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Biochemical Pharmacology 66 (2003) 1271–1278
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Effects of wogonin, a plant flavone from *Scutellaria radix*, on skin inflammation: *in vivo* regulation of inflammation-associated gene expression

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Received 22 January 2003; accepted 6 June 2003

Abstract

Flavonoids from plant origin show anti-inflammatory activity *in vitro* and *in vivo*. In addition to inhibition of inflammation-associated enzymes, such as cyclooxygenases (COX) and lipoxygenases, they have been found to regulate the expression of inflammation-associated proteins from *in vitro* experiments. In order to prove *in vivo* behavior and the potential for beneficial use against inflammatory skin disorders, the effect of wogonin (5,7-dihydroxy-8-methoxyflavone) on *in vivo* expression of several inflammation-associated genes was examined in the intact as well as in the inflamed mouse skin by reverse transcriptase–polymerase chain reaction analysis. When applied topically on the intact skin, only a high dose treatment of wogonin (1000 μg/ear/3 days) slightly increased COX-1 and fibronectin mRNA. On the other hand, wogonin at the doses of 250–1000 μg/ear/3 days potently lowered mRNA levels of COX-2 and tumor necrosis factor-α with less effect on intercellular adhesion molecule-1 and interleukin-1β in a sub-chronic skin inflammation model of tetradecanoylphorbol-13-acetate-induced ear edema (multiple treatment). The decrease of prostaglandin E_2 concentration (27.3–34.3%) was concomitantly observed in the wogonin-treated groups. A similar effect was also observed in an acute inflammation model of arachidonic acid-induced ear edema. From the present study, wogonin was proved to differentially regulate the expression of inflammation-associated genes *in vivo* and to become a useful therapeutic agent for skin inflammatory diseases mainly due to its modulation of the expression of proinflammatory molecules.

Keywords: Flavonoid; Wogonin; Skin inflammation; Cyclooxygenase; Tumor necrosis factor; Intercellular adhesion molecule

Human skin is a complex organ and various inflammatory disorders occur for a life-long time. Most of the skin disorders are well treated. But, it may not be feasible to successfully treat chronic inflammatory diseases, such as psoriasis and atopic dermatitis, partly due to their complex etiological origins and not completely understood disease process. Several different approaches have been used in these disorders, but still unsatisfactory to date. Recent studies have demonstrated that some inducible enzymes/cytokines and their reaction products are involved in chronic skin inflammatory diseases. COX-2, an inducible isoform of COX, is upregulated in chronic venous ulcers [1] as well as in well-known skin carcinogenesis. Inducible nitric oxide synthase (iNOS) is associated with psoriasis [2], and its reaction product, NO, is involved in various skin disorders [3]. And it was also shown that high concentrations of cytokines, such as tumor necrosis factor (TNF)-α, and adhesion molecules, including intercellular adhesion molecule (ICAM)-1, were found in the regional site of chronic skin inflammation [4,5]. Another examples are animal models of skin inflammation. A picryl chloride-induced contact hypersensitivity in mouse skin was related with NO production, presumably by iNOS [6]. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA)-induced dermal inflammation elevated the expression level of COX-2, leading to increased prostaglandin (PG) E_2 production [7]. And transgenic mice constitutively expressing COX-2 in the skin undergo abnormal differentiation of epidermis [8]. Therefore, an agent that modulates the activity and/or the expression of these

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Abbreviations: COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; TNF, tumor necrosis factor; ICAM, intercellular adhesion molecule; TGF, transforming growth factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PG, prostaglandin; IL, interleukin; AA, arachidonic acid; RT–PCR, reverse transcription–polymerase chain reaction; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunoabsorbant assay.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00463-5

biomolecules may have a potential to treat inflammatory skin disorders or at least affect the disease processes.

Flavonoids from plant origin show anti-inflammatory activity *in vivo* by oral administration or topical application on the skin [9]. As many flavonoids directly modulate the enzyme activities of phospholipase A_2, COX, and lipoxygenase [10], certain flavonoid derivatives were recently found to modulate the expression level of several inflammation-associated genes. For instance, the down-regulating capacity of COX-2 and/or iNOS was observed in cell culture by several flavonoid derivatives, including apigenin, luteolin, wogonin, and quercetin, among which wogonin was the most potent [11–14]. In addition, some of the flavonoid derivatives inhibited the production of proinflammatory cytokines, such as TNF-α [15]. Apigenin and quercetin inhibited cytokine-induced ICAM-1 production from human umbilical vein endothelial cells [16]. Baicalein inhibited the production of various cytokines from human peripheral blood mononuclear cells induced by super-antigen treatment [17]. Although these properties of flavonoids may be favorable for treating chronic skin inflammation, there have been few studies to prove these properties of flavonoids *in vivo* using experimental animal models of skin inflammation. Several plant constituents, including resveratrol (stilbene) and sylimarin (flavonolignan), were demonstrated to inhibit *in vivo* expression of COX-2, interleukin (IL)-1, c-*fos*, and transforming growth factor (TGF)-β1 in mouse skin [7,18]. However, up to date, wogonin was the only flavonoid proved to down-regulate COX-2 expression in mouse skin induced by TPA treatment [19]. Moreover, it is not understood yet whether flavonoids affect *in vivo* expression levels of all or some parts of proinflammatory genes in the intact or in the inflamed skin depending on types of inflammation. Therefore, in order to answer these questions, the effects of wogonin as a representative flavonoid on the expression of several inflammation-associated genes were investigated using animal models of skin inflammation. Anti-inflammatory potential of wogonin is also discussed.

1. Materials and methods

1.1. Chemicals

TPA, arachidonic acid (AA, 99%), and prednisolone were obtained from Sigma-Aldrich Co. Wogonin (5,7-dihydroxy-8-methoxyflavone, Fig. 1) was isolated from the methanol extract of the radix of *Scutellaria baicalensis* Georgy according to the previously described procedure [20]. The purity was >95% based on HPLC analysis.

1.2. Animals

Male ICR mice (6 weeks, specific pathogen free) were obtained from Japan SLC. Animals were fed with laboratory chow (Purina Korea) and water *ad libitum*. They were acclimatized in a specific pathogen-free animal facility under the conditions of 20–22°, 40–60% relative humidity, and 12 hr/12 hr (light/dark) cycle at least 3 days prior to experiment.

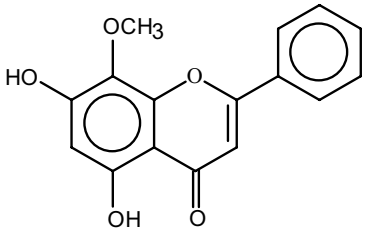


Fig. 1. Chemical structure of wogonin.

1.3. Application of flavonoid to the ears of mice and measurement of ear edema

For measuring ear edema and reverse transcription–polymerase chain reaction (RT–PCR) analysis, 5 mice/group were used. On the day of experiment (day 1), TPA (3 μg/20 μL acetone) was applied to the inner and outer surfaces of mouse ear for inducing sub-chronic inflammation according to the previously described [19]. Test compounds dissolved in oil-based vehicle were topically applied to the same site (50–200 μg/20 μL) at 1 and 12 hr after TPA treatment. Control group only received TPA and vehicle. On next day, same treatment regimen was carried out with TPA and test compounds. On day 3, TPA was applied, and 1 hr later, test compounds were treated. The thickness of both ears of mice was measured using engineering gauge (Lux Scientific Instrument) at 3 hr after final treatment of test compounds. Immediately after, mice were sacrificed. Ears of 3 randomly selected mice/group were removed and stored in RNA stabilization reagent (Qiagen) at −20° for RT–PCR analysis. For determination of PGE_2 concentration, a separate experiment was carried out using 4 mice/group. TPA and test compounds/vehicle were applied with the same treatment schedule as the above study and ears were removed 5 hr after final application of test compounds. A biopsy obtained by 4-mm punch was subjected to homogenization as described below. In order to examine the effect on the intact skin, wogonin in the vehicle was treated as same as above treatment schedule without TPA treatment. In this case, the control group received acetone and vehicle only. Ear thickness was measured in all animal groups, but no significant change was observed for 3 days.

For producing acute-type skin inflammation, 2% AA (10 μL/ear) dissolved in acetone was topically applied to ears of 5 mice/group based on the previously described procedure [21] and 5 min later, test compounds in oil-based vehicle were smeared to the same site. One hour after AA treatment, the ear thickness was measured. Mice were sacrificed and ear was removed. The following RT–PCR procedure was used.

1.4. RT–PCR analysis

After cutting into small pieces, ear samples were homogenized in guanidine thiocyanate-based RLT buffer (Qiagen) containing 1% β-mercaptoethanol for 30 s using Polytron homogenizer. Total RNA was extracted using RNeasy mini kit (Qiagen) according to the supplier's protocol. The concentration of RNA content was determined by measuring the absorbance at 260 and 280 nm and stored at −70° until RT–PCR analysis. cDNAs were synthesized using RT reaction at 42°, 50 min and 99°, 5 min in Gene Cycler thermal cycler (Bio-Rad). Primers were synthesized on the basis of the repeated mouse cDNA sequence for COX-1, COX-2, IL-1β, TNF-α, iNOS, ICAM-1, fibronectin, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The primer sequences used for PCR were as follows: COX-1: sense, 5′-TGC ATG TGG CTG TGG ATG TCA TCA A-3′, antisense, 5′-CAC TAA GAC AGA CCC GTC ATC TCC A-3′, 450 bp; COX-2: sense, 5′-ACT CAC TCA GTT TGT TGA GTC ATT C-3′, antisense, 5′-TTT GAT TAG TAC TGT AGG GTT AAT G-3′, 583 bp; IL-1β: sense, 5′-TGC AGA GTT CCC CAA CTG GTA CAT C-3′, antisense, 5′-GTG CTG CCT AAT GTC CCC TTG AAT C-3′, 387 bp; TNF-α: sense, 5′-ACA AGC CTG TAG CCC ACG-3′, antisense, 5′-TCC AAA GTA GAC CTG CCC-3′, 428 bp; iNOS: sense, 5′-CCC TTC CGA AGT TTC TGG CAG CAG C-3′, antisense, 5′-GGC TGT CAG AGC CTC GTG GCT TTG G-3′, 469 bp; ICAM-1: sense, 5′-TCG GAG GAT CAC AAA CGA AGC-3′, antisense, 5′-AAC ATA AGA GGC TGC CAT CAC G-3′, 471 bp; fibronectin: sense, 5′-GCA ACG TGT TAT GAC GAT GG-3′, antisense, 5′-CTA ACG GCA TGA AGC ACT CA-3′, 253 bp; G3PDH: sense, 5′-TGA AGG TCG GTG TGA ACG GAT TTG GC-3′, antisense, 5′-CAT GTA GGC CAT GAG GTC CAC CAC-3′, 983 bp. The amplification was performed at 94° for 15–60 s, at 50–68° for 30–60 s, and at 72° for 45–90 s with 25 cycles for IL-1β and 30 cycles for other genes under saturation, in 25 μL reaction mixture. After amplification, 5 μL of reaction mixture was analyzed on 1.5% agarose gel electrophoresis. The bands were visualized by ethidium bromide staining for 10 min. The band density was quantified by densitometric scanning using SigmaGel (Version 1.0, Jandel Sci.). The signal intensities were normalized by comparing with that of G3PDH and represented as relative ratios.

1.5. Measurement of PGE_2 concentration

As an index of skin COX activity, PGE_2 concentration was measured essentially following the previously described procedure [19]. In brief, a biopsy was homogenized in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 10 μM indomethacin. After centrifugation at 1500 *g* for 10 min, 50 mM of citrate buffer (pH 3.5) was added to the supernatant. The mixture was centrifuged again at 2500 *g* for 10 min. The resulting supernatant was applied to a 6 mL Sep-Pak C_{18} cartridge (Waters Associate) and eluted with 5 mL ethyl acetate containing 1% methanol. The eluent was dried under N_2 stream and PGE_2 concentration was measured with an enzyme-linked immunoabsorbant assay (ELISA) kit (Cayman Chem.) according to the manufacturer's instruction.

1.6. Statistical analysis

All results were represented as arithmetic mean ± SD. One-way ANOVA test was used for evaluation of statistical significance.

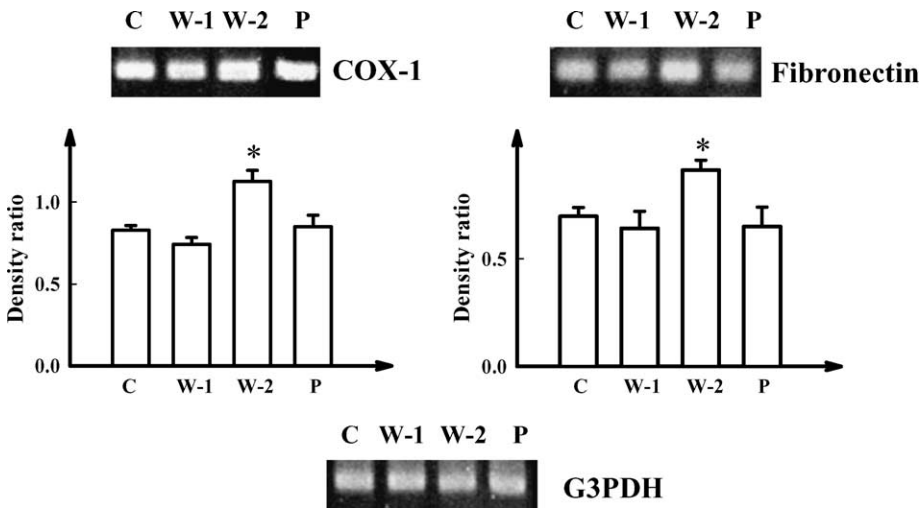
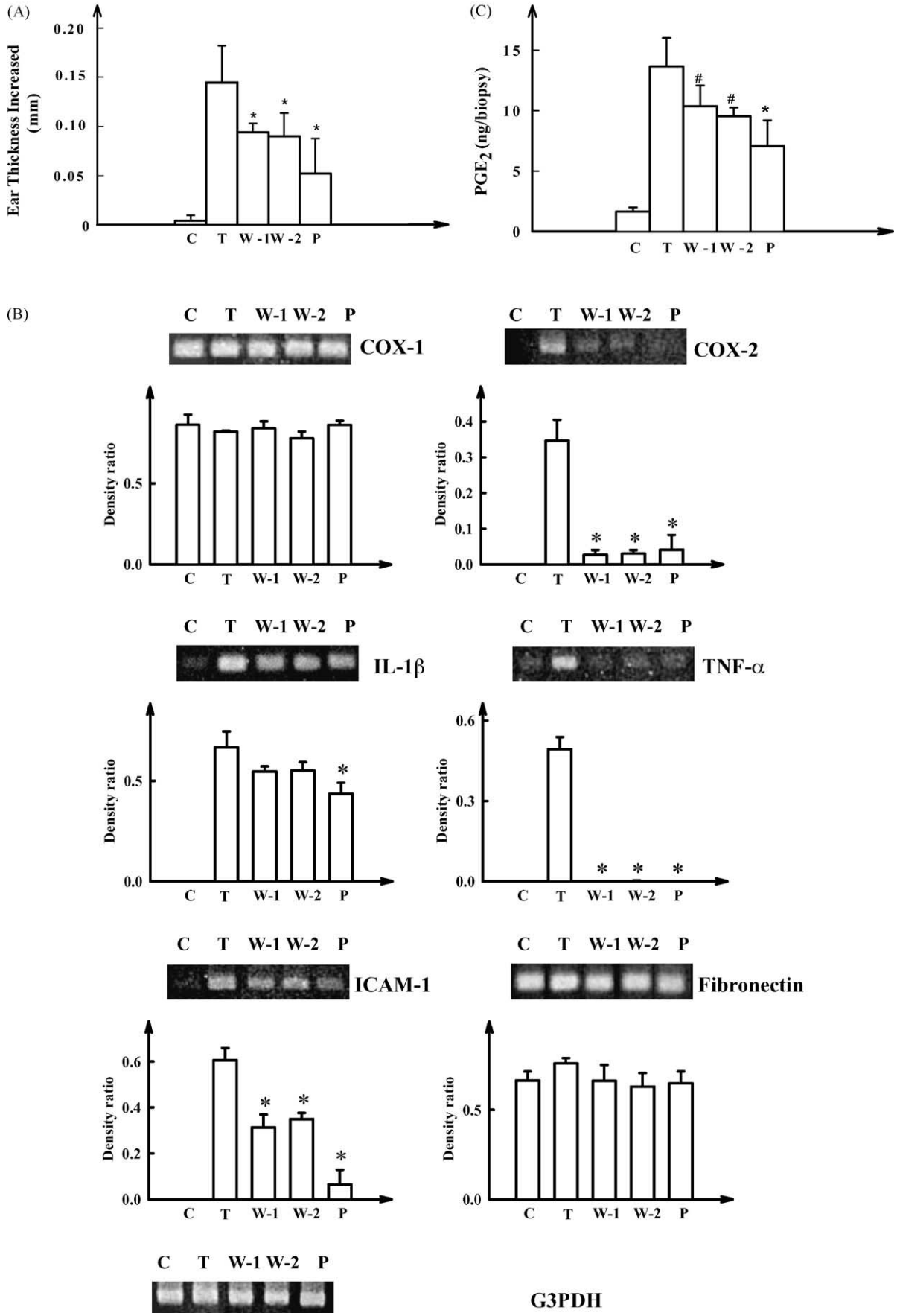


Fig. 2. Effect of wogonin on the intact skin. Test compounds or vehicle were topically applied to the intact ear skin of 5 mice/group for 3 consecutive days. The each ear sample removed from 3 mice/group was homogenized and RNA was obtained as described in Section 1. One representative PCR result among three analyses from each animal group was shown here. The ratio of band density compared to G3PDH was represented as arithmetic mean ± SD (N = 3). DNA bands for the inducible genes, including COX-2, IL-1β, TNF-α, ICAM-1, and iNOS, were not detected. C: control (vehicle), W-1: wogonin (250 μg/ear/3 days), W-2: wogonin (1000 μg/ear/3 days), P: prednisolone (250 μg/ear/3 days). *: $P < 0.05$, significantly different from the control group.


(A)
Ear Thickness Increased (mm)
0.20
0.15
0.10
0.05
0
C T W-1 W-2 P
(C)
PGE2 (ng/biopsy)
15
10
5
0
C T W-1 W-2 P
(B)
C T W-1 W-2 P
COX-1
COX-2
Density ratio
0.5
0.0
0.4
0.3
0.2
0.1
IL-1β
TNF-α
0.6
0.3
ICAM-1
Fibronectin
G3PDH

2. Results

2.1. Control study

Initially, the effect of wogonin on the expression level of several inflammation-associated genes of the intact skin was checked using RT–PCR, and an edematic activity was determined by conventional ear thickness measurement. The vehicle-treated intact ear skin did not provoke mRNA expression of the inducible genes, COX-2, IL-1β, TNF-α, ICAM-1, and iNOS. Only the constitutive genes, including COX-1, fibronectin, and G3PDH, were detected (Fig. 2). When topically applied to the intact ear skin, wogonin (total dose, 250 μg/ear/3 days) did not produce any significant change of ear thickness (data not shown) and mRNA expression of the genes checked. In contrast, when a higher dose of wogonin was applied (total dose, 1000 μg/ear/3 days), COX-1 mRNA slightly increased by 36.2% with concomitant increase of fibronectin gene expression (30.8%). Prednisolone (total dose, 250 μg/ear/3 days), a reference anti-inflammatory steroid, did not change the levels of mRNA transcripts of the constitutive and the inducible genes checked.

2.2. TPA-induced ear inflammation (multiple treatment, sub-chronic study)

Multiple treatment of TPA with vehicle for 3 days gradually increased the ear thickness with simultaneous changes of gene expression. As shown in Fig. 3A, a treatment of wogonin (250 μg/ear/3 days) showed 34.7% anti-inflammatory activity at 52 hr after initial TPA treatment when judged by reduction of ear thickness. Prednisolone showed 63.9% inhibition at the same dose. At a dose of 1000 μg/ear/3 days, wogonin showed a slightly higher anti-inflammatory activity (38.2% inhibition at 52 hr), but not significantly different from that of the low dose treatment. Multiple treatment of TPA for 3 consecutive days resulted in the expression of COX-2, IL-1β, TNF-α, and ICAM-1, while mRNA levels of the constitutive genes were not significantly changed. Under these conditions, wogonin at 250 and 1000 μg/ear/3 days almost completely reduced mRNA transcripts of COX-2 (91.3 and 92.2% reduction, respectively) and TNF-α genes (99.4 and 99.6% reduction) as shown in Fig. 3B. The same compound moderately reduced ICAM-1 mRNA with less reduction by a high dose (48.4 and 42.4% reduction at 250 and 1000 μg/ear/3 days, respectively), while mRNA level of IL-1β was weakly reduced (17.4–18.0%). Consistent with the reduced COX-2 mRNA, wogonin reduced PGE_2 concentration by 27.3 and 34.3% at doses of 250 and 1000 μg/ear/3 days, respectively, compared to the TPA-treated control group (Fig. 3C). Wogonin did not significantly affect the expression level of the constitutive genes, COX-1, fibronectin, and G3PDH. Prednisolone (250 μg/ear/3 days) showed similar suppressive pattern of mRNA expression of the inducible genes as wogonin, but with some different potency of suppression on ICAM-1 expression. Prednisolone potently reduced mRNA transcripts of COX-2 (88.2% reduction), TNF-α (99.9%), and ICAM-1 (89.4%) with less effect on IL-1β (34.5%). Prednisolone also showed potent reduction of PGE_2 concentration by 55.0%. No apparent change of the expression level of the constitutive genes was observed by prednisolone treatment. iNOS mRNA was not detected in the intact skin as well as in the TPA-treated skin.

2.3. AA-induced ear inflammation (acute study)

Topically applied AA provoked ear edema peaking in approximately 1 hr (Fig. 4A). AA induced COX-2 and IL-1β expression (Fig. 4B). In contrast to the TPA-treated inflammatory response, TNF-α and ICAM-1 were not induced in this acute-type inflammation. The expression level of the constitutive genes, COX-1, fibronectin, and G3PDH, remained unchanged. When treated 5 min after AA application, wogonin greatly reduced ear edema (61.5 and 66.6% inhibition at 50 and 200 μg/ear, respectively). In addition, wogonin potently reduced COX-2 mRNA (51.0 and 53.2% reduction at 50 and 200 μg/ear, respectively). A weak reduction of IL-1β mRNA was also observed, but a higher dose treatment of wogonin (200 μg/ear) showed less reduction (27.5 and 12.1% reduction at 50 and 200 μg/ear, respectively). Wogonin did not show any change of the expression level of the constitutive genes, COX-1, fibronectin, and G3PDH. On the other hand, prednisolone (50 μg/ear) applied 5 min after AA treatment weakly reduced ear edema (22.9% inhibition). This weak activity of prednisolone was parallel with the weak reduction of COX-2 mRNA (28.5%). Prednisolone did not change mRNA expression of IL-1β noticeably. iNOS mRNA

Fig. 3. Effect of wogonin on TPA-induced ear edema (multiple treatment). TPA and test compounds/vehicle were topically applied to the ear skin of 5 mice/group. Ear edema was measured at 52 hr after initial treatment of TPA. Immediately after, mice were sacrificed and ears were removed for RT–PCR analysis. For PGE_2 measurement, TPA and wogonin/vehicle were treated to 4 mice/group with the same treatment schedule as ear edema study. Mice were sacrificed at 54 hr after initial treatment of TPA and the ear biopsies were obtained by 4-mm punch. C: control (vehicle), T: TPA, W-1: wogonin (250 μg/ear/3 days), W-2: wogonin (1000 μg/ear/3 days), P: prednisolone (250 μg/ear/3 days). $^{\#}$: $P < 0.1$, *: $P < 0.05$, significantly different from the TPA-treated group. (A) Inhibition of ear edema. The ear thickness increased of the TPA-treated group were 0.14 ± 0.04 mm at 52 hr. $N = 10$. (B) RT–PCR analysis. One representative PCR result among three analyses from each animal group was shown here. The ratio of band density compared to G3PDH was represented as arithmetic mean ± SD ($N = 3$). iNOS band was not detected. (C) Inhibition of PGE_2 production. PGE_2 concentration of the skin homogenate was measured with ELISA. The control and the TPA-treated skin gave 1.7 ± 0.3 ng and 13.7 ± 2.4 ng PGE_2/biopsy, respectively. $N = 4$.

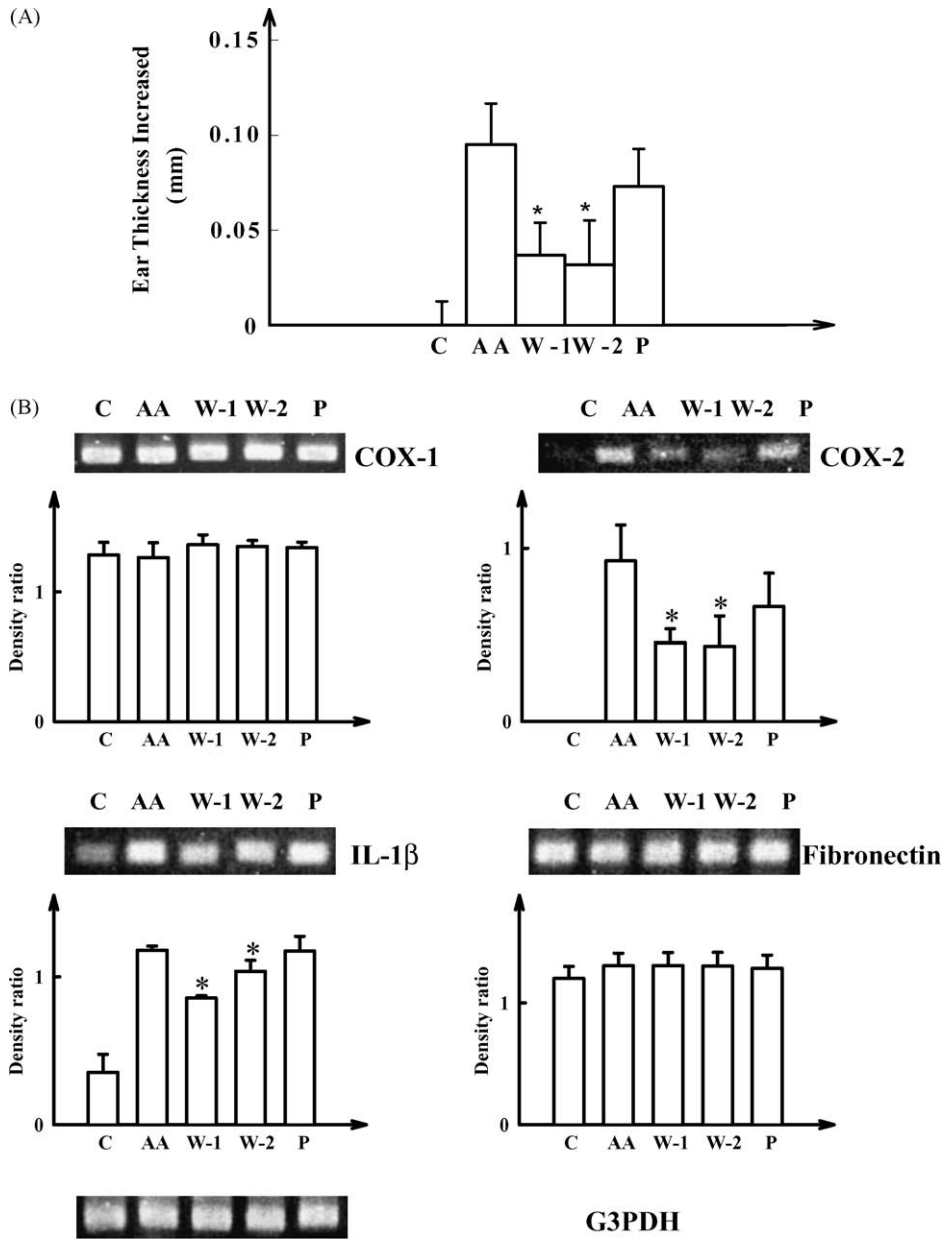


Fig. 4. Effect of wogonin on AA-induced ear edema. AA and test compounds/vehicle were topically applied to the ear skin of 5 mice/group. Ear edema was measured at 1 hr after AA treatment. Immediately after, mice were sacrificed and ears were removed for RT–PCR analysis. C: control (vehicle), AA: arachidonic acid, W-1: wogonin (50 μg/ear), W-2: wogonin (200 μg/ear), P: prednisolone (50 μg/ear). *: $P < 0.05$, significantly different from the AA-treated group. (A) Inhibition of ear edema. The ear thickness of the AA-treated group was 0.09 ± 0.02 mm. N = 10. (B) RT–PCR analysis. One representative PCR result among three analyses from each animal group was shown here. The ratio of band density compared to G3PDH was represented as arithmetic mean ± SD (N = 3). TNF-α, ICAM-1, and iNOS bands were not detected.

was not detected in the intact skin as well as in the AA-treated skin.

3. Discussion

Many lines of evidence from *in vitro* studies make clear that flavonoids are not just inhibitors of inflammation-associated enzymes, but expression regulators of inflammation-associated genes. Therefore, in order to find *in vivo* behavior of flavonoid, RT–PCR technique was used to measure mRNA level of several inflammation-associated genes, including COX-1, COX-2, IL-1β, TNF-α, ICAM-1, and fibronectin. When applied topically on the intact ear skin for 3 days, wogonin at a total dose of 1000 μg/ear/3 days slightly increased mRNA transcript of the constitutive genes, COX-1 and fibronectin. It was observed that quercetin (the most abundant flavonol in nature) also increased slightly mRNA transcript of COX-1 at the same dose (data not shown). As far as our best knowledge, this is the first report describing that certain flavonoids affect the expression level of the constitutive genes by application to the

intact skin. However, the physiological significance and the detailed molecular mechanism of the elevated expression of COX-1 and fibronectin by flavonoids are not understood. These points may be important and need to be investigated further since many cosmetic products contain plant extracts having flavonoids as their major constituents.

It is well known that TPA provokes an inflammatory response and initial hyperplasia of mouse skin. Even single treatment of TPA to mouse ear induced COX-2 expression, which was diminished by dexamethasone [22]. AA was also reported to induce COX-2 expression on mouse ear [22]. In another study, TPA induced the expression of COX-2, TNF-α, TGF-β1, and c-*fos* on mouse skin [18]. These previous findings are well correlated with the present investigation showing the expression of COX-2, IL-1β, TNF-α, and ICAM-1 by TPA, and COX-2 and IL-1β by AA. Wogonin showed the significant effects on the expression of these genes. In TPA-induced inflammation, wogonin potently reduced COX-2 and TNF-α gene expression while ICAM-1 and IL-1β were weakly affected. The same results were also observed in an acute-type model of AA-induced inflammation. Therefore, all findings from the present study strongly suggest that wogonin differentially affects the expression of inflammation-associated genes *in vivo*, although the possibility of wogonin to alter mRNA stability is not totally excluded.

It was previously observed that topically applied wogonin (250–1000 μg/3 days) produced dose-dependent inhibitory effects on TPA-induced ear edema, COX-2 expression, and PGE_2 production of the dorsal skin of mice [19]. In this present investigation, treatment of wogonin with the low (250 μg/3 days) and the high dose (1000 μg/3 days) showed strong suppressive activity on induction of inflammation-associated genes, including COX-2, but did not produce any noticeable difference between wogonin-treated groups although the high dose treatment exhibited slightly higher inhibition of ear edema and PGE_2 production. The inconsistent results obtained from these two studies may be partly explained by the difference of skin tissues used. The penetration of wogonin through ear skin may be easier than that through the dorsal skin. Thus, the low dose treatment of wogonin might be enough to show maximum suppression of proinflammatory gene expression. The different treatment schedules and the different times for end point measurement might also cause the different results. In the previous study [19], wogonin was treated 2 hr after final TPA treatment. And COX-2 expression was measured by Western blotting at 8 hr since the time to attain maximum COX-2 expression was 6–8 hr after final TPA treatment. In the present study, wogonin was treated 1 hr after final TPA treatment and mRNA expression was checked by RT–PCR at 4 hr since the average time for maximum expression of proinflammatory genes was found as 1–4 hr after final TPA treatment in the preliminary experiment.

In respects of down-regulation of inflammation-associated genes, wogonin behaves very similarly with a steroidal anti-inflammatory drug, prednisolone, despite of some different sensitivity. However, the cellular action mechanisms of these two compounds are thought to be different. Steroidal anti-inflammatory drugs are known to exert anti-inflammatory activity mainly by binding to their glucocorticoid receptor [23]. Indeed, a steroidal receptor antagonist (Ru-486) reversed the suppressing effect of prednisolone on COX-2 and iNOS expression in lipopolysaccharide-induced RAW 264.7 cells. In contrast, the suppressive effect of wogonin was not changed in the presence of Ru-486 [14]. And it was shown from the present study that prednisolone weakly inhibited AA-induced ear edema whereas wogonin potently inhibited. All these findings indicate that *in vivo* action mechanisms of prednisolone and wogonin are quite different. Although wogonin is known to regulate the activation of transcription factors, such as activator protein-1 and nuclear transcription factor-κB [24,25], it is not clear why this compound reduces mRNA levels of COX-2 and TNF-α genes more strongly than those of IL-1β and ICAM-1. This difference may occur from the inappropriate sampling time due to the different maximum induction time for these inducible genes. However, as noted above, the time to reach maximum mRNA expression of these inducible genes was 1–4 hr, and the expression levels of these genes were well maintained through 4–6 hr after final TPA treatment (data not shown). Our sampling time (4 hr) was within the range of maximum expression. So the possibility due to the different maximum expression time could be excluded. The other possible explanation is that the different sensitivity may occur from the difference of intrinsic sensitivity of wogonin in suppressing transcription of certain genes due to their different transcription factors and/or signal transduction pathways involved depending on each gene. For instance, COX-2 gene has binding sites for transcriptional factors of activator protein-1 and -2, nuclear transcription factor-κB, NF-IL6, and CRE [26,27], while IL-1β gene has some different sites for transcription factors of nuclear transcription factor-κB, NF-IL6, LPS-IL-1-inducible-STAT, and CRE [28]. Therefore, *in vivo* susceptibility of wogonin may be different depending on the inducible genes affected and needs to be further studied.

Previously, wogonin showed *in vivo* anti-inflammatory activity on several animal models of inflammation, including carrageenan-induced paw edema and adjuvant-induced arthritis in rats, by oral administration [29]. Wogonin reduced TPA-induced skin inflammation in mice by topical application [30]. Wogonin was also reported to reduce TNF-α production in lipopolysaccharide plus galactosamine-injected mice [31]. Among varieties of flavonoid molecules, wogonin was found to be one of the most potent suppressors of COX-2 and iNOS expression from macrophages in culture [12–14]. Therefore, from the results of the present investigation, it is suggested that the modulation of proinflammatory gene expression may be one of the *in vivo* action mechanisms of anti-inflammation by wogonin.

And certain flavonoids may become useful therapeutic agents not only for acute inflammation but also for chronic inflammatory diseases especially in the skin. In addition, many plant extracts having flavonoids are used in cosmetics and they are proved to reduce skin inflammation. However, regardless of single compound or mixture, *in vivo* regulatory effect on proinflammatory gene expression of skin inflammation has been rarely demonstrated. Therefore, further characterization of the effects of structurally diverse flavonoids on different animal models of skin inflammation is necessary to establish the detailed action mechanism(s) and pharmacological significance.

Acknowledgments

This research was supported by the research grant (02-PJ2-PG3-20905-0006) by the Ministry of Health and Welfare, South Korea, and greatly acknowledged.

References

[1] Abd-El-Aleem SA, Ferguson MW, Appleton I, Bhowmick A, McCollum CN, Ireland GW. Expression of cyclooxygenase isoforms in normal human skin and chronic venous ulcers. J Pathol 2001;195: 616–23.

[2] Bruch-Gerharz D, Fehsel K, Suschek C, Michel G, Ruzicka T, Kolb-Bachofen V. A proinflammatory activity of interleukin-8 in human skin: expression of the inducible nitric oxide synthase in psoriatic lesions and cultured keratinocytes. J Exp Med 1996;184:2007–12.

[3] Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V. Nitric oxide in human skin: current status and future prospects. J Invest Dermatol 1998;110:1–7.

[4] LaDucca JR, Caspari AA. Targeting tumor necrosis factor alpha. New drugs used modulate inflammatory diseases. Dermatol Clin 2001;19: 617–35.

[5] Veale D, Rogers S, Fitzgerald O. Immunolocalization of adhesion molecules in psoriatic arthritis, psoriatic and normal skin. Br J Dermatol 1995;132:32–8.

[6] Morita H, Hori M, Kitano Y. Modulation of picryl chloride-induced contact hypersensitivity reaction in mice by nitric oxide. J Invest Dermatol 1996;107:549–52.

[7] Zhao J, Sharma Y, Agarwal R. Significant inhibition by the flavonoid antioxidant silymarin against 12-*O*-tetradecanoylphorbol 13-acetate-caused modulation of antioxidant and inflammatory enzymes, and cyclooxygenase-2 and interleukin-1 expression in SENCAR mouse epidermis: implications in the prevention of stage 1 tumor promotion. Mol Carcinog 1999;26:321–30.

[8] Neufang G, Furstenberger G, Heidt M, Marks F, Muller-Decker K. Abnormal differentiation of epidermis in transgenic mice constitutively expressing cyclooxygenase-2 in skin. Proc Natl Acad Sci USA 2001;98:7629–34.

[9] Kim HP, Son KH, Chang HW, Kang SS. Effects of naturally occurring flavonoids on inflammatory responses and their action mechanisms. Nat Prod Sci (Korea) 2000;6:170–8.

[10] Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, editor. The flavonoids: advances in research since 1986. London: Chapman & Hall; 1993. p. 619–52 [chapter 15].

[11] Krol W, Czuba ZP, Threadgill MD, Cunningham BD, Pietse G. Inhibition of nitric oxide (NO) production in murine macrophages by flavones. Biochem Pharmacol 1995;50:1031–5.

[12] Kim HK, Cheon BS, Kim YH, Kim SY, Kim HP. Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure–activity relationships. Biochem Pharmacol 1999;58:759–65.

[13] Liang Y-C, Huang Y-T, Tsai S-H, Shiau S-Y, Chen C-F, Lin J-F. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. Carcinogenesis 1999;20:1945–52.

[14] Chi YS, Cheon BS, Kim HP. Effect of wogonin, a plant flavone from *Scutellaria radix*, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cells. Biochem Pharmacol 2001;61:1195–203.

[15] Cho JY, Kim PS, Park J, Yoo ES, Baik KU, Kim Y-K, Park MH. Inhibitor of tumor necrosis factor-α production in lipopolysaccharide-stimulated RAW 264.7 cells from *Amorpha fruticosa*. J Ethnopharmacol 2000;70:127–33.

[16] Gerritsen ME, Carley WW, Ranges GE, Shen C-P, Phan SA, Ligon GF, Perry CA. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. Am J Pathol 1995;147:278–92.

[17] Krakauer T, Li BQ, Young HA. The flavonoid baicalin inhibits superantigen-induced inflammatory cytokines and chemokines. FEBS Lett 2001;500:52–5.

[18] Jang M, Pezzuto JM. Effects of resveratrol on 12-*O*-tetradecanoylphorbol-13-acetate-induced oxidative events and gene expression in mouse skin. Cancer Lett 1998;134:81–9.

[19] Park BK, Heo MY, Pak H, Kim HP. Inhibition of TPA-induced cyclooxygenase-2 and skin inflammation in mice by wogonin, a plant flavone from *Scutellaria radix*. Eur J Pharmacol 2001;425:153–7.

[20] You KM, Jong H, Kim HP. Inhibition of cyclooxygenase/lipoxygenase from human platelets by polyhydroxylated/methoxylated flavonoids isolated from the several medicinal plants. Arch Pharmacol Res 1999;22:18–24.

[21] Young JM, Spires DA, Bedord CJ, Wagner B, Ballaron SJ, De Young LH. The mice ear inflammatory response to topical arachidonic acid. J Invest Dermatol 1984;82:367–71.

[22] Sanchez T, Moreno JJ. Role of prostaglandin H synthase isoform in murine ear edema induced by phorbol ester application on skin. Prostaglandins Other Lipid Mediat 1999;57:119–31.

[23] Goppelt-Struebe M. Molecular mechanisms involved in the regulation of prostaglandin biosynthesis by flucocorticoids. Biochem Pharmacol 1997;53:1389–95.

[24] Chang Y-L, Shen J-J, Wung B-S, Cheng J-J, Wang D-L. Chinese herbal remedy wogonin inhibits monocyte chemotactic protein-1 gene expression in human endothelial cells. Mol Pharmacol 2001;60:507–13.

[25] Kim H, Kim YS, Kim SY, Suk K. The plant flavonoid wogonin suppresses death of activated C6 rat glial cells by inhibiting nitric oxide production. Neurosci Lett 2001;309:67–71.

[26] Adderley SR, Fitzgerald DJ. Oxidative damage of cadiomyocytes is limited by extracellular regulated kinase 1/2-mediated induction of cyclooxygenase-2. J Biol Chem 1999;274:5038–46.

[27] Appleby SB, Ristimaki A, Neilson K, Narko K, Hla T. Structure of the human cyclooxygenase-2 gene. Biochem J 1994;302:723–7.

[28] Tsukada J, Saito K, Waterman WR, Webb AC, Auron PE. Transcription factors NF-IL-6 and CREB recognize a common essential site in the human prointerleukin 1β gene. Mol Cell Biol 1994;14:7285–97.

[29] Kubo M, Matsuda H, Tanaka M, Kimura Y, Okuda H, Higashino M, Tani T, Namba K, Arichi S. Studies on *Scutellaria radix*. VII. Anti-arthritic and anti-inflammatory actions of methanol extract and flavonoid components from *Scutellaria radix*. Chem Pharm Bull 1984;32:2724–9.

[30] Yasukawa K, Takido M, Takeuchi M, Nakagawa S. Effect of chemical constituents from plants on 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation in mice. Chem Pharm Bull 1989;37:1071–3.

[31] Dien MV, Takahashi K, Mu MM, Koide N, Sugiyama T, Mori I, Yoshida T, Yokochi T. Protective effect of wogonin on endotoxin-induced lethal shock in D-galactosamine-sensitized mice. Microbiol Immunol 2001;45:751–6.