Biochemical Pharmacology, Vol. 42, No. 9, pp. 1673–1681, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

INHIBITION OF MAMMALIAN 5-LIPOXYGENASE AND CYCLO-OXYGENASE BY FLAVONOIDS AND PHENOLIC DIETARY ADDITIVES

RELATIONSHIP TO ANTIOXIDANT ACTIVITY AND TO IRON ION-REDUCING ABILITY

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(*Received* 11 *April* 1991; *accepted* 4 *July* 1991)

Abstract—We investigated the ability of various plant flavonoids (a) to inhibit 5-lipoxygenase and cyclo-oxygenase activities in rat peritoneal leukocytes, (b) to inhibit lipid peroxidation in rat liver microsomes, and (c) to stimulate DNA degradation caused by the antibiotic bleomycin in the presence of ferric ions. These compounds were compared with a range of synthetic phenolic substances including carnosol, vanillin, vitamin E and its analogue trolox c. The flavonoids were potent inhibitors of non-enzymatic peroxidation in membranes but this was not significantly correlated with their ability to inhibit either pathway of eicosanoid synthesis, suggesting that their mode of inhibition of 5-lipoxygenase/cyclo-oxygenase is not simply due to interception of peroxyl radicals generated at the active site of the enzymes. Many of the flavonoids and other compounds (including carnosol, vitamin E and trolox c) stimulated Fe^{3+}/bleomycin-dependent DNA degradation. Those flavonoids which stimulated DNA degradation at low concentrations but which inhibited it at higher concentrations ("biphasic" effect, possibly caused by changing relative contributions of ability to reduce ferric-bleomycin or to chelate iron ions from the bleomycin) were selective inhibitors of 5-lipoxygenase compared to cyclo-oxygenase. In contrast, those flavonoids that did not stimulate DNA degradation at all proved to be cyclo-oxygenase selective inhibitors. Compounds that increased Fe^{3+}/bleomycin-dependent DNA damage up to a maintained plateau were non-selective inhibitors of both 5-lipoxygenase and cyclo-oxygenase. Thus, a combination of iron-chelating and iron ion-reducing properties appears to be required for selective 5-lipoxygenase inhibition by phenolic compounds. Carnosol, vitamin E and trolox c were also found to be 5-lipoxygenase inhibitors of varying potency, and all were less active as cyclo-oxygenase inhibitors.

Leukotrienes, prostanoids and other eicosanoids play important roles in human metabolism [1]. Their biosynthesis involves the controlled and stereospecific insertion of molecular oxygen into various positions in arachidonic acid, catalysed by lipoxygenase or cyclo-oxygenase enzymes [1]. Since increased production of leukotrienes has been implicated in several human diseases, there has been considerable interest in the development of 5-lipoxygenase inhibitors for therapeutic use [1, 2]. Many phenolic compounds found in plants, especially flavonoids, are powerful inhibitors of both 5-lipoxygenase and cyclo-oxygenase [3–6]. In a recent survey of the action of flavonoids upon these enzymes in rat peritoneal leukocytes, some compounds were found to be selective as lipoxygenase inhibitors, some as cyclo-oxygenase inhibitors, some inhibited both enzymes and some were poorly active against either enzyme [3]. These selectivities suggested that different structure-dependent mechanisms determine inhibition of the two enzymes by the flavonoids, and prompted further studies.

Both cyclo-oxygenase and lipoxygenase catalyse controlled stereospecific free radical peroxidation of arachidonic acid at their active sites. Polyunsaturated fatty acids or fatty acyl side chains in membranes can also be peroxidized in the absence of enzymes by exposure to reactive oxygen species and/or to transition metal ions in a free radical chain reaction known as lipid peroxidation (reviewed in Ref. 7). The products formed are complex and non-stereospecific, but there are relationships between enzymic and non-enzymic peroxidation. Thus, low levels of lipid peroxides can activate both cyclo-oxygenase and lipoxygenase enzymes and thereby increase eicosanoid formation [8, 9]. Many lipoxygenase inhibitors, including some flavonoids, also inhibit lipid peroxidation [4, 10–13]. Similarly, some inhibitors of lipid peroxidation, such as vitamin E, have been shown to inhibit lipoxygenases [14]. Inhibitors of lipid peroxidation often act by scavenging chain-propagating peroxyl (lipid-O_2·) free radicals [7, 15], and it is therefore widely believed that lipoxygenase inhibition by such chain-breaking antioxidants is due to scavenging of similar radicals that are formed within the active site of the enzymes [4, 9, 16–18].

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Table 1. Inhibition of microsomal lipid peroxidation by flavonoids and related phenolic compounds

Compound	Number of —OH groups	Inhibition of lipid peroxidation IC_{50} (μM ± SEM)
Aglycone flavonoids		
Flavone	0	675 ± 41
3-Hydroxyflavone	1	20 ± 3.6
Chrysin	2	45 ± 2
Galangin	3	3 ± 0.3
Naringenin	3	465 ± 47
Fisetin	4	1.5 ± 0.1
Kaempferol	4	1.5 ± 0.3
Hypolaetin	5	<5
Quercetin	5	1.5 ± 0
Morin	5	2.5 ± 0.2
Gossypetin	6	<5
Myricetin	6	1.5 ± 0
Glycosides		
Naringin	2	Inactive
Rutin	4	30 ± 2
Hypolaetin-8-glucoside	4	~5
Gossypin	5	<5
Other compounds		
NDGA	4	0.3 ± 0.1
Gossypol	6	0.1 ± 0

IC_{50} values are based on 2–3 experiments in which the compounds were tested at 4–8 concentrations in duplicate. In some cases (hypolaetin, hypolaetin-8-glucoside, gossypetin and gossypin), due to scarcity of material only one experiment was performed with each concentration tested in duplicate, and an upper limit for the inhibitory activity has been shown, and no SEM is given. Test system was Fe^{3+}-ascorbate-induced liver microsomal lipid peroxidation, as described in Materials and Methods.

However, recent studies suggest that the capacity of various catechols to inhibit soybean lipoxygenase I is related to their ability to reduce ferric iron at the active site to the catalytically inactive ferrous form [19, 20]. Many flavonoids and other plant phenolics have iron ion-reducing ability [19–22] and, in some cases, this causes them to stimulate free radical damage to substrates other than lipids [13, 23]. For example, several plant phenolics (including the flavonoids quercetin and myricetin) were found to accelerate iron ion-dependent damage to DNA by the anti-tumour antibiotic bleomycin, presumably by reducing ferric bleomycin to the ferrous form and allowing it to complex with oxygen and attack the DNA [13].

In the present paper, we examine the relationship between iron ion-reducing ability, ability to inhibit lipid peroxidation and the action of plant phenolics upon cyclo-oxygenase and 5-lipoxygenase activities in rat peritoneal leukocytes. These cells were chosen because their activation causes the generation of products by both cyclo-oxygenase and 5-lipoxygenase pathways.

MATERIALS AND METHODS

Reagents. Hypolaetin, hypolaetin-8-glucoside, gossypetin and gossypin were prepared as described in Ref. 3. Other chemicals were from the Sigma Chemical Co. except for flavone, 3-hydroxyflavone, galangin, fisetin, myricetin and trolox c (which were from the Aldrich Chemical Co.), BW755c (which was a gift from the Wellcome Research Laboratories), carnosol (from Nestec Research, Switzerland) and Hanks balanced salt solution (HBSS, from Gibco).

Isolation of rat peritoneal leukocytes. Mixed peritoneal leukocytes were elicited from 200 g female Wistar rats by an i.p. injection of 10 mL of a solution of 6% (w/v) glycogen in sterile saline, followed 20 hr later by 60 mL ice-cold modified HBSS free of Ca^{2+} and Mg^{2+}. After 45 sec massage, the peritoneal washing was removed, centrifuged at 400 *g* for 10 min at 4° and the contaminating erythrocytes in the pellet lysed after resuspension in a small volume of HBSS by adding 9 vol. isotonic Tris-buffered ammonium chloride (0.83% w/v, pH 7.2) for 10 min at 37°. After a further centrifugation and washing, the cells were resuspended in HBSS containing 1.26 mM Ca^{2+} and 0.9 mM Mg^{2+}. Viability based on trypan blue exclusion was better tnan 95%.

Stimulation of release of eicosanoids and their radioimmunoassay (RIA). Aliquots of 0.5 mL mixed peritoneal leukocytes at 5×10^6 cells/mL were placed in 1.5 mL polypropylene microcentrifuge tubes and preincubated for 10 min at 37° with the compound of interest or its vehicle (usually methanol) added in 5 μL. To this was added 5 μL of ionophore A23187 (final concentration 1 μM), dissolved in dimethyl sulphoxide (vehicle alone added to controls) for a further 10 min incubation. After the cells had been pelleted in a bench centrifuge for 1.5 min, the supernatants were decanted and frozen. Aliquots of 25 or 5 μL of the thawed samples were analysed by RIA for TXB_2 or LTB_4 (indicators for

cyclo-oxygenase and 5-lipoxygenase, respectively) as described previously in Ref. 3.

Kinetic studies of cytochrome c reduction. Substances under test were dissolved in a small volume of ethanol and rapidly mixed with a solution of cytochrome *c* (30 μM, Sigma type VI horse heart ref. C7752) in degassed 50 mM HEPES–KOH buffer, at pH 7.4, in a cuvette. The change in A_{550} was recorded using a double beam spectrophotometer.

Concentrations of test substances were chosen to ensure pseudo-first order conditions if possible, and second order rate constants were determined from the variation in pseudo-first order rate constant with concentration of the test substance. Where such a calculation was not possible due to the rapidity of the reaction, the rate constant was estimated by dividing the pseudo-first order rate constant by the concentration of the test substance.

Assay of lipid peroxidation. Microsomal fractions were prepared from freshly homogenized rat livers as described in Ref. 24 and suspended in 0.25 M NaCl, pH 7.4, at 2.5–5.0 mg microsomal protein/mL. They were stored in aliquots at −20° for no longer than 3 weeks. Reaction mixtures contained the following reagents in a final volume of 1.0 mL: 0.5 mL phosphate–saline buffer (3.4 mM Na_2HPO_4/NaH_2PO_4 plus 0.15 M NaCl, adjusted to pH 7.4), phenolic compound dissolved in ethanol (or an equal volume of ethanol in the controls), 100 μM $FeCl_3$, 0.25 mg of microsomal protein and 100 μM ascorbic acid. Solutions of ascorbic acid and iron salts were made up fresh immediately before use. Reaction mixtures were incubated at 37° for 20 min and then subjected to the thiobarbituric acid (TBA) test as described in Ref. 24. The $(TBA)_2$-MDA chromogen was extracted into butan-1-ol and the A_{532} of the upper organic layer measured.

Bleomycin assay. Bleomycin-dependent degradation of calf thymus DNA was assayed essentially as described in Ref. 13. For this, 0.5 mL DNA (1 mg/mL) was mixed with 0.05 mL bleomycin sulphate (1.5 units/mL), 0.1 mL $MgCl_2$ (0.05 M), 0.05 mL Tris buffer (1.0 M, pH 7.4), 0.1 mL $FeCl_3$ (0.5 mM) and the reaction was started by addition of 0.1 mL of the test substance, dissolved in ethanol. Tubes were incubated at 37° for 1 hr, followed by the addition of 1 mL of 25% (w/v) HCl and 1 mL TBA reagent (1% w/v TBA in 0.05 M NaOH). The tubes were heated at 100° for 10 min to develop the MDA-$(TBA)_2$ chromogen which was measured at 532 nm after cooling. Ethanol, in which many of the phenols were dissolved, does not affect bleomycin-dependent DNA degradation [13, 25].

RESULTS

Rat liver microsomes undergo rapid non-enzymatic peroxidation when incubated in the presence of $FeCl_3$ and ascorbic acid, and this is a popular system for testing antioxidant activity [25, 26]. A range of flavonoids was tested for their ability to inhibit peroxidation and IC_{50} values were determined (Table 1). In general, inhibitory activity increased with the number of —OH substituents. NDGA and gossypol were also powerful inhibitors of peroxidation, as expected [13]. Figure 1 shows some representative graphs of the concentration-dependent inhibition of lipid peroxidation by eight of the flavonoids.

Several flavonoids have also been shown to inhibit 5-lipoxygenase and cyclo-oxygenase activities in rat peritoneal leukocytes (as measured by effects on the production of LTB_4 and TXB_2, respectively) and IC_{50} values have been determined [3]. Figure 2A shows that antioxidant activity (as measured in the microsomal system) and ability to inhibit lipoxygenase were not significantly correlated ($r = 0.37$, $P > 0.05$). There was also no correlation between antioxidant activity and ability to inhibit cyclo-oxygenase ($r = 0.05$, $P > 0.05$, Fig. 2B). In order to confirm the accuracy of our assays of antioxidant ability, we compared our IC_{50} values with those already published for the ability of flavonoids to inhibit peroxidation of a different lipid substrate, mitochondrial membranes [27]. A good correlation was obtained ($r = 0.83$, $P < 0.05$, Fig. 2C).

The flavonoids were then tested for pro-oxidant action towards non-lipid substrates by examining their ability to accelerate damage to DNA induced by a bleomycin–Fe^{3+} complex [13, 25]. Three kinds of action were observed. Some flavonoids did not promote DNA damage by bleomycin–Fe^{3+} at any of the wide range of concentrations tested. Flavonoids that did promote DNA damage produced two types of concentration-dependent effects. In the first type, the amount of DNA damage initially increased with flavonoid concentration, but then declined markedly with further increase in concentration. Figure 3A shows two examples of this "biphasic" effect, using myricetin and fisetin. In the second type, DNA damage increased with concentration up to a maximum, but then remained essentially constant or began to decrease only at exceptionally high concentrations. Figure 3B shows morin and kaempferol as examples of this "saturation" effect. Table 2 lists the compounds of each type tested.

The relationship between the effect of the flavonoids in the bleomycin assay and their actions on cyclo-oxygenase and lipoxygenase were then investigated (Table 2). There was a striking relationship between the effects of these naturally occurring phenolic substances in the two kinds of test. Thus, those compounds which were inactive in the bleomycin assay and which did not promote DNA damage were potent inhibitors of cyclo-oxygenase, with much less effect on 5-lipoxygenase (i.e. were cyclo-oxygenase selective). In contrast, the two flavonoids (morin and kaempferol) which demonstrated a "saturation" effect in the bleomycin assay were moderately potent inhibitors of both enzymes, and were therefore non-selective. Finally, the six flavonoids which demonstrated a "biphasic" effect on DNA damage proved to have variable potency as 5-lipoxygenase inhibitors, but were in all cases less potent as inhibitors of cyclo-oxygenase (i.e. were 5-lipoxygenase selective).

These results suggest that behaviour in the bleomycin assay is somehow correlated with the action of the plant phenolics upon lipoxygenase and cyclo-oxygenase. Our previous studies have identified many compounds that accelerate bleomycin-induced

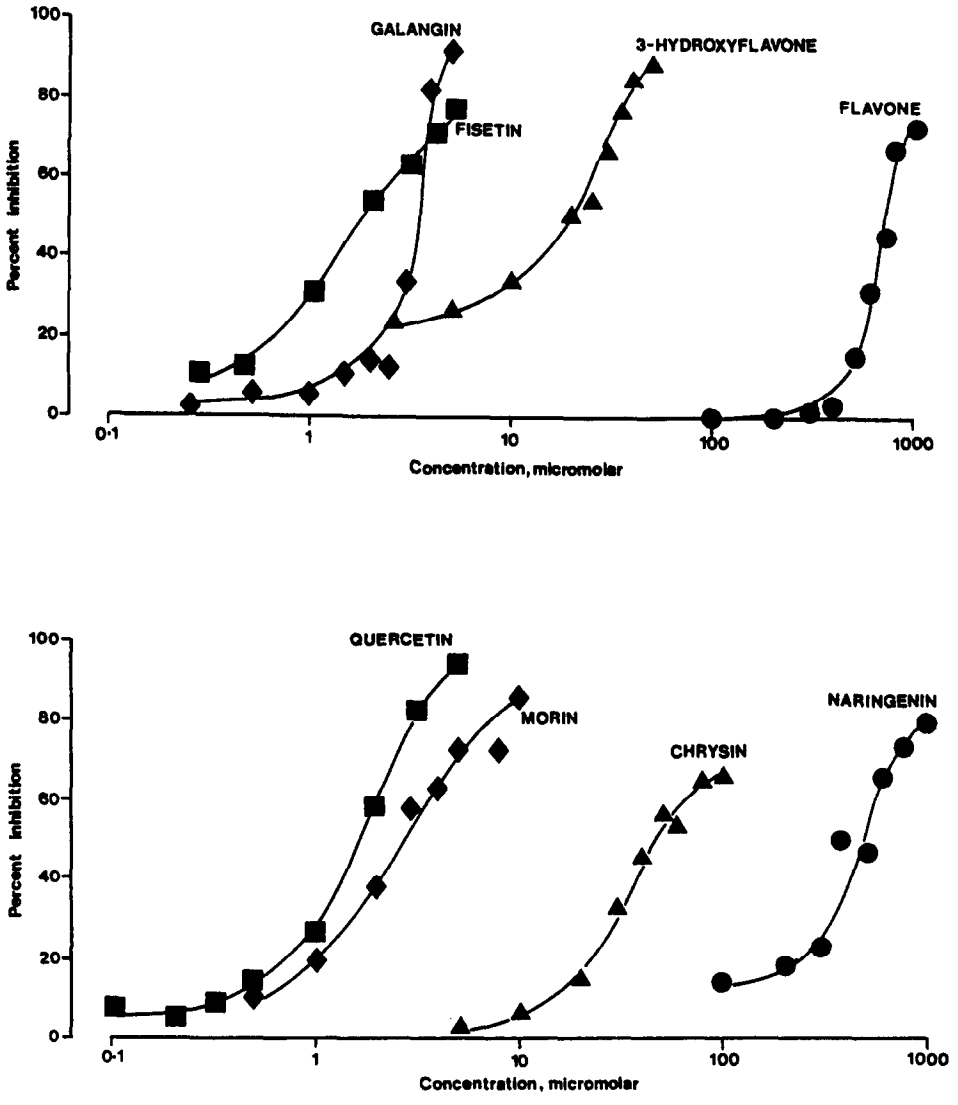

Fig. 1. Concentration-dependent inhibition of microsomal Fe^{3+}-ascorbate-dependent lipid peroxidation by various aglycone flavonoids. Results show mean values for 4–6 tests at each concentration; the bars showing SEM (up to 10% of mean) have been omitted for clarity.

DNA damage [13, 15, 25], and we therefore investigated the inhibition of rat leukocyte 5-lipoxygenase and cyclo-oxygenase by a range of these compounds together with others including some phenolic dietary additives whose effects on bleomycin-induced DNA damage have not previously been studied. Figures 3 and 4 show some representative data for the effects of these compounds on the bleomycin assay (Fig. 3C and D) and on 5-lipoxygenase and cyclo-oxygenase (Fig. 4). Table 3 summarizes the results obtained for all the compounds, including carnosol (an active component of rosemary extract) and trolox c (a synthetic water soluble "analogue" of α-tocopherol that has been proposed for use as an antioxidant [28]). These data establish for the first time that carnosol and propyl gallate are potent inhibitors of mammalian 5-lipoxygenase, with vitamin E and trolox c less potent. Furthermore, with the single exception of BW755c, all the compounds in Table 3 which promote DNA damage by bleomycin are relatively selective inhibitors of 5-lipoxygenase, in that they show lower capacity to inhibit cyclo-oxygenase.

It was notable that the three compounds which demonstrated a "biphasic" effect on bleomycin–Fe^{3+} DNA damage (NDGA, carnosol and propyl gallate, two of which are shown in Fig. 3C) proved to be potent and selective 5-lipoxygenase inhibitors, and that *p*-nitrophenol which had no effect on bleomycin–Fe^{3+} DNA damage was cyclo-oxygenase selective. In the case of the other seven compounds shown in Table 3, these were mostly 5-lipoxygenase selective with varying degrees of potency. As noted above, BW755c was an exception, as it was clearly more active against cyclo-oxygenase.

Compounds that were active in stimulating

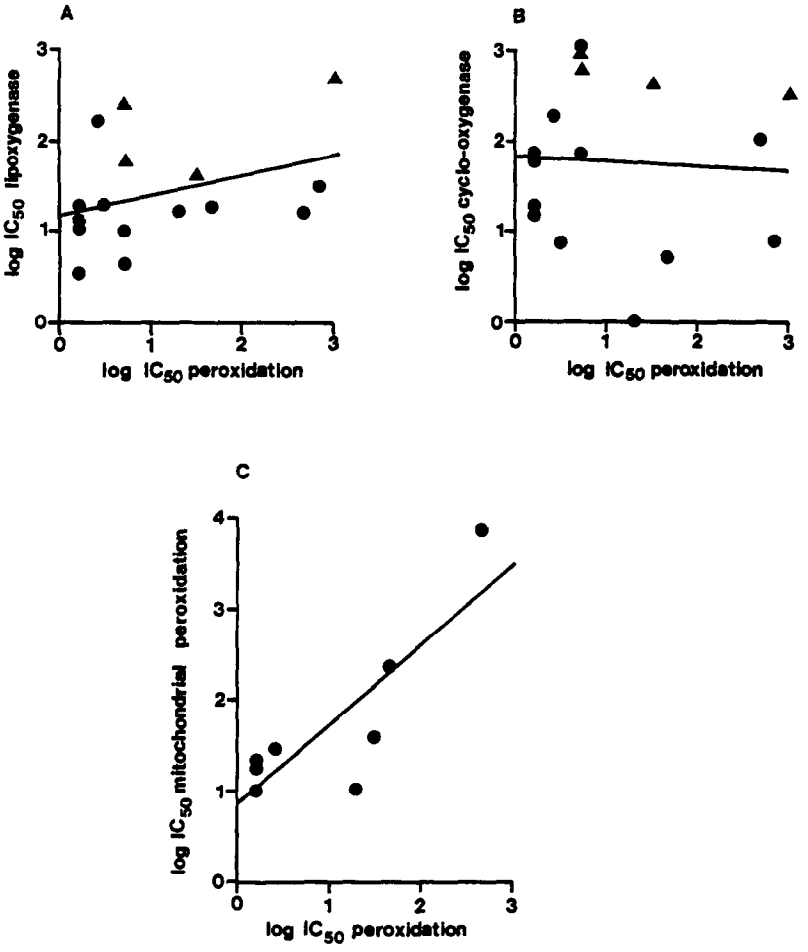

Fig. 2. Correlation plots of the activities of various flavonoid aglycones (●) and flavonoid glycosides (▲) as inhibitors of microsomal lipid peroxidation compared with their activities as inhibitors of leukocyte 5-lipoxygenase (A), leukocyte cyclo-oxygenase (B) and mitochondrial lipid peroxidation (C). The activities are shown on logarithmic scales in terms of the IC_{50} values expressed in micromolar units. Data for mitochondrial peroxidation are from Ref. 27.

bleomycin-dependent DNA damage were also tested for their ability to reduce ferricytochrome *c* to ferrocytochrome *c*, as measured by a rise in absorbance at 550 nm [22]. All compounds active in the bleomycin test were able to reduce cytochrome *c*, but there was no close correlation between the rate of cytochrome reduction and the ability to inhibit 5-lipoxygenase (Table 4). On the other hand, compounds such as galangin, naringin, naringenin, 3-hydroxyflavone, *p*-nitrophenol and vanillin which did not promote bleomycin-dependent DNA damage did not reduce ferricytochrome *c* (data not shown).

DISCUSSION

Because of the contribution of leukotrienes to tissue injury in human disease, there has been considerable interest in the development of lipoxygenase-specific inhibitors, and in their mechanisms of action [1–3]. Mechanistic studies have usually been performed upon plant lipoxygenases such as soybean lipoxygenase-I [2, 19, 20], since substantial quantities of pure enzyme can be obtained, but the extent to which plant lipoxygenases resemble human enzymes is not entirely clear. Despite the recent cloning and expression of human 5-lipoxygenase [29, 30], the enzyme is not yet widely available for study of inhibitors, so simple model systems that give predictive information about potential inhibitors would be of value. Moreover, measurements of lipoxygenase activities in cell systems involve expensive and time-consuming cell preparation and radioimmunoassays and can be complicated by inherent biological variability, so that they are not always suitable for the rapid screening of large numbers of putative inhibitors.

It is frequently proposed that inhibition of lipoxygenases is due to reaction of the inhibitor with free radicals generated at the active site of the enzyme [4, 9, 16–18]. However, we found no significant correlation between the ability of a range of plant phenolic compounds to inhibit 5-lipoxygenase

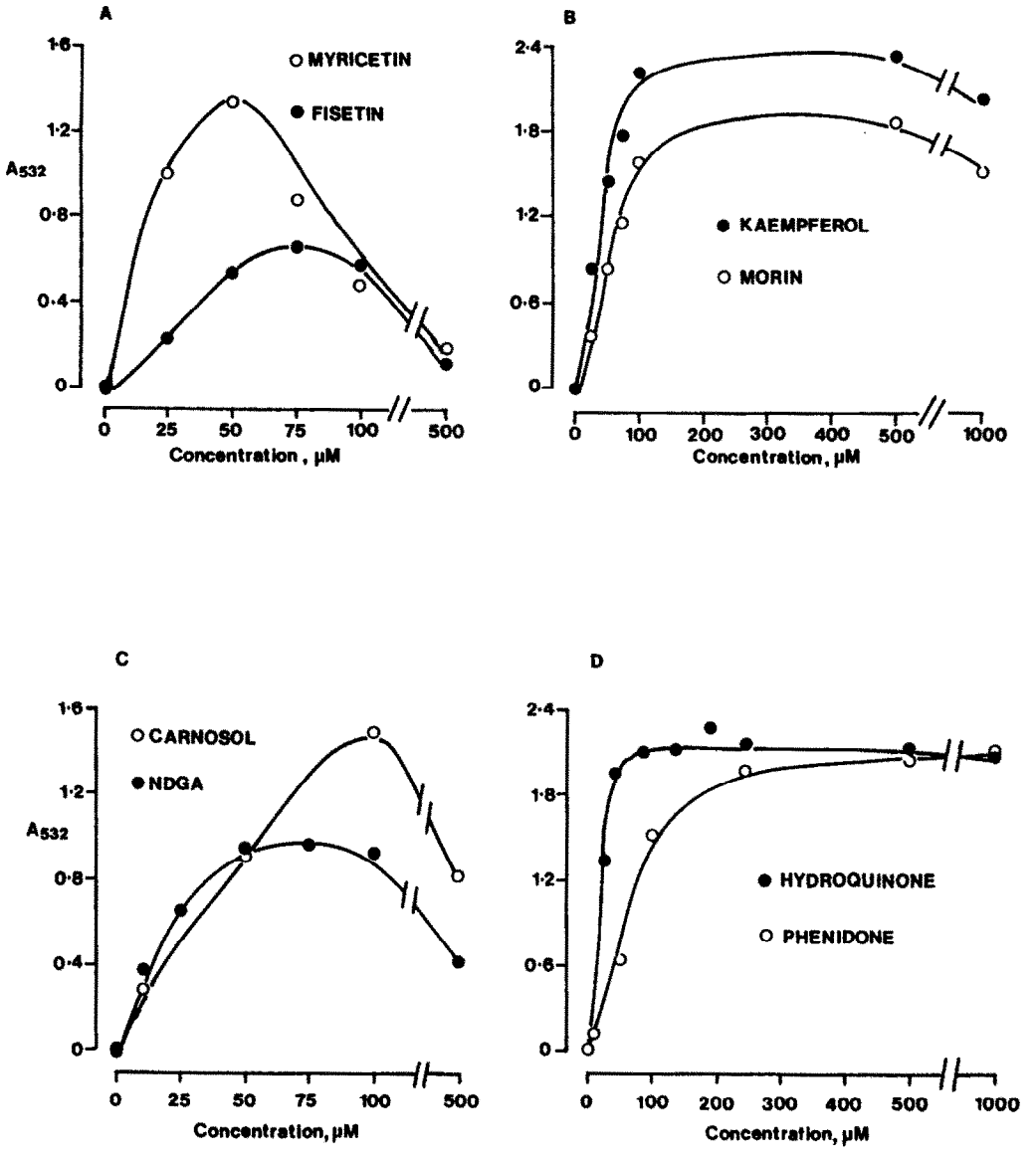

Fig. 3. Representative examples of the two types of concentration-dependent stimulation of DNA damage induced by a complex of Fe^{3+} and bleomycin in the presence of flavonoids (panels A and B) or other phenolic compounds (panels C and D). Panels A and C show "biphasic" effects of various concentrations of test substances, whereas panels B and D show the "saturating" effects of higher concentrations of the test substances.

and their ability to inhibit non-enzymatic lipid peroxidation in microsomal membranes (our data, Fig. 2) or in mitochondrial membranes [27]. Moreover, no correlation was observed between ability to inhibit cyclo-oxygenase (an enzyme also catalysing a free radical reaction) and antioxidant activity (Fig. 2). Many compounds of varying molecular size and differing chemistry seem to have access to the active sites of 5-lipoxygenase and cyclo-oxygenase in rat peritoneal leukocytes (as judged by the IC_{50} values in Tables 2 and 3), so that steric control of the ability to enter the active site does not seem to be a major determinant of inhibitory capacity. We conclude that factors other than antioxidant ability towards lipid-derived radicals are important in determining the ability of plant phenolics to inhibit 5-lipoxygenase and cyclo-oxygenase.

Free radical reactions are also involved in some human diseases (reviewed in Ref. 7) and there have been many proposals for the therapeutic use of antioxidants (reviewed in Ref. 15). Antioxidants are frequently characterized using lipid systems, yet inhibitors of lipid peroxidation can often show pro-oxidant effects (stimulation of free radical damage) to other substrates, such as carbohydrates or DNA [13, 15, 25]. One system frequently used to screen for pro-oxidant activity is the ability of the compound

Table 2. Pro-oxidant effects and inhibition of eicosanoid synthesis by flavonoids

Compound tested	Type of promotion of DNA damage by bleomycin*	Approximate IC_{50} (μM) for inhibition of: 5-lipoxygenase	cyclo-oxygenase	Selectivity†
Quercetin	A	4	16	LO
Gossypetin	A	10	Inactive	LO
Fisetin	A	11	80	LO
Myricetin	A	13	56	LO
Hypolaetin-8-glucoside	A	56	>1000	LO
Gossypin	A	250	630	LO
Kaempferol	B	20	20	NS
Morin	B	160	180	NS
3-Hydroxyflavone	NE	16	1	CO
Chrysin	NE	18	5	CO
Galangin	NE	20	7	CO
Flavone	NE	32	8	CO
Naringin	NE	≫500	320	CO

* A, biphasic stimulation curve, as in Fig. 3A; B, saturation effect (Fig. 3B); NE, no effect on DNA damage.

† NS, non-selective; LO, 5-lipoxygenase selective; CO, cyclo-oxygenase selective (based on relative inhibitory potencies as determined by IC_{50} value). Data for flavonoids from Ref. 3.

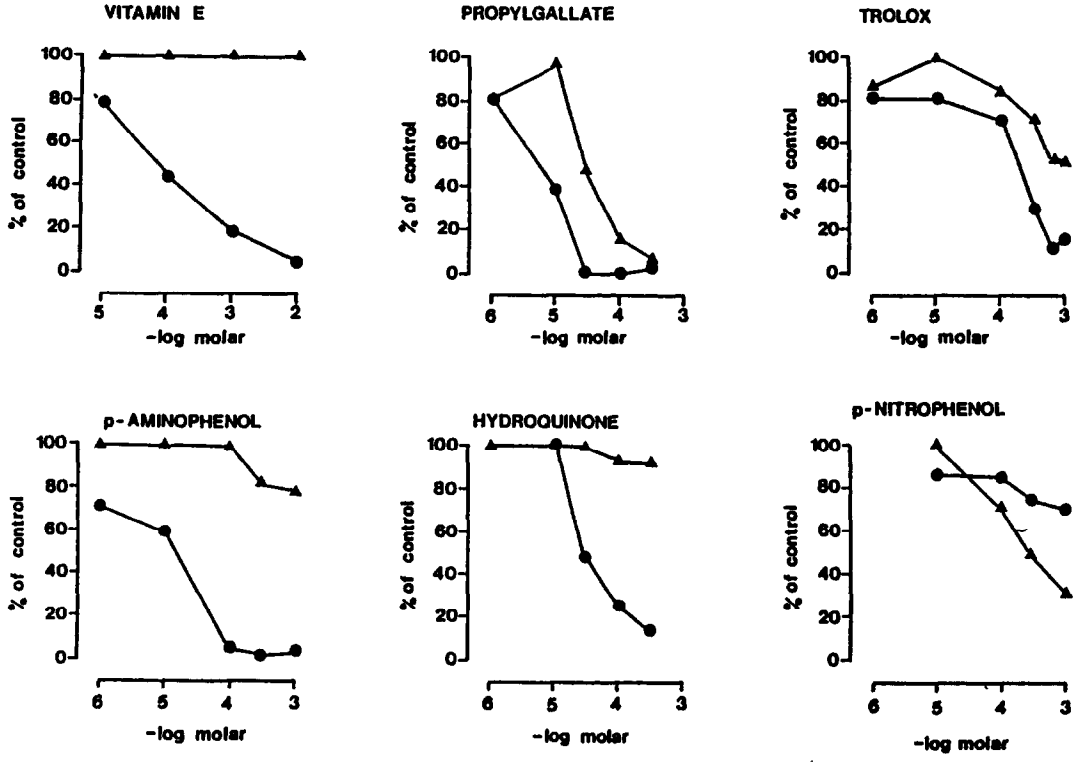

Fig. 4. Inhibition of 5-lipoxygenase (●) and cyclo-oxygenase (▲) activity in A23187-stimulated rat mixed peritoneal leukocyte suspensions, as measured by RIA of LTB_4 and TXB_2, respectively. Results show mean values for 3 tests at each concentration and are expressed as percentage of the amount of product generated in the absence of inhibitor.

to accelerate bleomycin-induced damage to DNA [13, 15, 25]. The anti-tumour antibiotic bleomycin binds both to ferric ions and to DNA. The resulting ternary complex produces DNA degradation if a reducing agent is added to convert Fe^{3+} to Fe^{2+} [31]. Iron ion-chelating agents can inhibit bleomycin-induced DNA degradation by withdrawing iron ions from the ternary complex [31].

We observed a remarkable qualitative correlation between the behaviour of plant flavonoids in the

Table 3. Action of various phenolic and other compounds on bleomycin-induced DNA damage and their inhibitory effects on 5-lipoxygenase and cyclo-oxygenase

Compound tested	Type of promotion of DNA damage by bleomycin*	Approximate IC_{50} (μM) for inhibition of: 5-lipoxygenase	cyclo-oxygenase	Selectivity†
NDGA	A	0.8	5	LO
Carnosol	A	2	16	LO
Propyl gallate	A	5	30	LO
Phenidone	B	2.5	80	LO
p-Aminophenol	B	15	>1000	LO
BW755c	B	25	8	CO
Hydroquinone	B	30	Inactive	LO
Catechol	B	40	65	LO
Vitamin E	B	63	Inactive	LO
Trolox c	B	170	1000	LO
p-Nitrophenol	NE	>1000	250	CO
Vanillin	NE	Inactive	Inactive	—

* A, biphasic stimulation curve, as in Fig. 3A; B, saturation effect (Fig. 3B); NE, no effect on DNA damage.

† LO, 5-lipoxygenase selective; CO, cyclo-oxygenase selective (based on relative inhibitory potencies as determined by IC_{50} value).

Table 4. Rate constants for cytochrome *c* reduction and IC_{50} values for 5-lipoxygenase inhibition in rat leukocytes

Compound	k_2 $M^{-1}sec^{-1}$	IC_{50} (μM)
Myricetin	*	13
Quercetin	*	3.5
Phenidone	*	2.5
BW755c	*	25
Kaempferol	4400	20
Morin	1540	160
Propyl gallate	940	5
p-Aminophenol	620	15
Carnosol	290	2
Trolox c	48	170
Catechol	12	74

* Indicates that reduction was too rapid to be measured in this assay system.

bleomycin assay and their ability to affect eicosanoid synthesis in rat peritoneal leukocytes (Table 2). Flavonoids that did not stimulate DNA damage were cyclo-oxygenase selective. Compounds that increased DNA damage when added at low concentrations but then inhibited it at higher concentrations ("biphasic effect", see Fig. 3) tended to be selective inhibitors of lipoxygenase. All such flavonoids possess a vicinal diol structure. The two flavonoids that increased DNA damage up to a maximum that remained constant as more compound was added (or decreased only at exceptionally high concentrations) were non-selective inhibitors. In a similar fashion, the three synthetic compounds that also exhibited "biphasic" effects on bleomycin-induced DNA damage proved to be potent and selective inhibitors of 5-lipoxygenase (Table 3).

Compounds that stimulate DNA damage by ferric bleomycin presumably do so by reducing it to the ferrous form. Indeed, these compounds were shown to reduce ferricytochrome *c*. It has already been proposed that iron ion-reducing capacity determines the ability of some phenols to inhibit soybean lipoxygenase-I [19, 20]. In the more physiological system we have used, however, other factors are clearly important in determining the overall profile of inhibitor action on the two enzymes since compounds that appeared to act simply as reducing agents in the bleomycin assay were not necessarily either potent as 5-lipoxygenase inhibitors or selective against 5-lipoxygenase versus cyclo-oxygenase. Those inhibitors which were both potent and selective inhibitors of 5-lipoxygenase seemed to have not only the ability to reduce the ferric bleomycin complex, but also the ability to inhibit the DNA degradation when added at slightly higher concentrations. This is probably due to their ability to bind iron ions and withdraw them from the bleomycin [31], so inhibiting DNA degradation.

It thus seems possible that selective inhibitors of 5-lipoxygenase of the flavonoid/catechol type possess a combination of iron ion-chelating and iron ion-reducing properties, although iron chelation alone is not sufficient to ensure 5-lipoxygenase inhibition as substances such as CP21 [32] do not have this property, at least in rat leukocytes (unpublished results). It may also be that the bleomycin assay will provide a very simple and inexpensive test-tube screen for lipoxygenase-selective redox inhibitors of the type used in the present experiments.

Acknowledgements—We are grateful to the Ono Pharmaceutical Company, Nestle plc and the Medical Research Council for research support. We also thank Professor R. C. Hider for useful discussions and for the donation of iron-chelating drugs.

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