DFP-treated animals (Green & Kitchen 1985). DFP does not enhance fentanyl entry in to the brain (Green & Kitchen 1985) and the lack of effect on plasma protein binding accords with this observation. The differential interaction of these two structurally related opioids may reflect the different proteins which they bind. Alfentanil is predominantly bound in plasma to α_1-acid glycoprotein whilst fentanyl binds red blood cells, glycoproteins and lipoproteins (Meuldermans et al 1982). It seems likely therefore that DFP is displacing alfentanil from α_1-acid glycoprotein sites, though the possibility of irreversible phosphorylation of the acceptor proteins cannot be excluded.

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Flavonoids, leucocyte migration and eicosanoids

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Abstract—Quercetin reduced the concentration of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) in the pleural exudate induced in rats by 1% carrageenan given intrapleurally. Leucocyte migration in the exudate was also reduced by the flavonoid. Inhibition of eicosanoids and leucocytes in the exudate was dose-related. Quercetin also reduced LTB_4 synthesis in cells stimulated with ionophore A23187, either ex-vivo or in-vitro. A similar, though less active, mode of action was found with quercitrin, while apigenin and luteolin reduced leucocyte accumulation and PGE_2 formation, but not LTB_4-formation.

It is now known that flavonoids, phenolic pigments widespread in the plant kingdom, can induce a wide range of biological effects, including enzyme inhibition, plant growth regulation and mutagenicity of bacterial testing strains (see Pagonis et al 1986).

Recently it has been observed in-vitro that some of these natural products are potent inhibitors of cyclic AMP phosphodiesterase (cAMP-PDE) in many human and animal tissues (Berets et al 1986). Flavonoids also inhibit platelet aggregation (Gryglewski et al 1985, 1987) and enzymes involved in arachidonic acid metabolism, i.e. cyclooxygenase and lipoxygenase enzymes, though various flavonoids differ in the action on cyclooxygenase and lipoxygenase pathways (Palmer & Salmon 1985).

We have evaluated in the rat the effects of quercetin, quercitrin, luteolin and apigenin on the concentration of PGE_2 and LTB_4 in experimentally induced inflammatory exudate and their relationship with leucocyte numbers migrating into the pleural cavity.

The activity of flavonoids was compared with that of indomethacin and compound BW 755C.

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Materials and methods

Male Wistar-Nossan rats (80–90 g) were used. Carrageenan pleurisy was induced as described previously (Capasso et al 1975). 0·2 mL of 1% carrageenan (Viscarin) suspension was injected intrapleurally into rats and 6 h after injection, the animals were killed using ether anaesthesia, exsanguinated from the carotid artery and exudate collected in polypropylene tubes at 0°C. A sample (10 μL) of the exudate was removed immediately for determination of the leucocyte count according to Hurley et al (1966) while the remainder was centrifuged at 2000 *g* for 5 min at 0°C to precipitate cells and debris. Enzymic activity was terminated with methanol–formic acid (1 mL 20 μL) and PGE_2 and LTB_4 extracted into $CHCl_3$ (2 mL) then evaporated to dryness under N_2. PGE_2 and LTB_4 were determined by radioimmunoassay. The specificity of these antisera and the procedure were reported by Autore et al (1987).

Quercetin (2–24 mg kg^{-1}), quercitrin (2–24 mg kg^{-1}), luteolin (2–24 mg kg^{-1}), apigenin (2–24 mg kg^{-1}), indomethacin (1–9 mg kg^{-1}) and compound BW 755C (10–50 mg kg^{-1}) were given intraperitoneally after intrapleural injection of carrageenan. The flavonoids were dissolved in ethanol and then diluted with 0·9% NaCl (saline); indomethacin (Indoxen) and BW 755C were dissolved in saline directly.

In some experiments a portion (500 μL) of the exudate, collected 6 h after carrageenan injection, was used to assess the capacity of the inflammatory cells to synthesize LTB_4 ex-vivo. The formation of LTB_4 by cells in the inflammatory exudate from drug-treated animals was assayed as follows. After 30 min pre-incubation of exudate (500 μL) at 37°C, ionophore A23187 (5 μg, 10 μL) was added and incubation continued for 5 min at 37°C. After centrifugation at 2000 *g* for 5 min, the supernatant was removed and assayed for LTB_4. In other experiments, 500 μL of pooled inflammatory exudate (collected 6 h after carrageenan injection and containing 5×10^6 cells) from control rats was incubated at 37°C for 30 min before addition of drug (1–100 μg,

Table 1. Effects of quercetin, quercitrin, apigenin, luteolin, BW 755C and indomethacin on leucocyte count and eicosanoid concentration in inflammatory exudate obtained after intrapleural injection of 0·2 mL of 1% carrageenan.

Treatment	Leucocytes $\times 10^6$	Inhibition (%)	LTB_4 ng mL^{-1}	Inhibition (%)	PGE_2 ng mL^{-1}	Inhibition (%)
Control	47·3 ± 6·3		4·75 ± 0·13		21·13 ± 3·2	
Quercetin						
2 mg kg^{-1}	33·51 ± 4·0[a]	29·1	3·27 ± 0·08[a]	31·1	20·30 ± 3·1	3·9
8 mg kg^{-1}	24·42 ± 3·0[c]	48·3	2·38 ± 0·07[c]	49·8	18·40 ± 2·7	12·1
24 mg kg^{-1}	19·10 ± 2·4[c]	59·6	1·00 ± 0·05[c]	78·9	15·54 ± 2·7	26·4
Quercitrin						
2 mg kg^{-1}	36·51 ± 3·7	22·8	4·75 ± 0·09	19·1	20·78 ± 2·6	1·6
8 mg kg^{-1}	28·93 ± 3·5[a]	38·8	3·50 ± 0·09	26·3	18·95 ± 2·6	6·3
24 mg kg^{-1}	22·70 ± 3·5[a]	52·0	2·68 ± 0·07[c]	43·3	16·83 ± 2·4	20·3
Apigenin						
2 mg kg^{-1}	34·40 ± 3·4[a]	27·2	4·78 ± 0·11	+0·6	18·35 ± 2·0	13·1
8 mg kg^{-1}	28·91 ± 3·0[a]	38·8	4·57 ± 0·10	3·7	14·41 ± 1·8	31·8
24 mg kg^{-1}	22·10 ± 2·7[c]	53·2	4·37 ± 0·09	8·0	9·39 ± 1·0[c]	55·5
Luteolin						
2 mg kg^{-1}	37·80 ± 4·0	20·0	4·51 ± 0·10	5·0	20·00 ± 2·2	5·3
8 mg kg^{-1}	30·40 ± 3·1[b]	35·7	4·63 ± 0·10	2·5	18·05 ± 1·8	14·5
24 mg kg^{-1}	22·47 ± 2·7[c]	52·4	4·47 ± 0·08	5·8	10·13 ± 1·6[b]	52·0
BW 755C						
10 mg kg^{-1}	39·85 ± 4·1	15·7	3·00 ± 0·10[a]	36·8	11·70 ± 1·3[b]	44·6
25 mg kg^{-1}	33·41 ± 3·5[a]	29·3	2·20 ± 0·10[c]	53·6	8·00 ± 1·0[c]	62·1
50 mg kg^{-1}	19·80 ± 2·7[c]	58·1	1·73 ± 0·09[c]	63·5	3·50 ± 0·07[c]	83·4
Indomethacin						
1 mg kg^{-1}	40·41 ± 3·0	14·5	4·87 ± 0·13	+2·1	17·10 ± 2·1	19·0
3 mg kg^{-1}	37·10 ± 2·7	21·5	4·63 ± 0·15	12·0	9·31 ± 1·8[c]	55·9
9 mg kg^{-1}	23·73 ± 1·1[c]	49·8	4·41 ± 0·07	7·1	3·75 ± 1·0[c]	82·2

Each value is the mean ± s.e., n = 6–8. *P* values, a < 0·05, b < 0·02, c < 0·01, by Student's *t*-test.

10 μL). After a further 15 min of incubation at 37°C, A23187 (5 μg, 10 μL) was added and incubation continued for a further 5 min. The mixture was then processed as described for LTB_4 synthesis ex-vivo. To confirm the identity of immunoreactive LTB_4, cell-free exudates from four animals treated with indomethacin (10 mg kg^{-1}) or four with BW 755C (100 mg kg^{-1}), or untreated, were subjected to extraction and thin layer chromatography before RIA as described by Capasso et al (1987).

Drugs. Flavonoids (quercetin, quercitrin dihydrate, apigenin, glucoside, luteolin) were obtained through the courtesy of Dr Carle Reinhold, Homburg Degussa Pharma, Frankfurt; carrageenan (Viscarin 402, Marine Colloids, Springfield, New Jersey, USA); ionophore A23187 (Calbiochem, Bishop's Stortford, Herts, [^{125}I] PGE_2 (NEN), [^{3}H] LTB_4 (Amersham International, Amersham, Bucks); indomethacin (indoxen, Merck, Sharp and Dohme); compound BW 755C (3-amino-1-[(*m*-trifluoromethyl) phenyl]-2-pyrazoline, Wellcome Research Laboratories, Beckenham).

Results

Inflammatory exudate from control rats 6h after intrapleural injection of carrageenan contained 47·3 ± 6·3 × 10^6 leucocytes mL^{-1} (n = 20). The concentrations of LTB_4 and PGE_2 in the exudate were 4·75 ± 0·13 ng mL^{-1} (n = 15) and 21·13 ± 3·2 ng mL^{-1} (n = 20), respectively.

Quercetin 2–24 mg kg^{-1} decreased, in a dose-related manner, the concentrations of both LTB_4 and PGE_2 and also reduced accumulation of cells in the exudate. However, quercetin was a more effective inhibitor of the lipoxygenase product LTB_4 than the cyclooxygenase product PGE_2 (Table 1). Quercetin also inhibited the synthesis of LTB_4 by cells stimulated with A23187 either ex-vivo or in-vitro (Tables 2, 3).

Quercitrin 2–24 mg kg^{-1} had an effect similar to but weaker than, that of quercetin on eicosanoid production and leucocyte migration (Tables 1, 2, 3).

Apigenin, 2–24 mg kg^{-1}, caused a dose-dependent reduction in the concentration of PGE_2 in the inflammatory exudate but the concentration of LTB_4 was not decreased either in-vivo or ex-vivo (Tables 1, 2). Apigenin also reduced the leucocyte count in the exudate. This profile of activity was shared by luteolin, but

Table 2. Effects of flavonoids and BW 755C on LTB_4 synthesized ex-vivo in exudate stimulated with A23187.

Treatment	LTB_4 ng mL^{-1}	Inhibition (%)
Control	20·10 + 2.0	
Quercetin		
2 mg kg^{-1}	14·40 ± 2·2[a]	28·3
8 mg kg^{-1}	10·73 ± 1·8[c]	46·6
24 mg kg^{-1}	4·31 ± 1·0[c]	78·5
Quercitrin		
2 mg kg^{-1}	16·46 ± 2·4	18·1
8 mg kg^{-1}	13·47 ± 2·0	32·9
24 mg kg^{-1}	11·51 ± 1·8[c]	42·7
Apigenin		
2 mg kg^{-1}	22·41 ± 2·93	+11·4
8 mg kg^{-1}	20·00 ± 2·00	0·4
24 mg kg^{-1}	19·41 ± 2·00	3·4
Luteolin		
2 mg kg^{-1}	20·97 ± 2·95	+4·3
8 mg kg^{-1}	20·11 ± 2·57	0
24 mg kg^{-1}	19·00 ± 2·37	5·4
BW 755C		
10 mg kg^{-1}	13·91 ± 2·2[a]	30·7
25 mg kg^{-1}	10·13 ± 2·0[b]	49·6
50 mg kg^{-1}	6·00 ± 1·0[c]	70·1

Footnote as for Table 1.

Table 3. Effects of flavonoids on LTB_4 synthesized in-vitro by cells stimulated with A23187.

Treatment	LTB_4 (ng 5×10^6 cells)	Inhibition (%)
Control	6·00 ± 1·1	
Quercetin		
1 μg mL^{-1}	4·60 ± 0·08	23·3
10 μg mL^{-1}	3·71 ± 0·07[a]	38·1
100 μg mL^{-1}	1·65 ± 0·04[a]	72·5
Quercitrin		
1 μg mL^{-1}	5·96 ± 0·09	0·6
10 μg mL^{-1}	4·80 ± 0·07	20·0
100 μg mL^{-1}	3·12 ± 0·06[b]	48·0
Apigenin		
1 μg mL^{-1}	6·05 ± 1·1	0
10 μg mL^{-1}	6·00 ± 1·0	0
100 μg mL^{-1}	5·81 ± 1·0	3·1
Luteolin		
1 μg mL^{-1}	5·70 ± 1·2	0
10 μg mL^{-1}	6·00 ± 0·09	0
100 μg mL^{-1}	5·81 ± 0·09	3·1
BW 755C		
1 μg mL^{-1}	4·10 ± 1·0[a]	31·6
10 μg mL^{-1}	3·20 ± 0·08[b]	46·6
100 μg mL^{-1}	1·70 ± 0·06[c]	71·6

Footnote as for Table 1.

it was less potent. BW 755C, 10–50 mg kg^{-1} i.p., inhibited the concentration of both the eicosanoids in inflammatory exudate, but it was a more effective inhibitor of PGE_2 than LTB_4. However, BW 755C, 100 mg kg^{-1}, completely inhibited synthesis of LTB_4 and PGE_2 (data not shown).

BW 755C, 10–50 mg kg^{-1}, also inhibited the accumulation of leucocytes in the exudate. The ex-vivo and in-vitro results also showed that BW 755C inhibited the synthesis of LTB_4 by cells stimulated with A23187.

Indomethacin caused a dose-dependent reduction in the concentration of PGE_2 in the inflammatory exudate but the concentration of LTB_4 was not decreased. Higher doses of indomethacin also lowered the leucocyte count (data not shown). Immunoreactive LTB_4 in the pleurisy exudates from control and indomethacin-treated (10 mg kg^{-1}) rats had identical chromatography mobility to that of authentic LTB_4. No immunoreactive material was detected in exudate from rats treated with BW 755C (100 mg kg^{-1}).

Discussion

Quercetin inhibits the metabolism of arachidonic acid via the lipoxygenase pathway in-vitro (Palmer & Salmon 1985) and this has been confirmed in the present study. The data also showed that administration of quercetin to rats reduced the concentration of the lipoxygenase product LTB_4 in inflammatory exudate. Quercetin also inhibited the cyclooxygenase product PGE_2 in the exudate, but, at low doses, lipoxygenase was the primary target of inhibition. A similar mode of action was shown for quercitrin, while luteolin and apigenin reduced the concentration of PGE_2 but not of LTB_4. Treatment of rats with compound BW 755C, a free radical scavenger, caused a reduction in eicosanoid concentration and leucocyte number in the exudate. Leucocyte accumulation was also inhibited by indomethacin at doses that reduced the concentration of PGE_2 but not that of LTB_4, in the exudate. Quercetin has been reported to possess 'free radical scavenging' or 'antioxidant' properties in several systems, e.g. inhibition of oxidation processes in microsomes and mitochondria, and inhibition of ascorbate-stimulated generation of lipid peroxidase in boiled liver microsomes from rats (see Gryglewski et al 1985). However, inhibition of lipoxygenase products by quercetin and quercitrin seems to depend on the prevention of the free radical initiation of oxidation of arachidonic acid (Dirks et al 1982) rather than on chelation of heavy metal ions by flavonoids (Sekiya et al 1982).

Recently Landolfi et al (1984) and Gryglewski et al (1985) have shown that the lipoxygenase inhibitory properties of flavonoids are reduced or abolished if the 3-hydroxyl group in the benzopyrone ring is blocked with a sugar (quercitrin), or removed (apigenin). Our results show that, among the four flavonoids studied, the most active is quercetin, followed by quercitrin, while apigenin and luteolin inhibit only PGE_2 formation.

The flavonoids tested also inhibited leucocyte migration in the animal model of acute inflammation described. This action is not related to inhibition of prostaglandin synthesis, since quercetin and quercitrin inhibit cell migration into the pleural cavity at doses that do not inhibit, or weakly inhibit, the formation of PGE_2. It is known that LTB_4 is a potent leucotactic agent, therefore the reduction of cell migration by quercetin and quercitrin could be ascribed, at least in part, to the inhibition of LTB_4 synthesis. However, this cannot be so with luteolin and apigenin which decrease leucocyte numbers without reducing the concentration of LTB_4; other factors, therefore, may be involved, e.g. complement pathway and chemotactic peptides (Salmon et al 1983).

Each flavonoid tested showed similar effects both in-vivo and ex-vivo or in-vitro although pronounced differences in their action on arachidonic metabolites were observed. Studies are needed to confirm the therapeutic potential of flavonoids as anti-inflammatory agents.

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