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Studies of Structure Activity Relationship of Flavonoids for the Anti-allergic Actions

Ho Cheong, Shi-Yong Ryu[1], Min-Ho Oak, Seung-Hoon Cheon, Gyurng-Soo Yoo and Kyeong-Man Kim

College of Pharmacy, Chonnam National University, Kwang-Ju 500-757, Korea and [1]Korea Institute of Chemical Technology, Yusung P.O. Box 107, Taejeon 305-606, Korea

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The structure activity relationship of flavonoids for anti-allergic actions was studied by determining the IC_{50} values for the degranulation. The hexosaminidase release from RBL-2H3 cells (degranulation marker) was employed as an estimate for the anti-allergic actions. Among 22 flavonoid compounds tested, luteolin, apigenin, diosmetin, fisetin, and quercetin were found to be most active with IC_{50} values less than 10 μM.

Key words : Allergy, Flavonoids, Degranulation, Hexosaminidase

INTRODUCTION

Immediate hypersensitivity reaction is initiated as allergen-induced immunoglobulin E (IgE) binds to the high affinity IgE receptor (FcεRI) located on mast cell surface. On re-exposure to the same allergen, IgEs initially bound to the FcεRI are cross-linked to give a signal for the subsequent intracellular signaling pathways (Kinet *et al.*, 1987; Shimizu *et al.*, 1989; Ra *et al.*, 1989). Intracellular calcium is increased and degranulation occurs to release various inflammatory mediators such as histamine, serotonin, and leukotrienes.

β-hexosaminidase is located in the secretory granules of mast cells where histamines are stored, and is released along with histamine when mast cells are immunologically activated (Schwartz *et al.*, 1981; Marquardt *et al.*, 1983). With this reason, hexosaminidase is considered as a degranulation marker, and has been widely used for the biochemical studies of allergy (Choi *et al.*, 1996). It is also used for the screening of anti-allergic agents (Fisher *et al.*, 1995).

Flavonoids are classified into flavones, flavanones, flavonols, anthocyanidins and isoflavones according to their structural features. Flavonoids have numerous physiological actions including anti-allergic actions (Fewtrell and Gomperts, 1977; Fanning *et al.*, 1983; Middleton *et al.*, 1984; Kim and Chung, 1990). In this study, we investigated the structure activity relationship of flavonoids focusing on flavones and flavonols. Anti-allergic activities were determined by determining the IC_{50} values of structurally related compounds for inhibiting degranulation of RBL-2H3 cells.

Correspondence to: Kyeong-Man Kim, College of Pharmacy, Chonnam National University, Kwang-Ju 500-757, Korea

MATERIALS AND METHODS

Antigens and antibodies

DNP-BSA (2, 4-dinitrophenylated bovine serum albumin) was kindly provided by Dr. Koda (College of Pharmacy, Gifu University, Japan). DNP-specific mouse monoclonal IgE was obtained from the hybridoma (HiDNP mIgE) provided by Dr. Kinet (Harvard Medical School, USA).

Flavonoids

Flavone, 3-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone, chrysin (5,7-dihydroxyflavone), baicalein (5, 6,7-trihydroxyflavone), 3,6-dihydroxyflavone, diosmin, fisetin (3,3',4',7-tetrahydroxyflavone), galangin (3,5,7-trihydroxyflavone), myricetin (3,3',4',5,5',7-hexahydroxyflavone), morin (2',3,4',5,7-pentahydroxyflavone), naringenin (4',5,7-trihydroxyflavanone), baichanin A, (±)-catechin, (+)-catechin, (-)-epicatechin were purchased from Aldrich, USA. Apigenin (4',5,7-trihydroxyflavone), luteolin (3',4',5,7-tetrahydroxyflavone), kaempferol (3, 4',5,7-tetrahydroxyflavone), quercetin (3,3',4',5,7-pentahydroxyflavone) was isolated from *Chrysanthemum indicum*. Diosmetin (3',5,7-trihydroxy 4'-methoxyflavone) was obtained by the acid hydrolysis of diosmin. They were dissolved in 100% DMSO, then diluted with Siraganian buffer for desired concentrations.

Hexosaminidase assay

The hexosaminidase assay was conducted according to the method of Choi *et al.* [6]. RBL-2H3 cells were grown in EMEM supplemented with 10% fetal bovine serum and gentamicin. A day before experiments, RBL-

2H3 cells were dispensed in 24 well plates at 2×10^5 cells per well using the medium containing 0.5 μg/ml mouse monoclonal IgE. After incubation overnight, cells were washed twice with 500 μl of siraganian buffer (119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$ 25 mM PIPES, 40 mM NaOH, pH 7.2), and 160 μl siraganian buffer was added. After 10 min incubation at 37°C for 10 min, cells were treated with test substances (20 μl), then stimulated with DNP-BSA antigen (100 ng/ml final) for 20 min at 37°C to induce degranulation. The reaction was stopped on the ice bath for 10 min and 100 μl of the supernatant was taken. It was centrifuged with table top centrifuge and 20 μl of the supernatant was transferred to 96 well plates, then incubated with 20 μl of substrate (1 mM *p*-nitrophenyl-N-acetyl-β-D-glucosaminide) for 1hr at 37°C. The reaction was stopped by adding 200 μl of stop solution (0.1 M $Na_2CO_3/NaHCO_3$). The absorbance was measured by ELISA reader at 405 nm.

Statistics

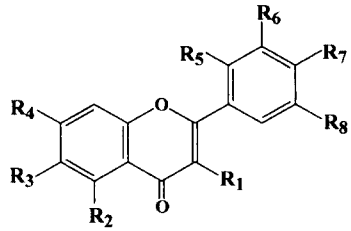
Student's *t*-tests were used for the statistical analysis.

RESULTS AND DISCUSSION

Figures 1-3 show the chemical structures and IC_{50} values of flavonoid analogs tested for the inhibition of hexosaminidase release from RBL-2H3 cells. Of the compounds we tested, flavones or flavonols were active (Fig. 1) but flavanones/isoflavones (Fig. 2) or catechins (Fig. 3) were not active.

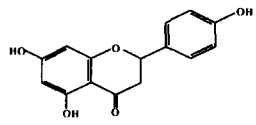
Among flavones and flavonols, luteolin was most active followed by almost indistinguishably apigenin, diosmetin, quercetin, 3,6-dihydroxyflavone, fisetin, and kaempferol (Fig. 1). Based on our experimental results, we could reach following conclusions for flavones and flavanols. For R1, R2, R6, and R8, either -H or -OH did not make big difference; -OH was much better than -H at R3, R4, and R7; sugar at R4 decreased the activity drastically; -H was better than -OH for R5.

The structure activity relationship of flavonoids for anti-allergic actions was extensively characterized by Kim and Chung (1990). For more than 20 flavonoids structural analogs, they conducted animal studies including 48hr passive cutaneous anaphylaxis. This was a great advance for the understanding the structure activity relationship of flavonoids for the anti-allergic activities. However, the technical limitation of experimental approach (animal studies) did not allow them to have precise determinations of relative potencies of each flavonoid. Here we employed an extremely delicate and stable *in vitro* experimental assay for the evaluation of anti-allergic activities. Using this assay, we could compare the relative and absolute anti-allergic activities of each flavonoid. These results would



Name	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	IC_{50} (μM)
Flavone	-H	-H	-H	-H	-H	-H	-H	-H	>100.0
3-hydroxyflavone	-OH	-H	-H	-H	-H	-H	-H	-H	>100.0
6-hydroxyflavone	-H	-H	-OH	-H	-H	-H	-H	-H	28.0
7-hydroxyflavone	-H	-H	-H	-OH	-H	-H	-H	-H	21.0
Chrysin	-H	-OH	-H	-OH	-H	-H	-H	-H	>100.0
Baicalein	-H	-OH	-OH	-OH	-H	-H	-H	-H	17.0
Apigenin	-H	-OH	-H	-OH	-H	-H	-OH	-H	4.5
Luteolin	-H	-OH	-H	-OH	-H	-OH	-OH	-H	1.8
Diosmetin	-H	-OH	-H	-OH	-H	-OH	$-OCH_3$	-H	
3,6-dihydroxyflavone	-H	-OH	-H	-OH	-H	-H	-H	-H	6.0
Diosmin	-H	-OH	-H	-OS*	-OH	-H	$-OCH_3$	-H	>100
Fisetin	-OH	-H	-H	-OH	-H	-OH	-OH	-H	3.3
Galangin	-OH	-OH	-H	-OH	-H	-H	-H	-H	40.0
Kaempferol	-OH	-OH	-H	-OH	-H	-H	-OH	-H	7.5
Quercetin	-OH	-OH	-H	-OH	-H	-OH	-OH	-H	3.0
Myricetin	-OH	-OH	-H	-OH	-H	-OH	-OH	-OH	6.7
Morin	-OH	-OH	-H	-OH	-OH	-H	-OH	-H	51.0

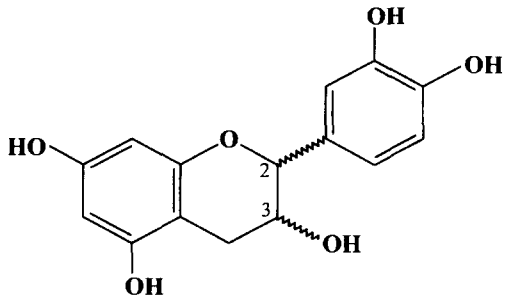
Fig. 1. Structure-activity relationship of flavones and flavonols. *represents sugar.



Naringenin - IC_{50} (μ M) >100 **Baichanin A - IC_{50} (μ M) >100**

[4, 5, 7-trihydroxyflavanone]

Fig. 2. Structure-activity relationship of flavanone and isoflavone.



(±)-catechin (2*RS*, 3*SR*) **IC_{50} (μ M) > 100**

(+)- catechin (2*RS*, 3*SR*) **IC_{50} (μ M) > 100**

(−)-epicatechin (2*R*, 3*R*)) **IC_{50} (μ M) > 100**

Fig. 3. Structure-activity relationship of catechins.

be utilized as valuable guide for the development of new anti-allergic drugs.

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