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Acute and chronic antiinflammatory effects of plant flavonoids

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Abstract

The antiinflammatory activities of 30 flavonoids isolated from several plants of the Compositae family were investigated using carrageenan-induced mouse paw edema and cotton pellet-induced rat granuloma. Compounds were administered with a unique dose of 75 mg/kg i.p. in the acute test with carrageenan and 25 mg/kg/day in the chronic granuloma test. Flavonoids inhibit the development of the induced granuloma, mostly when a catechol or guaiacol-like B ring is contained in the compound structure, jaceosidin being the most active flavonoid screened. Flavonoids significantly inhibited the maximum edema response in the acute test. We conclude that several of the isolated flavonoids tested here showed antiinflammatory effects, depending on the experimental model used.

Keywords: Flavonoids; Antiinflammatory activity; Carrageenan-induced mouse paw edema; Granuloma

1. Introduction

Flavonoids are a class of secondary metabolites widely distributed in the plant kingdom. A fairly large number of plants known to contain flavonoids are used in folk medicine, in some cases as antiinflammatory agents. Biological assays using isolated compounds revealed that flavonoids exhibit a wide range of effects on biological systems. They have been shown to exert antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet, antiallergic and antiinflammatory activities. Also, it was found that they increase capillary permeability and exert an inhibitory effect on protein exudation and leucocyte migration.

Biochemical investigations of the flavonoid mechanism of action have shown that these compounds inhibit a wide variety of enzymatic systems. The ability of certain flavonoids to inhibit both cyclooxigenase and 5-lipoxigenase pathways of the arachidonate metabolism may contribute to the antiinflammatory properties [1]. On the other hand, flavonoids are known to display many antioxidant properties including scavenging free radicals and preventing lipid peroxidation [2]. These activities seem to be directly related to the number of hydroxyl groups at ring B [3].

To gain deeper insight into the structure–activity relationships, four 3′,4′-dihydroxyflavonols differing in the substitution of the A and C rings were evaluated for their ability to inhibit chemiluminescence. It could be shown that an additional *o*-hydroxy group in the A ring, or a 6-methoxy group, has no significant influence, thus confirming the *o*-hydroxy group of the B ring as the most important structural feature for the radical scavenging activity [4]. Moreover, 3,4-dihydroxyphenylacetic acid and 3-hydroxyphenylacetic acid metabolites which arise from quercitin glycosides from the human intestinal microflora have been tested [5].

The results indicated that 3,4-dihydroxyphenylacetic acid reduced chemiluminescence considerably in an amount which was much more pronounced than that of the other metabolite and quercitin.

As part of a study program of the chemical composition of numerous species from several genus of the *Compositae* family occurring in the San Luis neighborhood, a semi-arid region of Argentina, we isolated a large number of flavonoids, as shown in Table 1. Some of these plants are used in popular medicine because of their antiinflammatory activities.

The inhibition of chemiluminescence derived from the radical scavenger activity of flavonoids [4] showed the therapeutic relevance of these compounds, persuading us to test other antiinflammatory assays. We determined the inhibitory effects of 30 flavonoids belonging to the flavonone (7), flavone (13) and flavonol (10) types in two in vivo inflammation models: cotton pellet-induced granuloma and carrageenan-induced mouse paw edema. These results pro-

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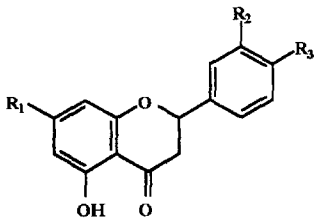
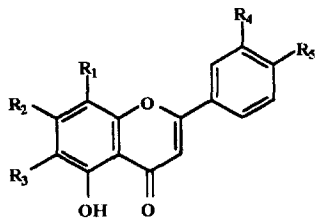
Table 1
Structures of compounds tested

Flavanones



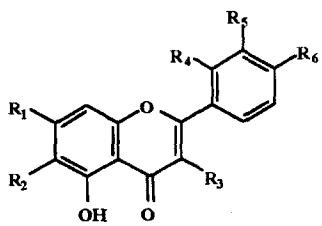
		R_1	R_2	R_3	Source
1	eridictyol	OH	OH	OH	a
2	7-*O*-methyleridictyol	OCH_3	OH	OH	a
3	hesperetin	OH	OH	OCH_3	p
4	hesperedin	ORutin	OH	OCH_3	q
5	sakuranetin	OCH_3	H	OH	a
6	homoeriodictyol	OH	OCH_3	OH	b
7	3′,4′-di-*O*-methyleriodictyol	OH	OCH_3	OCH_3	b

Flavones



		R_1	R_2	R_3	R_4	R_5	Source
8	7,3′-di-*O*-methylluteoline	H	OCH_3	H	OCH_3	OH	c
9	7,3′,4′-tri-*O*-methylluteoline	H	OCH_3	H	OCH_3	OCH_3	c
10	5,6,7,3′,4′-pentahydroxyflavone	H	OH	OH	OH	OH	r
11	nepetin (eupafolin)	H	OH	OCH_3	OH	OH	d
12	cirsiliol	H	OCH_3	OCH_3	OH	OH	e
13	jaceosidin	H	OH	OCH_3	OCH_3	OH	f
14	5-hydroxy-6,7,3′,4′-tetra-*O*-methylflavone	H	OCH_3	OCH_3	OCH_3	OCH_3	s
15	euparotin	H	OCH_3	OCH_3	OH	OCH_3	g
16	7-*O*-methylsudachietin	OCH_3	OCH_3	OCH_3	OCH_3	OH	h
17	pectolinaringenin	H	OH	OCH_3	H	OCH_3	i
18	navadensin	OCH_3	OH	OCH_3	H	OCH_3	i
19	genkwanin	H	OCH_3	H	H	OH	j
20	7,4′-di-*O*-methylapigenin	H	OCH_3	H	H	OCH_3	j

Flavonols



		R_1	R_2	R_3	R_4	R_5	R_6	Source
21	quercitin	OH	H	OH	H	OH	OH	k
22	3-*O*-methylquercitin	OH	H	OCH_3	H	OH	OH	k
23	rutin	OH	H	O-rutin	H	OH	OH	q
24	axillarin	OH	OCH_3	OCH_3	H	OH	OH	l
25	artemetin	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	l
26	7-*O*-isopentenylkaempferol	O-isopen.	H	OH	H	H	OH	m
27	rhamnazin	OCH_3	H	OH	H	OCH_3	OH	n
28	santin	OH	OCH_3	OCH_3	H	H	OCH_3	o
29	penduletin	OCH_3	OCH_3	OCH_3	H	H	OH	o
30	morin	OH	H	OH	OH	H	OH	q

Source: (a) *Tessaria dodoneaefolia* Hook et Arn.; (b) *Baccharis calliprinos* aff.; (c) *Petunia patagonica* (Speg) Milan; (d) *Gaillardia megapotamica* var. *Megapotamica*; (e) *Teucrium gricebachii* Hook et Arn.; (f) *Artemisia echegarayi* Hieron; (g) *Baccharis genistifolia* DC; (h) *Baccharis thymifolia* Hook et Arn.; (i) *Baccharis grisebachii* Hieron; (j) *Baccharis crispa* Sprengel; (k) *Grindelia pulchella* Dun; (l) *Tessaria absinthioides* Hook et Arn.; (m) *Pterocaulon alopecuroides* (L.) DC; (n) *Baccharis spartioides* (Hook et Arn.) Remy; (o) *Haplopapus scrobiculatus* (Nees) DC; (p) by hydrolysis of **4**; (q) purchased; (r) by demethylation of **11**; (s) by methylation of **11**.

vide support to strengthen the structure–activity relationships previously reported.

2. Results and discussion

In the cotton pellet model the inhibition data for flavanones, flavones and flavonols carrying a catechol or guaiacol-like B ring (3′,4′-dihydroxy or 3′-hydroxy-4′-methoxy or 3′-methoxy-4′hydroxy) were similar: **1**, **2**, **3**, **4**, **5**, **7**, **8**, **9**, **11**, **15**, **16**, **19**, **21**, **22**, **23**, **26**, and **27** (21–33%); more active were flavone **10** (37%) and jaceosidin **13** (44%), the most active flavonoid screened with a highly significant difference. However, morin **30**, having a resorcin-like B ring, exhibited an important inhibitory activity of 43% (Table 2). On the other hand, the 3′,4′-dimethoxy derivatives were less potent.

The flavonoids **5** and **19** (4′-hydroxy-7-*O*-methyl) were also effective in inhibiting granulomatous tissue formation. On the contrary, the *O*-methylation in position 4′ (**17** and **20**) cancels any inhibitory effect.

The inhibitory effect was also found to be independent of the presence of the double bond between C-2 and C-3. The flavonol **21** exhibited almost the same inhibitory potency as the flavonone **1** did. Similarly, the presence of a C-3–OH and its methylation or conjugation with sugar does not alter the activity. The flavones such as **10** and **11** or the flavonols **21** and **22** and glycoside **23** were equally potent in inhibiting cotton pellet-induced granuloma.

These findings demonstrate that flavonoids are capable of inhibiting the development of cotton pellet-induced granuloma, especially when a catechol or guaiacol-like B ring is present, while the involvement of ring A is still unclear.

Furthermore, flavonoids were tested in mice at different hours (1, 3, 5, and 7 h) for their activity against carrageenan-induced inflammation. The activities at each time of flavanones, flavones and flavonols are listed in Table 2. Flavanones **1** and **3**, flavones **10** and **20** and flavonols **21**, **22**,

Table 2
Antiinflammatory activity

Product	Inhibition (%) of induced granuloma (chronic test)	Inhibition (%) of carrageenan edema (acute test)			
		1 h	3 h	5 h	7 h
1	24*	30	44*	50	45
2	33*	31	48	17	29
3	28**	18	46*	17	25
4	30**	0	34	35	40
5	22*	0	36	27	16
6	14	0	12	23	40
7	27*	5	19	23	18
8	23*	16	29	38	15
9	23*	0	24	21	25
10	37**	21	23*	12	14
11	30*	5	35	40	35
12	26	16	31	38	12
13	44**	6	28	50	35
14	16	30	48	38	31
15	24	6	7	10	13
16	21*	24	25	45	27
17	0	5	38	35	32
18	10	0	3	10	3
19	26*	16	27	29	26
20	0	23	42*	22	28
21	26*	40	51*	50	46
22	31*	35	55	42*	31
23	24*	0	43	3	0
24	20*	0	15	9	0
25	0	0	23	29	4
26	22*	0	5	18	0
27	24*	39	54**	42*	40
28	13	20	44	27	30
29	0	0	18	14	4
30	43**	0	43*	54*	32
Dexamethaxone	50***	n.t.	n.t.	n.t.	n.t.
Phenylbutazone	n.t.	7	39**	61**	50*

Values represent percentage of edema reduction. Dunnet's *t*-test for unpaired data was applied for statistical evaluation ($n=6$ animals). Level of significance: * $p<0.05$; ** $p<0.005$; *** $p<0.001$ vs. control; n.t. not tested.

27 and **30** significantly inhibited the edema response at 7 h of carrageenan-induced mouse paw edema, which was in the same order of magnitude as that observed after phenylbutazone administration. Less pronounced inhibitive activities were observed with flavanones **2**, **3** and **5**, flavones **8**, **11**, **13**, **16**, **19** and **20**, and flavonol **28**. Flavonoids **6**, **7**, **9**, **10**, **12**, **17**, **25**, and **29** exhibited only a slight antiinflammatory effect without being significant and **15**, **18**, **24** and **26** showed no significant activity.

In the carrageenan model it was not possible to find a structure–activity relationship.

Summarizing all the flavonoids tested here, they appeared to have antiinflammatory activity depending on both their structure and the method used for the assay.

3. Experimental

3.1. Antiinflammatory activity

Acute antiinflammatory activity of compounds was determined against carrageenan-induced edema in the hindpaw of the mouse [6]. Rockland mice of both sexes (weight range 25–30 g) were divided into control, standard and test groups of six animals each. The compounds suspended in saline solution were administered to the test animals at 75 mg/kg intraperitoneally. Phenylbutazone (80 mg/kg) suspended in the same delivery system was given to the animals of the standard group. Edema was induced by subcutaneous injection of 0.05 ml of a 3.5% solution of carrageenan in saline solution in the subplantar region of the left hind paw in all the animals. The paw edema was measured plethysmographically (Ugo Basile plethysmograph) before injection and at intervals of 1, 3, 5 and 7 h after injection of carrageenan.

The edema volume is expressed in each animal as the difference found in the left hind paw compared with the control hind one. The percent edema inhibition was calculated for each group with respect to the control.

Chronic antiinflammatory activity was also investigated against a foreign body granuloma produced by implanting a pellet of cotton in a model of subchronic proliferative inflammation [7].

Female Wistar rats (weight range 120 g) were divided in three groups of six animals each, and granuloma was induced by implanting a small piece of sterile cotton (50 mg) into the dorsal area of the anesthetized rats.

The experimental group received 25 and 50 mg/kg subcutaneously of compounds daily for 6 days, the reference group was administered with 7 mg/kg of dexamethasone and controls received saline solution. At day 7, granulomas were excised, dried and weighed.

4. Statistical analysis

Dunnet's *t*-test for unpaired data was applied.

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