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Antiinflammatory Activity of Naturally Occurring Flavone and Flavonol Glycosides

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Our previous report demonstrated that certain flavonoid aglycones such as apigenin (flavone), quercetin, morin (flavonols), and biochanin A (isoflavone) showed *in vivo* antiinflammatory activity *via* topical and oral routes of administration. As a continual study, the various flavonoid glycosides have been evaluated in mouse ear edema assay using archidonic acid or croton-oil as a inflammagen. Flavonoids were orally administered (2 mg/mouse) and ear edema inhibition was measured. Significant antiinflammatory activities were found especially in flavone and flavonol glycosides (15-29% inhibition) although the flavonoid derivatives tested showed less antiinflammatory activity than hydrocortisone or indomethacin. Chalcone and flavanone derivatives were not significantly active. And in general, flavonol glycosides of kaempferol-type were found to have a higher oral antiinflammatory activity than that of flavonol glycosides of quercetin-type in mice.

Key words: Flavonoid, Anti-inflammatory activity, Mouse ear edema assay

INTRODUCTION

Flavonoids, known as nature's tender drugs have shown various biological/pharmacological activities (Havsteen, 1983). Many investigators have found that flavonoid aglycones and glycosides obtained from medicinal plants possessed antiinflammatory activities in several animal models of inflammation (Gabor, 1986; Lewis, 1989; Middleton and Kandaswami, 1992). However, there has been a few report to show anti-inflammatory activity of flavonoids based on their structural activity relationships. We have previously reported that among thirteen flavonoid aglycones tested, flavone and flavonol aglycones having hydroxyl groups in A and B rings of flavonoid molecules were revealed to show significant antiinflammatory activity against mouse ear edema induced by arachidonic acid (AA) or croton-oil (Kim *et al.*, 1993). Therefore, it may be worthy of studing the antiinflammatory activity of the flavonoid glycosides.

In this investigation, flavonoids isolated from the various medicinal plants have been tested using mouse ear edema assay in order to compare the anti-inflammatory activity with emphasis of flavone and flavonol glycosides.

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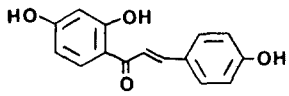
MATERIALS AND METHODS

Flavonoids

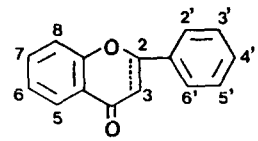
The chemical structures and origins of each flavonid derivative were represented in Fig. 1.

Mouse ear edema inhibition assay

For measuring antiinflammatory activities, arachidonic acid (AA) or croton-oil induced mouse ear edema assay were employed. With slight modification (Kim *et al.*, 1993) of original ear edema method by Tonneli *et al.* (1965), flavonoids (2 mg) finely suspended in 0.5% tween 80 (100 μl) were orally administered 1 hr prior to the topical application of 2% AA or 2.5% croton-oil dissolved in acetone (25 μl/ear) to both ears of male ICR mice (20-22 g) obtained from Experimental Animal Farm, Seoul National Univ., Korea. The ear thicknesses were measured using dial thickness gauge (Lux Scientific Instrument) 1 hr after AA treatment or 5 hr



KF-1: G.u.



Compounds	$\Delta^{2,3}$	3	5	6	7	8	3'	4'	Source
KF-2	No	H	H	H	H	H	H	H	Aldrich
KF-3[a]	No	H	H	H	OH	H	H	OH	G.u.
KF-4	No	H	OH	H	OH	H	H	OH	Sigma
KF-5	Yes	H	H	H	H	H	H	H	Aldrich
KF-6[b]	Yes	H	H	H	OH	H	OH	OH	A.j.
KF-7	Yes	H	OH	H	OH	H	H	OH	Aldrich
KF-8[a]	Yes	H	H	H	OR_1	H	H	OCH_3	C.z.
KF-9[a]	Yes	H	OH	H	OH	H	OH	OH	L.j.
KF-10[c]	Yes	H	OH	OH	OR_2	H	H	H	S.b.
KF-11[d]	Yes	H	OH	R_3	OCH_3	H	H	OH	Z.j.
KF-12[e]	Yes	H	OH	R_4	OCH_3	H	H	OH	Z.j.
KF-13	Yes	OH	H	H	H	H	H	H	Tokyo Kasei
KF-14	Yes	OH	OH	H	OH	H	H	OH	Tokyo Kasei
KF-15[f]	Yes	OR_3	OH	H	OH	H	H	OH	P.j.
KF-16[f]	Yes	OR_5	OH	H	OH	H	H	OH	P.j.
KF-17[g]	Yes	OR_6	OH	H	OH	H	H	OH	G.b.
KF-18[f]	Yes	OR_3	OH	H	OR_3	H	H	OH	P.j.
KF-19[h]	Yes	OR_7	OH	H	OR_3	R_8	H	OCH_3	E.k.
KF-20[i]	Yes	OR_9	OH	H	OR_7	H	H	OH	M.o.
KF-21	Yes	OH	OH	H	OH	H	OH	OH	Aldrich
KF-22[a]	Yes	OR_3	OH	H	OH	H	OH	OH	L.j.
KF-23[j]	Yes	OR_{10}	OH	H	OH	H	OH	OH	K.p.
KF-24[j]	Yes	OR_7	OH	H	OH	H	OH	OH	K.p.
KF-25[g]	Yes	OR_6	OH	H	OH	H	OH	OH	G.b.
KF-26[a]	Yes	OR_1	OH	H	OH	H	OH	OH	S.j.
KF-27[i]	Yes	OR_9	OH	H	OR_7	H	OH	OH	M.o.
KF-28[a]	Yes	OCH_3	OH	OCH_3	OCH_3	H	OH	OCH_3	V.r.

[a]Unpublished result, [b]Chamsuksai *et al.* (1981), [c]Chi *et al.* (1989), [d]Woo *et al.* (1979), [e]Woo *et al.* (1980), [f]Do *et al.* (1992), [g]Kang *et al.* (1990), [h]Kang *et al.* (1991), [i]Kang *et al.* (1988), [j]Jung *et al.* (1992).

R_1: rhamoglucose, R_2: glucuronic acid, R_3: glucose, R_4: 6‴-feruloylglucose, R_5: glucose-6-O-$COCH_2$-CH(OH)-CH_2COOH, R_6: 6‴-coumaroyl-glucorhamnose, R_7: rhamnose, R_8: prenyl, R_9: galctorhamnose, R_{10}: galactose.

G.u.: *Glycyrrhiza uralensis*, A.j.: *Albizzia julibrissin*, C.z.: *Chrysenthemum zawadskii*, L.j.: *Lonicera japonica*, S.b.: *Scutellaria baicalensis*, Z.j.: *Zizyphus jujuba*, P.j.: *Polygala japonica*, G.b.: *Ginkgo biloba*, E.k.: *Epimedium koreanum*, M.o.: *Melilotus officinale*, K.p.: *Kalopanoax pictum*, S.j.: *Sophora japonica*, V.r.: *Vitex rotundifolia*

Fig. 1. Chemical structures of flavonoids.

after croton-oil treatment. The animals were preconditioned in our animal housing at least for 1 week, under the conditions of 22± 1℃, 12 hr/12 hr (L/D) cycle and fed mouse lab chow and water *ad libitum*. The statistical analysis was performed by Student t-test.

RESULTS AND DISCUSSION

We previously reported the structural activity relationship of anti-inflammatory activity of 13 structurally different flavonoid aglycones and found that flavone and flavonol derivatives having hydroxyl groups in A and B ring showed an anti-inflammatory activity (Kim *et al.*, 1993). In order to elucidate the antiinflammatory activity of flavonoid glycosides, mainly flavone and flavonol glycosides were evaluated using the same animal model in this investigation. When the various flavonoid derivatives were orally administered at a dose of 2 mg/mouse and tested against AA or croton-oil induced mouse ear edema, chalcone and flavanone derivatives were not active in contrast to the potent activity exhibited by hydrocortisone in croton-oil induced edema and by indomethacin in AA induced edema (Table I). These results were well matched with our previous findings (Kim *et al.*, 1993) and the results of 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ear edema assay by Yasukawa *et al.* (1989). Table I clearly demonstrated that flavone and flavonol glycosides as well as flavonoid aglycones showed a significant anti-inflammatory activity against both AA and croton-oil induced

Table I. Antiinflammatory activity of flavonoids

Compounds[a]	% inhibition[b]	% inhibition[c]
Control	0	0
Hydrocortisone	24-34*[d]	55-70*
Indomethacin	45-68*	26-36*
Chalcone		
KF-1 (Isoliquiritigenin)	<15	16
Flavanone		
KF-2 (Flavanone)[e]	<15	<15
KF-3 (Liquiritigenin)	NT	<15
KF-4 (Naringenin)	NT	<15
Flavone		
KF-5 (Flavone)[e]	15	<15
KF-6 (7',3',4'-Trihydroxyflavone)	22*	<15
KF-7 (Apigenin)[e]	18*	<15
KF-8 (Linarin)	<15	<15
KF-9 (Luteolin)	25-29*	15-25*
KF-10 (Baicalin)	15-24*	16-27*
KF-11 (Spinosin)	22*	20-22*
KF-12 (6'''-Feruloylspinosin)	23*	20-25*
Flavonol		
KF-13 (Flavonol)[e]	<15	<15
KF-14 (Kaempferol)	18*	16*
KF-15 (Astragalin)	16-19*	16*
KF-16	15-22*	18-20*
KF-17	17-23*	19*
KF-18	18-24*	21-24*
KF-19 (Icariin)	26-29*	16*
KF-20 (Robinin)	24-26*	<15
KF-21 (Quercetin)	16-24*	12-23*
KF-22 (Isoquercitrin)	19*	23*
KF-23 (Hyperoside)	<15	19-25*
KF-24	<15	19*
KF-25	15-20*	<15
KF-26 (Rutin)	<15	15
KF-27 (Clovin)	18*	<15
KF-28 (Vitexicarpin)	21*	<15

[a] All compounds were orally administered (2 mg/mouse) except the control group treated with only vehicle and 3 mice were used per group.
[b] AA-induced edema assay
[c] Croton-oil induced edema assay
[d] Range of three separate experiments
[e] Data from Kim *et al.* (1993), *$p<0.01$, significantly different from control.
NT: not tested.

edema, in contrast to no activity expressed by naked flavone (KF-5) and flavonol (KF-13), which suggested that hydroxyl groups in A and B rings may be needed to exhibit antiinflammatory activity. Flavonol glycosides showed similar antiinflammatory activity to their aglycones (KF-15, 16, 17, 18, 19 to kaempherol, KF-22 to quercetin) by the oral treatment. KF-6 showed anti-inflammatory activity only against AA induced edema and KF-9 showed a higher antiinflammatory activity against AA-induced edema than that against croton-oil induced edema. This pattern might comform the previous finding (Kim *et al.*, 1993) that flavone derivatives such as flavone, chrysin and apigenin showed a higher activity against AA induced edema *via* oral and topical routes of administration than that against croton-oil induced edema. Flavonol glycosides, kaempherol glycosides (KF-15, 19, 20) and quercetin glycosides (KF-25, 27), also showed a higher activity against AA induced edema than croton-oil induced edema. Many authors demonstrated that flavonid derivatives possessed cyclooxygenase/lipoxygenase inhibitory activity (Welton *et al.*, 1988; Ferrandiz *et al.*, 1990), which showed anti-inflammation in AA induced edema because eicosanoids involved in this edema development (Amer *et al.*, 1985). Therefore, these glycosides may show anti-inflammatory activity, at least partly due to cyclooxygenase/lipoxygenase inhibition. And in general, kaempherol glycosides (KF-15 to KF-20) were found to show higher activity than quercetin glycosides (KF-22 to KF-27). However, no clear structural activity relationships depending on the positions or types of sugar substitution was found in anti-inflammatory activity of flavonoid glycosides. It may be thought that the differences of the activity by flavonol glycosides tested might be due to their differences of bioavailability and/or metabolism, because their aglycones are same as their glycosides (kaempherol and quercetin). All of these results could suggest that at least part of their anti-inflammatory activity by these medicinal plants may be due to their content of these flavonoid derivatives tested, although the activities were less than those of hydrocortisone or indomethacin.

In conclusion, flavonoid glycosides (flavone and flavonol) showed antiinflammatory activity by the oral treatment, similar to flavonoid aglycones, or even higher. Generally, flavonoid glycosides were found to show higher activities against AA-induced edema that croton-oil induced edema. Among flavonol glycosides, kaempferol-types of compounds were found to have a higher antiinflammatory activity than quercetin-types of compounds.

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