Inhibition of Cyclooxygenase/Lipoxygenase from Human Platelets by Polyhydroxylated/Methoxylated Flavonoids Isolated from Medicinal Plants

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Various flavonoid derivatives were previously reported to possess the inhibitory activity on cyclooxygenase/lipoxygenase. And these properties of flavonoids might contribute to their anti-inflammatory activity *in vivo*. In this study, several polyhydroxylated/methoxylated flavonoid derivatives such as oroxylin A, wogonin, skullcapflavone II, tectorigenin and iristectorigenin A were isolated from the medicinal plants. These compounds were evaluated for their inhibitory effects on cyclooxygenase/lipoxygenase from the homogenate of human platelets *in vitro*. It was found that isoflavones including daidzein and tectorigenin possessed the inhibitory activity on cyclooxygenase, although the potency of inhibition was far less than that of indomethacin. In addition, oroxylin A, baicalein and wogonin inhibited 12-lipoxygenase activity without affecting cyclooxygenase, which suggested that 5,6,7- or 5,7,8-trisubstitutions of A-ring of flavone gave favorable results. The IC_{50} values of oroxylin A and NDGA aginst 12-lipoxygenase were found to be 100 and 1.5 uM, respectively.

Key words : Flavonoid, Cyclooxygenase, Lipoxygenase, Platelet, Oroxylin A, Tectorigenin

INTRODUCTION

Flavonoids are widely distributed polyphenol compounds in nature. They are known as nature's tender drugs. Flavonoids were reported to possess a variety of biological activities such as anti-inflammation and anticancer (Havsteen, 1983; Middleton and Kandaswami, 1992; Kim *et al.*, 1996). Among these activities, anti-inflammation and enhanced microcirculation of flavonoids are suggested to be mediated, at least in part, by inhibition of cyclooxygenase (CO) and/or lipoxygenase (LO) since the products of CO/LO, eicosanoids, exert a wide spectrum of biological activities including inflammation. From many previous investigations, mainly flavones/flavonols and some prenylated flavonoids were revealed to inhibit these eicosanoid generating enzymes depending on the concentrations (Baumann *et al.*, 1980; Kimura *et al.*, 1986; Welton *et al.*, 1988; Reddy *et al.*, 1991). Quercetin, but not other flavonols, showed the weak inhibitory activity on CO from blood platelets (Ferrandiz *et al.*, 1990). Some flavones such as chrysin, 3-hydroxyflavone and galangin were reported to be CO inhibitors when measured thromboxane B_2 (TXB_2) formation from the A-23187 stimulated leukocytes (Laughton *et al.*, 1991). Recently, Kim *et al.* (1998) found that amentoflavone, a biflavonoid, was a selective CO inhibitor comparable to indomethacin. In contrast to a few reports describing the inhibitory activity on CO, various flavonoid derivatives inhibited lipoxygenases. Flavonol derivatives including fisetin, kaempferol and quercetin were found to be inhibitors of 5-LO (Laughton *et al.*, 1991). Cirsiliol was a relatively specific 5-LO inhibitor (Yamamoto *et al.*, 1984). Several prenylated flavones such as artonin E showed potent inhibitory activity on 5-, 12 and 15-LOs (Kimura *et al.*, 1986; Reddy *et al.*, 1991). Baicalein, but not other flavones from Scutellaria radix, was reported to be a selective 12-LO inhibitor (Sekiya and Okuda, 1982). All of these previous investigations indicated that some flavonoids inhibited the eicosanoid generating enzyme activities, showing more preferred inhibition on LO rather than CO. And a 2,3-double bond in C-ring of flavonoid molecule might be important for inhibition of CO/LO while the potencies of inhibition were depending on the hydroxylation and methoxylation patterns of A and B rings of flavonoids. Therefore, it may be meaningful to elucidate the inhibitory potential of polyhydroxylated/methoxylated flavonoids having a C-2,3-double bond on CO/LO. In this investigation, flavone/flavonol/isoflavone derivatives having a C-2,3-double bond as well as polyhydroxyl/methoxyl groups were isolated

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from the several medicinal plants. And their effects were evaluated on CO/LO from human platelets as a part of our continuing efforts for finding new anti-inflammatory flavonoid derivatives. The structural-activity relationships were also described.

MATERIALS AND METHODS

Materials

Plant materials used in this study were purchased from Kyung Dong crude drug market in Seoul, Korea. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian 200 MHz NMR using TMS as an internal standard. Mass spectrum was obtained with Hewlett Packard GC/MS system. The purity of the compounds was checked using TLC (Kiesel gel F254 glass plate, Merck). [^{14}C] Arachidonic acid (AA, 55 mCi/mmol) was obtained from NEN (Boston, MA). Prostaglandin E_2 (PGE_2), TXB_2, $PGF_{2\alpha}$, 5-, 12-, and 15-hydroxyeicosatetraenoic acid (HETE) were the products of Cayman Chem. Co. (Ann Arbor, MI). Quercetin and apigenin were the products of Aldrich Chem. Co. (Milwakee, MI). Daidzein was isolated according to the previously described (Lee *et al.*, 1994). Arachidonic acid (99%), indomethacin and nordihydroguaiaretic acid (NDGA) were from Sigma Chem. Co. (St. Loius, MO). All other chemicals were the highest grade reagents available.

Isolation of flavonoid derivatives (Fig. 1)

Viticis fructus (*Vitex rotundifolia*) was extracted with n-hexane and discarded. The residue was re-extracted with acetone twice and evaporated to dryness. Vitexicarpin was obtained using SiO_2 column with chloroform: methanol (99:1) as a mobile phase following the previously published procedure of Shin *et al.* (1994).

Vitexicarpin (3',5-dihydroxy-3,4',6,7-tetramethoxyflavone): Recrystallized from acetone and directly compared with an authentic sample., ^{1}H-NMR (DMSO-d_6) δ 12.60 (1H, s, 5-OH), 9.37 (1H, s, 3'-OH), 7.59 (1H, d, *J*=2.0 Hz, H-2'), 7.58 (1H, dd, *J*=2.4 and 9.0 Hz, H-6'), 7.10 (1H, d, *J*=9.3 Hz, H-5'), 6.86 (1H, s, H-8), 3.74, 3.81, 3.87 and 3.92 (12H, 4s, 4×-OCH_3)., ^{13}C-NMR (DMSO-d_6) δ 155.56 (C-2), 137.94 (C-3), 178.20 (C-4), 151.71 (C-5), 131.58 (C-6), 158.63 (C-7), 91.24 (C-8), 151.61 (C-9), 105.56 (C-10), 122.19 (C-1'), 115.05 (C-2'), 146.33 (C-3'), 150.26 (C-4'), 111.82 (C-5'), 120.33 (C-6'), 55.56, 56.40, 59.64, 59.97 (4×-OCH_3).

Scutellaria radix was refluxed in methanol for 5 hrs. This procedure was repeated three times. After evaporation, the residue was partitioned in diethyl ether/water. Ether fraction was evaporated and redissolved in small amount of ether to allow crystallization. Crystals

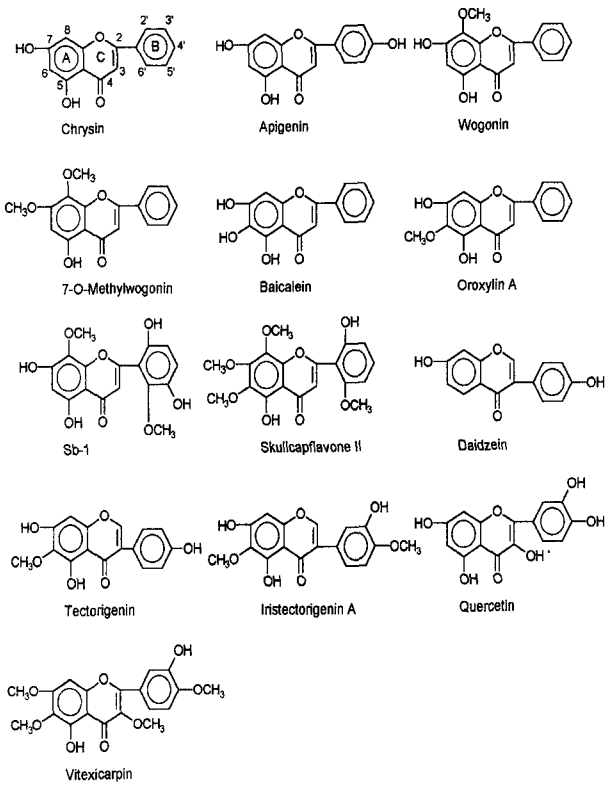

Fig. 1. Chemical structures of flavonoids used in this study.

from ether fraction containing baicalein and wogonin were dissolved in small amount of methanol and chromatographed on RP-18 column (40~63 um, Merck) using methanol:water (4:1) as a mobile phase to yield baicalein. The supernatant obtained from ether fraction after crystallization was dried and poured onto SiO_2 column. Using chloroform:methanol gradient (97:3→ 95:5), oroxylin A, wogonin, skullcapflavone II, chrysin and 2',5,5',7-tetrahydroxy-6',8-dimethoxyflavone (sb-1) were successfully isolated. Wogonin was methylated using methyl iodide in the presence of K_2CO_3 at 30°C for 3 days to yield 7-O-methylwogonin according to the procedure of Lee *et al.* (1994).

Oroxylin A (5,7-dihydroxy-6-methoxyflavone): Recrystallized from acetone, yellow needles, m.p.=186~188 °C, ^{1}H-NMR (DMSO-d_6) δ 12.96 (1H, s, 5-OH), 7.59~8.12 (5H, m, phenyl), 7.0 (1H, s, H-8), 6.67 (1H, s, H-3), 3.78 (3H, s, -OCH_3). Peaks of 7.59~8.12 were decoupled., MS m/z 284 (M^+), 269 (M^+-CH_3), 241 (M^+-CH_3-CO), 167, 139.

Wogonin (5,7-dihydroxy-8-methoxyflavone): Recrystallized from acetone, yellow needles, m.p.=201~204°C, ^{1}H-NMR (DMSO-d_6) δ 12.51 (1H, s, 5-OH), 10.83 (1H, brs, 7-OH), 7.57~8.10 (5H, m, phenyl), 7.0 (1H, s, H-6), 6.31 (1H, s, H-3), 3.85 (3H, s, -OCH_3)., ^{13}C-NMR (DMSO-d_6) δ 163.0 (C-2), 105.0 (C-3), 182.1 (C-4), 157.3 (C-5), 99.0 (C-6), 149.6 (C-7), 127.7 (C-8), 156.2 (C-9), 103.6 (C-10), 130.8 (C-1'), 126.2 (C-2'

and 6'), 129.2 (C-3' and 5'), 132.0 (C-4').

Skullcapflavone II (2',5-dihydroxy-6,6',7,8-tetramethoxyflavone): Recrystallized from acetone, yellow needles, m.p.=171~174°C, ^{1}H-NMR (DMSO-d_6) δ 12.64 (1H, s, 5-OH), 10.17 (1H, s, 2'-OH), 7.32 (1H, t, *J*=8.4 Hz, H-4'), 6.62 (2H, d, *J*=8.4 Hz, H-3' and 5'), 6.34 (1H, s, H-3), 4.0, 3.81, 3.78 and 3.74 (12H, 4s, 4×-OCH_3)., MS m/z 374 (M^+), 359 (M^+-CH_3), 211, 183.

Chrysin (5,7-dihydroxyflavone): Recrystallized from acetone, yellow needles, directly compared with an authentic sample from Aldrich Chem. Co., ^{1}H-NMR (DMSO-d_6) δ 12.85 (1H, s, 5-OH), 7.59-8.11 (5H, m, phenyl), 7.0 (1H, s, H-3), 6.54 (1H, d, *J*=1.6 Hz, H-8), 6.23 (1H, d, *J*=1.6 Hz, H-6).

2',5,5',7-Tetrahydroxy-6',8-dimethoxyflavone (sb-1): Recrystallized from acetone, yellow powders, m.p.> 240°C, ^{1}H-NMR (DMSO-d_6) δ 12.57 (1H, s, 5-OH), 10.79, 9.46 and 9.06 (3H, 3s, 3×-OH), 6.90 and 6.59 (2H, 2d, J=8.8 Hz, H-3'and 4', not assigned), 6.33 and 6.29 (2H, 2s, H-3 and 6, not assigned), 3.75 and 3.72 (6H, 2s, 2×-OCH_3)., ^{13}C-NMR (DMSO-d_6) δ 161.7 (C-2), 111.0 (C-3), 181.8 (C-4), 157.1 (C-5), 98.9 (C-6), 150.3 (C-7), 127.5 (C-8), 156.2 (C-9), 103.5 (C-10), 114.7 (C-1'), 148.2 (C-2'), 111.5 (C-3), 119.7 (C-4'), 142.4 (C-5'), 145.9 (C-6'), 60.7 and 60.3 (2×-OCH_3).

Baicalein (5,6,7-trihydroxyflavone): Recrystallized from ether, yellow needles, m.p.>240°C, Directly compared with an authentic sample from Biomol Res. Lab. (PA, USA)., ^{1}H-NMR (DMSO-d_6) δ 7.41~7.86 (5H, m, phenyl), 7.27 (1H, s, H-8), 6.50 (1H, s, H-3)., Peaks of 7.41~7.86 were decoupled.

7-O-Methylwogonin (5-hydroxy-7,8-dimethoxyflavone): Recrystallized from acetone, yellow needles, m.p.=183~184°C, ^{1}H-NMR (DMSO-d_6) δ 12.67 (1H, s, 5-OH), 7.62~8.12 (5H, m, phenyl), 7.06 (1H, s, H-3), 6.64 (1H, s, H-6), 3.94 and 3.86 (6H, 2s, 2×-OCH_3).

From Belamcandae rhizoma (*Belamcanda chinensis*), methanol extract was obtained and dried. The residue was partitioned in chloroform/water. Chloroform fraction was evaporated and poured onto SiO_2 column. Chloroform:methanol gradient (97:3→95:5) was used to yield iristectorigenin A and tectorigenin according to the procedure of Lee *et al.* (1989) and Yamaki *et al.* (1990).

Tectorigenin (4',5,7-trihydroxy-6-methoxyisoflavone): Recrystallized from acetone, slightly yellowish needles, m.p.=228~230°C, ^{1}H-NMR (DMSO-d_6) δ 8.36 (1H, s, H-2), 7.39 (2H, d, *J*=8.4 Hz, H-2' and 6'), 6.83 (2H, d, *J*=8.4 Hz, H-3' and 5'), 6.52 (1H, s, H-8), 3.76 (3H, s, -OCH_3)., ^{13}C-NMR (DMSO-d_6) δ 154.1 (C-2), 121.7 (C-3), 180.6 (C-4), 152.7 (C-5), 131.5 (C-6), 153.2 (C-7), 93.7 (C-8), 157.4 (C-9), 104.7 (C-10), 121.1 (C-1'), 130.1 (C-2'), 114.9 (C-3'), 157.4 (C-4'), 114.9 (C-5'), 130.1 (C-6'), 59.8 (-OCH_3).

Iristectorigenin A (3',5,7-trihydroxy-4',6-dimethoxyisoflavone): Recrystallized from acetone, slightly yellowish needles, m.p.=234~236°C, ^{1}H-NMR (DMSO-d_6) δ 13.10 (1H, s, 5-OH), 8.40 (1H, s, H-2), 7.15 (1H, d, *J* =1.8 Hz, H-2'), 7.0 (1H, dd, *J*=1.8 and 8.0 Hz, H-6'), 6.84 (1H, d, *J*=8.0 Hz, H-5'), 6.53 (1H, s, H-8), 3.81 and 3.77 (6H, 2s, 2×-OCH_3)., ^{13}C-NMR (DMSO-d_6) δ 154.31 (C-2), 121.60 (C-3), 180.53 (C-4), 152.65 (C-5), 131.32 (C-6), 157.43 (C-7), 93.72 (C-8), 153.25 (C-9), 104.73 (C-10), 121.75 (C-1'), 113.16 (C-2'), 147.19 (C-3'), 146.63 (C-4'), 115.15 (C-5'), 121.56 (C-6'), 59.78 and 55.53 (-OCH_3).

Preparation of platelet homogenate from human blood

From nonsmoking male healthy volunteers who had not taken nonsteroidal anti-inflammatory drugs for recent two weeks, blood was withdrawn with 3.8% sodium citrate (10% v/v) and immediately centrifuged to obtain platelet rich plasma (PRP). PRP was centrifuged again at 1,000 g for 10 min and the precipitated platelets were washed twice with 25 mM Tris-HCl buffer containing 1 mM EDTA (pH 7.4). The platelet homogenate were obtained by sonication for 3 sec three times on ice using probe sonicator. After determination of protein concentration with Bio-Rad protein assay kit, the platelet homogenate was used as CO/LO source without further purification.

Cyclooxygenase assay

Incubation mixture consisted of 0.01 μCi [^{14}C]AA and 100 μg protein of platelet homogenate in 100 mM Tris-HCl buffer, pH 8.0, with 5 mM EDTA, 2 mM reduced glutathione, 50 mM L-tryptophan and 2 μM hemoglobin with or without testing compounds. Indomethacin or each flavonoid was dissolved in DMSO and diluted to appropriate concentration with above buffer solution. Each compound tested was added to the incubation mixture before adding platelet homogenate. Same amount of DMSO was added in control incubation mixture. Final concentration of DMSO was 0.1% (v/v). Total incubation mixture was 100 μl/tube. The mixture was incubated at 37°C for 20 min, and the reaction was terminated with 50 μl ice-cold 0.15 N HCl. Chloroform:methanol (2:1), 900 μl, was immediately added and the products were extracted by vigorous vortexing. The organic layer was obtained and evaporated with N_2. After dissolving the residue in small amount of chloroform:methanol (2:1), CO products were separated twice with TLC using ethylacetate:acetic acid (99:1) as a mobile phase according to the procedure of Kim *et al.* (1998). TLC plate was autoradiographed for 4~7 days. The radioactive spots comigrated with authentic standard TXB_2 were scraped out and counted with LSC (Pharmacia 1209). All assays were performed in triplicate and

data were represented as mean±S.D. Same experiments were run at least twice and they gave the similar results.

Lipoxygenase assay

The standard enzyme assay system consisted of platelet homogenate (20 μg protein/tube) and 0.01 μCi [^{14}C]AA in 100 mM Tris-HCl buffer, pH 7.4 containing 1 mM EDTA and 2 mM reduced glutathione with or without testing compound. The incubation and extraction procedure was essentially same as the procedure of CO assay, except incubation time of 15 min. LO products were separated on TLC using petroleum ether:diethyl ether:acetic acid (50:50:1) as a mobile phase. After autoradiography, the spots corresponding to 12-HETE were scraped out and radioactivity was counted.

RESULTS

For elucidating the effects on CO/LO, polyhydroxylated/methoxylated flavonoid derivatives were isolated from the several medicinal plants. Wogonin and related flavones were isolated from Scutellaria radix. The spectral results of oroxylin A, wogonin, skullcapflavone II, chrysin and baicalein were compared and identified according to the reports of Yun-Choi *et al.* (1992). 2',5,5',7-Tetrahydroxy-6',8-dimethoxyflavone (sb-1) was identified with a comparison of the spectral data of Kimura *et al.* (1984). Tectorigenin and iristectorigenin A were isolated from *Belamcanda chinensis*. The spectral results of these isoflavones were compared with the previously published results (Morita *et al.*, 1972; Lee *et al.* 1989). The spectral results of all flavonoid derivatives isolated were in agreement with the previously published data.

For testing the effects of flavonoids on CO/LO, platelet homogenate from healthy human volunteers was used throughout this study. In order to optimize the enzyme assay conditions, preliminary experiments were carried out. When EDTA (0~20 mM) was added in CO assay system, 5 mM EDTA was found to be optimal. CO activity was dependent on protein concentration (platelet homogenate) up to 100 μg protein/tube and, at the protein concentration of 100 μg/tube, linearity was found depending on the incubation time up to 30 min (data not shown). Therefore, optimum assay system for measuring CO activity contained 100 μg protein/tube in the presence of 5 mM EDTA and the incubation was continued for 20 min. For LO assay, same incubation condition with 20 ug protein/tube was used in the presence of 1 mM EDTA. The incubation was continued for 15 min. Under these conditions, the major end products found were thromboxane B_2 (TXB_2) and 12-HETE from CO and LO assay, respectively (Fig. 2). The percent inhibition of enzyme

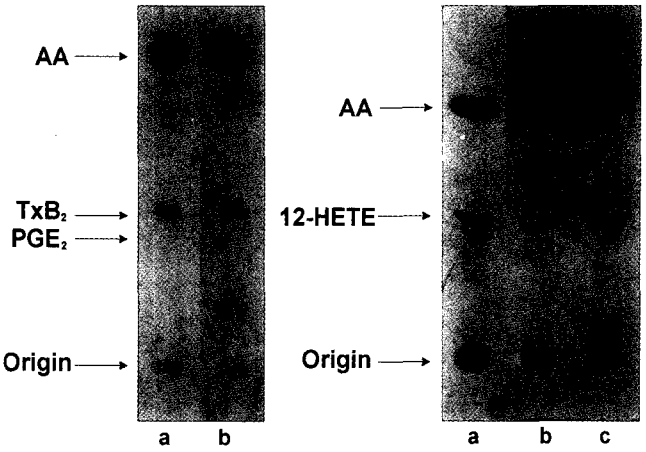

Fig. 2. Autoradiogram of CO/LO reaction. Left panel represented the results of CO reaction: (a) [^{14}C]AA in the presence of platelet homogenate, (b) in the presence of platelet homogenate with 100 μM indomethacin. Right panel represented the results of LO reaction: (a) [^{14}C]AA in the presence of platelet homogenate, (b) in the presence of platelet homogenate with 100 μM NDGA, (c) in the presence of platelet homogenate with 100 μM oroxylin A.

activity was calculated based on these products. Maximum yields of conversion of [^{14}C]AA to TXB_2 and 12-HETE were always less than 5% in both reactions.

Under these assay conditions, indomethacin, a known CO inhibitor, potently inhibited formation of TXB_2, but

Table I. Effects of polyhydroxylated/methoxylated flavonoids on CO/LO from human blood platelets[a]

	% Formation of TXB_2[b]	% Formation of 12-HETE[b]
Control	100.0±10.1[c]	100.0±10.4[d]
Indomethacin	24.0±4.6*	110.4±17.5
NDGA	49.3±8.6*	19.1±1.9*
Flavones		
Apigenin	89.6±10.1	95.0±11.5
Wogonin	104.7±4.0	82.8±7.7
7-O-methylwogonin	102.7±9.3	87.0±7.5
Chrysin	105.5±6.7	82.3±10.6
Baicalein	104.0±9.7	35.8±4.0*
Oroxylin A	91.0±10.5	42.2±3.3*
Sb-1	132.2±14.6	117.2±6.4
Skullcapflavone II	98.3±6.4	106.5±4.0
Isoflavones		
Daidzein	84.3±9.5	86.6±9.3
Tectorigenin	82.2±12.5	117.5±4.2
Iristectorigenin A	105.7±3.7	90.0±7.0
Flavonols		
Quercetin	122.4±10.4	67.1±9.1*
Vitexicarpin	127.0±14.1	114.5±7.8

[a]One of two separate experimental results were shown here., All compounds were measured for their inhibitory activity of CO/LO at 100 μM.
[b]Arithmatic mean ±SD (n=3), *: $p<0.001$, significantly different from control.
[c]Mean cpm ±SD was 1,420±143., [d]Mean cpm±SD was 2,168±226.

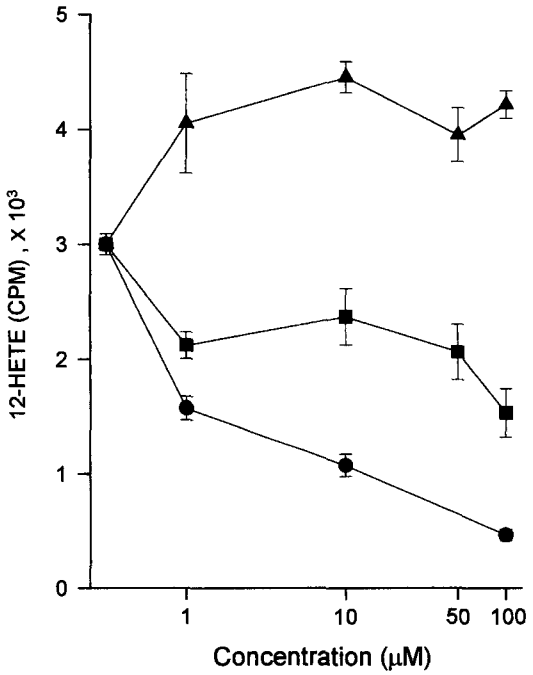

Fig. 3. Concentration dependent inhibition of 12-lipoxygenase by oroxylin A. Platelet homogenate without an inhibitor gave 2,998±102 cpm of 12-HETE., NDGA (●), oroxylin A (■), sb-1 (▲).

not 12-HETE, while NDGA, essentially a dual inhibitor of CO and LO, inhibited formation of TXB_2 as well as 12-HETE being more potent on LO (Table I). When flavonoid derivatives (100 μM) were added in the incubation mixture, several flavonoid derivatives (100 μM) inhibited these enzymes. Quercetin used as a reference flavonoid, clearly inhibited LO activity (32.9 %), but not CO activity. However, apigenin did not show the significant inhibitory activity on both enzymes, although weak inhibition (10.4%) of CO was observed. Among the flavonoids isolated, daidzein and tectorigenin weakly inhibited CO enzyme activity (less than 20%) at 100 μM. In contrast, baicalein and oroxylin A strongly inhibited LO activity. In addition, wogonin, a structurally similar flavone with oroxylin A, weakly inhibited LO activity. Fig. 2 represented the autoradiographic results of the inhibitory activities of reference compounds and oroxylin A. Oroxylin A showed the concentration-dependent inhibition of LO activity (IC_{50}=100 μM) along with NDGA (IC_{50}=1.5 μM) as shown in Fig. 3. Polymethoxylated flavonoids such as sb-1 and vitexicarpin rather enhanced these enzyme activities.

DISCUSSION

Numerous researchers have studied the effects of flavonoids on CO/LO partly for finding an active ingredient in crude drugs as well as for a hope to find a lead compound for pharmacological use. These studies have led to the findings of some flavonoid derivatives as inhibitors of CO/LO. However, the inhibitory activity was not sufficiently potent and selective for a clinical trial except several prenylated derivatives having potent inhibitory activity on 5-LO (Kimura *et al.*, 1986). We have recently found that amentoflavone, previously reported as a $sPLA_2$ inhibitor (Chang *et al.*, 1994), showed potent and selective inhibition of CO (Kim *et al.*, 1998). For our continual search, polyhydroxylated/methoxylated flavonoid derivatives were isolated and evaluated for their inhibitory activity of CO/LO.

In this investigation, CO and LO enzyme activities were separately measured under each optimal condition since it is sometimes difficult to interpretate the experimental results when CO/LO activities were measured in the same assay tube. For example, inhibitors of CO (i.e. indomethacin) inhibited formation of TXB_2 but increased the production of 12-HETE, and 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and 12-HETE affected CO activity (Siegel *et al.*, 1979; Croset *et al.*, 1983). Our assay was carried out within the linear range of enzyme activity under the conditions employed. From the results, isoflavones such as daidzein and tectorigenin were found to inhibit TXB_2 formation of CO, albeit less potent compared with the inhibitory activity of indomethacin. These results, however, may be important since there has been no report concerning isoflavonoids having inhibitory activity on CO/LO and tectorigenin may be an active principle in *Belamcanda chinensis* used as an anti-inflammatory agent in Asia. But structurally similar iristectorigenin A having more substituents on B-ring was not active at 100 μM. Highly hydroxylated/methoxylated flavonoids such as vitexicarpin, sb-1 and skullcapflavone-II did not show inhibition of CO. The inhibitory activity of quercetin on 12-LO was correlated with the previous results of Ferrandiz *et al.* (1990) and Laughton *et al.* (1991). On LO activity, 5,6,7- or 5,7,8-trisubstituted flavones without B-ring 2substituents such as baicalein, oroxylin A and wogonin showed the inhibition. Sekiya and Okuda (1982) previously described that baicalein was a selective 12-LO inhibitor while oroxylin A did not show the inhibitory activity. However, our results clearly demonstrated that oroxylin A as well as baicalein selectively inhibited 12-LO activity. Wogonin was less active, but the inhibition was apparent. All these active ones were flavones having 5,6,7- or 5,7,8-trihydroxylated/methoxylated substitutions, in which 6-hydroxy or methoxyl group is more favorable than 8-methoxyl substitution (baicalein and oroxylin A vs wogonin). It is, however, noted that oroxylin A was less active on LO compared with the inhibitory activity of baicalein. Chrysin having 5,7-dihydroxyl groups also weakly reduced formation of 12-HETE. B-ring substitution may not be favorable in

these molecules (chrysin vs apigenin; oroxylin A vs sb-1). Similarly polymethoxylated flavonoids such as vitexicarpin, sb-1 and skullcapflavone-II were inactive, and sb-1 and vitexicarpin rather enhanced the activities of CO/LO. 7-O-Methylation did not produce a favorable result (wogonin vs 7-O-methylwogonin). Gil *et al.* (1994) found that some polyhydroxylated/methoxylated flavones/flavonols including quercetagetin and scutellarein were PLA_2 inhibitors and they showed *in vivo* anti-inflammatory activity. Our study has shown that polyhydroxylated/methoxylated flavones/flavonols such as vitexicarpin, sb-1 and skullcapflavone-II did not possess the inhibitory activity of CO/LO. From the results obtained in this investigation, it may be suggested that anti-inflammatory and antiathmatic activity of Scutellaria radix seem to be mediated, at least partly, by flavones having inhibitory activity of LO.

In conclusion, isoflavones such as daidzein and tectorigen weakly inhibited CO activity. The potency of inhibition was far less than that of indomethacin. Baicalein and oroxylin A isolated from Scutellaria radix possessed the potent and selective inhibitory activity on 12-LO. 5,6,7- or 5,7,8-Trisubstitutions of A-ring of flavone were important for their activity on 12-LO. B-ring substitution (s) was not favorable in these molecules. The anti-inflammatory and antiathmatic activities of Scutellaria radix and *Belamcanda chinensis* may be at least in part due to these flavonoids having CO/LO inhibition.

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