Arch. Pharm. Res. Vol. 16, No. 1, pp. 18-24, 1993

Antiinflammatory Activity of Flavonoids: Mouse Ear Edema Inhibition

Hee Kee Kim, Soon Young Namgoong and Hyun Pyo Kim

College of Pharmacy, Kangweon National University, Chuncheon 200-701, Korea

(Received Octeober 20, 1992)

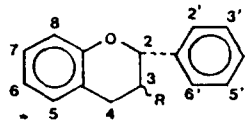
In this investigation, the various flavonoid aglycones were evaluated for their inhibitory activities against croton-oil or arachidonic acid induced mouse ear edema by oral or topical administration. The compounds tested were thirteen derivatives of flavan-3-ol (catechin and epicatechin), flavanone (flavanone and naringenin), flavone (flavone, chrysin and apigenin), flavonol (flavonol, galangin, quercetin and morin) and isoflavone (biochanin A and 2-carbethoxy-5,7-dihydroxy-4'-methoxyisoflavone), along with hydrocortisone, indomethacin, 4-bromophenacyl bromide, nordihydroguaiaretic acid and phenidone as positive controls. As a general, 5,7-dihydroxy-flavonols having hydroxyl group(s) in B-ring and biochanin A (isoflavone) were found to show broad inhibitory activities (14-52%) against croton-oil or arachidonic acid induced ear edema by oral or topical application at the dose of 2 mg/mouse, although they showed less activity than hydrocortisone (26-88%) or indomethacin (36-80%). Flavonoid aglycones tested showed higher activity when applied topically than by the oral administration. It was also found that they inhibited arachidonic acid induced edema more profoundly than croton-oil induced edema by topical application. In arachidonic acid induced edema when applied topically, flavone derivatives such as flavone, chrysin and apigenin were revealed to be the good inhibitory agents in addition to flavonols and isoflavones. When quercetin and biochanin A were selected for evaluating in carrageenan induced rat pleurisy model (5 hr and 24 hr), both flavonoids showed antiinflammatory activity at the dose of 70 mg/kg by the oral administration. All of these results revealed that flavonoid aglycones, especially 5,7-dihydroxy-flavonols having hydroxyl group(s) in B-ring and biochanin A (isoflavone) possessed *in vivo* antiinflammatory activity.

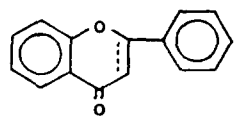
Key words: Antiinflammation, Mouse ear edema inhibition, Flavonoid, Flavone, Flavonol, Isoflavonoid, Quercetin, Biochanin A

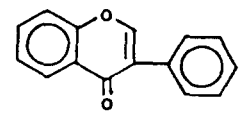
INTRODUCTION

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, antiallergic 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), a few study was reported about *in vivo* antiinflammatory activities of the various flavonoid aglycones mainly based on the structure-activity relationships. In this report, we have studied *in vivo* antiinflammatory activity of flavonoid aglycones using mouse ear edema test. Mouse ear edema inhibition test was known to be a simple and reproducible system to find antiinflammatory agents. But, it should be mentioned that activities expressed in ear edema bioassay are varied depending on the inflammagens and administration routes used. In croton-oil induced edema, steroidal antiinflammatory drug (SAID) types of compounds show sensitive results, while nonsteroidal antiinflammatory drug (NSAID) types of compounds and cyclooxygenase (CO)/lipoxygenase (LO) inhibitors are sensitive in arachidonic acid induced edema (Amer *et al.*, 1985; Berkenkopf *et al.*, 1985; Bouclier *et al.* 1990). Previous reports (Gabor, 1980; Della Loggia *et al.*, 1986; Welton *et al.*, 1988; Lewis, 1989) found that the various flavonoid aglycones and glycosides showed the antiinflammatory activities using

Correspondence to: Hyun Pyo Kim, College of Pharmacy, Kangweon National University, Chuncheon 200-701, Korea







	$\Delta^{2,3}$	2	3	4	5	6	7	8	2′	3′	4′	5′	6′
Flavan-3-ol													
(−)-Epicatechin	No	–	OH	H	OH	H	OH	H	H	OH	OH	H	H
(+)-Catechin	No	–	OH	H	OH	H	OH	H	H	OH	OH	H	H
Flavanone													
Flavanone	No	–	H	–	H	H	H	H	H	H	H	H	H
Naringenin	No	–	H	–	OH	H	OH	H	H	H	OH	H	H
Flavone													
Flavone	Yes	–	H	–	H	H	H	H	H	H	H	H	H
Chrysin	Yes	–	H	–	OH	H	OH	H	H	H	H	H	H
Apigenin	Yes	–	H	–	OH	H	OH	H	H	H	OH	H	H
Flavonol													
Flavonol	Yes	–	OH	–	H	H	H	H	H	H	H	H	H
Galangin	Yes	–	OH	–	OH	H	OH	H	H	H	H	H	H
Quercetin	Yes	–	OH	–	OH	H	OH	H	H	OH	OH	H	H
Morin	Yes	–	OH	–	OH	H	OH	H	OH	H	OH	H	H
Isoflavone													
Biochanin A	Yes	H	–	–	OH	H	OH	H	H	H	R_1	H	H
Ethoxybiochanin A	Yes	R_2	–	–	OH	H	OH	H	H	H	R_1	H	H

R_1=-OCH_3, R_2=-$COOCH_2CH_3$

Fig. 1. Chemical structures of flavonoid derivatives.

mouse ear edema test, but there is a lack of knowledge between these two inflammagens (croton-oil and arachidonic acid) used and administration routes for the antiinflammatory activity of flavonoids. Therefore, it may be neccessary to evaluate antiinflammatory activities of flavonoid aglycones using these two different inflammagens and different routes of administration (oral and topical), based on the structure-activity relationships. And it may be also valuable to compare these *in vivo* antiinflammatory activities with known CO/LO inhibitory activities of flavonoids (Sekiya and Oknda, 1982; Welton *et al.*, 1988; Ferrandiz *et al.*, 1990; Laughton *et al.*, 1991), since eicosanoid mediators produced by CO/LO are believed to be involved in inflammation, immunoregulation and platelet aggregation.

In this investigation, using croton-oil and arachidonic acid induced mouse ear edema test, antiinflammatory activities of thirteen flavonoid aglycones were studied and structure-activity relationships were discussed.

METERIALS AND METHODS

Materials

All flavonoid aglycones used in this study were purchased from Aldrich Chem. Co. (USA) except flavonol (Tokyo Kasei Chem. Co., Japan). The chemical structures of flavonoids used in this study were represented in Fig. 1. Croton-oil, arachidonic acid (99%), λ-carrageenan, hydrocortisone, indomethacin, 4-bromophenacyl bromide (4-BPB), nordihydroguaiaretic acid (NDGA), phenidone and May-Grunwald stain were supplied by Sigma Chem. Co. (USA). Giemsa's staining solution was purchased from BDH (England). RPMI 1640 media and fetal calf serum (FCS) were obtained from GIBCO (USA).

Animals

Mice (ICR) and rats (Sprague-Dawley) were obtained from Animal Farm (Seoul National Unversity) and Yuhan Pharmaceutical Res. Center (Korea), respectively. Animals were maintained in an animal chamber equipped with laminar air flow (Fine-Bio Tech., Korea) at a temperature of 22± 1℃ and relative humidity of 55± 10% throughout the study.

Croton-oil induced ear edema inhibiton

In order to determine the proper concentration and contact time of croton-oil treatment, various concentrations of croton-oil in acetone (25 μl/ear) were topically applied to ears of male mice (20-22 g) according to the procedure of Tonneli *et al.* (1965). And ear thicknesses were measured using dial thickness gauge (Lux Scientific Instrument) at regular time intervals. Thickness increases were calculated compared to the control group (vehicle only).

For measuring systemic antiinflammatory activity of flavonoids, the compounds finely suspended in 0.5% tween 80 (100 μl/mouse) were oraally administered

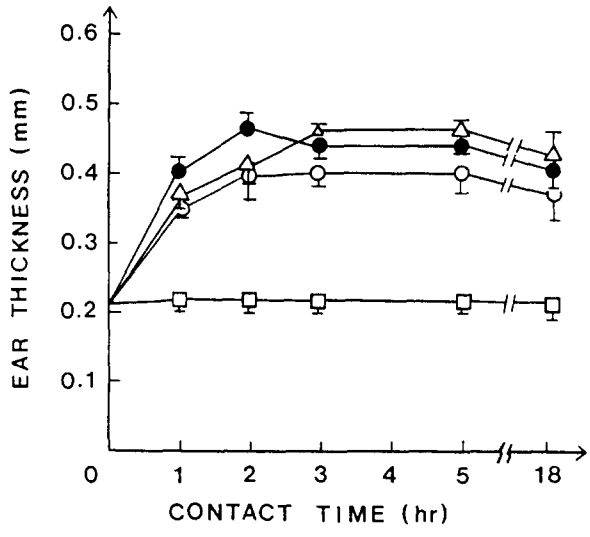


Fig. 2. Time course and dose response of croton-oil induced mice ear edema. Control (□), 1% croton-oil (○), 2.5% croton-oil (●), 5% croton-oil (△), Each data point and bar represents mean± SD (n=6).

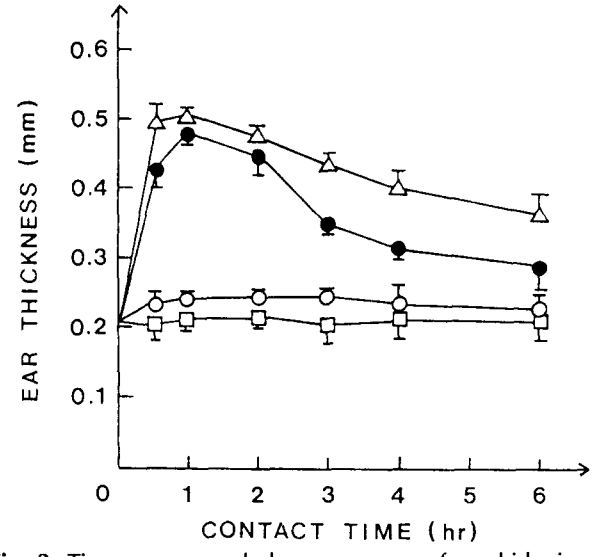


Fig. 3. Time course and dose response of arachidonic acid (AA) induced mice ear edema. Control (□), 0.4% AA (○), 2% AA (●), 10% AA (△), Each data point and bar represents mean± SD (n=6).

(vehicle alone administered to control) and 2.5% croton-oil dissolved in acetone (25 μl/ear) was applied topically 1 hr later. And ear thickness was measured 5 hr after croton-oil treatment. For measuring topical antiinflammatory activity of flavonoids, the compounds dissolved in acetone (25-50 μl/ear) were applied to mice ear. Thirty minutes after, 2.5% croton-oil was topically applied to mice ear and thickness was measured 5 hrs later. Percent inhibition of ear edema was calculated compared to the control group treated with vehicle and croton-oil only. For the compounds showing significant antiinflammatory activity at the dose of 2 mg/mouse, IC_{25} or IC_{50} was calculated by measuring inhibition from at least 3 different doses.

Arachidonic acid induced ear edema inhibition

All procedures of arachidonic acid induced ear edema inhibition were exactly same as the procedures of croton-oil induced edema inhibition test, except 2% arachidonic acid used as an inflammagen instead of croton-oil and the contact time was 1 hr.

Rat carrageenan pleurisy inhibition

According to the procedure of Schrier *et al.* (1990), rat pleurisy inhibition test was carried out. The test compounds dissolved in DMSO (0.1 ml) were orally administered to female SD rats (100-120 g) at the dose of 70 mg/kg. Three hours later, 0.2 ml of 1% λ-carrageenan solution (sterile saline) was injected intrapleurally. Rats were sacrificed with ethyl ether 5 and 24 hr after injection of carrageenan solution. The chest was opened by lateral incision. RPMI 1640 (3 ml) with 10% FCS was injected into pleural cavity and pleural fluid was aspirated using pasteur pipette. This washing procedure was repeated again and exudates were combined. After the volumes of pleural exudates were recorded, cell pellets were obtained by centrifugation at 200 g for 5 min. Total cell numbers were counted using Naubauer hemocytometer after red blood cells were lyzed with hemolytic solution (0.85% NH_4Cl, 0.1 M HEPES). Differential counts for polymorphonuclear leucocytes (PMNs) and mononuclear cells were carried out after May Grunwald-Giemsa staining.

Statistical analysis

Student t-test was used for the statistical analysis.

RESULTS

In order to determine proper concentration and contact time of inflammagens, curves of Fig. 2 and 3 were constructed for croton-oil and arachidonic acid ear edema. And the results were well correlated with others (Tonneli *et al.*, 1965; Young *et al.*, 1984; Chang *et al.*, 1985). From these curves, 2.5% croton-oil (contact time: 5 hrs) and 2% arachidonic acid (contact time: 1 hr) were selected and used for further experiments.

In croton-oil ear edema inhibition as represented in Table I, quercetin, morin and biochanin A showed significant edema inhibition with oral administration of flavonoids (2 mg/mouse). In this experiment, hydrocortisone showed potent inhibition, whereas indomethacin showed moderate inhibition comparatively. And 4-BPB, NDGA and phenidone did not show antiinflammatory activity. With topical application of flavonoids (2 mg/ear), quercetin, morin and biochanin A also showed significant inhibition and exceptions were apigenin

Table I. Croton-oil induced ear edema inhibition by flavonoids

Group	Thickness increased[a,b] Mean± SD	Thickness increased[a,c] Mean± SD
Control	0.22± 0.01 (0)[d]	0.25± 0.01 (0)
Hydrocortisone	0.07± 0.01 (68)**	0.03± 0.01 (88)**
Indomethacin	0.14± 0.01 (36)**	0.14± 0.01 (44)**
4-BPB	0.21± 0.02 (5)	0.25± 0.01 (0)
NDGA	0.23± 0.01 (−5)	0.18± 0.01 (28)**
Phenidone	0.22± 0.01 (0)	0.25± 0.01 (0)
Flavan-3-ols		
Epicatechin	0.21± 0.01 (5)	0.25± 0.01 (0)
Catechin	0.22± 0.01 (0)	0.25± 0.01 (0)
Flavanones		
Flavanone	0.22± 0.02 (0)	0.26± 0.01 (−5)
Naringenin	0.23± 0.02 (−5)	0.25± 0.01 (0)
Flavones		
Flavone	0.23± 0.02 (−5)	0.26± 0.00 (−5)
Chrysin	0.24± 0.01 (−9)	0.24± 0.01 (4)
Apigenin	0.19± 0.02 (14)	0.18± 0.01 (28)**
Flavonols		
Flavonol	0.21± 0.02 (5)	0.25± 0.01 (0)
Galangin	0.22± 0.02 (0)	0.26± 0.02 (−5)
Quercetin	0.16± 0.01 (27)**	0.19± 0.01 (24)**
Morin	0.19± 0.01 (14)*	0.22± 0.01 (12)
Isoflavones		
Biochanin A	0.17± 0.01 (23)**	0.19± 0.01 (24)**
Ethoxy-biochanin A[e]	0.22± 0.01 (0)	0.26± 0.01 (−5)

[a] Scale: mm.
[b] All compounds were orally administered at the dose of 2 mg/mouse (n=6).
[c] All compounds were topically applied to mice ear at the dose of 2 mg/ear (n=6).
[d] All values in parenthesis represent percent inhibition from control.
[e] Abbreviation of 2-carbethoxy-5,7-dihydroxy-4′-methoxyisoflavone.
*p<0.01, **p<0.001, Significantly different from the control group.

Table II. Arachidonic acid induced ear edema inhibition by flavonoids

Group	Thickness increased[a,b] Mean± SD	Thickness increased[a,c] Mean± SD
Control	0.27± 0.02 (0)[d]	0.25± 0.02 (0)
Hydrocortisone	0.20± 0.02 (26)*	0.12± 0.04 (52)**
Indomethacin	0.13± 0.01 (52)**	0.05± 0.01 (80)**
4-BPB	0.32± 0.02 (−18)	0.35± 0.02 (−37)*
NDGA	0.34± 0.02 (−27)*	0.13± 0.02 (48)**
Phenidone	0.32± 0.02 (−18)	0.04± 0.02 (84)**
Flavan-3-ols		
Epicatechin	0.25± 0.02 (7)	0.18± 0.02 (28)*
Catechin	0.24± 0.03 (11)	0.26± 0.04 (−4)
Flavanones		
Flavanone	0.24± 0.03 (11)	0.16± 0.05 (36)
Naringenin	0.27± 0.01 (0)	0.15± 0.01 (40)**
Flavones		
Flavone	0.23± 0.03 (15)	0.07± 0.01 (72)**
Chrysin	0.24± 0.02 (11)	0.12± 0.01 (52)**
Apigenin	0.22± 0.01 (18)*	0.11± 0.06 (56)*
Flavonols		
Flavonol	0.24± 0.03 (11)	0.21± 0.02 (16)
Galangin	0.23± 0.03 (15)	0.09± 0.01 (64)**
Quercetin	0.21± 0.03 (22)*	0.12± 0.04 (52)**
Morin	0.23± 0.01 (15)*	0.12± 0.04 (52)**
Isoflavones		
Biochanin A	0.23± 0.01 (15)*	0.14± 0.02 (44)**
Ethoxy-biochanin A[e]	0.25± 0.01 (7)	0.13± 0.02 (48)**

[a] Scale: mm.
[b] All compounds were orally administered at the dose of 2 mg/mouse (n=8).
[c] All compounds were topically applied to mice ear at the dose of 2 mg/ear (n=6).
[d] All values in parenthesis represent percent inhibition from control.
[e] Abbreviation of 2-carbethoxy-5,7-dihydroxy-4′-methoxyisoflavone.
*p<0.01, **p<0.001, Significantly different from the control group.

and NDGA.

Against arachidonic acid induced edema, apigenin, quercetin, morin and biochanin A showed significant inhibition (Table II) when flavonoids were orally administered (2 mg/mouse). Hydrocortisone and indomethacin showed activity, but, in this case, indomethacin was much more active than hydrocortisone. 4-BPB, NDGA and phenidone did not show antiinflammatory activity, but increased edema thickness. Flavone inhibited 15% of ear edema but not statistically significant. When these flavonoids were topically applied, all flavonoids tested except catechin, flavanone and flavonol, showed the significant inhibitory activities. Hydrocortisone, indomethacin, NDGA and phenidone also showed activity.

The IC_{25} or IC_{50} values of the several selected flavonoids were calculated from at least three different doses and represented in Table III.

When quercetin and biochanin A were tested in carrageenan induced rat pleurisy, these two flavonoids reduced number of total cells infiltrated in pleural cavity at 5 hr (Table IV) and 24 hr (Table V) after carrageenan injection. Biochanin A reduced exudate volume significantly at 24 hr and also showed pattern of reduction of exudate volume at 5 hr, but not statistically significant, in contrast to the finding that quercetin reduced exudate volume only at 24 hr. Indomethacin reduced both exudate volume and number of cells infiltrated in 5 hr pleurisy, while indomethacin reduced only number of cells infiltrated in 24 hr pleurisy. It

Table III. Relative activity of several flavonoids

Compounds	croton-oil induced edema		AA-induced edema	
	oral[a]	topical[a]	oral[a]	topical[b]
Hydrocortisone	0.06	0.004	2.1	2.0
Indomerthacin	0.90	0.3u	0.09	0.08
NDGA	−[c]	1.80	−	2.40
Phenidone	−	−	−	0.06
Flavone	−	−	−	0.49
Apigenin	−	1.57	4.7	−
Quercetin	1.95	2.08	4.3	1.85
Biochanin A	2.78	1.67	6.0	2.38

[a] IC_{25} (mg/mouse or mg/ear).
[b] IC_{50} (mg/mouse).
[c] Data not available.

Table IV. Effects of quercetin and biochanin A on 5 hr carrageenan pleurisy

Group	Exudate Vol. (ml) Mean± SD		Total No. of cells (×10⁷) Mean± SD		A	B
Control	5.25± 0.05		0.71± 0.01		−[b]	−
Carrageenan	6.90± 0.33	(0)[a]	9.43± 1.11	(0)	0	0
Hydrocortisone	6.70± 0.50	(12)	6.88± 1.85⁺	(29)	26	70
Indomethacin	6.40± 0.30⁺	(29)	5.13± 0.62**	(49)	46	94
Quercetin	6.92± 0.29	(−1)	6.80± 1.87⁺	(30)	35	−30
Biochanin A	6.70± 0.50	(12)	6.48± 1.12⁺	(38)	32	53

Five rats (70 mg of test compound/rat) per group.
[a] Values in parenthesis represent percent inhibition, which were calculated as 100−[(Test group-control)/(Carrageenan treated group-control)]×100, eq. Actual exudate volume for carrageenan treated group was 6.90−5.25=1.65 (ml). A: Percent reduction of number of PMNs compared to carrageenan treated group. B: Percent reduction of number of mononuclear cells compared to carrageenan treated group.
[b] Percent mononuclear cells for the control group were always more than 97%. **p<0.001, ⁺p<0.05, Significantly different from the carrageenan treated groups.

was also found that indomethacin reduced number of mononuclear cells more profoundly than PMNs. Interstingly indomethacin actually increased exudate volume in 24 hr pleurisy. However, hydrocortisone reduced both exudate volume and number of cells infiltrated in 24 hr pleurisy.

DISCUSSION

We evaluated thirteen flavonoid aglycones for the antiinflammatory activity against mouse ear edema induced by croton-oil or arachidonic acid, in comparison with hydrocortisone (SAID), indomethacin (NSAID, CO inhibitor), 4-BPB (PLA_2 inhibitor), NDGA and phenidone (CO/LO inhibitor).

In croton-oil induced ear edema test, most active

Table V. Effects of quercetin and biochanin A on 24 hr carrageenan pleurisy

Group	Exudate Vol. (ml) Mean± SD		Total No. of cells (×10⁷) Mean± SD		A	B
Control	5.35± 0.05		0.83± 0.02		−[b]	−
Carrageenan	5.83± 0.24	(0)[a]	9.20± 0.40	(0)	0	0
Hydrocortisone	5.40± 0.11*	(90)	5.10± 0.79**	(45)	49	50
Indomethacin	6.56± 0.54⁺	(−152)	6.45± 0.86**	(30)	18	99
Quercetin	5.42± 0.20⁺	(85)	7.27± 0.39**	(23)	21	21
Biochanin A	5.43± 0.24	(83)	7.29± 0.82*	(21)	23	23

Five rats (70 mg of test compound/rat) per group.
[a] Values in parenthesis represent percent inhibition, which were calculated as 100−[(Test group-control)/(Carrageenan treated group-control)]×100, eq. Actual exudate volume for carrageenan treated group was 5.83−5.35=0.48 (ml). A: Percent reduction of number of PMNs compared to carrageenan treated group. B: Percent reduction of number of mononuclear cells compared to carrageenan treated group.
[b] Percent mononuclear cells for the control group were always more than 97%. **p<0.001, *P<0.01, ⁺p<0.05, Significantly different from the carrageenan treated groups.

one was hydrocortisone as expected. 4-BPB and phenidone did not show activity. But NDGA, nonspecific lipoxygenase inhibitor, showed moderate activity by topical application. This antiinflammatory activity does not seem to be the activity by LO inhibition, but by antioxidative activity of NDGA (Ahnfelt-Ronne and Nielson, 1987; Maloff *et al.*, 1987; Faure *et al.*, 1990). This might be conformed by the fact that phenidone, potent CO/LO inhibitor, did not show activity in the same test. Among flavonoids, quercetin, morin and biochanin A showed weak, but significant activity, by both oral and topical application. Apigenin showed significant inhibiton when applied topically. Therefore it is suggested that 5,7-dihydroxy-flavone or -flavonol derivatives having at least one hydroxyl group in B-ring and biochanin A (isoflavone) possess antiinflammatory activity against croton-oil induced mouse ear edema. This kind of structure-activity relationship was similar to the previous finding of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mice ear edema inhibiton (Yasukawa *et al.* 1989). This similarity between croton-oil induced edema and TPA-induced edema may be reasonable in that croton-oil used as a inflammagen is extracted from the seed oil of *Croton tiglium* L., which contains phorbol esters (Lubach and Kietzmann, 1992). And it is interesting to note that biochanin A (isoflavone) showed antiinflammatory activity, because there has been a few report mentioning *in vivo* antiinflammatory activity of isoflavonoids (Gabov, 1986).

In arachidonic acid induced ear edema, the situation was totally different. When flavonoids were topically applied at the dose of 2 mg/ear, all flavonoids tested, except catechin, flavanone and flavonol, showed inhi-

bition. Especially, flavone derivatives such as flavone, chrysin and apigenin showed potent activity in arachidonic acid induced ear edema test. Flavone, which was known as a CO inhibitor (Welton *et al.*, 1988; Laughton *et al.*, 1991), was the most active one among flavonoids. There have been several reports concerning inhibition of CO/LO activities by flavonoids (Sekiya *et al.*, 1992; Yoshimoto *et al.*, 1983; Landolfi *et al.*, 1984; Welton *et al.*, 1988; Ferrandiz *et al.*, 1990; Laughton *et al.*, 1991). Flavonoids such as cirsiliol and quercetin were found to be relatively selective inhibitors of 5-lipoxygenase from rat leukemic cell (Yoshimoto *et al.*, 1983). Baicalein was revealed to be a most potent inhibitor of lipoxygenase in nature (Sekiya and Okuda, 1992). And flavone derivatives such as flavone, chrysin, and apigenin were revealed to be CO inhibitors and flavonols were to be LO inhibitors in human platelets (Landolfi *et al.*, 1984). All of these previous findings indicated that flavonoids not only inhibited CO/LO activity *in vitro*, but also showed inhibiton in cell level. And, from our results of this study, it is suggested that flavonoid aglycones including flavones, flavonols and isoflavones may show *in vivo* antiinflammatory activity against arachidonic acid induced mice ear edema, at least, partly due to CO/LO inhibition. Among positive controls, the order of potency was phenidone> Indomethacin>hydrocortisone>NDGA. 4-BPB was not active. In oral administration, among flavonoids tested, only apigenin, quercetin, morin and biochanin A were active, which indicated that bioavailability or biodegradation may be a crucial factor to express antiinflammatory activities of flavonoid aglycones. Therefore, it is worthy of mentioning that when flavonoid derivatives are evaluated for antiinflammatory activity using mouse ear edema test, routes of administration should be well considered.

In order to investigate *in vivo* antiinflammatory activity of biochanin A in another kind of model, we employed rat carrageenan pleurisy test, which is a widely accepted model by many researchers. According to our results (Table IV and V), hydrocortisone was more active in 24 hr pleurisy and indomethacine was more active in 5 hr pleurisy. And it was found that indomethacin increased exudate volume and reduced mononuclear cells in 24 hr pleurisy. This was well correlated with the previous results (Piper *et al.*, 1974; Baruth *et al.*, 19985; Welton *et al.*, 1986). Quercetin was more active in 24 hr pleurisy than 5 hr pleurisy for reducing the exudate volume. Quercetin reduced cell number in 5 hr and 24 hr pleurisy. However, biochanin A reduced the exudate volume and number of cells infiltrated in 5 hr and 24 hr pleurisy.

All of above results indicated that quercetin (flavonol) and biochanin A (isoflavone) showed broad antiinflammatory activity against croton-oil and arachidonic acid induced edema by oral and topical route of administration, and in carrageenan-induced rat pleurisy. These findings suggested that, in addition to CO/LO inhibition, other mechanisms such as antioxidant/antiradicalar actions (Afanasev *et al.*, 1989) and neutrophil modulation (Busse *et al.*, 1984; Kenny *et al.*, 1990) should be involved in antiinflammation by these flavonoids. And it was clearly established that isoflavonoid, biochanin A, possessed antiinflammatory activity.

ACKNOWLEDGEMENT

This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1991.

LITERATURE CITED

Amer, E. C., Rule, B. L., Rudnick, M. S. and Galbraith, W., Arachidonic acid-induced ear edema: Correlation of swelling with production of lipoxygenase (LO) products. *Agents and Actions,* 16, 600-601 (1985).

Ahnfelt-Ronne, I. and Nielsen, O. H., The antiinflammatory moiety of sulfasalazine, 5-aminosalicylic acid, is a free radical scavenger. *Agents and Actions,* 21, 191-194 (1987).

Afanasev, I. B., Dorozhiko, A. I., Brodskii, A. V., Kostyuk, V. A. and Potapovitch, A. I., Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.,* 38, 1763-1769 (1989).

Busse, W. W., Kopp, D. E. and Middleton, E., Flavonoid modulation of human neutrophil function. *J. Allergy Clin. Immunol.,* 73, 801-809 (1984).

Bouclier, M., Cavey, D., Kail, N. and Hensby, C., Experimental models in skin pharmacology. *Pharmacol. Rev.,* 42, 127-154 (1990).

Berkenkopf, J., Casmer, C. and Watnick, A., Drug activities in arachidonic acid and croton-oil induced inflammations. *Agents and Actions,* 16, 601-602 (1985).

Baruth, H., Berger, L., Bradshaw, D., Cashin, C. H., Coffey, J. W., Gupta, N., Konikoff, J., Roberts, N. A. and Wyler-plaut, R., Caprofen, In Rainsford, K. D. and Path, M. R. C. (eds), *Anti-inflammatory and antirheumatic drugs.* Vol. II, CRC Press, Boca Raton, 1985, pp. 33-47.

Chang, J., O'Neill-Davis, L., Lamb, B., Calson, R. P. and Lewis, A. J., The utility of arachidonic acid treated mouse ear inflammatory model as a potential screen for lipoxygenase (LO) inhibitors. *Agents and Actions,* 16, 600 (1985).

Della Loggia, R., Tubaro, A., Dri, P., Zilli, C. and Del Negro, P., The role of flavonoids in the antiinflammatory activity of *Chamomilla recutita.* In Cody *et al.* (eds), *Plant Flavonoids in Biology and Medicine: Biochemical, pharmacological, and structure-activity relationships.* Alan R. Liss, New York 1986, pp. 481-484.

Faure, M., Lissi, E., Torres, R. and Videla, L. A., Antioxi-

dant activities of lignans and flavonoids. *Phytochemistry,* 29, 3773-3775 (1990).

Ferrandiz, M. L., Ramachandran Nair, A. G. and Alcaraz, M. J., Inhibition of sheep platelet arachidonate metabolism by flavonoids from Spanish and Indican medicinal herbs. *Pharmazie,* 45, 206-208 (1990).

Gabor, M., Anti-inflammatory and anti-allergic properties of flavonoids. In Cody *et al.* (eds), *Plant flavonoids in biology and medicine: Biochemical, pharmacological, and structure-activity relationships.* Alan R. Liss, New York, 1986, pp. 471-480.

Harsteen, B., Flavonoids, A class of natural products of high ı harmacological potency. *Biochem. Pharmacol.,* 32, 1141-1148 (1983).

Kenny, M. T., Balistreri, F. J. and Tomey, H. L., Flavonoid modulation of murine neutrophil cytokinesis. *Immunopharmacol. Immunotoxicol.,* 12, 527-541 (1990).

Landolfi, R., Mower, R. L. and Steiner, M., Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Structure-activity relations. *Biochem. Pharmacol.,* 33, 1525-1530 (1984).

Laughton, M. J., Evans, P. J., Moroney, M. A., Hoult, J. R. S. and Halliwell, B., Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem. Pharmacol.,* 42, 1673-1681 (1991).

Lewis, D. A., Flavonoids. In Lewis, D. A. (eds.), *Anti-inflammatory drugs from plant and marine sources. Agents and Actions, Suppl.* Vol. 27, Berkhauser Verlag, Basel, 1989, pp. 137-165.

Lubach, D. and Kietzmann, M., Anti-inflammatory effect and thinning of the skin: Models for testing topically applied corticoids on human skin. In Maibacu, H. I. (eds.), *Topical Corticoids,* S. Karger, 1992, pp. 26-41.

Maloff, B. L., Peifer, D., Cooke, M. and Ackerman, N. R., Inhibition of LTB_4 binding to human neutrophils by nordihydroguaiaretic acid. *Agents and Actions,* 21, 358-360 (1987).

Piper, R. J., Sanda, M., Chinea, G. and Oronsky, A. L., Leukocyte chemotaxis *in vivo.* II. Analysis of the selective inhibition of neutrophil or mononuclear cell accumulation. *J. Lab. Clin. Med.,* 84, 394-406 (1974).

Schrier, D. J., Lesch, M. E., Wright, C. D. and Gilbertson, R. B., The antiinflammatory effects of adenosine receptor agonists on the carrageenan-induced pleural inflammatory response in rats. *J. Immunol.,* 145, 1874-1879 (1990).

Sekiya, K. and Okuda, H., Selective inhibition of platelet lipoxygenase by baicalein. *Biochem. Biophys. Res. Comm.,* 105, 1090-1095 (1982).

Tonneli, G., Thiabault, L. and Ringler, I., A bioassay for the concomitant assessment of the antiphlogistic and thymolytic activities of topically applied corticoids. *Endocrinol.,* 77, 625-634 (1965).

Welton, A. F., Tobias, L. D., Fiedler-nagy, C., Anderson, W., Hope, W., Meyers, K. and Coffey, J. W., Effect of flavonoids on arachidonic acid metabolism. In Coby *et al.* (eds), *Plant Flavonoids in Biology and Medicine: Biochemical, pharmacological, and structure-activity relationships.* Alan R. Liss, New York, 1986, pp. 231-242.

Welton, A. F., Hurley, J. and Will, P., Flavonoids and arachidonic acid metabolism. In Cody *et al.* (eds), *Plant Flavonoids in Biology and Medicine II: Biochemical, cellular, and medicinal properties.* Alan R. Liss, New York, 1988, pp. 301-312.

Yasukawa, K., Takido, M., Takeuchi, M. and Nakagawa, S., Effect of chemical constituents from plants on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation in mice. *Chem. Pharm. Bull.,* 37, 1071-1073 (1989).

Yoshimoto, T., Furukawa, M., Yamamoto, S., Horie, T. and Watanabe-Kohno, S., Flavonoids: Potent inhibitors of arachidonate 5-lipoxygenase. *Biochem. Biophys. Res. Comm.,* 116, 612-618 (1983).

Young, J. M., Spires, D. A., Bedord, C. J., Wagner, B., Ballaron, S. J. and De Yound L. H., The mice ear inflammatory response to topical arachidonic acid. *J. Invest. Dermatol.,* 82, 367-371 (1984).