Antioxidant Profile of Mono- and Dihydroxylated Flavone Derivatives in Free Radical Generating Systems

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Flavone, Antioxidant, Free Radical, Human Neutrophil, Superoxide Generation

A number of free radical generating systems were used to investigate the antioxidant properties and structure-activity relationships of a series of monohydroxylated and dihydroxylated flavones. Ortho-dihydroxylated flavones showed the highest inhibitory activity on enzymic and non-enzymic microsomal lipid peroxidation as well as on peroxyl radical scavenging. Most flavones were weak scavengers of hydroxyl radical, while ortho-dihydroxylated flavones interacted with superoxide anion generated by an enzymic system or by human neutrophils. This series of compounds did not exert cytotoxic effects on these cells. Scavenging of superoxide and peroxyl radicals may determine the antioxidant properties of these active flavones.

Introduction

There is an increasing interest in the study of antioxidant compounds and their role in human health. Antioxidants may protect cells against free radical induced damage in diverse disorders including ischemic conditions, atherosclerosis, rheumatoid disease, lung hyperreactivity or tumour development (Sies, 1991; Bast *et al.*, 1991; Halliwell *et al.*, 1992; Rice-Evans and Diplock, 1993; Das and Maulik, 1994) .

Flavonoid derivatives have shown antioxidant, or pro-oxidant activities. They can inhibit peroxidative processes in lipid membranes mainly by scavenging free radicals (Bors and Saran, 1987; Fraga *et al.*, 1987; Sichel *et al.*, 1991; Cotelle *et al.*, 1992; Laughton *et al.*, 1991; Nègre-Salvayre and Salvayre, 1992; Carini *et al.*, 1992; Morel *et al.*, 1993; Grinberg *et al.*, 1994; Mira *et al.*, 1994; Bors *et al.*, 1990) , whereas some flavonoids exert prooxidant actions mainly by their iron-reducing ability and are able to generate oxygen radicals in some *in vitro* systems (Hodnick *et al.*, 1988; Laughton *et al.*, 1989) . It is interesting to note that flavonoids are components of many vegetables present in the human diet.

In previous work we studied the antioxidant and free radical scavenging properties of polyhydroxylated and polymethoxylated flavonoids mainly of natural origin (Huguet *et al.*, 1990; Mora *et al.*, 1990; Cholbi *et al.*, 1991; Ríos *et al.*, 1992; Sanz *et al.*, 1994) . Nevertheless the high number of substituents present in these natural compounds makes difficult to identify structure/activity relationships due to the simultaneous influence of multiple functional groups.

In this study we have investigated the antioxidative potential of a series of synthetic flavones which possess a reduced number of hydroxyl substituents to identify the structural features relevant for the antioxidative efficacy. The structure of these compounds appear in Table I. We have assessed their ability to interact with reactive oxygen species like hydroxyl and superoxide, which was generated by an enzymic reaction and also by human neutrophils. Inhibition of lipid peroxidation, a free radical mediated process, was studied using rat liver microsomes and non-enzymic and enzymic inducing systems, while to form peroxyl radicals we thermally activated the azo-initiator 2,2′-azo-bis-(2-amidinopropane).

Abbreviations: NBT, nitroblue tetrazolium; PBS, phosphate buffered saline; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; IC_{50} inhibitory concentration 50%; SOD, superoxide dismutase.

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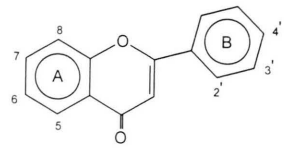
Table I. Structure of the flavones tested.



Compound	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$
7-Hydroxyflavone	H	H	OH	H	H	H	H
2′-Hydroxyflavone	H	H	H	H	OH	H	H
4′-Hydroxyflavone	H	H	H	H	H	H	OH
5,2′-Dihydroxyflavone	OH	H	H	H	OH	H	H
5,4′-Dihydroxyflavone	OH	H	H	H	H	H	OH
7,2′-Dihydroxyflavone	H	H	OH	H	OH	H	H
7,4′-Dihydroxyflavone	H	H	OH	H	H	H	OH
3′,4′-Dihydroxyflavone	H	H	H	H	H	OH	OH
6,7-Dihydroxyflavone	H	OH	OH	H	H	H	H
7,8-Dihydroxyflavone	H	H	OH	OH	H	H	H
8-Hydroxy-7-methoxyflavone	H	H	OCH_3	OH	H	H	H

Materials and methods

Drugs

Flavonoids were purchased from Apin Chem. Ltd. (Oxon, UK). 2,2′-Azo-bis-(-2-amidinopropane) dihydrochloride was obtained from Park Scientific Ltd., (Northampton, UK). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Liver microsomal preparations

Male Wistar rats (200–250 g) were killed by cervical dislocation and liver microsomes prepared as previously described (Slater and Sawyer 1971). Aliquots of microsomal suspensions in 1.15% (w/v) KCl were stored at −80 °C.

Lipid peroxidation induced by Fe^{2+}/ascorbate

Reaction mixtures contained 2 mg microsomal protein/ml in 0.1 M Tris[hydroxymethyl]aminomethane-HCl, pH 7.4. Peroxidation was induced by $FeSO_4$ (5 μM) and ascorbate (500 μM). The samples were incubated in triplicate at 37 °C for 20 minutes and products of lipid peroxidation were detected by the thiobarbituric acid method measuring the absorbance at 535 nm (Mansuy *et al.*, 1986) . Appropriate controls were performed to discard any possible interference with the assay (Halliwell, 1990) .

Lipid peroxidation induced by CCl_4/NADPH (Slater and Sawyer, 1971)

Reaction mixtures contained 1.5 mg microsomal protein, the NADPH-generating system (0.2 mM $NADP^+$, 4 mM glucose-6-phosphate, 0.6 units glucose-6-phosphate dehydrogenase) in the same buffer as above. Peroxidation was started by addition of CCl_4 (0.02 M). After 15 minutes incubation at 37 °C thiobarbituric acid-reactive substances were determined as above (Mansuy *et al.*, 1986; Halliwell, 1990) .

Lipid peroxidation induced by Fe^{3+}-ADP/NADPH

Final concentrations were: 0.25 mg microsomal protein, 10 mM KH_2PO_4–KOH buffer, pH 7.4, 1.7 mM ADP and 100 μM Fe^{3+} (pre-mixed before addition). Addition of NADPH (400 μM) was followed by incubation at 37 °C for 20 minutes. Lipid peroxidation products were detected by the thiobarbituric acid method as above (Mansuy *et al.*, 1986; Halliwell, 1990) .

Peroxyl radical scavenging

The reaction was carried out at 45 °C for 90 min in reaction mixtures (1 ml) of 50 mM

KH_2PO_4–KOH pH 7.4, 0.68 mM lysozyme, different concentrations of flavones and 10 mM 2,2′-azo-bis-(-2-amidinopropane) dihydrochloride. Aliquots of 50 µl were added to 950 µl of a suspension of *Micrococcus lysodeikticus* (0.3 mg/ml) in Dulbecco's buffer and the change of absorbance was followed at 450 nm during the first minute (Lissi and Clavero, 1990) .

Hydroxyl radical generation

Hydroxyl radical (·OH) was generated by incubation for 60 minutes at 37 °C in the presence of 20 µM $FeCl_3$, 1.4 mM H_2O_2, 2.8 mM deoxyribose and 100 µM EDTA in 1 ml of 10 mM KH_2PO_4–KOH buffer, pH 7.4. Deoxyribose degradation by hydroxyl radical was estimated using the thiobarbituric acid method (Laughton *et al.*, 1989) .

Superoxide radical generation

Superoxide was generated by oxidation of hypoxanthine (100 µM) with xanthine oxidase grade I (0.066 U; one unit converts 1 µmol of xanthine to uric acid per min at pH 7.5 at 25 °C) in 1 ml of 10 mM KH_2PO_4–KOH buffer, pH 7.4 and was detected by nitroblue tetrazolium (NBT, 100 µM) followed spectrophotometrically at 550 nm (Halliwell, 1985). Influence on enzyme activity was followed by uric acid formation from xanthine (15 min incubation at 25 °C) and absorbance was measured at 295 nm (Payá *et al.*, 1992) .

Preparation of human neutrophils

Erythrocytes were sedimented in 2.0% (w/v) dextran (Mw 526,000) in 0.9% NaCl at room temperature and the supernatant was centrifuged at 1200 *g* for 10 min at 4 °C. Remaining erythrocytes were lysed by hypotonic treatment. The pellet was resuspended in phosphate-buffered saline solution (PBS), and neutrophils were purified by ficoll-hypaque sedimentation. Using the trypan blue exclusion test viability was greater than 95%. The cells were resuspended in PBS containing 1.26 mM Ca^{2+} and 0.9 mM Mg^{2+}.

Superoxide generation by human neutrophils

2.5×10^6 Cells/ml were preincubated for 5 min at 37 °C with test compound and NBT (100 µM) and then they were stimulated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA, 1 µM) for 10 min. Tubes were centrifuged at 1200 *g* for 10 min at 4 °C and the pellets were treated with 500 µl of dimethyl sulfoxide:HCl (95:5, v/v) and sonicated. Absorbance was measured at 540 nm in a microtiter plate reader (Rice-Evans *et al.*, 1991) .

Cytotoxicity studies

The cytoplasmic marker enzyme lactate dehydrogenase was determined in supernatants after incubation of cells with test compounds or vehicle by measuring the rate of oxidation of 180 µM NADH, using 0.63 M sodium pyruvate dissolved in pH 7.5 50 mM phosphate buffer as substrate and recording the rate of absorbance change at 340 nm (Bergmeyer and Bernt, 1974) .

Statistical analysis

The two-way analysis of variance (ANOVA) was performed, followed by the Dunnett's t-test for unpaired samples.

Results

Ortho-dihydroxylated flavones exhibited the highest inhibitory activity on lipid peroxidation irrespective of the inducing system used (Table II). The presence of a free catechol group was necessary for inhibition of non-enzymic lipid peroxidation and blockade of the hydroxyl at C-7 by methylation dramatically reduced the activity. In contrast, the resulting compound inhibited enzymic lipid peroxidation. A number of flavones unable to inhibit non-enzymic lipid peroxidation showed potent inhibitory efects when the lipoperoxidative process was induced by CCl_4/NADPH, while they exerted weak effects or were inactive on the Fe^{3+}-ADP/NADPH system, suggesting that they could scavenge CCl_4-derived free radicals. Another possibility would be that they could interact with the microsomal enzymic system responsible for the metabolism of this xenobiotic, a previous step leading to CCl_4 activation (Cheeseman, 1981; Cheeseman, 1982).

Orthodihydroxylated flavones also inhibited lysozyme inactivation by peroxyl radicals although they were less potent than on lipid peroxidation (Table III). A similar behaviour was observed with

Table II. Effect of flavones on microsomal lipid peroxidation.

Compounds	Fe^{2+}/ascorbate %I 100 μM	IC_{50} (μM)	CCl_4/NADPH %I 100 μM	IC_{50} (μM)	Fe^{3+}-ADP/NADPH %I 100 μM	IC_{50} (μM)
7-Hydroxyflavone	5.4±0.9**	ND	39.2±3.9**	ND	5.0±1.6	ND
2′-Hydroxyflavone	4.3±0.9*	ND	72.0±2.8**	3.7±0.8	11.5±1.4	ND
4′-Hydroxyflavone	4.8±0.9*	ND	33.4±1.6**	ND	5.5±0.7	ND
5,2′-Dihydroxyflavone	4.4±1.1*	ND	81.0±0.6**	1.9±0.2	29.0±1.0**	ND
5,4′-Dihydroxyflavone	6.5±0.7**	ND	61.4±1.2**	9.2±3.3	5.4±0.7	ND
7,2′-Dihydroxyflavone	7.2±0.3**	ND	69.3±1.2**	18.4±1.4	28.4±3.7**	ND
7,4′-Dihydroxyflavone	5.4±0.8**	ND	76.5±1.1**	18.6±7.3	12.2±5.9*	ND
3′,4′-Dihydroxyflavone	96.7±0.2**	7.3±0.3	86.1±1.0**	3.4±1.0	94.4±0.5**	16.3±3.9
6,7-Dihydroxyflavone	96.8±0.2**	10.0±0.6	76.2±2.5**	9.4±1.2	95.7±0.5**	21.9±4.8
7,8-Dihydroxyflavone	96.9±3.3**	5.1±0.7	92.5±0.6**	2.7±0.5	97.4±0.2**	15.0±8.5
8-Hydroxy-7-methoxyflavone	23.6±1.5**	ND	78.0±1.0**	21.3±3.1	78.1±3.1**	26.2±4.6
Propyl gallate	96.9±1.5**	4.9±0.1	98.6±1.2**	4.5±0.6	95.4±2.2**	8.0±0.1

Absorbances due to the adduct formed between products of lipid peroxidation and thiobarbituric acid were measured at 535 nm. Percentage of inhibition due to flavonoid was calculated after deducting the basal level of peroxidation (without stimulus) which gave an absorbance value of 0.017±0.02 (mean±SE, n = 9) in the absence of flavonoid. In the presence of flavonoid, this basal level was not significantly modified. Absorbance values in control groups (without flavonoid) were: Fe^{2+}/ascorbate = 0.749±0.005; CCl_4/NADPH = 0.125±0.002; Fe^{3+}-ADP/NADPH = 0.843±0.024 (mean±SE, n = 12). ND = not determined. Results are expressed as mean ±SE of percentages of inhibition (% I) from 6–12 determinations. An appropriate range of five concentrations was used to calculate the inhibitory concentration 50% (IC_{50}) .*P<0.05, **P<0. 01.

the reference compound, propyl gallate. In contrast to the inhibition of non-enzymic lipid peroxidation, blockade of the hydroxyl at C-7 did not reduce the potency of the compound. With a lower potency, 7,4′-dihydroxyflavone and 7,2′-dihydroxyflavone also behaved as scavengers of the peroxyl radical.

As seen in Fig. 1, most flavones tested were weak scavengers of the hydroxyl radical and only 5,2′-dihydroxyflavone and 3′,4′-dihydroxyflavone were inactive. Interestingly, 6,7-dihydroxyflavone and 7,8-dihydroxyflavone increased deoxyribose degradation indicating possible pro-oxidant effects. In the presence of ascorbate there is a higher generation of the hydroxyl radical and 6,7-dihydroxyflavone was inactive, whereas 7,8-dihydroxyflavone behaved as a weak inhibitor of deoxyribose degradation (Fig. 2). The most active

Table III. Effect of flavones on peroxyl radical scavenging.

Compounds	% Inhibition (100 μM)	IC_{50} (μM)
7-Hydroxyflavone	17.8±1.3**	ND
2′-Hydroxyflavone	26.4±3.4**	ND
4′-Hydroxyflavone	20.2±1.7**	ND
5,2'-Dihydroxyflavone	0.6±1.3	ND
5,4′-Dihydroxyflavone	12.3±1.4**	ND
7,2′-Dihydroxyflavone	62.6±1.3**	75.6±3.4
7,4′-Dihydroxyflavone	46.9±1.7**	113.8±19.2
3′,4′-Dihydroxyflavone	98.9±2.2**	22.5±3.4
6,7-Dihydroxyflavone	99.7±0.5**	39.7±5.9
7,8-Dihydroxyflavone	99.8±1.5**	24.6±0.5
8-Hydroxy-7-methoxyflavone	99.7±3.3**	26.7±1.6
Propyl gallate	100.0±1.8**	33.9±4.8

The change of absorbance was followed at 450 nm. Percentages of inhibition were calculated by comparison of dA/min values for control and flavonoid groups. Results are expressed as mean ±SE of percentages of inhibition from 6 determinations. **P<0.01. dA/min in control group (without flavonoid) was 0.453±0.008. ND = not determined.

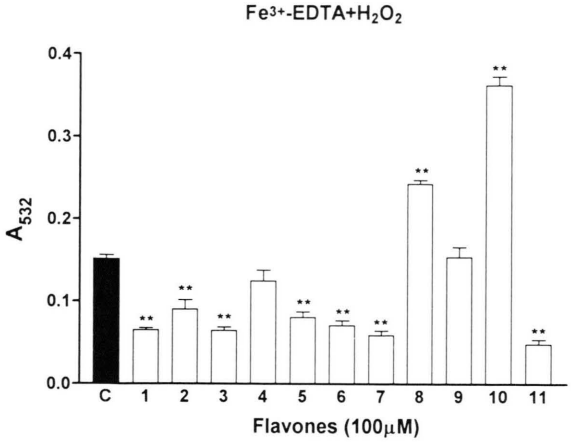


Fig. 1. Effect of flavones on deoxyribose degradation by Fe^{3+}–EDTA+H_2O_2. Mean ± SE from 6–24 determinations. **P<0.01. **C**: control, **1**: 7-hydroxyflavone, **2:** 2′-hydroxyflavone, **3**: 4′-hydroxyflavone, **4:** 5,2′-dihydroxyflavone, **5:** 5,4′-dihydroxyflavone, **6**: 7,2′-dihydroxyflavone, **7**: 7,4′-dihydroxyflavone, **8**: 3′,4′-dihydroxyflavone, **9**: 6,7-dihydroxyflavone, **10**: 7,8-dihydroxyflavone, **11**: 8-hydroxy-7-methoxyflavone. The reference scavengers were mannitol (50 mM) and dimethylsulfoxide (20 mM) which gave percentages of inhibition of 60.0 ± 1.4** and 83.3 ± 3.3**, respectively.

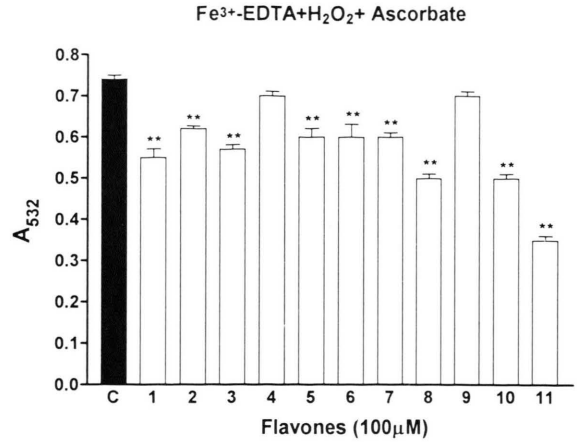


Fig. 2. Effect of flavones on deoxyribose degradation by Fe^{3+}–EDTA+H_2O_2+Ascorbate. Mean ± SE from 6–10 determinations. **P<0.01. **C**: control, **1**: 7-hydroxyflavone, **2:** 2′-hydroxyflavone, **3**: 4′-hydroxyflavone, **4:** 5,2′-dihydroxyflavone, **5:** 5,4′-dihydroxyflavone, **6**: 7,2′-dihydroxyflavone, **7**: 7,4′-dihydroxyflavone, **8**: 3′,4′-dihydroxyflavone, **9**: 6,7-dihydroxyflavone, **10**: 7,8-dihydroxyflavone, **11**: 8-hydroxy-7-methoxyflavone. The reference scavengers were mannitol (50 mM) and dimethylsulfoxide (20 mM) which gave percentages of inhibition of 89.0 ± 0.3** and 100.0 ± 1.8**, respectively.

compound was 8-hydroxy-7methoxyflavone, which at 100 μM inhibited the reaction by 67.9±2.2%, P<0.01 (without ascorbate) and 52.0±1.5%, P<0.01 (with ascorbate).

Only two monohydroxylated flavones, 7-hydroxyflavone and 4′-hydroxyflavone showed some inhibitory activity on NBT reduction by superoxide generated by hypoxanthine/xanthine oxidase

Table IV. Effect of flavones on superoxide generated by hypoxanthine/xanthine oxidase.

Compounds	% Inhibition (100 μM)	IC_{50} (μM)
7-Hydroxyflavone	31.0±3.3**	ND
2′-Hydroxyflavone	4.4±2.6	ND
4′-Hydroxyflavone	16.8±3.3**	ND
5,2′-Dihydroxyflavone	2.2±2.2	ND
5,4′-Dihydroxyflavone	4.7±1.7	ND
7,2′-Dihydroxyflavone	22.2±1.2**	ND
7,4′-Dihydroxyflavone	64.0±1.5**	ND
3′,4′-Dihydroxyflavone	94.4±0.4**	3.6±0.4
6,7-Dihydroxyflavone	98.7±0.5**	3.7±0.3
7,8-Dihydroxyflavone	97.8±0.4**	12.2±3.2
8-Hydroxy-7-methoxyflavone	0.0±0.7	ND
Propyl gallate	88.9±0.3	14.7±0.8
SOD	88.9±0.3** (100 U/ml)	2.4±0.3 (U/ml)

The change of absorbance was followed at 560 nm. Percentages of inhibition were calculated by comparison of dA/min values for control and flavonoid groups. Results are expressed as mean ±SE of percentages of inhibition from 6–12 determinations. **P<0.01. dA/min in control group (without flavonoid) was 0.440±0.006. ND = not determined.

(Table IV). Nevertheless ortho-dihydroxylated derivatives inhibited this reaction with IC_{50} in the μM range. At these concentrations they did not show any influence on xanthine oxidase activity. In contrast, 7,4′-dihydroxyflavone is an inhibitor of this enzyme at concentrations between 5 to 100 μM with an IC_{50} value of 44.7±6.8 μM. Thus, this assay did not permit to determine its possible superoxide scavenging properties.

We also tested this series of flavones as scavengers of superoxide using intact human neutrophils stimulated with TPA. As shown in Table V, 7,4′-dihydroxyflavone exhibited a slight effect, indicating that it is not a scavenger of superoxide. Two ortho-dihydroxylated compounds, 3′,4′-dihydroxyflavone and 7,8-dihydroxyflavone, which scavenged superoxide in the xanthine oxidase system were also active in human neutrophils. Nevertheless 6,7-dihydroxyflavone, which potently acted as scavenger of superoxide in enzyme preparations, showed a lower effect in neutrophils. The flavones tested did not exert cytotoxic effects on human neutrophils, as they did not induce lactic dehydrogenase release (data not shown).

Discussion

The presence of free hydroxyl groups at C-5 and C-7, besides the catechol group in the B ring has been reported to increase the antilipoperoxidative activity of flavone derivatives in enzymic and non-enzymic *in vitro* systems (Mora *et al.*, 1990; Cholbi *et al.*, 1991; Sanz *et al.*, 1994). In the present work, the results obtained with flavones possessing only one or two hydroxyl groups, have confirmed the importance that free catechol groups in the rings A (6,7-dihydroxyflavone and 7,8-dihydroxyflavone) or B (3′,4′-dihydroxyflavone) have for activity.

Flavones could inhibit lipid peroxidation either by interfering with the initiation or the propagation phases of this process (Halliwell, 1990). The most active flavones on microsomal lipid peroxidation also behaved as scavengers of peroxyl radicals, suggesting that these ortho-dihydroxylated compounds would act as chain-breaking antioxidants with donation of a hydrogen atom to intermediate peroxyl and alkoxyl radicals and formation of a more stable flavone radical. Indeed, in certain conditions aroxyl radicals of flavones have been generated and the semiquinone formed from ortho-dihydroxyl groups was quite stable (Bors and Saran, 1987).

Table V. Effect of flavones on superoxide generated by human neutrophils.

Compounds	% Inhibition (100 μM)	IC_{50} (μM)
7-Hydroxyflavone	4.8±2.8	ND
2′-Hydroxyflavone	− 3.3±3.5	ND
4′-Hydroxyflavone	9.4±7.2	ND
5,2′-Dihydroxyflavone	− 1.5±0.3	ND
	−38.6±6.6**	ND
5,4′-Dihydroxyflavone	12.8±0.6	ND
7,2′-Dihydroxyflavone	16.7±3.9*	ND
7,4′-Dihydroxyflavone	73.9±2.5**	26.3±5.9
3′,4′-Dihydroxyflavone	29.9±5.8**	ND
6,7-Dihydroxyflavone	90.4±1.6**	12.6±2.2
7,8-Dihydroxyflavone	7.5±1.2	ND
8-Hydroxy-7-methoxyflavone		
Propyl gallate	70.8±3.1**	50.8±3.6
Fraxetin	84.7±1.6**	6.7±3.7
SOD	93.4±2.3** (100 U/ml)	11.1±2.1 (U/ml)

Absorbances were measured at 540 nm. Percentages of inhibition were calculated by comparison of absorbance values for control and flavonoid groups. Results are expressed as mean ±SE of percentages of inhibition from 6 determinations. *$P<0.05$. **$P<0.01$. Absorbance in control group (without flavonoid) was 0.504±0.010. ND = not determined.

The hydroxyl radical is a extremely reactive species which can be originated by a Fenton-type reaction (Halliwell, 1990) . This radical can be formed from superoxide, *e.g.* produced by phagocytic cells and contribute to the production of cell damage during inflammatory processes (Weiss, 1989) . The observed inhibitory effects on deoxyribose degradation exerted by the flavones tested could be due in part to the scavenging of superoxide, since superoxide dismutase inhibits the generation of hydroxyl radical by the system Fe^{3+}–EDTA+H_2O_2 (Laughton *et al.*, 1989) . On the other hand, ascorbate increases the rate of hydroxyl radical production by redox-cycling iron. In this system the generation of hydroxyl radical is not inhibited by superoxide dismutase (Laughton *et al.*, 1989) and the flavones tested showed a lower inhibitory effect, suggesting that they are weak scavengers of hydroxyl radical. The scavenging of this radical by flavones will have physiological relevance if the concentrations of these compounds obtained *in vivo* are high enough to compete with biological molecules.

It has been suggested that complexation of Fe^{3+} with EDTA can facilitate the oxidation of phenols by iron leading to the production of superoxide and thus to a higher generation of hydroxyl radical (Laughton *et al.*, 1989) . Nevertheless we have not observed pro-oxidant effects of these flavones in suspensions of intact cells, which are a more physiological system.

The antioxidant properties of flavones may be related to scavenging of superoxide, which has been reported to depend on the presence of free hydroxyl groups in the B ring (Sichel *et al.*, 1991). Nevertheless our results indicate that such a structural feature is not essential for this activity. For scavenging of superoxide, a catechol (Huguet *et al.*, 1990) or pyrogallol structure of the B ring is necessary and the activity is also favoured by the presence of a hydroxyl group at C-6 (Cotelle *et al.*, 1992). In our study, we have confirmed the importance of catechol groups in the B ring observed for polysubstituted flavones (Huguet *et al.*, 1990) and also in the A ring, since 6,7-dihydroxyflavone and 7,8-dihydroxyflavone are scavengers of superoxide. These structural features are also supported by our data on the generation of superoxide by human neutrophils. In this system, it does not seem to be relevant for activity the presence of free hydroxyl groups at C-5 and C-7, structural determinants suggested for polyhydroxylated flavones (Limasset *et al.*, 1993). None of the flavones seemed to affect directly the process of superoxide generation by human neutrophils since their potency or efficacy did not increase in this cell system as compared with the xanthine oxidase assay.

Activation of phagocytes induces the release of lysosomal enzymes and the generation of reactive oxygen species which play an important role in the defence against infection (Weiss, 1989). This process can also contribute to damage tissue components in inflammatory conditions. Thus, the control of phagocyte activation and generation of superoxide can represent a key step in the modulation of inflammatory states. It is interesting to note that these flavones have not exerted cytotoxic effects on human neutrophils and that orthodihydroxylated derivatives, mainly 3′,4′-dihydroxyflavone and 7,8-dihydroxyflavone possess antioxidant properties *in vitro*. Our results thus confirm and extend previous findings on structure/activity relationships for the flavonoid class. On the other hand, our data can also provide a basis to understand better the mechanism of action of some flavonoid compounds *in vivo*, like 7,8-dihydroxyflavone which exerts hepatoprotective effects in mice (Payá *et al.*, 1993) probably dependent on its antioxidant action.

Acknowledgements

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