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Effects of the Constituents of *Paeonia lactiflora* Root on Arachidonate and NO Metabolism

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Abstract – In order to establish the anti-inflammatory cellular mechanism of the paeony root (*Paeonia lactiflora*, Pall, Paeoniaceae), the constituents including paeoniflorin, albiflorin, (+)-catechin, paeonol, benzoic acid and methyl gallate were evaluated for their effects on arachidonate and NO metabolism. Among the compounds tested, only paeonol weakly inhibited cyclooxygenase-2-mediated PGE_2 production from LPS-treated RAW 264.7 cells. (+)-Catechin and methyl gallate weakly inhibited inducible nitric oxide synthase-mediated NO production from the same cell line. In particular, methyl gallate significantly inhibited 5-lipoxygenase from RBL-1 cells with an IC_{50} of 8.4 μM. These results suggest that the inhibition of these components on arachidonate and NO metabolism may contribute at least in part to anti-inflammatory mechanism of the paeony root.

Keywords □ *Paeonia lactiflora*, cyclooxygenase, nitric oxide synthase, lipoxygenase, methyl gallate

INTRODUCTION

Among various proinflammatory chemical mediators, eicosanoids (prostaglandin, PG and leukotriene, LT) synthesized from arachidonic acid (AA) play an important role in many inflammatory disorders. Cyclooxygenases (COX) and lipoxygenases (LOX) are the enzymes that synthesize eicosanoids. Especially, COX-2 (an inducible isoform of COX) and 5-LOX produce high amounts of PGs and LTs, respectively, in the inflammatory lesion. In addition, nitric oxide (NO) synthesized from arginine by nitric oxide synthase (NOS) is also involved in some inflammatory disorders. In particular, iNOS (inducible NOS) produces massive amount of NO in certain cell types including macrophages (Gallin and Snyderman, 1999). Thus, it is worthy to evaluate the effects of potential anti-inflammatory agents on AA and NO metabolism.

The paeony root (*Paeonia lactiflora*, Pall, Paeoniaceae) has been frequently prescribed in Chinese medicine for enhancing blood circulation, inhibiting inflammation, etc. (Huh, 1966). From this plant material, several constituents such as paeoniflorin, albiflorin, and benzoylpaeoniflorin were isolated (Wu *et al.*, 1996). And many previous investigations have demonstrated that the paeony root exhibited hepatoprotection (Qi, 1991), improved memory function (Ohta and Matsumoto, 1993) and inhibited platelet aggregation (Lin *et al.*, 1999). Some of the anti-inflammatory actions of this plant material and the constituents responsible were also described. For instance, the whole extract inhibited COX (Prieto *et al.*, 2003), and inhibited monocyte chemotactic protein (MCP)-1 and –3 secretion from human nasal fibroblasts (Leem *et al.*, 2004). As the constituents, paeoniflorin showed anti-inflammatory action (Takagi and Harada, 1969) and memory improvement (Ohta and Matsumoto, 1993). Paeoniflorin, desbenzoylpaeoniflorin and paeonone inhibited experimental contact hypersensitivities and passive cutaneous anaphylaxis reaction (Yamahara *et al.*, 1982). Recently, antioxidative activity underlining antigenotoxic property of the several constituents including paeoniflorin, gallic acid and methyl gallate was also reported (Lee *et al.*, 2005). These previous reports may explain some anti-inflammatory property of the paeony root. However, no report has been available to describe the effects of its constituents on AA and NO metabolism despite a pivotal role of these enzymatic pathways in inflammatory condition. Therefore, to define the anti-inflammatory activity further, the constituents from the

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paeony root including paeoniflorin, albiflorin, catechin, paeonol, benzoic acid and methyl gallate were examined for their effects on AA and NO metabolism in the present investigation.

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). Nordihydroguaiaretic acid (NDGA), A23187, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and LPS (*Escherichia coli* 0127:B8) were purchased from Sigma Chem. (St. Louis, MO). LipofecAMINE PLUS, DMEM and other cell culture reagents including FBS were products of Gibco BRL (Grand Island, NY). Protein assay kit was purchased from Bio-Rad Lab. (Hercules, CA). Paeoniflorin, albiflorin, (+)-catechin, paeonol, benzoic acid and methyl gallate (Fig. 1) were isolated from the roots of *P. lactiflora* and identified according to the previously described (Kang *et al.*, 1993). 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 PGE_2 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% CO_2 at 37°C based on the previously described procedures (Chi *et al.*, 2001). Briefly, cells were plated in 96-well plates (2×10^5 cells/well). After pre-incubation for 2 h, test compounds and LPS (1 μg/ml) were added and incubated for 24 h. Cell viability was assessed with MTT assay as described previously (Mossman, 1983). From the media, NO and PGE_2 concentrations were measured. For determination of NO concentration, the stable conversion product of NO, nitrite (NO_2^-), was measured using Griess reagent and optical density was checked at 550 nm. PGE_2 concentration in the medium was measured using ELISA kit for PGE_2 (Cayman Chem. Co.) according to the manufacturer's recommendation.

Effects on 5-LOX

In order to evaluate the inhibitory activity against 5-LOX, rat basophilic leukemia cells (RBL-1) purchased from ATCC were cultured in RPMI 1640 with 10% FBS, 2 mM glutamine and 1% antibiotics under 5% CO_2 at 37°C. The cells were plated in 96-well plate for 2 h. The tested compounds were added and preincubated for 10 min. For 5-LOX activation, 3 μM of A-23187 (ionophore) was added and the cells were incubated further for 15 min with a slight modification of the previously described procedure (Tries *et al.*, 2002). Media was collected and the concentration of 5-LOX products, cysteinyl leukotrienes ($LTC_4/D_4/E_4$), was measured using ELISA kit (Cayman Chem. Co.) according to the manufacturer's recommended pro-

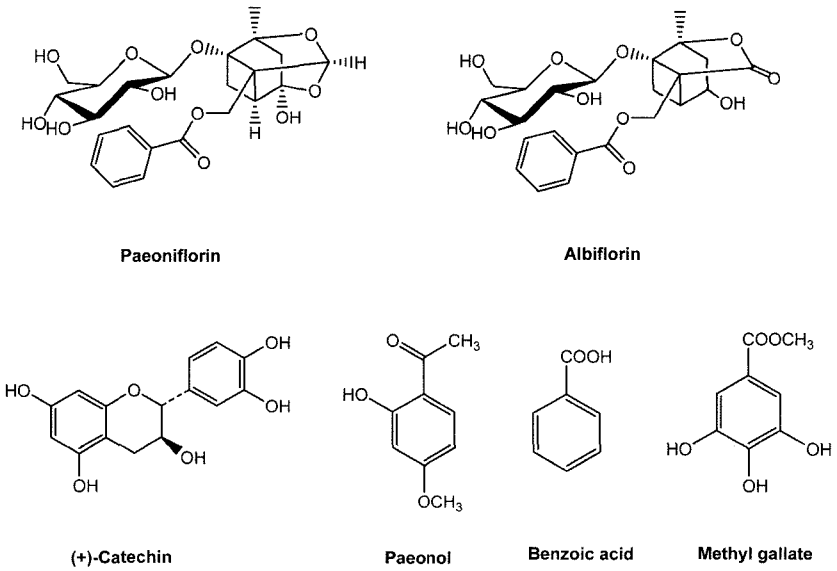

Fig. 1. The chemical structures of the constituents studied.

cedures.

Statistical analysis

Experimental values were represented as arithmetic mean ± SD. One-way ANOVA followed by Dunnet test was used to determine the statistical significance. All experiments were performed at least twice and they gave the similar results.

RESULTS AND DISCUSSION

LPS treatment (1 μg/ml) to mouse macrophage-like cell line, RAW 264.7, induced COX-2 and iNOS for 24 h incubation (Chi *et al.*, 2001). In this study, the concentrations of PGE_2 and NO increased to 27.9 ± 0.8 nM and 46.2 ± 0.5 μM from the basal levels of 1.9 ± 0.5 nM and 0.8 ± 0.2 μM, respectively, by LPS treatment (n = 3). Under this condition, the constituents were added simultaneously with LPS, and PGE_2 and NO concentrations were measured from the culture media as indices of COX-2 and iNOS activities. From this experiment, it was found that only paeonol among the constituents tested possessed weak inhibitory activity against COX-2-mediated PGE_2 production (17.4% inhibition at 100 μM) (Fig. 2). Other constituents did not show an inhibition at the concentrations up to 100 μM. Against iNOS-mediated NO production, catechin and methyl gallate showed weak inhibition. As shown in Fig. 3, catechin inhibited NO production (16.0% at 100 μM). Methyl gallate showed 17.9% inhibition at 100 μM. These constituents did not show a cytotoxic effect on RAW cells at the concentrations up to 100 μM for 24 h incubation. Under the same experimental condition, the reference compounds, NS-398 (selective COX-2 inhibitor) and AMT (iNOS inhibitor) showed 63.3% and 35.9% inhibition at 0.1 μM against COX-2- and iNOS-mediated PGE_2 and NO production, respectively.

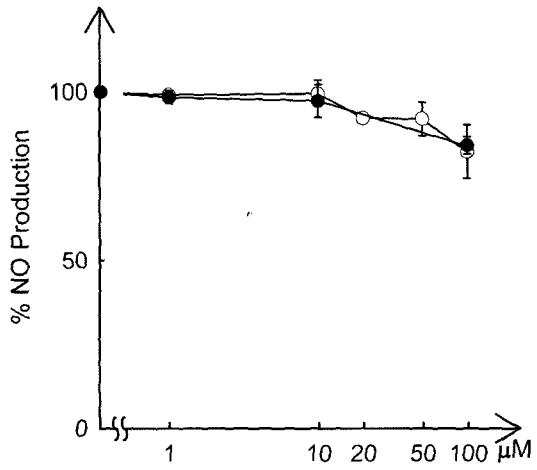

Fig. 3. Effect on iNOS-mediated NO production. Methyl gallate (○), Catechin (●), Data points and bars represent arithmetic mean ± SD (n = 3). No significantly different inhibition compared with the control group was observed (P < 0.005).

RBL-1 cells produced 2,393.8 ± 434.2 pg/ml of cysteinyl LTs upon activation with A23187, compared to the basal level of 6.2 ± 1.5 pg/ml (n = 3). On this 5-LOX activity, only methyl gallate showed a significant inhibition. As shown in Fig. 4, methyl gallate showed the concentration-dependent inhibition

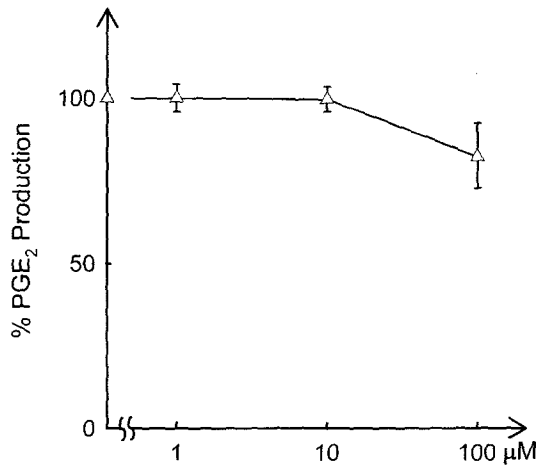

Fig. 2. Effect of paeonol on COX-2-mediated PGE_2 production. Data points and bars represent arithmetic mean ± SD (n = 3). No significantly different inhibition compared with the control group was observed (P < 0.005).

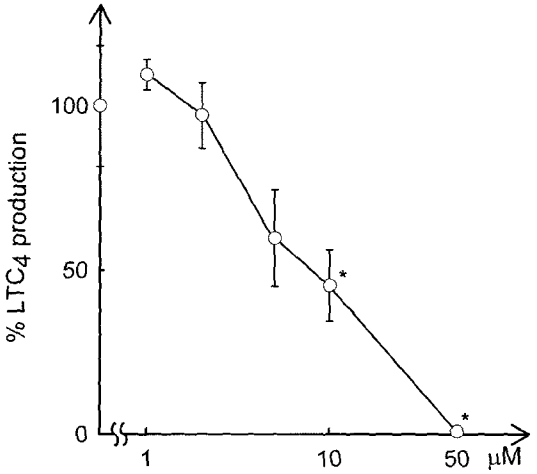

Fig. 4. Effect of methyl gallate on 5-LOX-catalyzed LT production. Data points and bars represent arithmetic mean ± SD (n = 3). *: P < 0.005, significantly different from the A23187-treated control.

with an IC_{50} of 8.4 μM. Other constituents did not show a significant inhibition at the concentrations up to 100 μM. The reference molecule, NDGA (nonspecific LOX inhibitor), showed 42.2% inhibition at 0.1 μM. Recent investigation has demonstrated that methyl gallate inhibited COX-2 and 5-LOX from mouse bone marrow-derived mast cells (Kim *et al.*, 2006). The 5-LOX inhibitory action of methyl gallate is well correlated with the present study, but the discrepancy in the sensitivity on COX-2-catalyzed reaction may be due to the different cells used, macrophages and mast cells.

From the results, it was demonstrated that paeonol weakly inhibited COX-2-mediated PGE_2 synthesis. Catechin and methyl gallate weakly inhibited iNOS-mediated metabolism. In particular, methyl gallate considerably inhibited 5-LOX activity. And 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose from *P. lactiflora* was previously found to inhibit COX-2 and iNOS (Lee *et al.*, 2003). Therefore, it is concluded that these constituents may contribute to anti-inflammatory action of paeony root at least in part by the inhibition of eicosanoid and NO synthesis.

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