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Flavonoids: Potential Antiinflammatory Agents

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Abstract – Flavonoids are widely distributed polyphenol compounds in plant kingdom and known to possess varieties of biological/pharmacological activities *in vitro* and *in vivo*. A search for antiinflammatory/immunoregulatory flavonoids as potential therapeutic agents has been continued, since serious side effects of currently used nonsteroidal and steroidal antiinflammatory drugs limit their long term uses for the inflammatory disorders. In this reserch, various flavonids were isolated and tested for their *in vivo* antiinflammatory activity and *in vitro* inhibitory activity of lymphocyte proliferation. Using a mouse ear edema assay, it was found that certain flavones/flavonols possess mild antiinflammatory activity and a C-2,3-double bond might be essential. Isoflavones were less active. These flavonoids inhibited *in vitro* lymphocyte proliferation, relatively specific for T-cell proliferation (IC_{50}=1-10 μM) and the inhibition was reversible. We have also tested several biflavonoid derivatives, since we recently found that biflavones were phospholipase A_2 inhibitors. It was demonstrated that biflavones such as ochnaflavone and ginkgetin inhibited lymphocyte proliferation induced by both concanavaline A and lipopolysaccharide. The inhibition was irreversible in contrast to that of flavones/flavonols. And antiinflammatory activity of biflavonoids are discussed.

Key words – flavonoids, antiinflammatory activities.

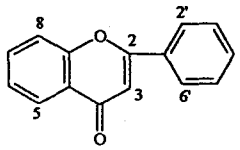
Introduction

Flavonoids, one of the most abundant class of compounds in plants, have been known to be the nature's tender drugs to show various biological/pharmacological activities such as antimicrobial, antifungal, antiviral, antihepatotoxic, antimutagenic, antiinflammatory, anti-allergic effects, etc. (Harsteen, 1983). Among these activities, antiinflammation by flavonoids has been continuously elucidated not only for establishing antiinflammatory principles in the medicinal plants, but also for developing a new class of antiinflammatory agents. However, in contrast to the numerous reports describing the antiinflammatory flavonoids as active principles of the medicinal plants using various experimental animal models (Gabor, 1986 ; Lewis, 1989), only a few studies were published about *in vivo* antiinflammatory activities of various flavonoid derivatives mainly based on the structur activity relationships. Recently, Panthong *et al.* (Panthong *et al.*, 1994) reported the antiinflammatory activity of several methoxylated flavones/flavonols.

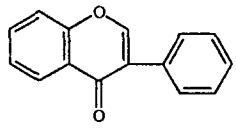
For several years, we have studied antiinflammatory activity of various flavonoids *in vivo* and *in vitro* in order to elucidate the structure activity relationships of various flavonoids and to develop potential an-

tiinflammatory agents based on flavonoid molecules. In this review, the antiinflammatory bioassay performed in our labs were presented and discussed with the experimental results of other investigators.

The flavonoid derivatives used in this



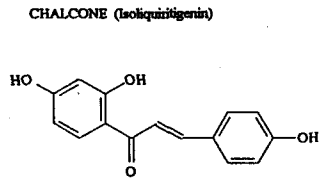
Flavanone
Flavone
Falavonol

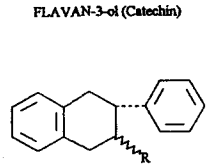


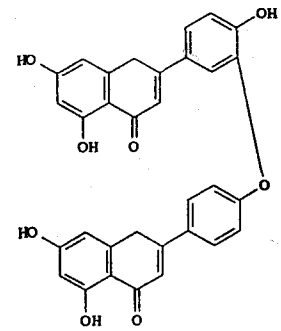
Isoflavone

Compounds	$\Delta^{2,3}$	3	5	6	7	8	2'	3'	4'	5'
Flavanone	no	H	H	H	H	H	H	H	H	H
Liquiritigenin	no	H	H	H	OH	H	H	H	H	H
Flavone	Yes	H	H	H	H	H	H	H	H	H
Apigenin	Yes	H	OH	H	OH	H	H	H	OH	H
Vitexin	Yes	H	OH	H	OH	R_1	H	H	OH	H
Isovitexin	Yes	H	OH	R_1	OH	H	H	H	OH	H
baicalin	Yes	H	OH	OH	OR_2	H	H	H	H	H
Spinosine	Yes	H	OH	R1	OCH_3	H	H	H	OH	H
Flavonol	Yes	OH	H	H	H	H	H	H	H	H
Kaempferol	Yes	OH	OH	H	OH	H	H	H	OH	H
Astragalin	Yes	OR_1	OH	H	OH	H	H	H	OH	H
Icariin	Yes	OR_3	OH	H	OR_1	R_4	H	H	OH	H
Quercetin	Yes	OH	OH	H	OH	H	H	OH	OH	H
Hyperoside	Yes	OR_5	OH	H	OH	H	H	OH	OH	H
Clovin	Yes	OR_6	OH	H	OR_3	H	H	OH	OH	H
Morin	Yes	OH	OH	H	OH	H	OH	H	OH	H
Myricetin	Yes	OH	OH	H	OH	H	H	OH	OH	OH
Biochanin A (isoflavone)	Yes	2-H	OH	H	OH	H	H	H	OCH_3	H

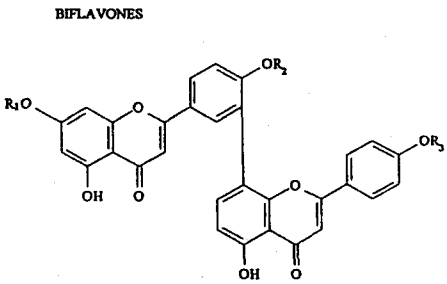
R_1 : glucose, R_2 : glucuronic acid, R_3 : rhamnose, R_4 : prenyl, R_5 : galactose, R_6 : galactorhamnose.







ochnaflavone



R1=R2=R3=H (amentoflavone)
R1=R3=H, R2=CH3 (bilobetin)
R1=R2=CH3, R3=H (ginkgetin)
R1=H, R2=R3 (isoginkgetin)
R1=R2=R3=CH3 (sciadopitysin)

Fig. 1. Chemical structures of flavonoids.

study were obtained from following three sources and the chemical structures of some representative flavonoids were shown in Fig. 1. Flavonoids such as apigenin and quercetin were purchased from commercial sources. Flavonoids such as kaempferol and quercetin glycosides, and biflavones were isolated from various plant sources according to the previous reports (see reference; *). Flavonoids such as 7-O-methyl biochanin A were chemically synthesized as published (Lee *et al.*, 1994).

In vivo antiinflammatory activity of flavonoids

Using commercially available 13 flavonoid aglycones, their anti-edematic activities were evaluated *via* oral and topical application in mouse ear edema bioassay according to the slightly modified method of (Kim *et al.*, 1993) of the original procedure of Tonneli *et al.* (Tonneli et al., 1965). Flavan-3-ols and flavanones were not active in croton oil induced edema at the dose of 100 mg/kg, p.o. and 2 mg/ear, topical. Certain flavones/flavonols such as apigenin, quercetin and morin showed weak antiinflammatory activity (12-28% inhibition) in the croton oil induced mouse ear edema *via* oral and topical application. Biochanin A, an isoflavone, showed antiinflammatory activity (23-24% inhibition). In addition, these flavonoids were found to be active in arachidonic acid (AA)-induced mouse ear edema test (15-22% inhibition) *via* oral administration. Especially, via topical application, most flavones/flavonols showed potent inhibition (40-72% inhibition) in AA-induced ear edema test. The ED_{25} or ED_{50} values of several flavonoid derivatives were represented in Table 1 (Kim *et al.*, 1993). Using the rat carrageenan (CGN)-induced pleurisy test according to the procedure of Schrier *et al.* (Schrier *et al.*, 1990), quercetin and biochanin A were also found to show the anti-inflammatory activity.

Table 1. Relative Activity of Several Flavonoids.

Compounds	croton-oil induced ear edema		AA-induced ear edema	
	oral[a]	topical[a]	oral[a]	topical[b]
Hydrocortisone	0.06	0.004	2.1	2.0
Indomethacin	0.90	0.30	0.09	0.08
NDGA	-[c]	1.80	-	2.40
Phenidone	-	-	-	0.06
Flavone	-	-	-	0.49
Apigenin	-	1.57	4.7	1.14
Quercetin	1.95	2.08	4.3	1.85
Biochanin A	2.78	1.67	6.0	2.38

[a]The represented values are ED_{25}(mg/mouse or mg/ear) of ICR mice (21±1mg). [b]ED_{50}(mg/ear), [c]Data not available.

These results indicated that a C-2,3-double bond is essential for *in vivo* antiinflammatory activity of flavonoids and the potencies of antiinflammatory activity depend on the patterns and numbers of hydroxylation(s) on A/B-ring. 5,7-hydroxylation on A-ring and 4'-hydroxylation on B-ring were favorable. The potent inhibitory activities of topically applied flavones/flavonols against AA-induced mouse ear edema suggested that these flavonoids actually behave like cyclooxygenase (CO)/lipoxygenase (LO) inhibitors *in vivo* as well as *in vitro*, because topically applied arachidonic acid converts to prostaglandins and leukotriens by CO/LO in the dermal area, which induce erythema and edema. These results might be supported by the fact that certain flavones/flavonols such as 3-hydroxyflavone, kaempferol, fisetin, and quercetin inhibit CO/LO (Laughton *et al* 1991; Ferrendiz *et al.*, 1990; Sekiya *et al.*, 1982). Topically applied flavone, which was known as a CO inhibitor (Welton *et al.*, 1988), was most active among flavonoids tested in AA-induced ear edema.

In order to investigate antiinflammatory activities of the giycoside derivatives, we have isolated 18 flavones/flavonol glycosides, mainly apigenin, kaempferol, and quercetin

glycosides, from various plants. And evaluated their antiinflammatory activities using, mouse ear edema bioassy. Glycosides were orally administered to mouse (100 mg/kg), and it was found that the antiinflammatory activities of flavone/flavonol glycosides are to their aglycones by oral treatment (Lee *et al.*, 1993). In general, flavonoid glycosides showed a higher activity against AA-induced ear edema than croton oil induced edema. However, no clear structure activity relationship was found depending on the positions or types of sugar substitutions. It may be thought that the differences in the activities of glycosides tested might be due to their differences in bioavailability and/or metabolism, because their aglycones are same as their glycosides (kaempferol and quercetin). It is worth to mention that C-glycosides of apigenin (flavone), vitexin, and isovitexin, showed higher activity compared to O-glycosides of flavones/flavonols (Yoo *et al.*, 1995).

Gabor (Gabor, 1986) found that sophoricoside (isoflavone glycoside) possess antiinflammatory activity against CGN-paw edema. Huh *et al.* (Huh *et al.*, 1987) reported that daidzein showed antiinflammatory activity. Kim and Chung (Kim and Chung, 1990) demonstrated the inhibitory effects of daidzein on type I-IV hypersensitivity reactions in experimental animals. Biochanin A (isoflavone) showed antiinflammatory activity comparable to quercetin (Kim *et al.*, 1993). Although all of these previous results indicated that certain isoflavonoids may show antiinflammatory activity, the structure activity relationship of isoflavonoids was not established. Therefore, for elucidating the antiinflammatory activity of isoflavonoids, 7 isoflavones having a C-2,3-double bond were isolated from *Pueraria* radix and six 7-O-alkyl derivatives of biochanin A were chemically synthesized. When the antiinflammatory activities of these derivatives were compared, it was demonstrated that certain isoflavones such as daidzein and puerarin possess activity although they were generally less active than flavones/flavonols (Lee *et al.*, 1994). When the 7-O-alkyl derivatives were topically applied to AA-induced edema, 7-O-ethyl and isopropylbiochanin A showed activities (52-54%, 2 mg/ear) comparable to biochanin A (51%), while 7-O-isobutyl and allylbiochanin A showed far reduced activity. A certain size limitation of 7-position substituents would be suspected and 7-O-substitution of isoflavone may not be favorable for the activity.

All of these results suggest that certain flavonoids possess *in vivo* antiinflammatory activity and a C-2,3-double bond is essential. Their activities depend on the patterns and numbers of hydroxylation, and glycosidic substitutions. However, they are generally far less active than the currently used antiinflammatory drugs, NSAID and SAID, presumably because of low bioavailability.

Inhibition of lymphocyte proliferation by flavonoids

Lymphocytes are one of the main cells participating in the inflammatory and immunomodulatory reactions. Among inflammatory disorders in human, lymphocyte proliferation is thought to be an important pathological cause in the chronic inflammatory disorders such as rheumatoid arthritis. Therefore, it may be valuable to find out the effects of flavonoids on lymphocyte proliferation.

There have been several important findings that flavonoids including quercetin, tangerein, kaempferol, and their glycosides isolated from *Ginkgo biloba* show suppression of concanavaline A (Con A) or phytohaemaglutinin (PHA)-induced lymphocyte proliferation (Berg and Daniel, 1988 ; Mookerjee *et al.*, 1986 ; Pignol *et al.*, 1988). Hirano *et al.* (Hirano *et al.*, 1989) reported that several flavone and isoflavone deri-

vatives showed the suppressive activity against the Con-A induced human lymphocyte proliferation. Moreover, Schwartz *et al.* (Schwartz *et al.*, 1982), and Schwartz and Middleton (Schwartz and Middleton, 1984) clearly demonstrated the suppressive effects of several flavonoids including quercetin on the generation and effector function of cytotoxic T-lymphocyte. All of these results indicated that certain flavonoid derivatives suppressed lymphocyte proliferation. However, it is improbable to establish the structure activity relationships of flavonoids and specificity of suppression. Therefore, we have examined about 50 flavonoid derivatives and demonstrated their suppressive effects depending on the flavonoid molecules and types of stimulation.

The suppressive activity of flavonoids against T and B-cell proliferation *in vitro* was studied (Namgoong *et al.*, 1994). Chalcone, flavan-3-ols and flavanone derivatives were not active against both the Con A and lipopolysaccharide (LPS)-stimulated lymphocyte proliferation up to the concentration of 10 μM. Flavones/flavonols having a C-2,3-double bond were active only against T-cell proliferation and their potencies depend on the hydroxylation patterns. Apigenin, kaempferol, and quercetin having either 4'- or 3',4'-hydroxyl group(s) on B-ring was active, but morin having 2',4'-hydroxyl groups on B-ring was not active. Flavones/flavonols also inhibited mixed lymphocyte reaction (MLR) at the same concentration ranges. Because cytotoxic T-lymphocytes are believed to be involved in mixed lymphocyte, flavones/flavonols are suggested to be the relatively selective inhibitors of T-cell proliferation. An exception among the flavonoids tested was myricetin having 3',4',5'-hydroxyl groups on B-ring, which showed the inhibitory activity against B-cell proliferation. Fig. 2 demonstrated the inhibitory activity of several derivatives. It was also found that the inhibition by flavones/flavonols was fully reversible (Lee *et al.*, 1995), which is well correlated with the results of Mookerjee *et al.* (Mookerjee *et al.*, 1986).

When flavone/flavonol glycosides were tested, nothing was found to be inhibitory up to 10 μM. We do not know at present whether

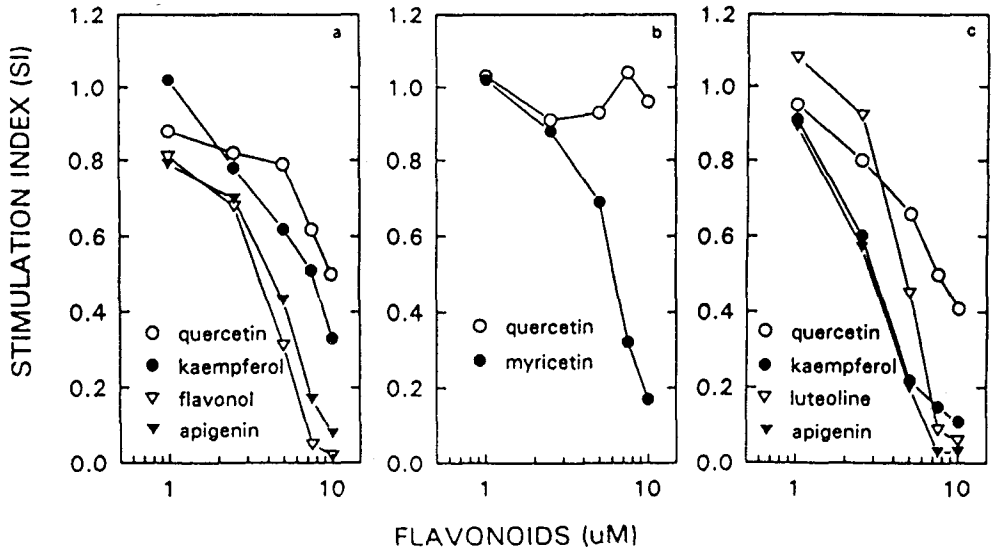


Fig. 2. Suppression of lymphocyte proliferation by flavonoids
(a) Con A induced proliferation, (b) LPS induced proliferation
(c) Mixed lymphocyte culture reaction.

this phenomenon is due to the fact that flavonoid glycosides hardly penetrate cell membrane or the intact glycosides have no activity on lymphocytes because of a steric hinderence by glycosidic portion. Isoflavones tested were shown to inhibit T-cell proliferation, but not B-cell proliferation as in flavones/flavonols. Generally, they were less active than flavones/flavonols (Namgoong *et al.*, 1994).

What is the mechanism of inhibition against lymphocyte proliferation by flavonoids ? Mookerjee *et al.* (Mookerjee *et al.*, 1986) suggested that lymphoblastosis inhibition by quercetin was due to the inhibition of thymidine uptake. And several other authors reported that some flavonoids inhibit protein kinase C and protein tyrosine kinase (Ferriola et al., 1989 ; Chang and Geahlen, 1992; Middleton and Kandaswami, 1992). However, any single mechanism does not fit to the structure activity relationship of inhibition of lymphocyte proliferation by flavonoids. It is still a long way to unveil the mechanism of action of flavonoids.

Biflavonoids : potential antiinflammatory agents

Biflavonoid is one of the classes of naturally occurring bioflavonoids. There are many diverse families of biflavonoids, chalcone dimer, flavonone dimer and flavone dimer, etc. Certain biflavonoids were previously reported to possess the inhibitory activities to phosphodiesterase (Ruckstuhl *et al.*, 1979), lens aldose reductase (Iwu *et al.*, 1990), mast cell histamine release (Chang *et al.*, 1994), and anticancer activity (Lin *et al.*, 1989). However, there is no report describing the antiinflammatory activity and the effects on lymphocyte proliferation. Therefore, we have isolated 9 biflavones from three plant extracts and evaluated their biological activities.

Table 2. Irreversible Inhibition of Biflavonoids against Lymphocyte Proliferation.

S.I. Compounds	Washing	Exp. 1 (24 hrs)	Exp. 2 (48 hrs)
Control	-	1.0±0.12	1.0±0.16
	+	1.03±0.31	0.93±0.21
Quercetin	-	0.43±0.14 *	0.58±0.18 *
	+	1.06±0.49 *	0.83±0.23 *
Apigenin	-	0.13±0.16 *	0.04±0.20 *
	+	1.10±0.30 *	0.81±0.32 *
Ochnaflavone	-	0.03±0.02	0.04±0.03
	+	0.04±0.02	0.0±0.03
Isocryptomerin	-	0.02±0.01	0.08±0.04
	+	0.02±0.02	0.10±0.04

Flavonoids were preincubated at 10 μM for the indicated time without mitogen.
Con A was added after washing the lymphocyte three times and incubated without adding each flavonoid. *: P<0.001, Significantly different between groups.

When we tested 9 biflavones for the inhibitory activity against lymphocyte proliferation, biflavones such as ochnaflavone, ginkgetin, isocryptomerin were active (IC_{50}= 0.1-10 μM), while amentoflavone and sciadopitysin were not (Lee *et al.*, 1995). The active biflavones were found to be general inhibitors of lymphocyte proliferation, i.e. T, B-cell proliferation and mixed lymphocyte reaction (MLR), compared to flavones/flavonols mainly effecting T-cells as mentioned above. They showed the similar inhibition profiles at the same concentration ranges. In addition, biflavones were irreversible inhibitors of lymphocyte proliferation as represented in Table 2. Although we could not derive any structure activity relationships because of limited numbers of biflavones tested, it was evident that biflavones showed totally different action profiles compared to the conventional flavones/flavonols.

Previously, we reported that ochnaflavone and several other biflavones were phospholipase A_2 (PLA_2) inhibitors (Chang *et al.*, 1994). Ochnaflavone was revealed to be a relatively specific group II PLA_2 inhibitor and

its inhibitory action was non-competitive. Because PLA_2 was believed to be involved in various inflammatory disorders, evaluation of that activity is meaningful. And, in fact, our preliminary experiments showed that biflavones possess antiinflammatory activity *in vivo*, which might suggest the potential use of biflavonoids on inflammatory disorders.

Conclusion

The various flavonoids were evaluated for their antiinflammatory activity *in vivo* and effects on lymphocyte proliferation *in vitro*. Certain flavones/flavonols having a C-2,3-double bond showed the significant antiinflammatory activity and the suppressive activity on lymphocyte proliferation. Potencies for these activities were depending on the patterns and numbers of hydroxylation, and glycosidic substitution(s) on the flavonoid molecules. Especially, biflavones may be the potential agents for treating inflammatory/immunological disorders such as rheumatoid arthritis, not only because they could inhibit lymphocyte proliferation but also because they were found to be inhibitors of PLA_2.

Acknowledgements

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