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Inhibition of Prostaglandin E_2 Production by Flavone and its Related Compounds

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Abstract. *We have previously reported that among 12 major ingredients of Sairei-to, Scutellariae radix inhibited prostaglandin E_2 (PGE_2) production by lipopolysaccharide (LPS)-activated mouse macrophage-like RAW264.7 cells more efficiently than other ingredients, and wogonin, a major flavonoid from Scutellariae radix, showed greater inhibitory activity and membrane permeability than baicalein and baicalin. Here the effects of six other flavonoids, with similar structures, on membrane permeability and PGE_2 production were investigated. 7-Methoxyflavone inhibited the LPS-stimulated PGE_2 production to the greatest extent, followed by flavone>wogonin (5,7-dihydroxy-8-methoxyflavone)>> 7,8-dimethoxyflavone>chrysin (5,7-dihydroxyflavone)> baicalein (5,6,7-trihydroxyflavone)>>chromone. 7-Methoxyflavone also showed the highest membrane permeability, followed by flavone>chrysin>7,8-dimethoxy-flavone>wogonin>baicalein. When PGE_2 inhibitory activity was expressed per molecule incorporated into the cells, wogonin produced the greatest inhibition, further substantiating its anti-inflammatory potency.*

Scutellariae radix is one of the major ingredients in Kampo medicines or traditional Chinese herbal medicines. Various analytical methods, such as high performance liquid chromatography (HPLC), thin layer chromatography, and mass spectrometry have been used to identify or quantify the marker components for quality control and standardization purposes for medicinal plants (1). These methods are useful to ensure their consistent pharmacological and biological activity and stability. *Scutellariae* radix, one of 12 major ingredients of Sairei-to, possesses a broad spectrum of biological activities (2-4) and contains baicalin, baicalein and wogonin as major flavonoids. However, the biological significance of these flavonoids is unclear. We have previously investigated the potency of baicalin, baicalein and wogonin in inhibiting lipopolysaccharide (LPS)-stimulated prostaglandin E_2 (PGE_2) production in conjunction with their membrane permeability assessed by HPLC, and found that wogonin and all the other ingredients failed to inhibit the expression of cyclooxygenase-2 (COX-2) at both protein and mRNA levels (5). The metabolic pharmacokinetics of baicalin and baicalein has been reported (6, 7). Here seven related compounds were investigated for their inhibitory activity on PGE_2 production by LPS-stimulated mouse macrophage-like RAW264.7 cells, in relation to their membrane permeability. The compounds used in this study were chromone, flavone, 5,7-dihydroxyflavone (chrysin), 5,6,7-trihydroxyflavone (baicalein), 5,7-dihydroxy-8-methoxyflavone (wogonin), 7-methoxyflavone and 7,8-dimethoxyflavone (structures shown in Figure 1).

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Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: chromone, flavone, baicalein, wogonin, chrysin, 7-methoxyflavone, 7,8-dimethoxyflavone, fetal bovine serum (FBS) and LPS from *Escherichia coli* (serotype 0111:B4) (Sigma-Aldrich Co., St. Louis, MO, USA); Dulbecco's modified Eagles medium (DMEM) (GIBCO BRL, Grand Island, NY, USA).

Cell culture. RAW264.7 cells that had been established from the peritoneal fluid of BALB/c mice and shown the phenotype characteristics of monocytes and macrophages (supplied by Professor Ohmori, Meikai University) were cultured in DMEM supplemented with 10% heat-inactivated FBS, under a humidified 5% CO_2 atmosphere.

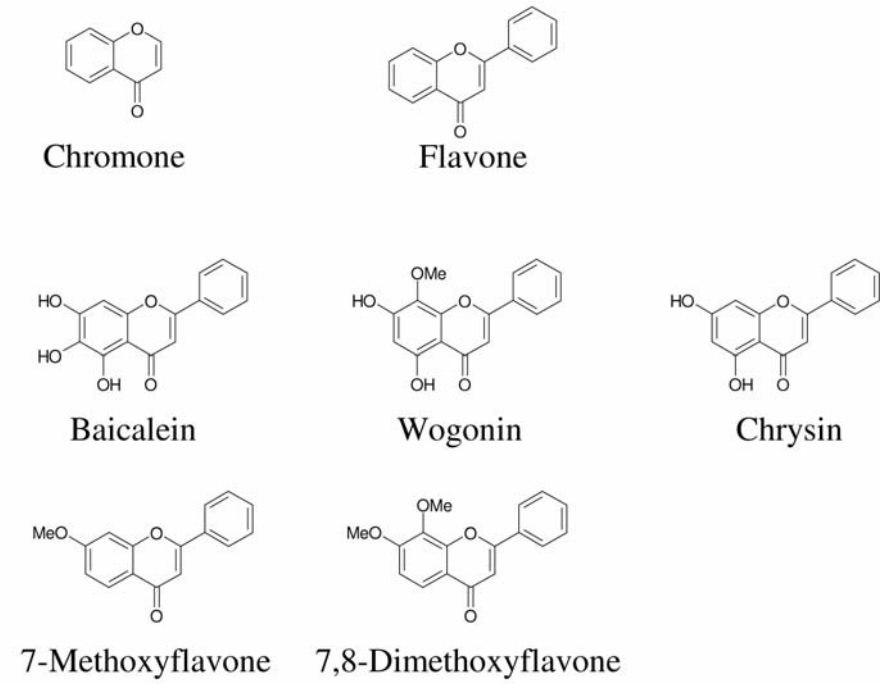

Figure 1. *Chemical structures of chromone, flavone, baicalein, wogonin, chrysin, 7-methoxyflavone and 7, 8-dimethoxyflavone.*

Measurement of PGE_2 production. RAW264.7 cells were subcultured in 24-well plates and incubated for 24 hours without or with *Scutellariae* radix components (baicalein or wogonin) (0.1, 1, 10 μM) or its related compounds (chromone [1, 10 μM], flavone, chrysin, 7-methoxyflavone and 7,8-dimethoxyflavone [0.1, 1, 10 μM] in the presence of LPS (100 ng/ml). RAW264.7 cells untreated with LPS were included for comparison. The culture medium supernatant was collected by centrifugation and for the PGE_2 concentration determined by EIA kit (Cayman Chemical Co, Ann Arbor, MI, USA).

HPLC analysis. RAW264.7 cells (5×10^6) were incubated for 6 hours with 1 μg of flavone, baicalein, wogonin, chrysin, 7-methoxyflavone or 7,8-dimethoxyflavone. The cells were then washed three times with phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS(–)), lysed with lysis buffer (10 mM Tris-HCl [pH 7.6], 1% Triton® X-100, 150 mM NaCl, 5 mM EDTA-2Na) and deproteinized with an equivalent volume of acetonitrile. After centrifugation for 5 minutes at 10,000 ×g, the supernatant was collected and stored at –40˚C until HPLC determination. Ten μl of supernatant were injected into an HPLC system (ODS-HG-5; 4.5 mm ×150 mm, Develosil, Nomura Chemical Co. Ltd., Aichi, Japan), eluted with a mobile phase of 50% acetonitrile in water containing 0.2% phosphate at a flow rate of 1 ml/min, and then detected with UV at 274 nm or at 258 nm (7,8-dimethoxyflavone).

Protein determination. The protein in the cell lysate was determined by a Protein Assay Kit (Bio-Rad, Hercules, CA, USA).

Results

Inhibition of LPS-stimulated PGE_2 production. The untreated RAW264.7 cells produced only background level (0.11 ng/ml) of PGE_2 in the culture medium. Upon stimulation with LPS, PGE_2 production was elevated to 15.33 ng/ml (Figure 2). Six of the flavonoids (except chromone) dose-dependently reduced the LPS-stimulated PGE_2 production. Among them, 7-methoxyflavone was the most potent (IC_{50}=0.73 μM), followed by flavone (IC_{50}=0.75 μM)>wogonin (IC_{50}=0.82 μM)>>7,8-dimethoxyflavone (IC_{50}=7.39 μM)>chrysin (IC_{50}=8.15 μM)> baicalein (IC_{50}>10 μM) (Figure 2). Chromone was essentially inactive (IC_{50}>10 μM).

Intracellular uptake of Scutellariae components. 7-Methoxyflavone was accumulated in the cells to the highest concentration (6.69±0.11 nmol/mg cellular protein), followed by flavone (6.54±0.62)>chrysin (6.47±1.33)>7,8-dimethoxyflavone (4.99±0.12)>wogonin (1.31±0.29)> baicalein (0.32±0.10) (Figure 3).

Discussion

We previously reported that the *Scutellariae* radix extract component wagonin inhibited PGE_2 production by LPS-stimulated RAW264.7 cells most potently, followed by baicalein and then baicalin, in the same order as their decreasing membrane permeability (wogonin>baicalein> baicalin) (5). In the present study, the ability to inhibit the LPS-stimulated PGE_2 production was shown for the first time to decline in the order of 7-methoxyflavone> flavone>wogonin >7,8-dimethoxyflavone> chrysin >>baicalein >>chromone. The membrane permeability decreased in the order of 7-methoxyflavone>flavone>chrysin >7,8-dimethoxyflavone>

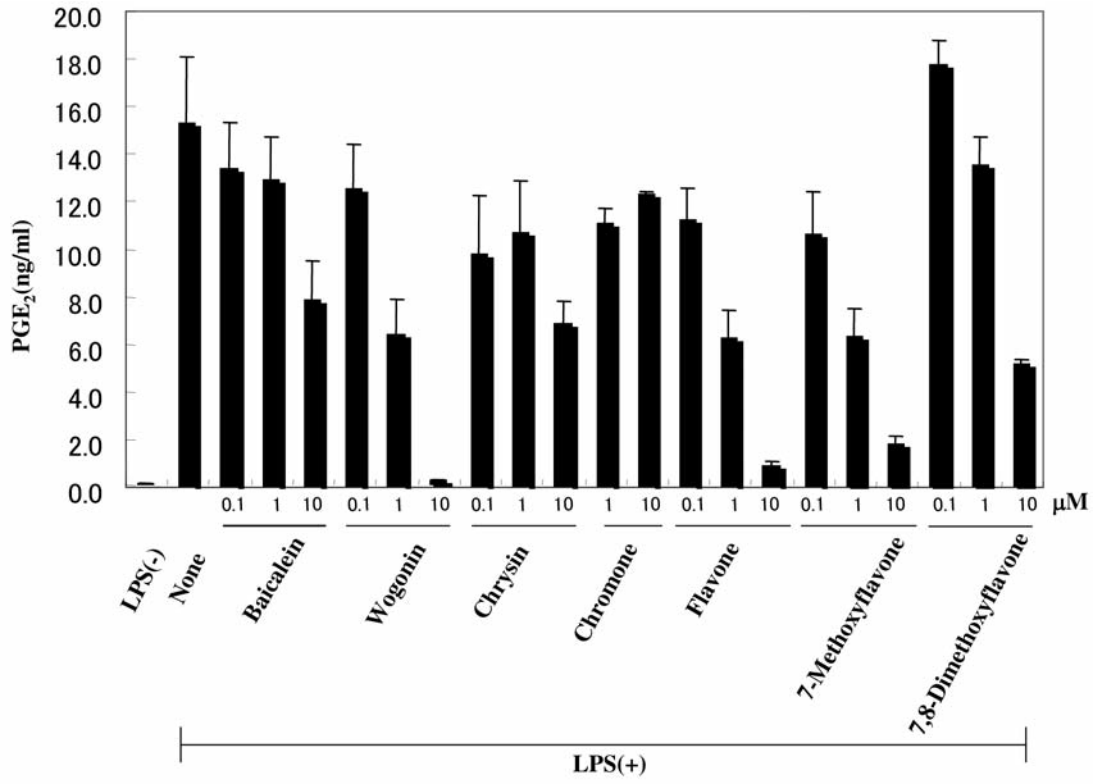

Figure 2. *Inhibition by components of Scutellariae radix of LPS-stimulated PGE_2 production in RAW264.7 cells incubated for 24 hours. Each value represents the mean±SD from three independent experiments.*

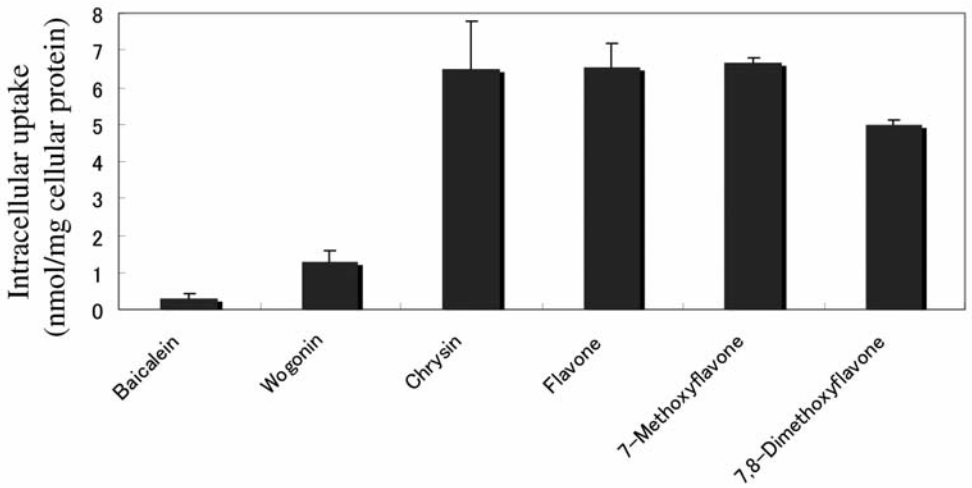

Figure 3. *Intracellular uptake of components of Scutellariae radix. RAW264.7 cells (5×10^6) were treated for 6 hours with 1 μg/ml of flavonoid. Each value represents the mean±SD from three independent determinations.*

wogonin >baicalein. The high uptake of 7-methoxyflavone may thus be due to its higher lipophilicity. These results indicated that the increase in the number of hydroxyl groups in the molecule (flavone>chrysin>baicalein) reduced both the membrane permeability and the PGE_2 production. On the other hand, the addition of a methoxy group did not apparently affect the membrane permeability and PGE_2 inhibitory activity of flavone (compare flavone *vs*. 7-methoxyflavone or

7,8-dimethoxyflavone). The addition of a methoxyl group at the C-8 position of chrysin slightly enhanced the PGE_2 inhibitory activity (compare chrysin *vs*. wogonin).

When PGE_2 inhibitory activity was expressed per molecule incorporated into the cells, wogonin (1÷1.31÷0.82=0.93) showed the greatest inhibitory activity, followed by 7-methoxyflavone (1÷6.69÷0.73=0.20), flavone (1÷6.54÷0.75= 0.20)>7,8-dimethoxyflavone (1÷4.99÷7.39=0.027)>chrysin (1÷6.47÷8.15=0.019), further substantiating the possible anti-inflammatory potency of wogonin.

Chrysin as well as baicalin and baicalein, possesses diverse biological activities such as anti-oxidant, anti-allergy, anti-inflammatory and anti-cancer action (8-10). It has been proposed that chrysin acts as an agonist of the peroxisome proliferator-activated receptor (PPAR)-γ which results in the down-regulation of key pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and COX-2 (11). But wogonin, baicalein and oroxylin A, which are the main active constituents of *Scutellariae* radix, showed much stronger inhibitory activities of PGE_2 production than that of chrysin (10, 12-16). Further studies are required to elucidate more precisely the point of action of these flavonoids.

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