- Marone, G., Spadaro, G., Palumbo, C., and Condorelli, G., The anti-IgE/anti-FcepsilonRIalpha autoantibody network in allergic and autoimmune diseases. *Clin. Exp. Allergy*, 29, 17-27 (1999).
- McMillan, R. M. and Walker, E. R., Designing therapeutically effective 5-lipoxygenase inhibitors. *Trends pharmacol. Sci.*, 13, 323-330 (1992).
- Moon, T. C., Murakami, M., Ashraf, M. D., Kudo, I., and Chang, H. W., Regulation of cyclooxygenase-2 and endogenous cytokine expression by bacterial lipopolysaccharide that acts in synergy with c-kit ligand and Fc epsilon receptor I crosslinking in cultured mast cells. *Cell. Immunol.*, 185, 146-152 (1998).
- Moon, T. C., Murakami, M., Kudo, I., Son, K. H., Kim, H. P., Kang, S. S., and Chang, H. W., A new class of COX-2 inhibitor, rutaecarpine from *Evodia rutaecarpa*. *Inflamm. Res.*, 48, 621-625 (1999).
- Murakami, M., Matsumoto, R., Austen, K. F., and Arm, J. P., Prostaglandin endoperoxide synthase-1 and -2 couple to different transmembrane stimuli to generate prostaglandin D₂ in mouse bone marrow-derived mast cells. *J. Biol. Chem.*, 269, 22269-22275 (1994).
- Murakami, M., Austen, K. F., and Arm, J. P., The immediate phase of c-kit ligand stimulation of mouse bone marrow-derived mast cells elicits rapid leukotriene C₄ generation through posttranslational activation of cytosolic phospholipase A₂ and 5-lipoxygenase. *J. Exp. Med.*, 182, 197-206 (1995).
- Murakami, M., Kambe, T., Shimbara, S., and Kudo, I., Functional coupling between various phospholipase A₂s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. *J. Biol. Chem.*, 274, 3103-3115 (1999).
- Murakami, M. and Kudo, I., Diversity and regulatory functions of mammalian secretory phospholipase A₂s. *Adv. Immunol.*, 77, 163-194 (2001).
- O'Banion, M. K., Winn, V. D., and Young, D. A., cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. *Proc. Natl. Acad. Sci., U.S.A.*, 89, 4888-4892 (1992).
- Okayama, Y., Bradding, P., Tunon-de-Lara, M., Holgata, S. T., and Church, M. K., Cytokine production by human mast cells. *Chem. Immunol.*, 61, 114-134 (1995).
- Osakabe, N., Takano, H., Sanbongi, C., Yasuda, A., Yanagisawa, R., Inoue, K., and Yoshikawa, T., Anti-inflammatory and anti-allergic effect of rosmarinic acid (RA); inhibition of seasonal allergic rhinoconjunctivitis (SAR) and its mechanism. *Biofactors*, 21, 127-131 (2004).
- 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).
- Singh, V. P., Patil, C. S., and Kulkarni, S. K., Effect of 5-lipoxygenase inhibition on events associated with inflammatory bowel disease in rats. *Indian j. Exp. Biol.*, 42, 667-673 (1994).
- Son, J. K., Son, M. J., Lee, E. K., Moon, T. C., Son, K. H., Kim, C. H., Kim, H. P., Kang, S. S., and Chang, H. W., Ginkgetin, a biflavone from *Ginkgo biloba* leaves, inhibits cyclooxygenases-2 and 5-lipoxygenase in mouse bone marrow-derived mast cells. *Biol. Pharm. Bull.*, 28, 2181-2184 (2005).
- Son, M. J., Moon, T. C., Lee, E. K., Son, K. H., Kim, H. P., Kang, S. S., Son, J. K., Lee, S. H., and Chang, H. W., Naturally occurring biflavonoid, ochanflavone, inhibits cyclooxygenases-2 and 5-lipoxygenase in mouse bone marrow-derived mast cells. *Arch. Pharm. Res.*, 29, 282-286 (2006).
- Stevens, R. L. and Austen, K. F., Recent advances in the cellular and molecular biology of mast cells. *Immunol. Today*, 10, 381-386 (1989).
- Tokita, K., Uchida, Y., and Yamamoto, T., Roles of leukocytosis and cysteinyl leukotriene in polymorphonuclear leukocyte-dependent plasma extravasation. *J. Leukoc. Biol.*, 80, 1308-1319 (2006).
- Vane, J. R., Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biology*, 231, 232-235 (1971).
- Wang, J. M., He, H. B., Zhu, Y. Q., Shi, D. W., Li, Y. M., and Zhang, Y., Reversed phase HPLC determination of danshensu and protocatechuic aldehyde in *Salvia miltiorrhiza*. *Acta Acad. Med. Shanghai*, 18, 27-32 (1991).
- Whittle, B. J., Higgs, G. A., Eakins, K. E., Moncada, S., and Vane, J. R., Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature*, 284, 271-273 (1980).
- Wu, Y. J., Hong, C. Y., Lin, S. J., Wu, P., and Shiao, M. S., Increase of vitamin E content in LDL and reduction of atherosclerosis in cholesterol-fed rabbits by a water-soluble antioxidant-rich fraction of *Salvia miltiorrhiza*. *Arterioscler.*

- Thromb. Vasc. Biol.*, 18, 481-486 (1998).
- Yamaguchi, M., Sayama, K., Yano, K., Lantz, C. S., Noben-Trauth, N., Ra, C., Costa, J. J., and Galli, S. J., IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. *J. Immunol.*, 162, 5455-5465 (1999).