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# In vitro and in vivo inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin $E_2$ production

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**Abstract**

Flavonoids are widely distributed in plants, but their biological functions are still unclear. In the present study, in vitro and in vivo experiments were performed to demonstrate the inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide (NO) and prostaglandin $E_2$ production in RAW 264.7 macrophages, primary peritoneal macrophages, and Balb/c mice, respectively. In vitro results showed that wogonin and quercetin dose-dependently suppressed lipopolysaccharide-induced NO production in RAW 264.7 macrophages and primary peritoneal macrophages without a notable cytotoxic effect on either cell types associated with a decrease in inducible nitric oxide synthase (iNOS) protein expression in both cells. Rutin, at 80 μM only, had a slight but obvious inhibitory effect on lipopolysaccharide-induced NO production in primary peritoneal macrophages. Both wogonin and quercetin attenuated lipopolysaccharide-induced prostaglandin $E_2$ production in vitro. Intravenous injection of lipopolysaccharide (10 mg/kg, i.v.) resulted in a time-dependent induction of NO production in serum, and pretreatment with the L-arginine analog *N*-nitro-L-arginine methyl ester (L-NAME) blocked this induction. Intravenous pretreatment of Balb/c mice with rutin, wogonin or quercetin for 1 h followed by lipopolysaccharide treatment significantly inhibited lipopolysaccharide-induced NO production, but no inhibition of prostaglandin $E_2$ production was found. A decrease in iNOS protein, but not cyclooxygenase-2 protein, was detected in liver and lung specimens of lipopolysaccharide-treated Balb/c mice in the presence of rutin, wogonin or quercetin. In conclusion, data obtained both in vitro and in vivo suggest that wogonin and quercetin exert inhibitory activity on lipopolysaccharide-induced NO production through suppression of iNOS expression. 

*Keywords:* Rutin; Wogonin; Quercetin; Lipopolysaccharide; Nitric oxide (NO); Prostaglandin $E_2$

## 1. Introduction

Nitric oxide (NO) is a messenger and effector molecule in a variety of tissues (Palmer et al., 1988; Lowenstein et al., 1996). It has been identified as a neurotransmitter in the central nervous system and as a potent vasorelaxant in physiologically regulating blood pressure by modulating muscular tone (Hibbs et al., 1987; Moncada et al., 1992). NO is considered a deleterious molecule in the process of inflammation and sepsis (Wheeler and Bernard, 1999). Exposure to outer bacterial toxins such as lipopolysaccharide or lipoteicholic acid stimulates cellular inflammatory responses and releases factors including NO, prostaglandin $E_2$, cytokines, tumor necrosis factor-α and eicosanoid mediators to promote inflammatory responses (Penglis et al., 2000; Yamashita et al., 2000). Therefore, agents that block bacterial toxin-induced NO production might be beneficial in the treatment of inflammatory responses. The production of NO is regulated by intracellular nitric oxide synthases, and

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three types of nitric oxide synthases, endothelium NO synthase (eNOS), neural NO synthase (nNOS), and inducible NO synthase (iNOS), have been identified. After exposure to endogenous and exogenous stimulators such as bacterial toxin or viral infection, iNOS is induced quantitatively in various cells such as macrophages, smooth muscle cells, and hepatocytes, triggers several deleterious cellular responses, and causes some diseases including inflammation, sepsis, and stroke (Duval et al., 1996; Marletta, 1993; Nathan, 1992). Therefore, NO production induced by iNOS may reflect the degree of inflammation and provides a measure to assess the effect of drugs on the inflammatory process.

Prostaglandin $E_2$ is overproduced at sites of inflammation as a result of the activation of inducible cyclooxygenase-2, an enzyme in the conversion of arachidonic acid to prostaglandin $H_2$, prostacyclin, and thomboxane $A_2$ (Fu et al., 1990; Picot et al., 1994). Elevation of cyclooxygenase-2 protein has been demonstrated to be associated with the occurrence of some human diseases such as colon carcinoma (Shao et al., 2000). Specific cyclooxygenase-2 inhibitors effectively attenuate the symptoms of inflammation and reduce the rate of cancer occurrence (Levy, 1997; Wong et al., 1998). Therefore, there is a great interest in studying the effects and usefulness of drugs on cyclooxygenase-2.

Flavonoids exist as either simple or complex glycosides in many plants (Kuhnau, 1976), and humans are estimated to consume approximately 1 g flavonoids/day. Our previous studies, as well as those of others, have demonstrated that flavonoids exhibit a wide variety of interesting biological activities such as antioxidant and free radical scavenging activities, inhibition of 12-*o*-tetradecanoylphorbol 13-acetate-induced tumor promotion, and apoptosis-inducing activity (Mora et al., 1990; Chen et al., 1999). Although flavonoids have been studied extensively, the application of flavonoids in treating human diseases is still uncommon because of their high effective concentration and poor absorption after oral intake. Rutin, wogonin, and quercetin are structurally related flavonoids and exist extensively in the diet. Quercetin is derived from rutin through hydrolyzation by glucosidase in the gastrointestinal tract (Manach et al., 1997) and has a wide range of biological activities including inhibition of $Na^+/K^+$-ATPase, protein kinase C, tyrosine kinase, etc. (Kang and Liang, 1997). Our recent studies demonstrated that quercetin and wogonin had significant inhibitory activity on lipopolysaccharide-induced NO production by blocking iNOS gene expression in RAW 264.7 macrophages (Chen et al., 2000, 2001a,b). However, the in vivo action of these compounds on lipopolysaccharide-induced NO production is still undefined. A series of experiments were performed using RAW 264.7 macrophages, primary peritoneal macrophages, and Balb/c mice to demonstrate the differential inhibitory activity of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production.

## 2. Materials and methods

### 2.1. Agents

Three structurally related polyphenolic compounds (rutin, wogonin, and quercetin), sulfanilamine, naphthylethylenediamine dihydrochloride, *N*-nitro-L-arginine methyl ester (L-NAME), lipopolysaccharide, nitroblue tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate were obtained from Sigma (St. Louis, MO). The prostaglandin $E_2$ enzyme-linked immunosorbent assay (ELISA) kits were purchased from Cayman Chemical (Ann Arbor, MI). Fetal bovine serum, penicillin, streptomycin, L-glutamine, and RPMI media 1640 were purchased from Gibco Laboratories (Grand Island, NY).

### 2.2. Cell culture

RAW 264.7, a mouse macrophage cell line, was obtained from the American Type Culture Collection. Cells were cultured in RPMI-1640 medium supplemented with 2 mM glutamine, antibiotics (100 U/ml of penicillin and 100 U/ml of streptomycin), and 10% heat-inactivated fetal bovine serum, and maintained at 37 °C in a humidified incubator containing 5% $CO_2$. Thioglycollate-elicited peritoneal macrophages were obtained from specific pathogen-free male Balb/c mice at 5–8 weeks of age by injection of 3.0 ml of sterile 3% thioglycollate solution (Difco, Detroit, MI) for 4 days before lavage with 10 ml of phosphate-buffered saline. The peritoneal macrophages were centrifuged at 1500 rpm and washed once with RPMI-1640 supplemented with 10% fetal bovine serum. The cells were resuspended in RPMI-1640 at a density of $2 \times 10^6$ cells/ml. Viability was determined by Trypan blue exclusion. The cells were plated and incubated for 2–3 h at 37 °C in humidified 5% $CO_2$/95% air to allow macrophage adherence. The plates were then washed once with warm RPMI-1640 to remove non-adherent cells. This procedure resulted in an adherent cell population that was 85–95% macrophages, as determined by morphology and esterase staining.

### 2.3. In vivo study

Balb/c mice were injected with or without lipopolysaccharide (10 mg/kg, i.v.), and blood was collected from the retro-orbital sinus into heparin-containing tubes at different time periods. In the rutin-, wogonin-, and quercetin-treated groups, mice were injected with rutin, wogonin, and quercetin (6 mg/kg for rutin, 3 mg/kg for wogonin, and 3 mg/kg for quercetin, i.v.) for 1 h followed by lipopolysaccharide (10 mg/kg, i.v.) injection for a further 24 h. Blood was collected from the retro-orbital sinus into heparin-containing tubes. For nitrite and prostaglandin $E_2$ measurement, plasma samples were assayed by Griess reaction using known concentration of potassium nitrite as a calibration curve, and prostaglandin $E_2$ ELISA kits were used for prostaglandin $E_2$ analysis.

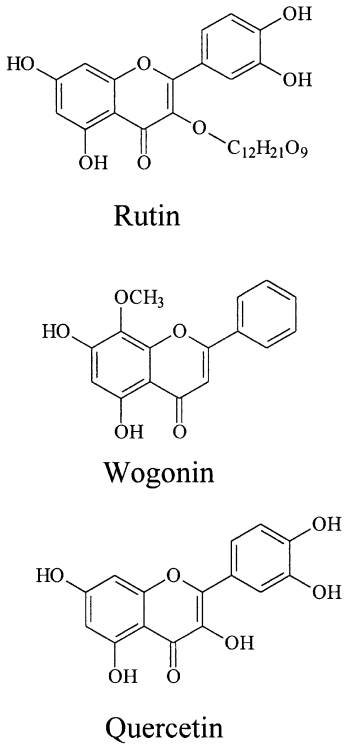


Fig. 1. Chemical structures of rutin, wogonin, and quercetin examined in the present study.

### 2.4. Nitrite assay

The nitrite concentration in the medium was measured as an indicator of NO production according to the Griess reaction (Chen et al., 2000). One hundred microliters from each supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water); absorbance of the mixture at 550 nm was determined with an ELISA plate reader (Dynatech MR-7000; Dynatech Laboratories).

### 2.5. Western blot analysis

RAW 264.7 macrophages and primary peritoneal macrophages were treated with lipopolysaccharide (50 ng/ml for RAW 264.7 macrophages and 5 mg/ml for primary peritoneal macrophages) in the presence or absence of different doses of rutin, wogonin, and quercetin for 12 h. Total cellular extracts or total proteins extracted from the liver and lung specimens were prepared according to our previous paper (Chen et al., 2001c). In brief, 50 μg cellular proteins or 100 μg liver or lung proteins were separated on 8% sodium dodecyl sulfate (SDS)-polyacrylamide minigels and transferred to Immobilon polyvinylidenedifluoride membranes (Millipore). The membrane was incubated overnight at 4 °C with 1% bovine serum albumin and then incubated with anti-iNOS, anti-cyclooxygenase-2 or anti-α-tubulin monoclonal antibodies (Transduction Laboratories). Expression of protein was detected by staining with nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

### 2.6. Measurement of prostaglandin $E_2$ production

RAW 264.7 cells were subcultured in six-well plates and were incubated with the indicated compounds for 12 h. From the supernatant of the culture medium, 100 μl was collected for the determination of prostaglandin $E_2$ concentration using prostaglandin $E_2$ ELISA kits (Cayman Chemical).

### 2.7. Statistics

The values are expressed as means ± S.E. The significance of differences from the respective controls for each experimental test condition was assayed by using Student's *t*-test for each paired experiment. * $P<0.05$ and ** $P<0.01$ were regarded as indicating significant differences.

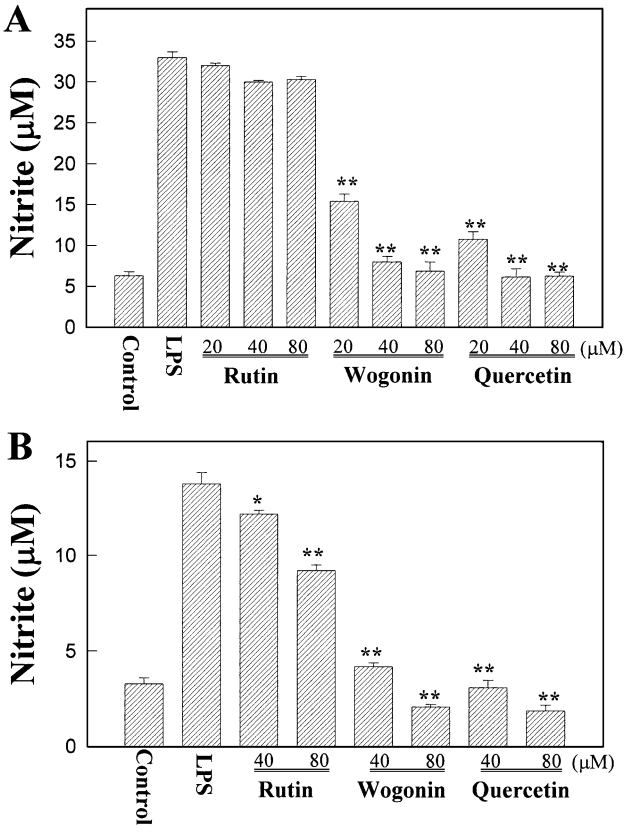


Fig. 2. Effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitrite production in RAW 264.7 macrophages (A) and primary peritoneal macrophages (B). Both cells were treated with or without lipopolysaccharide (LPS, 50 ng/ml for RAW 264.7 macrophages; 5 μg/ml for primary macrophages) or lipopolysaccharide plus different concentrations of rutin, wogonin, and quercetin. At the end of incubation, 100 μl of the medium was removed for measuring nitrite production. Control values were obtained in the absence of lipopolysaccharide or flavonoids. Data were derived from three independent experiments and are expressed as means ± S.E. * $P<0.05$ and ** $P<0.01$ indicate significant differences from the lipopolysaccharide-treated group.

## 3. Results

### *3.1. Inhibition of lipopolysaccharide-induced NO and prostaglandin $E_2$ production by rutin, wogonin, and quercetin in RAW 264.7 macrophages*

The chemical structures of rutin, wogonin, and quercetin are shown in Fig. 1. The effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO production in RAW 264.7 macrophages were investigated by measuring the accumulation of nitrite, estimated by the Griess reaction, in the culture medium. Rutin, wogonin, and quercetin at 80 μM did not interfere with the reaction between nitrite and Griess reagents (data not shown). Unstimulated macrophages, after 12 h of incubation in culture medium, produced background levels of nitrite (Fig. 2). When the cells were incubated with the indicated compounds alone, the concentration of nitrite in the medium was maintained at a background level similar to that in the unstimulated samples. After treatment with lipopolysaccharide (50 ng/ml) for 12 h, nitrite concentrations in the medium increased remarkably by about 20-

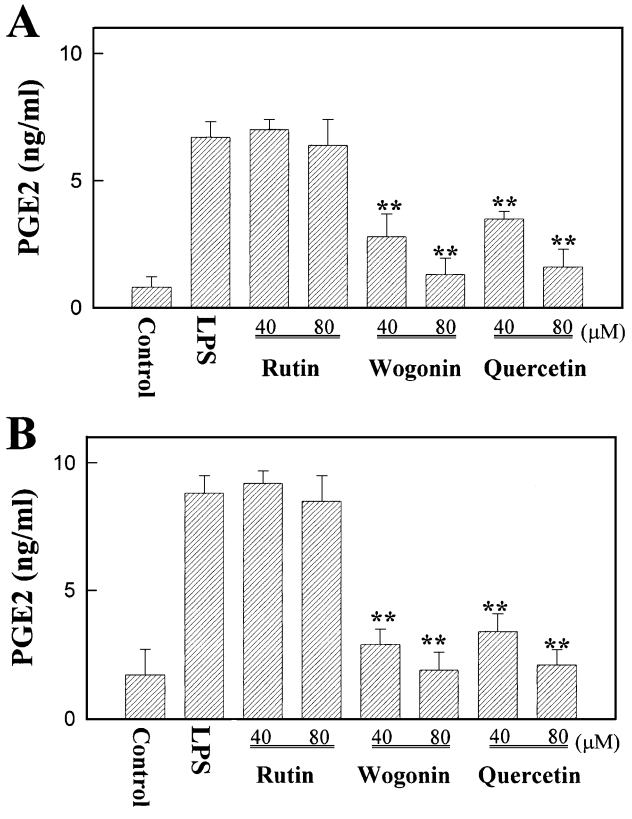


Fig. 3. Effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced prostaglandin $E_2$ production in RAW 264.7 macrophages (A) and primary peritoneal macrophages (B). Both cells were treated with or without lipopolysaccharide (LPS, 50 ng/ml for RAW 264.7 macrophages; 5 μg/ml for primary macrophages) or lipopolysaccharide plus different concentrations of rutin, wogonin, and quercetin. At the end of incubation, 100 μl of the medium was removed for measuring prostaglandin $E_2$ production as described in Materials and methods. Data were derived from three independent experiments and are expressed as means ± S.E. $^{**}P<0.01$ indicates significant differences from the lipopolysaccharide-treated group.

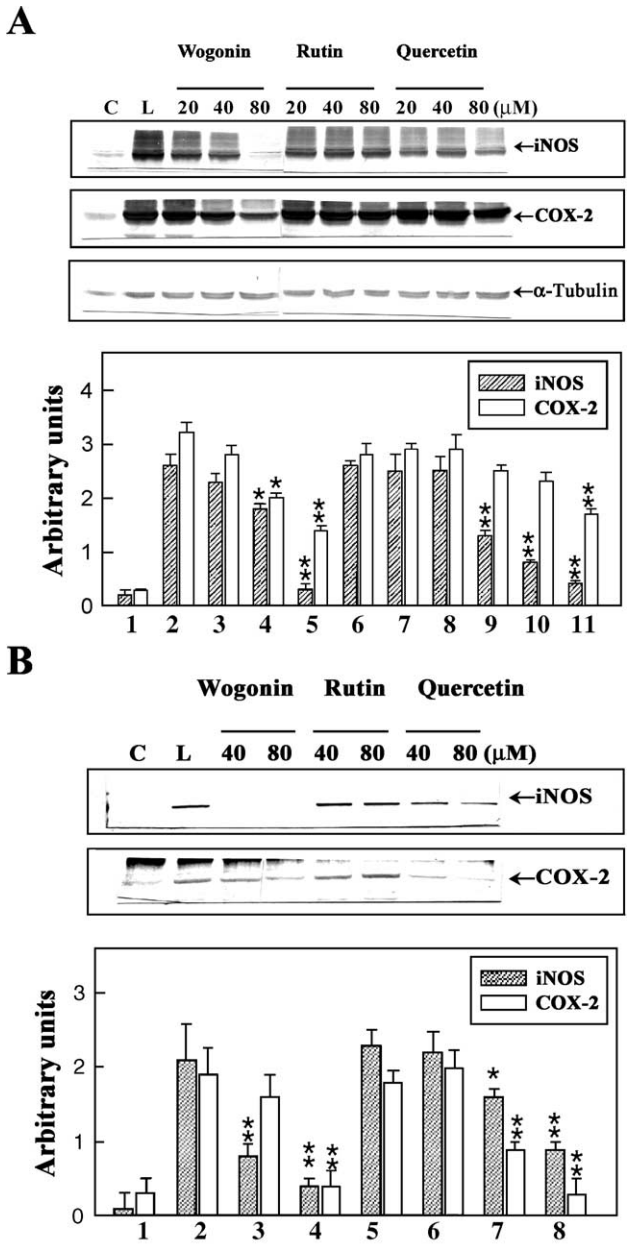


Fig. 4. Rutin, wogonin, and quercetin showed inhibitory activity on lipopolysaccharide-induced iNOS and cyclooxygenase-2 protein expression in RAW 264.7 macrophages (A) and primary peritoneal macrophages (B). Both cells were treated as described in Fig. 3. Equal amounts of total protein (50 μg/lane) were applied to detect the expression of iNOS, cyclooxygenase-2 and α-tubulin proteins by Western blotting using specific antibodies. C: Control; L: lipopolysaccharide-treated group. Bar charts at the lower panel of (A) and (B) show the band intensity of iNOS and cyclooxygenase-2 proteins by densitometry (IS-1000 Digital Imaging System). Data were derived from three independent experiments and are described as means ± S.E. $^{*}P<0.05$ and $^{**}P<0.01$ indicated significant differences from the lipopolysaccharide-treated group.

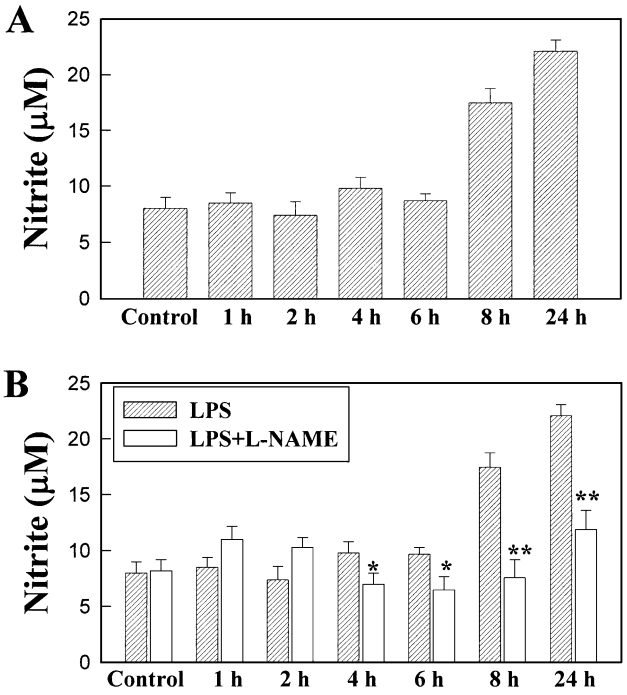


Fig. 5. A time-dependent increase in NO production after intravenous injection of lipopolysaccharide (10 mg/kg, i.v.) in Balb/c mice. (A) Balb/c mice were injected intravenously with 10 mg/kg of lipopolysaccharide for the indicated time periods, and the amount of NO in the serum was analyzed by Griess reaction. (B) Balb/c mice were injected intravenously with 10 mg/kg of lipopolysaccharide in the presence or absence of 1-h pre-injection of the L-arginine analog L-NAME (4 mg/kg, i.v.) for different time periods. The amount of NO in the serum was analyzed by Griess reaction.

fold ( ~ 30 μM). When RAW 264.7 macrophages were treated with different concentrations of wogonin and quercetin together with lipopolysaccharide (50 ng/ml) for 12 h, a significant concentration-dependent inhibition of nitrite production was detected (Fig. 2A). Examination of the cytotoxicity of rutin, wogonin, and quercetin in RAW 264.7 macrophages by thiazolyl blue (MTT) assay indicated that all three compounds, even at 80 μM, did not affect the viability of RAW 264.7 cells (data not shown). Therefore, inhibition of lipopolysaccharide-induced nitrite production by wogonin and quercetin was not the result of a possible cytotoxic effect on these cells. On analysis of prostaglandin $E_2$ production by ELISA, unstimulated RAW 264.7 macrophages in culture medium for 12 h produced a basal amount of prostaglandin $E_2$ in the medium as a control. After treatment with lipopolysaccharide (50 ng/ml) for 12 h, the medium concentration of prostaglandin $E_2$ increased significantly, compared with that of the control group. This increase was inhibited by co-treatment of cells with different concentrations (40 and 80 μM) of wogonin and quercetin (Fig. 3A). However, lipopolysaccharide-induced prostaglandin $E_2$ production was not inhibited by rutin, even at 80 μM, the highest concentration examined.

### *3.2. Inhibitory activity of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production in thioglycollate-elicited peritoneal macrophages*

In order to further demonstrate the inhibitory effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production, primary peritoneal macrophages were obtained from thioglycollate-treated Balb/c mice. The adherent cultured cells were more than 95% macrophages, as determined by morphology and esterase staining. Upon lipopolysaccharide (5 μg/ml) treatment for 12 h, nitrite production in the medium was increased (Fig. 2B). Wogonin and quercetin had an obvious inhibitory effect on lipopolysaccharide-induced NO production. Rutin caused a slight inhibition of lipopolysaccharide-induced NO production in primary peritoneal macrophages. On analysis of prostaglandin $E_2$ production in thioglycollate-elicited macrophages, wogonin and quercetin inhibited lipopolysaccharide-induced prostaglandin $E_2$ production to a basal level, but rutin did not inhibit prostaglandin $E_2$ production (Fig. 3B).

### *3.3. Alternative inhibitory effects of rutin, quercetin, and wogonin on lipopolysaccharide-induced iNOS and cyclooxygenase-2 proteins by Western blotting*

Western blot analysis demonstrated that unstimulated RAW 264.7 macrophages and thioglycollate-elicited macrophages expressed only a small amount of iNOS and cyclo-

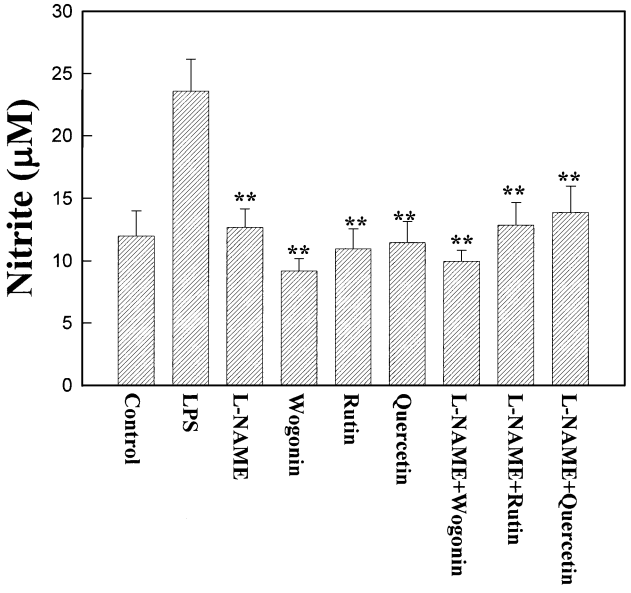


Fig. 6. In vivo effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO production in the serum of Balb/c mice. Balb/c mice were pretreated with rutin, wogonin, and quercetin in the presence or absence of L-NAME (4 mg/ml, i.v.) for 1 h followed by injection of lipopolysaccharide (10 mg/kg, i.v.) for 24 h. The amounts of NO in the serum were analyzed by Griess reaction. Data were derived from three independent experiments and are expressed as means ± S.E. ** $P < 0.01$ indicates significant differences from the lipopolysaccharide-treated group.

oxygenase-2 proteins. Rutin, wogonin, and quercetin treatment alone did not affect the basal expression of iNOS and cyclooxygenase-2 proteins. Upon lipopolysaccharide (50 ng/ml for RAW 264.7 and 5 μg/ml for primary peritoneal macrophages) treatment for 12 h, the expression of iNOS and cyclooxygenase-2 proteins increased in these cells. The increase in iNOS protein expression was significantly inhibited by co-treatment of cells with quercetin and wogonin for 12 h in RAW 264.7 and primary peritoneal macrophages. Rutin at similar concentrations, however, did not inhibit lipopolysaccharide-induced iNOS protein expression (Fig. 4). Quercetin and wogonin, at the dose of 80 μM, inhibited lipopolysaccharide-induced cyclooxygenase-2 protein expression in RAW 264.7 and primary peritoneal macrophages.

### *3.4. In vivo inhibitory effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production in Balb/c mice*

In vitro data for RAW 264.7 and primary peritoneal macrophages indicated that wogonin and quercetin are potent inhibitors of lipopolysaccharide-induced NO and prostaglandin $E_2$ production. Furthermore, in vivo experiments were performed in Balb/c mice. Fig. 5A shows a time-dependent induction of NO production in the serum of lipopolysaccharide (10 mg/kg, i.v.)-treated Balb/c mice, and the maximal induction of NO was detected at 24 h post-treatment. Pretreatment with the L-arginine analog L-NAME (4 mg/kg, i.v.) for 1 h inhibited lipopolysaccharide-induced NO production (Fig. 5B). The same animal model was used to study the in vivo effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO production. Intravenous injection of Balb/c mice with rutin, wogonin, and quercetin (6 mg/kg for rutin, 3 mg/kg for wogonin, and 3 mg/kg for quercetin, i.v.) with or without L-NAME (4 mg/kg, i.v.) for 1 h followed by lipopolysaccharide (10 mg/kg, i.v.) treatment was able to decrease lipopolysaccharide-induced NO production in the serum (Fig. 6). However, no significant inhibition by rutin, wogonin, and quercetin of lipopolysaccharide-induced prostaglandin $E_2$ production was found in vivo (data not shown), and L-NAME did not show a synergistic inhibitory effect on lipopolysaccharide-induced NO production with rutin, wogonin, and quercetin. Western blotting was performed to identify the expression of iNOS and cyclooxygenase-2 proteins in the liver and lung specimens of Balb/c mice under various treatments. Injection of lipopolysaccharide (10 mg/kg, i.v.) for 24 h induced iNOS and cyclooxygenase-2 proteins in the liver and lung specimens of Balb/c mice ($n = 3$) (Fig. 7). Pretreatment of Balb/c mice with rutin, wogonin, and quercetin significantly inhibited lipopolysaccharide-induced iNOS protein expression in the liver and lung specimens; however cyclooxygenase-2 protein expression remained unchanged in both tissues ($n = 3$).

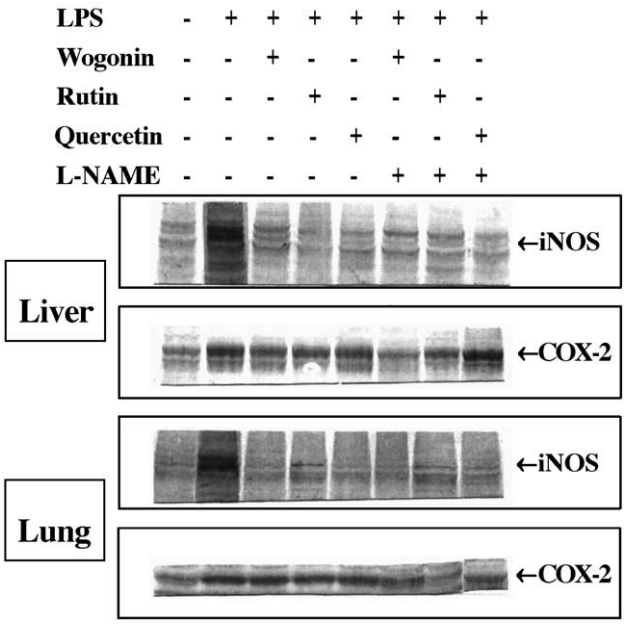


Fig. 7. In vivo inhibitory effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced iNOS and cyclooxygenase-2 protein expression in liver and lung specimens of Balb/c mice. Balb/c mice were treated as described in Fig. 6, and the proteins (100 μg/lane) in liver and lung of Balb/c mice were extracted. Detection of iNOS and cyclooxygenase-2 proteins in the liver and lung was performed using specific antibodies as described in Materials and methods.

## 4. Discussion

The present study demonstrated the in vitro and in vivo activity of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production in RAW 264.7 macrophages, primary peritoneal macrophages, and Balb/c mice, respectively. In vitro results showed that quercetin and wogonin inhibited lipopolysaccharide-induced NO and prostaglandin $E_2$ production associated with a decrease in iNOS and cyclooxygenase-2 proteins expression, but inhibition did not occur in rutin-treated macrophages. In vivo results demonstrated that rutin, quercetin, and wogonin suppressed lipopolysaccharide-induced NO but not prostaglandin $E_2$ production in the presence or absence of L-NAME. This study provides molecular evidence of the in vitro and in vivo effects of rutin, quercetin, and wogonin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production, and we propose that wogonin and quercetin are effective inhibitors of lipopolysaccharide-induced NO production in vitro and in vivo.

Several natural polyphenolic compounds such as epigallocatechin, curcumin, oroxylin A, and wogonin have been demonstrated to inhibit lipopolysaccharide-induced NO production by blocking iNOS gene expression (Lin et al., 1999). Rutin is one of the major flavonoids in *Sophorae Flos Immaturus*, which has been used in Chinese medicine as a remedy for hypertension and uterine bleeding. A previous study indicated that heavy metals enhance the antioxidant and anti-inflammatory activity of rutin by

complex formation (Afanaseva et al., 2001); however, in an in vitro assay, the glycoside rutin appeared to be ineffective (Aherne and O'Brien, 2000). In the present study, rutin was ineffective in inhibiting lipopolysaccharide-induced NO and prostaglandin $E_2$ production in RAW 264.7 cells and primary peritoneal macrophages. Interestingly, rutin was able to block lipopolysaccharide-induced NO production and iNOS expression in vivo. Morand et al. (2000) reported that rutinose located at the C3 site of rutin will be destroyed in the blood, and that rutin will be converted to quercetin. Therefore, it is possible that in vivo inhibition by rutin of lipopolysaccharide-induced NO production might be through the hydrolyzation of rutin to quercetin by glucosidase in vivo.

Quercetin is a prototypical polyphenolic plant flavonoid and can be derived from rutin though hydrolyzation by glucosidase. The biological activity of quercetin has been studied extensively. Quercetin is a potent antioxidant and anti-inflammatory agent and prevents cisplatin-induced cytotoxicity in LLC-PK1 cells in vitro and tubular injury induced by acute renal ischemia in vivo (Kuhlmann et al., 1998). Rangan et al. (1999) reported that quercetin inhibited the lipopolysaccharide-induced production of cytokines such as interleukin-1β and tumor necrosis factor-α by blocking nuclear factor-kappa B activation. In addition to beneficial effects, quercetin was also shown to be a strong mutagen without microsomal activation, but its mutagenic activity is increased significantly after microsomal activation (Bjeldanes, 1977). Wogonin is one of the major components of the Chinese herb *Scutellaria baicalensis*, and several biological functions have been demonstrated recently, including anti-hepatitis B virus and antioxidant activity (Gao et al., 2001; Huang et al., 2000). Chi et al. (2001) and our previous study (Chen et al., 2001a) demonstrated that wogonin had potent inhibitory activity on lipopolysaccharide-induced NO and prostaglandin $E_2$ production. In this study, wogonin and quercetin exerted potent inhibitory activity on lipopolysaccharide-induced NO and prostaglandin $E_2$ production, accompanied by a decrease in iNOS and cyclooxygenase-2 protein expression in macrophages. However, the inhibitory potency of wogonin and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production seemed stronger than that on iNOS and cyclooxygenase-2 protein expression. Direct and indirect iNOS and cyclooxygenase-2 enzyme activity assays indicated that wogonin and quercetin did not inhibit iNOS or cyclooxygenase-2 enzyme activity (Chen et al., 2001a,b). We propose that the suppression of NO and prostaglandin $E_2$ by wogonin and quercetin was mainly mediated by inhibition of iNOS and cyclooxygenase-2 protein expression; other factors involved still remain to be studied.

In vivo study showed that wogonin and quercetin inhibited lipopolysaccharide-induced NO but not prostaglandin $E_2$ production. Several studies have demonstrated that complex signaling pathways are involved in prostaglandin $E_2$ production stimulated by lipopolysaccharide (Pistritto et al., 2000; Franco and Doria, 1998). Therefore, it is possible that alternative inhibitory effects of wogonin and quercetin on lipopolysaccharide-induced prostaglandin $E_2$ in vitro and in vivo may be due to several factors, such as IL-10 and transforming growth factor-beta 1, etc., that participate in prostaglandin $E_2$ production in vivo. We propose that in vivo and in vitro lipopolysaccharide induces prostaglandin $E_2$ production through distinct pathways, and that in vitro inhibitors of lipopolysaccharide-induced prostaglandin $E_2$ production might not be effective in vivo.

The large amount of NO produced in response to bacterial lipopolysaccharide or cytokines plays an important role in endotoxemia and inflammatory conditions (Stoclet et al., 1998; Gidday et al., 1998). Therefore, drugs that inhibit NO production by inhibiting iNOS gene expression or its enzyme activity have beneficial therapeutic effects in the treatment of sepsis (Suh et al., 1998). iNOS enzyme inhibitors such as L-NAME are well-known nonspecific NOS inhibitors that effectively attenuate NO production induced by lipopolysaccharide and cytokines. In vitro and in vivo studies indicate that NO functions as an anti-inflammatory mediator. For instance, release of NO, by adding exogenous NO donors, decreases cytokine-induced endothelial cell activation, inhibits endothelial–leukocyte interaction, and attenuates vascular inflammation (De Caterina et al., 1995; Khan et al., 1996). Peng et al. (1998) recently reported that treatment with L-NAME in combination with lipopolysaccharide/interferon-γ decreases stimulated NO production, but augments lipopolysaccharide/interferon-γ-induced iNOS expression 2.5-fold. In this study, L-NAME was able to attenuate lipopolysaccharide-induced NO production in the serum of Balb/c mice, and treatment of Balb/c mice with rutin, wogonin, and quercetin in the presence of L-NAME resulted in inhibition of lipopolysaccharide-induced NO production. Results indicate that the inhibitory action of rutin, quercetin, and wogonin on lipopolysaccharide-induced NO production is compatible but not synergistic with the action of L-NAME in vivo.

In conclusion, this study provides evidence for the in vitro and in vivo effects of rutin, wogonin, and quercetin on lipopolysaccharide-induced NO and prostaglandin $E_2$ production. We propose that wogonin and quercetin are potential inhibitors of lipopolysaccharide-induced NO production and merit practical application.

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