

Inhibition of Inducible Nitric Oxide Synthase and Cyclooxygenase-2 Activity by 1,2,3,4,6-Penta-O-galloyl- β -D-glucose in Murine Macrophage Cells

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Activated macrophages express inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), and produce excessive amounts of nitric oxide (NO) and prostaglandin E₂ (PGE₂), which play key roles in the processes of inflammation and carcinogenesis. The root of *Paeonia lactiflora* Pall., and the root cortex of *Paeonia suffruticosa* Andr., are important Chinese crude drugs used in many traditional prescriptions. 1,2,3,4,6-penta-O-galloyl- β -D-glucose (PGG) is a major bioactive constituent of both crude drugs. PGG has been shown to possess potent anti-oxidant, anti-mutagenic, anti-proliferative and anti-invasive effects. In this study, we examined the inhibitory effects of 1,2,3,4,6-penta-O-galloyl- β -D-glucose (PGG) isolated from the root of *Paeonia lactiflora* Pall. on the COX-2 and iNOS activity in LPS-activated Raw 264.7 cells, COX-1 in HEL cells. To investigate the structure-activity relationships of gallic acid and gallic acid for the inhibition of iNOS and COX-2 activity, we also examined (-)-epigallocatechin gallate (EGCG), gallic acid, and gallacetophenone. The results of the present study indicated that PGG, EGCG, and gallacetophenone treatment except gallic acid significantly inhibited LPS-induced NO production in LPS-activated macrophages. All of the four compounds significantly inhibited COX-2 activity in LPS-activated macrophages. Among the four compounds examined, PGG revealed the most potent in both iNOS (IC₅₀ \approx 18 μ g/mL) and COX-2 inhibitory activity (PGE₂: IC₅₀ \approx 8 μ g/mL and PGD₂: IC₅₀ \approx 12 μ g/mL), respectively. Although further studies are needed to elucidate the molecular mechanisms and structure-activity relationship by which PGG exerts its inhibitory actions, our results suggest that PGG might be a candidate for developing anti-inflammatory and cancer chemopreventive agents.

Key words: 1,2,3,4,6-Penta-O-galloyl- β -D-glucose, Inducible nitric oxide synthase, Cyclooxygenase-2, Anti-inflammatory activity

INTRODUCTION

There are multiple lines of compelling evidence supporting that nitric oxide (NO) and prostaglandins (PGs) are involved in various pathophysiological processes including inflammation and carcinogenesis, and inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) are mainly responsible for the production of large amounts of these mediators (Schmidt and Walter, 1994; and Simon, 1999).

Nitric oxide synthase (NOS) is an important enzyme involved in the regulation of inflammation, vascular tone, neurotransmission, tumor cells and other homeostasis of human body. NO is a radical produced from L-arginine via NOS and also an important cellular second messenger. NO plays a dual role as both a beneficial and detrimental molecule in the process of inflammation. Molecular cloning and sequencing analysis have revealed the existence of at least three main types of NOS isoforms (Marletta, 1993). Both neuronal NOS and endothelial NOS are constitutively expressed, whereas iNOS is inducible in response to interferon- γ , lipopolysaccharide (LPS) and a variety of pro-inflammatory cytokines (Szabo, 1995). The induced iNOS catalyzes the formation and release of a large amount of NO, which plays a key role in the pathophysiology of a

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variety of diseases including septic shock (Liu and Hotchkiss, 1995; Tunctan *et al.*, 1998). Therefore, NO production catalyzed by iNOS may reflect the degree of inflammation and provides a measure by which effects of drugs on the inflammatory process can be assessed.

Cyclooxygenase (COX) which has two isoforms, COX-1 and COX-2, is the enzyme that catalyzes the rate-limiting step in prostaglandin synthesis, converting arachidonic acid into prostaglandin H₂, which is then further metabolized to prostaglandin E₂ (PGE₂), PGF_{2 α} , PGD₂ and other eicosanoids (Funk, 2001; Cao and Prescott, 2002). COX-1 is constitutively expressed in many tissues and plays a role in tissue homeostasis (Amiram *et al.*, 1988). COX-2 which can be expressed in a variety of cells and tissues is an inducible isoform the expression of which is stimulated by growth factors, inflammatory cytokines, carcinogens, and tumor promoters, implying a role for COX-2 in both inflammation and control of cell growth (Alice *et al.*, 1989; Dean *et al.*, 1991; Subbaramaiah *et al.*, 1996). Thus, compounds that inhibit the activity or expression of COX-2 might be an important target for anti-inflammation or cancer chemoprevention.

1,2,3,4,6-Penta-O-galloyl- β -D-glucose (PGG) is structurally similar to (-)-epigallocatechin gallate (EGCG) (Fig. 1). It has been known that PGG is a potent chemopreventive agent in tumor promoter-mediated oxidative processes (Bhimani *et al.*, 1993) and block oxidative stress and proliferation of human hepatocellular carcinoma cells, and induce cancer cell apoptosis (Bhimani *et al.*, 1993; Pan *et al.*, 1999; Oh *et al.* 2001). PGG has anti-tumor promoting activity in two-stage carcinogenesis experiments with mouse skin (Fujiki *et al.*, 1992) and anti-invasive activity in highly metastatic mouse melanoma (Ho *et al.*, 2002). The root of *Paeonia lactiflora* Pall. and the root cortex of *Paeonia suffruticosa* Andr. are well known traditional Chinese crude drugs used as both analgesic and anti-inflammatory agents. During preliminary phytochemical analysis, PGG has been shown to be a major bioactive constituent from the crude drugs (Sato, *et al.*, 1997). However, the detailed mechanisms of anti-inflammatory effects of the crude drugs have not been determined yet.

On the basis of the consideration of selective COX-2 inhibitors or iNOS inhibitors as anti-inflammatory and cancer chemopreventive agents and in order to elucidate in details the mechanisms of the anti-inflammatory effects of the crude drugs, we examined PGG isolated from the root of *Paeonia lactiflora* Pall. on the activities of COX-1, COX-2 and iNOS.

MATERIALS AND METHODS

Materials

The root of *Paeonia lactiflora* was purchased from herb

markets in Seoul, Korea and voucher specimens have been deposited at Herbarium of Natural Products Research Institute, Seoul National University, Seoul, Korea. [^{1-¹⁴C}] arachidonic acid (56 mCi/mmol), 5-S-[5,6,8,9,11,12,14,15-³H(N)] hydroxyl-6,8,11,14-eicosatetraenoic acid (182 Ci/mmol), [5,6,8,9,11,12,14,15-³H(N)] thromboxanes B₂ (152 Ci/mmol), [5,6,8,9,12,14,15-³H(N)] prostaglandin D₂ (115 Ci/mmol), [5,6,8,9,12,14,15-³H(N)] prostaglandin F (170 Ci/mmol) were purchased from NEN Life Science (Boston, MA). Fetal bovine serum (FBS) was purchased from Invitrogen (Grand Island, NY). All the other reagents were from Sigma (St. Louis, MO).

Purification of PGG

The roots of *Paeonia lactiflora* (8 kg) were extracted three times with methanol and the methanol extracts were partitioned into *n*-hexane, ethyl acetate, *n*-butanol, and water fractions. The ethyl acetate fraction was chromatographed over a silica gel column eluting with a gradient of cyclo-hexane/isopropyl alcohol mixtures (100:0 \rightarrow 0:100) and then PGG (53 mg) was purified by reverse phase C18 column using water/acetonitrile mixtures (100:0 \rightarrow 0:100). The structure of PGG was identified by ¹H-NMR, ¹³C-NMR, and electron ionization mass spectrometer spectral data together with published data. Purified PGG was dissolved in DMSO.

Cell culture

The mouse macrophage cell line (Raw 264.7 cells) and human erythroleukemia cell line (HEL cells) were purchased from the American Type Culture Collection. Cells were grown at 37°C in a humidified atmosphere (5% CO₂) in DMEM and RPMI-1640 medium containing 5% fetal bovine serum.

MTT assay

Cytotoxicity was measured by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan (Mosmann, 1983). Briefly, cells were seeded at a density 5 \times 10⁴ cells/mL in 96 well-plates. After incubating for 24 h, cells were treated with test samples. The cells were incubated for an additional 24 h. A 50 μ L aliquot of the MTT solution (5 mg/mL) was added to each well of the assay plate, which was then incubated for a further 4 h at 37°C. The medium was then removed by aspiration and MTT-formazan production was solubilized in 200 μ L DMSO. The extent of MTT reduction to formazan within the cells was quantified by measuring absorbance at 540 nm using an ELISA reader. Each experiment was performed in triplicate.

iNOS assay in LPS-induced Raw 264.7 cells

Raw 264.7 macrophage cells were cultured in a 150

cm² tissue culture flask until confluent. Cultured 264.7 macrophages were plated at 5×10^5 cells/well in a 24-well plate and preincubated for 4 h at 37°C in a humidified atmosphere (5% CO₂). The cells were replaced with new media, and then incubated the medium with 1 µg/mL LPS and test samples. After an additional 20 h incubation, the media were removed and analyzed for nitrite accumulation as an indicator of NO production by Griess reaction. Briefly, 50 µL of Griess reagent (0.1% naphthylethylenediamine and 1% sulfanilamide in 5% H₃PO₄ solution) were added to 200 µL of each supernatant from LPS and sample-treated cells in triplicate. The plates were incubated for 5 min, and then were read at 540 nm against a standard curve of sodium nitrite. The amount of nitrite in the samples was calculated from a sodium nitrite standard curve freshly prepared in culture medium.

COX-1 enzyme assay in HEL cells

The COX-1 activity was determined by using human erythroleukemic cell line HEL naturally express COX-1 protein, but not COX-2 (Berg *et al.*, 1997). Cultured HEL cells were plated at 1×10^6 cells per well in a 24-well plate and incubated for 24 h at 37°C in a humidified atmosphere (5% CO₂) with test samples or vehicle solvent (final 0.5% DMSO). The plate contents were centrifuged, the supernatant was removed and the cells were resuspended in 200 µL of PBS. The resuspended cells were incubated for 10 min at 37°C in the presence of 1 µM calcium ionophore (A23187) and [1-¹⁴C]arachidonic acid solution (0.05 µCi) and then acidified with 10 µL of 1 M citric acid to pH 3.5. After shaking the 24-well plate, 200 µL of the reaction mixture was transferred to a microcentrifuge tube and extracted twice with 500 µL of ethyl acetate to isolate the metabolic products of cyclooxygenase. The ethyl acetate layers were dried and resuspended in 10 µL of ethyl acetate and then applied to aluminium foil-backed silica gel TLC sheets. TLC was performed at room temperature using a solvent containing chloroform-methanol-acetic acid-water (v/v, 90:10:1:1). The [¹⁴C]-labeled prostanoids, were detected by autoradiography, by placing the TLC plate in contact with a Fuji BAS-III imaging plate for 24 h. The imaging plate was scanned using a BAS-1500 bio-imaging analyzer (Fuji Film) and the radioactivities of the prostanoid bands were quantified. The inhibitory effects of test samples on COX-1 activity were determined by measuring the amount of ¹⁴C-labeled prostanoids produced versus the DMSO control, and are expressed as percentages of the control.

COX-2 enzyme assay in LPS-induced Raw 264.7 cells

The COX-2 activity was determined by using murine macrophages, Raw 264.7 cells (Liang *et al.*, 1994). Raw

264.7 macrophages were cultured in a 150 cm² tissue culture flask until confluent. Cultured Raw 264.7 macrophages were plated at 2×10^5 cells/well in a 24-well plate and preincubated for 24 h at 37°C in a humidified atmosphere (5% CO₂). Cells were incubated for 24 h, added to an aspirin solution (final concentration, 0.4 µg/mL) so as to exclude the basal COX-1 effect and further incubated for 6 h. Aspirin pretreated cells were then added to the test samples (final 0.5% DMSO) and LPS (2.5 µg/mL) for 18 h to induce COX-2 (Osullivan *et al.*, 1992). The inhibitory effects of the test samples upon COX-2 were determined using the COX-1 assay method as described above. The inhibitory activity was expressed as IC₅₀ (50% inhibitory concentration) and each experiment was performed at least twice.

Statistical analysis

All values are expressed as mean±S.D. *P* values were calculated from the Students *t*-test, based on comparisons with appropriate control samples tested at the same time.

RESULTS AND DISCUSSION

Previous study has shown the inhibitory effect of PGG on tumor promotion by teleocidin, and PGG is as effective as (-)-epigallocatechin-3-gallate (Fujiki *et al.*, 1992). PGG, in which the glucose core is saturated with five galloyl groups, is structurally similar to (-)-epigallocatechin gallate (EGCG). To investigate the structure-activity relationships of gallate and gallic acid moieties for the inhibition of iNOS and COX activity, we also examined EGCG, gallic acid, and gallacetophenone. The chemical structures of PGG and the other compounds used in this study are shown in Fig. 1.

We first tested the effect of these compounds on cell viability by MTT assay. Murine macrophages, Raw 264.7, were treated with different concentrations of PGG, EGCG, gallic acid, or gallacetophenone. As shown in Fig. 2, gallic acid appeared to be most potent and PGG was second at concentration of 100 µg/mL. However, gallacetophenone and EGCG showed relatively weak inhibitory effects on cell viability. Although gallic acid and gallacetophenone are similar in chemical structure, there is some difference in the viability of Raw 264.7 cells. It was reported that three adjacent phenolic hydroxyl groups of gallic acid were responsible for cytotoxicity and its carboxyl group was not responsible, but seemed to be implicated in distinguishing between normal cells and tumor cells (Inoue *et al.*, 1994). Previous study has shown that PGG has anti-tumor promoting activity in two-stage carcinogenesis experiments with mouse skin (Fujiki *et al.*, 1992). During experiments, 3-O-tetragalloylquinic acid markedly prolonged the life

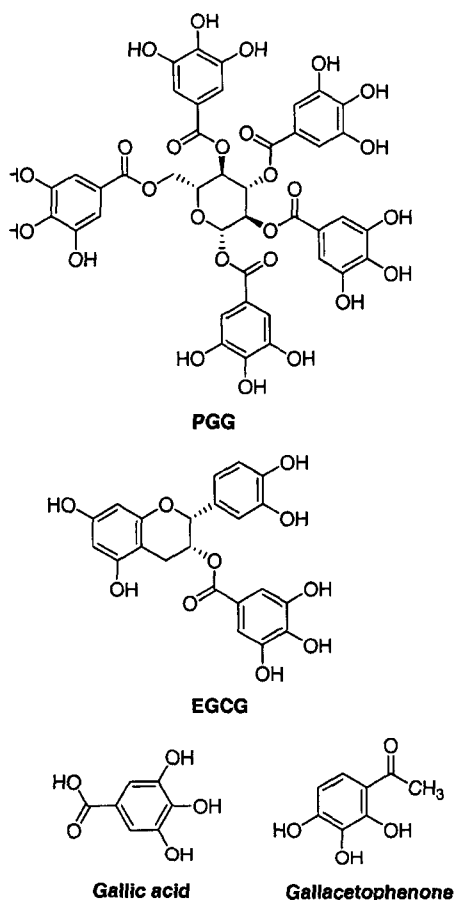


Fig. 1. Structures of PGG, EGCG, gallic acid, and gallacetophenone

span of mice and cured one out of six mice, but further galloylation of this compound did not lead to an increase in the anti-tumor activity. PGG is more active than other galloylglucoses (Miyamoto *et al.*, 1987). In another study, theaflavin-3,3'-O-digallate, which has two gallic acid moieties, exhibited the strongest inhibition of TPA-induced effects and anti-proliferative activity on tumor cells (Chen *et al.*, 1999; Liang *et al.*, 1999). These observations suggest that a high level of galloylation of PGG and hydroxyl groups of gallic acid moieties may be essential for the cytotoxic effects of the phenolic compounds.

As shown in Fig. 2, cell viability was almost not changed by the presence of PGG, EGCG, gallic acid, or gallacetophenone up to the concentration of 20 $\mu\text{g/mL}$. Thus, the concentration of the phenolic compounds in the range of 0.8–20 $\mu\text{g/mL}$ were chosen in the subsequent experiments. Their relative efficacy for inhibiting iNOS activity was evaluated to determine the anti-inflammatory effects of PGG and the structure-function relationships of phenolic compounds. NO production was monitored in Raw 264.7 cells stimulated by LPS in the presence or absence of PGG, EGCG, gallic acid, or gallacetophenone for 18 h. As shown in Fig. 3, LPS (1 $\mu\text{g/mL}$) increased the level of NO in

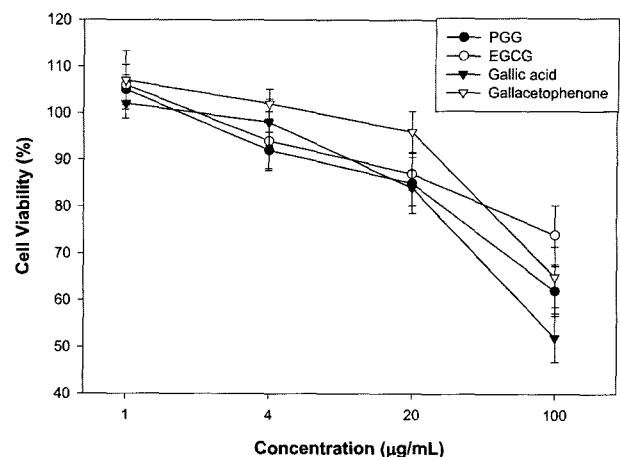


Fig. 2. Effects of PGG, EGCG, gallic acid, and gallacetophenone on murine macrophage cell growth. Raw 264.7 cells were treated with various concentrations of PGG, EGCG, gallic acid, or gallacetophenone dissolved in DMSO. After incubation for 24 h, cell density was assessed by MTT assay.

culture medium by 25-fold as compared to control. PGG (420 $\mu\text{g/mL}$) significantly inhibited LPS-inducible NO production in a concentration-dependent manner (i.e. 4869%) (Fig. 3). On a weighted basis, PGG exhibited the strongest inhibitory effect with an $\text{IC}_{50} \approx 18 \mu\text{g/mL}$ and EGCG was second with an $\text{IC}_{50} \approx 20 \mu\text{g/mL}$. Gallacetophenone showed the slight inhibition of LPS-induced NO production only at the highest (40% at 20 $\mu\text{g/mL}$). It is interesting to note that PGG, which has five gallic acids, showed strong inhibitory effect, but gallic acid showed almost no effect on iNOS activity in macrophage cells. Previous study has reported that EGCG inhibits the induction of iNOS in murine peritoneal macrophages activated with LPS and that the galloyl group and the hydroxyl group at the 3' position on EGCG are responsible for its strong anti-inflammatory property (Lin and Lin, 1997). This suggests that the gallate structure and hydroxyl groups of PGG and EGCG, not gallic acid moieties, may play a critical role in the inhibition of iNOS activity. Among the four compounds, PGG and EGCG showed the strong inhibitory effects on iNOS activity in macrophage cells, and these polyphenols have phenol rings that act as electron traps to scavenge peroxy radicals, superoxide anions, and hydroxyl radicals and prevent oxidation of iron (Ho *et al.*, 1992; Katiyar *et al.*, 1993; Lin *et al.*, 1996). Therefore, we suggest that in addition to the inhibition of iNOS activity, these compounds may block peroxynitrite and nitrite production through inhibition of oxidative reactions.

Another enzyme that plays a pivotal role in mediating inflammation is COX. There are two isoforms of COX, designated as COX-1 and COX-2 (Vane *et al.*, 1998). We next investigated whether the four compounds might affect COX-1 activity in human erythroleukemia cell line,

HEL cells. PGG and the other compounds showed almost no effect on COX-1 activity at the concentration of 20 $\mu\text{g}/\text{mL}$ (Fig. 3). COX-1 is constitutively expressed in most cell types, including platelets. COX-1 plays a role in the production of prostaglandins involved in protection of the gastric mucosal layer and thromboxanes in platelets. Thus, inhibition of COX-1 may explain their unwanted side effects, such as gastric and renal damage (Schlondorff, 1993). Shigyaku-san, a Chinese herbal medicine, has been used for treatment of gastritis and peptic ulcers in Japan. Shigyaku-san inhibits the activity of gastric H^+ , K^+ -ATPase, which is an important enzyme for the final step of acid secretion in the stomach (Sachs *et al.*, 1976). Among the components of Shigyaku-san, only the root of *Paeonia lactiflora* Pall. showed strong inhibitory effect of H^+ , K^+ -ATPase. PGG which is purified from the crude drug was a potent inhibitor of H^+ , K^+ -ATPase. PGG may be responsible for inhibition of acid secretion by the root of *Paeonia lactiflora* Pall (Ono *et al.*, 2000). Therefore, these may explain why COX-1 activity could not be affected by PGG.

COX-2 is absent from most healthy tissues but is induced by LPS, certain serum factors, cytokines and growth

factors, and is a predominant COX at sites of inflammation. COX-2 generally mediates elevations of prostaglandins associated with inflammation, pain, and pyresis (Hawkey, 1999). We investigated whether PGG, EGCG, gallic acid, and gallacetophenone might affect COX-2 activity in LPS-activated macrophage cells. Among the four compounds examined, PGG was shown to be the most active compound in LPS-activated macrophages. At concentrations of 0.8, 4, and 20 $\mu\text{g}/\text{mL}$, PGG inhibited PGE_2 production by 35%, 41%, 68% ($\text{IC}_{50} \approx 8 \mu\text{g}/\text{mL}$) and PGD_2 production by 22%, 39%, 55% ($\text{IC}_{50} \approx 12 \mu\text{g}/\text{mL}$), respectively (Fig. 5). On a weighted basis, the inhibition of PGE_2 production by COX-2 activity is in the following order: PGG > gallacetophenone > EGCG > gallic acid. These compounds significantly inhibited COX-2 activity in concentration-dependent manners. Gallacetophenone and EGCG exhibited strong inhibitory effects in PGE_2 production with $\text{IC}_{50} \approx 13 \mu\text{g}/\text{mL}$ and $\text{IC}_{50} \approx 18 \mu\text{g}/\text{mL}$, respectively, but weak inhibitory effect in PGD_2 production with 35% and 34% at 20 $\mu\text{g}/\text{mL}$, respectively. Gallic acid inhibited slightly in only PGE_2 production with 34% at 20 $\mu\text{g}/\text{mL}$. It has been suggested that EGCG may play a role in preventing carcinogenesis

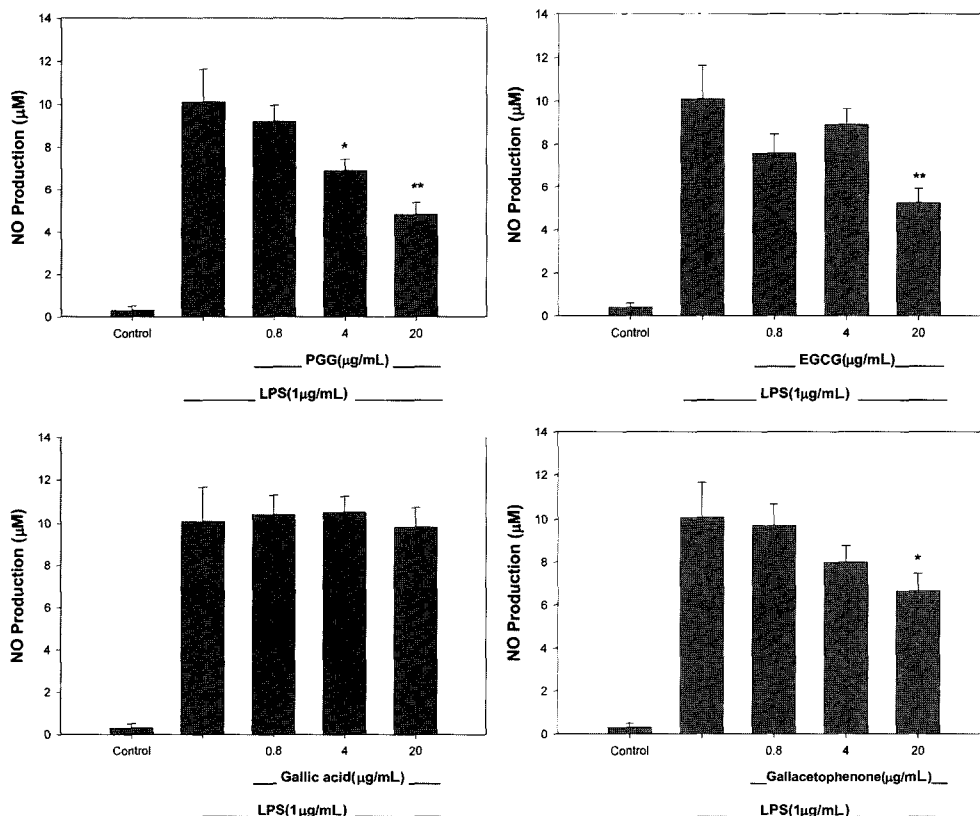


Fig. 3. Effects of PGG, EGCG, gallic acid, and gallacetophenone on LPS-induced NO production in Raw 264.7 macrophages. Raw 264.7 cells were treated with various concentrations of PGG, EGCG, gallic acid, or gallacetophenone dissolved in DMSO for 1 h prior to the addition of LPS (1 $\mu\text{g}/\text{mL}$), and the cells were further incubated for 18 h. Control cells were incubated with vehicle alone. The concentrations of NO in culture medium were monitored as described in the Materials and Methods. The values are expressed as means \pm S.D. of triplicate tests. * $P < 0.05$, ** $P < 0.01$ vs. LPS-treatment control values.

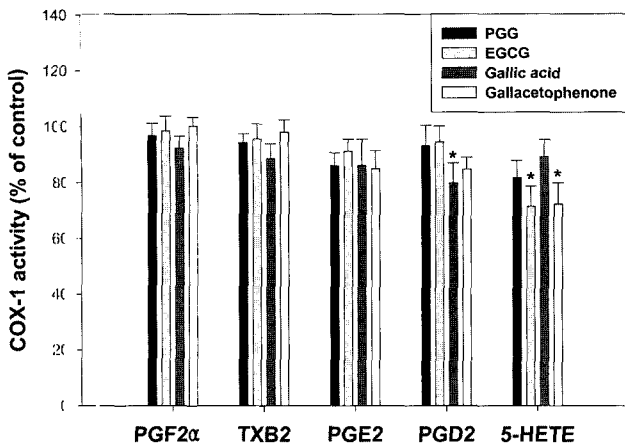


Fig. 4. Effects of PGG, EGCG, gallic acid, and gallacetophenone on COX-1 activity in HEL cells. Cells were treated with 20 μg/mL of PGG, EGCG, gallic acid, or gallacetophenone dissolved in DMSO, and the cells were incubated for 24 h. Control cells were incubated with vehicle alone. The measurement of PGF₂α, Thromboxane B₂ (TXB₂), PGE₂, PGD₂, 5-hydroxyeicosatetraenoic acid (5-HETE) was monitored as described in the Materials and Methods. The values are expressed as means±S.D. of two independent experiments performed in duplicate. *P<0.05 vs. control values.

and anti-inflammation (Lin and Lin, 1997). However, the effects of EGCG on PGE₂ production still remain uncertain.

Previous study has demonstrated that EGCG up-regulates COX-2 expression and PGE₂ production in LPS-untreated macrophages (Park *et al.*, 2001). In this study, EGCG also did not alter amounts of COX-2 and PGE₂ production at high concentration of LPS (1 mg/mL). In contrast, the results of the present study indicated that EGCG is a potent inhibitor on COX-2 activity in LPS (2.5 μg/mL)-treated macrophages (Fig. 5). Although massive LPS treatment inhibits the effects of EGCG on COX-2 activity, EGCG shows inhibitory effects of COX-2 activity in low concentration of LPS-treated macrophages. Because PGG with five gallate group was more active than EGCG with a gallate group in the inhibitory action against COX-2 activity, our results suggest that the gallate group plays an important role in the inhibition of COX-2 activity.

NO is also known to affect tumor progression by regulating the angiogenesis, possibly by stimulating the production of vascular endothelial growth factor (VEGF). Thus, NO donors caused increased VEGF production when treated to glioblastoma or hepatocellular carcinoma cell lines (Chin *et al.*, 1997). The pro-angiogenic activity of NO has also been observed in other experimental systems (Ziche *et al.*, 1994; Morbidelli *et al.*, 1996; Jenkins *et al.*, 1995). In another study, colon cancer cells genetically engineered to constitutively express COX-2 acquired increased

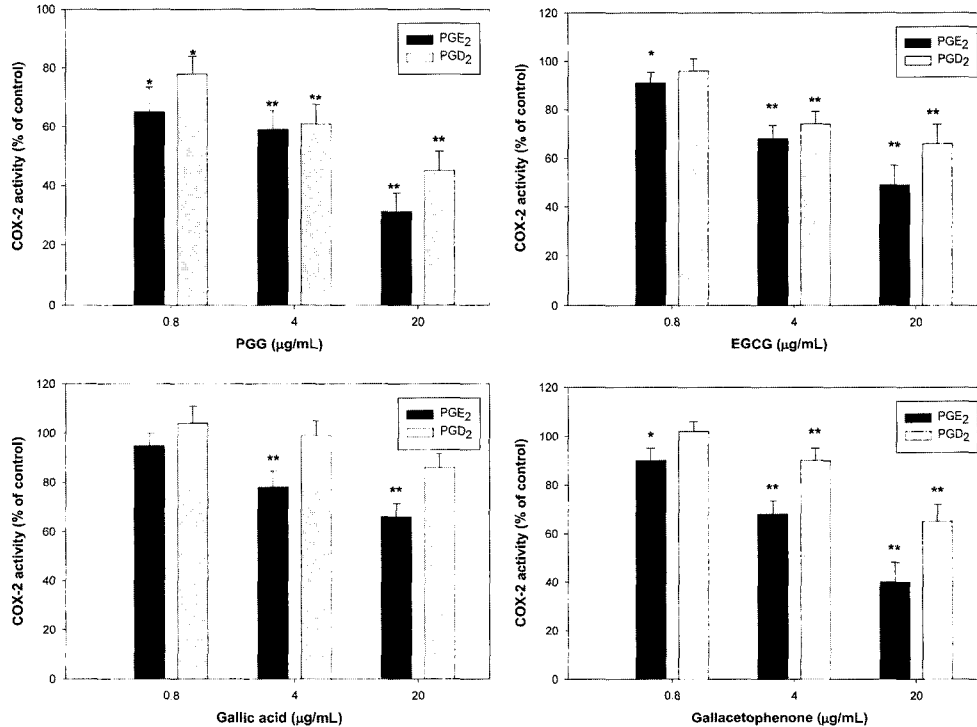


Fig. 5 Effects of PGG, EGCG, gallic acid, and gallacetophenone on LPS-induced COX-2 induction in Raw 264.7 macrophages. Raw 264.7 cells were treated with various concentrations of PGG, EGCG, gallic acid, or gallacetophenone dissolved in DMSO for 1 h prior to the addition of LPS (2.5 μg/mL), and the cells were further incubated for 18 h. Control cells were incubated with vehicle alone. The measurement of LPS-induced PGE₂ and PGD₂ production in culture medium was monitored as described in the Materials and Methods. The values are expressed as means±S.D. of two independent experiments performed in duplicate. *P<0.05, **P<0.001 vs. LPS-treatment control values.

invasiveness and metastatic potential as revealed by elevated expression and activation of matrix metalloproteinase-2, which was again reversed by treatment with sulindac sulfide (Tsuji *et al.*, 1997). COX-2 expression in colon cancer cells also promoted angiogenesis of co-cultured endothelial cells by stimulating the production of angiogenic factors (Tsuji *et al.*, 1998). In contrast, COX-2 inhibitor, celecoxib, reduced proliferation and induced apoptosis in angiogenic endothelial cells (Leahy *et al.*, 2002). PGG inhibited the invasion of highly metastatic mouse melanoma by reducing matrix metalloproteinase-9 (Ho *et al.*, 2002). In agreement with the above notion, we also found that PGG efficiently blocked VEGF-induced human umbilical vein endothelial cell proliferation and capillary-like tube formation of endothelial cell on Matrigel (Lee *et al.*, unpublished observation). All these findings suggest that PGG may contribute to the inhibition of angiogenesis by reducing NO and PGE₂ production through inhibition of iNOS and COX-2 activities.

In summary, the results of the present study indicated that PGG, EGCG, and gallacetophenone, but not gallic acid, pretreatment significantly inhibited LPS-induced NO production in LPS-activated macrophages. PGG and the other compounds showed almost no effect on COX-1 activity but all of the four compounds significantly inhibited COX-2 activity in LPS-activated macrophages. Of those compounds tested, PGG was the most potent inhibitor of both iNOS and COX-2 in concentration-dependent manner and EGCG also was a potent inhibitor of COX-2 activity in LPS-activated macrophages. Although further studies are needed to elucidate the molecular mechanisms by which PGG exerts its inhibitory actions, PGG may be useful in the prevention and treatment of inflammation or cancer associated with iNOS and COX-2.

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