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Evaluation of Some Flavonoids as Potential Bradykinin Antagonists

Hye Sook Yun-Choi[1], Sung Hyun Chung[2] and Young Joo Kim[1]

[1]*Natural Products Research Institute, Seoul National University, Seoul 110-460 and* [2]*College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*

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Fourteen flavonoids were evaluated for their effects as potential bradykinin (BK) antagonists. The compounds were evaluated in several *in vitro* and *in vivo* (oral administration) systems; inhibition of BK induced contractions in isolated rat ileum and uterus, antagonistic effects of BK induced plasma extravasation, reduction of acetic acid induced writhing nociception and protection from endotoxic shock. Skullcapflavone II (**3**), baicalein (**5**), 5-methoxyflavone (**11**), 6-methoxyflavone (**12**) and 2'-methoxyflavone (**14**) showed effects in all the tests although the order of potency were somewhat varied.

Key words: Potential bradykinin (BK) antagonists, Skullcapflavone II, Baicalein, 5-Methoxyflavone, 6-Methoxyflavone, 2'-Methoxyflavone

INTRODUCTION

A variety of factors including tissue damage, allergic reactions, viral infections and other inflammatory events activate a series of proteolytic reactions leading to the production of bradykinin(BK) and related kinins in blood and tissues. Once released, a nonapeptide, BK (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) participates in a wide range of physiological and pathological processes; It is involved in various inflammatory conditions e.g. thermal and chemical injury (Clark, 1979), allergic reactions (Proud *et al.*, 1983), rheumatoid, psoriatic gouty arthritis(Hamberg *et al.*, 1978; Sharma *et al.*, 1983), asthma etc. (Christian *et al.*, 1987). BK is a potent algesic agent, comparable in potency to substance P, and many times more potent than serotonin, histamine or acetylcholine (Collier and Lee, 1963). In addition to its algesic and pro-inflammatory actions, BK exhibits a vasodilator action and because of its ability to lower blood pressure (Haddy *et al.*, 1970; Mason and Melmon, 1965), BK has been implicated in the pathogenesis of several shock syndromes, particularly septic or endotoxic shock (Hirsch *et al.*, 1974; Robinson *et al.*, 1975). Since the first synthesis of specific competitive BK B_2 receptor antagonists by the replacement of L-proline at the position 7 of BK sequence with D-phenylalanine (Vavrek and Stewart, 1985), a large number of related peptide analogues of BK (Burch *et al.*, 1990; Bathon and Proud, 1991) have been synthesized. And, more recently, several BK analogues containing modified amino acids have been found far more potent than the previous analogues and, in addition, selective for B_2 receptors (Hock *et al.*, 1991; Kyle *et al.*, 1991). The discovery of the B_2 antagonists has made remarkable contributions to the characterization of B_2 receptors which are far more ubiquitous than B_1 receptors and mediate most of the physiological or pathological effects of BK. B_2 antagonists block the majority of the biological effects of BK and in the initial trials, BK antagonists provided encouraging results in the relief of cold symptoms caused by the rhinovirus, in the suppression of pain from burns, and in the treatment of allergic asthma (Steranka *et al.*, 1989; Burch *et al.*, 1990). However, the currently available antagonists are peptide analogues of BK and most of them have very short half-lives *in vivo* and it is considered that the availability of a stable and specific non-peptidic BK antagonist could provide a possible new form of therapeutic potential.

Scutellariae radix have been used for the treatment of inflammatory diseases, suppurative dermatitis, allergic diseases, fever etc. in oriental traditional medicine and various biological activities including anti-thrombotic, anti-allergic, anti-inflammatory activities were reported with solvent extracts or with the constituents (Kubo *et al.*, 1984, 1985) More recently, the separations of seven flavonoid components were reported from a solvent fraction prepared from the extract of this plant which showed to inhibit BK-induced contractions in rat ileum or uterus, antagonize BK-induced plasma extravasation, protect mice from endotoxic

Correspondence to: Hye Sook Yun-Choi, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea.

shock and reduce acetic acid induced writhing in mice (Chung *et al.*, 1991; Yun-Choi *et al.*, 1992). In the present study, the flavonoids from Scutellariae radix and several other flavonoids were evaluated for their potential as BK antagonists.

MATERIALS AND METHODS

Materials

Bradykinin (BK) acetate salt and lipopolysaccharide (LPS, from *Escherichia coli*, serotype 055 : B5) were purchased from Sigma Chem. Comp., U.S.A. and Evan's blue (CH-9470 Buchs) was purchased from Fluka AG, Switzerland. Oroxylin A, wogonin, skullcapflavone II, 5,2'6'-trihydroxy-7,8-dimethoxyflavone and baicalein were isolated from Scutellariae radix as described previously (Yun-Choi *et al.*, 1992). Other flavonoids evaluated in this study were purchased from Carl Roth GmbH & Co., Germany.

Animals

Sprague-Dawley rats 160-200 g, ICR male mice, 20-25 g (for vascular permeability and writhing tests) and ddy male mice, 20-25 g (for endotoxic shock test) were used for the experiments.

Isolated Rat Ileum and Uterus Contraction Assay

Terminal ileum was isolated from male rat and 2-3 cm segment of ileum was suspended in 10 ml tissue bath filled with modified Kreb's solution (composition, g/l; KH_2PO_4 0.16, KCl 0.35, $CaCl_2$ 0.25, $MgSO_4$ $7H_2O$ 0.29, NaCl 6.9, $NaHCO_3$ 2.1, glucose 1.98) at 36℃ and bubbled with 95% O_2-5% CO_2. Uterine preparation was obtained from virgin rat treated with diethyl stilbesterol (0.1 mg/ml ethanol/Kg, s.c.) 24 hr prior to the experiment and suspended in 10 ml organ bath containing modified de Jalon's solution (composition, g/l; NaCl 9.0, KCl 0.42, $CaCl_2$ 0.045, glucose 0.5, $NaHCO_3$ 0.5, $MgCl_2$ 0.005) at 31℃ and bubbled with 95% O_2-5% CO_2. After the resting period of about 60 min, isotonic contractions induced by the addition of 50 ul of bradykinin (BK, 10 ug/ml) were measured under a resting load of 1 g and recorded on a kymograph. 0.1 ml of test sample solution was added to the bath 1 min prior to BK and the antagonistic effect (% inhibition) of the test compound was evaluated comparing with the contraction induced by BK alone.

BK Induced Vascular Permeability Test

Test compounds (200 mg/kg) were given orally to ICR mice 25 hr and 1 hr prior to the injection of Evan's blue (10 mg/10 ml saline/kg, *i.v.*). Subsequent intradermal injection of BK (20 ug/100 ul saline) into the back skin was followed to induce the extravasation of dye, bound to albumin. One hour after the BK injection, the appropriate area of the skin was removed and the diameter of the pigmented area was measured.

Writhing Test

Test compounds were given as above to ICR mice and 0.7% acetic acid (10 ml/kg) was injected intraperitoneally. Starting from 10 min after the injection, the numbers of the writhing syndrome were recorded for 10 min.

Endotoxic Shock Test

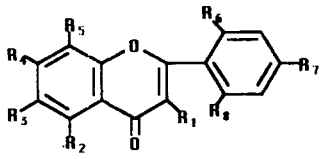
Test compounds were given as above to ddy mice and lipopolysaccharide (LPS, endotoxin, 30 mg/10 ml saline/kg) was injected into the tail vein. The animals were then observed up to 30 hrs after the LPS injection and the survival rates were calculated.

RESULTS AND DISCUSSION

Flavonoids are a group of compounds with benzo-γ-pyrone structure and are widely distributed in the plant kingdom. Thousands of flavonoids have been isolated and their structures have been determined (Harborne, 1988). Scutellariae plants were known to contain a large number of flavonoids and in addition, many of them are with unusual A- and/or B-ring substitutions. Actually, more than half of the flavonoids of Scutellariae plants are with unusual 5-methoxy in A-ring and/or 2'-oxygenation in B-ring substitutios (Yun-Choi, 1992). Several flavonoids isolated from Scutellariae radix were reported to be inhibitory against BK induced contractions in the previous paper (Yun-Choi *et al.*, 1992), and skullcapflavone II (**3**), which was most inhibitory against BK-induced contractions in rat isolated ileum and uterus, also contains 2',6'-dioxygenated substitutions. Nine additional related flavonoids were also selected with the structural interests and with the easy recruitment of the compounds for the present study.

It was known that BK receptors in rat uterus are B_2 types while rat ileum contains both B_1 and B_2 types (Regoli and Barabe, 1980). However, since some of the BK analogues, which selectively blocked BK induced contractions in rat uterus but not in guinea pig ileum, were found not specific BK antagonists but also inhibited vasopression induced contraction more potently than BK induced contraction (Burch *et al.*, 1990), both rat isolated ileum and uterus were employed for the primary screening for the BK antagonistic effects. BK antagonistic activities of flavonoids were evaluated in both rat ileum and uterus in the present study and the results are tabulated in Table II. The effects of comp. **1-5**, which were already reported (Yun-Choi,

Table I. Various flavonoids tested



		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	oroxylin A	H	OH	OCH_3	OH	H	H	H	H
2	wogonin	H	OH	H	OH	OCH_3	H	H	H
3	skullcapflavone II	H	OH	OCH_3	OCH_3	OCH_3	OCH_3	H	OH
4	5,2',6'-trihydroxy-7,8-dimethoxyflavone	H	OH	H	OCH_3	OCH_3	OH	H	OH
5	baicalein	H	OH	OH	OH	H	H	H	H
6	3-hydroxyflavone	OH	H	H	H	H	H	H	H
7	datiscetin	OH	OH	H	OH	H	OH	H	H
8	kaempferol	OH	OH	H	OH	H	H	OH	H
9	5-hydroxyflavone	H	OH	H	H	H	H	H	H
10	apigenin	H	OH	H	OH	H	H	OH	H
11	5-methoxyflavone	H	OCH_3	H	H	H	H	H	H
12	6-methoxyflavone	H	H	OCH_3	H	H	H	H	H
13	chrysin dimethylether	H	OCH_3	H	OCH_3	H	H	H	H
14	2'-methoxyflavone	H	H	H	H	H	OCH_3	H	H

Table II. Effects of various flavonoids against BK-induced contractions at isolated rat ileum and uterus

comp.	conc. (μg/ml)	ileum		uterus	
		20	40	20	40
1		–	23	–	34
2		63	76	–	14
3		62	93	53	90
4		14	52	14	37
5		–	42	–	32
6		56	84	–	16
7		29	68	–	22
8		54	81	–	12
9		26	71	–	12
10		–	48	–	13
11		66	93	–	49
12		87	98	41	75
13		38	88	56	73
14		58	98	60	87

Isolated ileum and uterus were pretreated with comp. (20 or 40 μg/ml) for 1 min prior to the addition of BK (50 ng/ml) and the average % inhibition was calculated from minimum five determinations.

et al., 1992), were also included in Table II for comparison. All the compounds showed at least some antagonistic effects in ileum. In isolated uterus, skullcapflavone II (**3**) and 2'-methoxyflavone (**14**) were most inhibitory showing 90 % and 87 % inhibition at 40 ug/ml respectively. 5-Methoxyflavone (**11**), 6-methoxyflavone(**12**), and chrysin dimethylether (**13**) showed 49 %, 75% and 73% inhibition respectively and moderate antagonistic effects (more than 30% inhibition) were observed with oroxylin A (**1**), 5,2',6'-trihydroxy-7,8-dimethoxyflavone (**4**) and baicalein (**5**) at 40 ug/ml. The flavonoids were also tested in three *in vivo* models. Several flavonoids were excluded from some experiments either because of low activity or shortage of the particular compound. For all the *in vivo* tests, flavonoids and aspirin which was employed as a positive control compound, were given orally (200 mg/kg) to mice twice (24 hr and 1 hr prior to each experiment) to see both of the long term and short term effects. Intradermal injection of BK into the skin of animals that have received *i.v.* injection of dye was observed resulting in intense blueing due to the extravasation of the dye. An increased capillary permeability is one of the cardinal features of acute inflammation and BK has been implicated to be one of the important mediators of inflammation (Proud, 1988). Actually, the ability of BK to increase capillary permeability to circulating dye by contracting endothelial cells was found to be one of its most striking pharmacological properties (Holdstock *et al.*, 1957) and various B_2 antagonists were observed to block the BK induced extravasation (Schachter *et al.*, 1987; Griesbacher and Lembeck, 1987). Mice were pretreated with flavonoids, the size of BK induced plasma extravasated and stained area was measured and the results are summerized in Table III. The flavonoids **3**, **5**, **11**, **12**, and **14**, which showed more than 30% antagonistic effects against BK-induced contractions in isolated uterus, again reduced the plasma extravasation. The diameters of the extravasated areas were reduced with the above flavonoids by 34-48%, while those were reduced with aspirin by 25%. BK has also been postulated to have a major role

Table III. Antagonistic effects of various flavonoids against BK-induced plasma extravasation

comp.	No. of animals	diameter of extravasation (mm, mean± s.d.)	inhibition (%)
control	13	11.4± 0.9	–
aspirin	9	8.5± 1.3	25
1	5	9.5± 0.3	17
2	5	8.3± 0.3	27
3	8	7.3± 1.6	36
4	4	9.2± 0.5	19
5	4	6.8± 1.0	40
6	5	10.7± 0.7	6
8	5	10.7± 1.0	6
9	5	11.1± 1.6	3
11	6	7.5± 1.8	34
12	6	5.9± 0.2	48
14	7	6.8± 1.1	40

Comp. (200 mg/kg, *p.o.*) were given 25 hr and 1 hr before the injection of Even's blue (10 mg/kg, *i.v.*) and subsequent injection of BK (100 μl, 2 mg/10 ml, *i.d.*). The diameter of the pigmented area was measured 1 hr after the BK injection.

Table IV. The effects of various flavonoids on the acetic acid induced writhing in mice

comp.	no. of animals	no. of writhing (mean± s.d.)	inhibition (%)
control	17	24± 5	–
aspirin	14	12± 3	50
1	8	19± 3	21
2	8	17± 5	29
3	8	7± 2	71
5	8	13± 5	46
6	7	20± 4	17
8	10	15± 4	37
9	7	20± 4	17
11	8	9± 2	62
12	8	7± 4	71
14	8	1± 1	96

Comp. (200 mg/kg, p.o.) were given 25 hr and 1 hr before the injection of 0.7% acetic acid (10 ml/kg, *i.p.*) and the numbers of writhing were counted for 10 min starting from 10 min after the injection.

Table V. The effects of various flavonoids on endotoxic shock

comp.	no. of dead / no. of total	survival (%)
control	13 / 15	13
3	1 / 8	87
5	2 / 8	75
6	4 / 7	43
9	4 / 7	43
11	2 / 8	75
12	3 / 7	57
14	0 / 8	100

Comp. (200 mg/kg, p.o.) were given 25 hr and 1 hr before the injection of lipopolysaccharide (30 mg/kg).

in pain production and antagonists of BK were observed to reduce abdominal writhing reflecting nociception elicited with *i.p.* injection of 0.7% acetic acid (Steranka *et al.*, 1987). Treatment with flavonoids **3**, **5**, **11**, **12**, and **14** also reduced the numbers of writhing induced by acetic acid (Table IV). 2'-Methoxyflavone (**14**) almost completely inhibited (96% inhibition) the induction of the writhing symptoms indicating their significant analgesic effects. Compound **3** (skullcapflavone II) and **12** (6-methoxyflavone) also showed strong analgesic effects (71% inhibition each). Compounds **5** and **11** were as much effective (46% and 62% inhibition respectively) as aspirin (50% inhibition). BK has also been implicated in the pathogenesis of septic shock. Although various complicated factors are involved in the endotoxic or septic shock conditions, high blood level of BK was observed in the course of septic shock and BK antagonists were reported to attenuate the shock symptoms, especially to reduce the mortality rate caused by endotoxin (Weipert *et al.*, 1988, Wilson *et al.*, 1989). The effects of flavonoids in the endotoxic shock model of mice are summerized in Table V. Species differencies were noticed among different mice during our experiments. Ddy mice were more sensitive to endotoxin than ICR mice and intravenous injection of lipopolysaccharide (30 mg/kg) to ddy mice resulted in death to most of the mice and only 13% of survival was observed at 30 hr of injection. Flavonoid **14** completely blocked the endotoxic effects and 100% of animals survived up to 30 hr and comp. **3**, **5**, **11** and **12** showed 87%, 75%, 75% and 57% of survival rates respectively. Comp. **6** and **9**, which were rather inactive in isolated uterus and in mice models of plasma extravasation or writhing symptoms, also showed about 40% protection of mice from endotoxic shock. From the above results, it was noted that flavonoids **3**, **5**, **11**, **12**, and **14** showed effects in all the animal systems tested although the order of potency were somewhat varied. The results also indicated that a methoxy group at $C_{2'}$ position of flavonoid ($R_6=OCH_3$) was favored for the BK-antagonistic effects as shown with skullcapflavone II (**3**) and 2'-methoxyflavone (**14**). A methoxy substitution at C_5 or C_6 position ($R_2=OCH_3$ or $R_3=OCH_3$) was also favored (**11**, **12** and **13**). Dioxygen functions at C_5 and C_6 (R_2 and R_3) and at $C_{2'}$ and $C_{6'}$ (R_6 and R_8) also seemed to give somewhat favorable effects (**1**, **4** and **5**). They were moderate antagonists against BK in rat uterus, although their effects *in vivo* were variable.

Although the above flavonoids are of low potency comparing with the known BK antagonists with peptide structures, the present compounds were consid-

ered worth to investigate since they, with their non-peptide structure, are supposed to be stable in the physiological conditions. Of course, the possibility can not be ruled out that they are non-specific and only functional antagonists of BK which exert their effects by inhibiting one of the post-receptor signal transduction events of BK and it also should be clarified whether they are competing with BK at the receptor sites.

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REFERENCES CITED

Burch, R. M., Farmer, S. G. and Steranka, L. R., Bradykinin receptor antagonists. *Med. Res. Rev.*, 10, 237-269 (1990).

Bathon, J. M. and Proud, D., Bradykinin antagonists. *Annu. Rev. Pharmacol. Toxicol.*, 31, 129-162 (1991).

Christian, S. C., Proud, D. and Cochrane, C. G., Detection of tissue kallikrein in the bronchoalveolar lavage fluid of asthmatic subjects. *J. Clin. Invest.*, 79, 188-197 (1987).

Chung, S. H., Choi, J. J., Yoo, K. S. and Yun-Choi, H. S., Bradykinin antagonistic activities of Scutellariae radix. *Kor. J. Pharmacogn.*, 22, 192-196 (1991).

Clark, W. G., Kinins and the peripheral and central nervous systems, In Erdos, E. G. (ed.), *Handbook of Experimental Pharmacology, Vol. 25* (supplement), Springer-Verlag, 1979, pp. 312-356.

Collier, H. O. and Lee, I. R., Nociceptive responses of guinea pigs to intradermal injections of bradykinin and kallidin-10. *Brit. J. Pharmacol.*, 21, 151 (1963).

Griesbacher, T. and Lembeck, F., Effect of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle in the rabbit. *Br. J. Pharmac.*, 92, 333-340 (1987).

Haddy, F. J., Emerson, T. E. Jr., Scott, J. B. and Daugherty, R. M. Jr., The effect of kinins on the cardiovascular system: in Erdos, E. G. (ed.), *Handbook of Experimental Pharmacology, Vol. 25*, Springer-Verlag, 1970, pp. 362-384.

Hamberg, U., Vahtera, E. and Moilanen, L., Functionally active $alpha_2$-macroglobulin and kinin release in synovial fluids of rheumatoid arthritis. *Agents and Actions*, 8, 50 (1978).

Harborne, J. B., The flavonoids, *Advances in research since 1980*, Chapman and Hall, London, 1988.

Hirsch, E. F., Nakajima, T., Oshima, G., Erdos, E. G. and Herman, C. M., Kinin system responses in sepsis after trauma in man. *J. Surg. Res.*, 17, 147-153 (1974).

Hock, F. J., Wirth, K., Albus, U., Linz, W., Gerhards, H. J., Hoe 140 a new potent and long acting bradykinin-antagonist; *in vitro* studies. *Br. J. Pharmacol.*, 102, 769-773 (1991).

Holdstock, D. J., Mathias, A. P. and Schachter, M., A comparative study of kinin, kallidin, and bradykinin. *Brit. J. Pharmacol.*, 12, 149-158 (1957).

Kubo, M., Matsuda, H., Kimura, Y., Okuda, H. and Arichi, S., Scutellariae radix. X. Inhibitory effects of various flavonoids on histamine release from rat peritoneal mast cells *in vitro*. *Chem. Pharm. Bull.*, 32, 5051-5054 (1984).

Kubo, M., Matsuda, H., Tani, T., Arichi, S., Kimura, Y. and Okuda, H., Studies on Scutellariae radix. XII. Anti-thrombic actions of various flavonoids from Scutellariae radix. *Chem. Pharm. Bull.*, 33, 2411-2415 (1985).

Kyle, D. J., Martin, J. A., Farmer, S. G., Burch, R. M., Design and conformational analysis of several highly potent bradykinin receptor antagonists. *J. Med. Chem.*, 34, 1230-1233 (1991).

Mason, D. T. and Melmon, K. L., Effects of bradykinin on forearm venous tone and vascular resistance in man. *Circulat. Res.*, 17, 106-113 (1965).

Proud, D., Kinin formation: Mechanisms and role in inflammatory disorders. *Ann. Rev. Immunol.*, 6, 49-83 (1988).

Proud, D., Togias, A., Nacleria, R. M., Crush, S. A., Norman, P. S. and Lichtenstein, L. M., Kinins are generated *in vivo* following nasal airway chalenge of allergic individuals with allergen. *J. Clin. Invest.*, 72, 1678-1685 (1983).

Regoli, D. and Barabe, J., Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, 32, 1-46 (1980).

Robinson, J. A., Klodnycky, Loeb, H. S. Racic, M. R. and Gunnar, R. M., Endotoxin, prekallikrein, complement and systemic vascular resistance. *Am. J. Med.*, 59, 61-67 (1975).

Schachter, M., Uchida, Y., Longridge, D. J., Labedz, T., Whalley, E. T., Vavrek, R. J. and Stewart, J. M., New synthetic antagonists of bradykinin. *Br. J. Pharmacol.*, 92, 851-855 (1987).

Sharma, J. N., Zeithlin, I. J., Deodhar, S. D. and Buchanan, W. W., Detection of kallikrein-like activity in inflamed synovial tissue. *Arch. Int. Pharmacodyn.*, 262, 279-286 (1983).

Steranka, L. R., Farmer, S. G. and Burch, R. M., Antagonists of bradykinin receptors. *FASEB J.*, 3, 2019-2025 (1989).

Vavrek, R. J. and Stewart, J. M., Competitive antagonists of bradykinin. *Peptides*, 6, 161-164 (1985).

Weipert, J., Hoffmann, H., Siebeck, M. and Whalley, E. T., Attenuation of arterial blood pressure fall in endotoxin shock in the rat using the competitive bradykinin antagonist Lys-Lys-(Hyp^2, Thi^{5,8}, DPhe^7)-BK (B4148). *Br. J. Pharmacol.*, 94, 282-284 (1988).

Wilson, D. D., de Garavilla, L., Kuhn, W., Togo, J., Burch, R. M. and Steranka, L. R., D-Arg-(Hyp^3-D-Phe^7)-bradykinin, a bradykinin antagonist, reduces mortality in a rat model of endotoxic shock. *Circ. Shock*, 27, 93-101 (1989).

Yun-Choi, H. S., Flavonoid components in plants of the Genus Scutellaria. *Kor. J. Pharmacogn.*, 23, 201-210 (1992).

Yun-Choi, H. S., Yoo, K. S., Chung, S. H., Yang, H. S. Choi, J. J. and Kim, Y. J., Flavonoids with bradykinin antagonistic effects from Scutellariae radix. *Kor. J. Pharmacogn.*, 23, 234-239 (1992).