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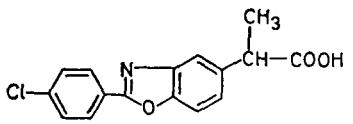
The pharmacology of benoxaprofen (2-[4-chlorophenyl]-α-methyl-5-benzoxazole acetic acid), LRCL 3794, a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthesis

C. H. CASHIN*, W. DAWSON** AND E. ANN KITCHEN

Pharmacology Department, Lilly Research Centre Limited, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, U.K.

Benoxaprofen is a potent and long-acting anti-inflammatory and antipyretic compound. Its anti-inflammatory activity has been demonstrated in carrageenan-induced oedema, in cellulose pellet granuloma and in both developing and established adjuvant arthritis tests in rats. Its antipyretic activity is greater than either aspirin or paracetamol in tests inducing pyrexia with yeast or 'E' pyrogen in rats and rabbits. Benoxaprofen has analgesic activity in tests where pain is accompanied by inflammation but not in other experimental models of pain. The weak prostaglandin synthetase inhibiting properties of this compound differentiate it from other acid anti-inflammatory compounds. The low ulcerogenic potential of benoxaprofen seen in animal models may be related to its relative inability to inhibit PG synthetase.

Acidic antirheumatic drugs like aspirin and indomethacin have as their three main properties their anti-inflammatory, antipyretic and analgesic actions. Many also inhibit prostaglandin biosynthesis (Flower, Gryglewski & others, 1972) which is an action proposed as a possible explanation for the therapeutic effects of these drugs (Vane, 1971).



I

In this report, we describe the properties of benoxaprofen (I), (2-[4-chlorophenyl]-α-methyl-5-benzoxazole acetic acid), which is the most active of a series of 2-aryl-5-benzoxazoyl-alkanoic acids in reducing carrageenan-induced oedema in rats (Dunwell, Evans & others, 1975) and in reversing or preventing the development of adjuvant-induced arthritis in rats. Ulcerogenic potential has been assessed in addition to anti-inflammatory, analgesic and antipyretic activity as this is commonly a limiting factor in the clinical use of non-steroidal anti-inflammatory drugs. Effects on prostaglandin production have been assessed directly in homogenates of ram seminal vesicle and indirectly in isolated tissue preparations.

* Present address: Dept of Pharmacology, Roche Products Ltd, Broadwater Road, Welwyn Garden City, AL7 3AY.
** Correspondence.

MATERIALS AND METHODS

Animals

Rats. Adjuvant arthritis and ulcerogenic tests were carried out using female Sprague-Dawley rats (Tuck). Wistar rats, bred in our laboratories were used in all other tests.

Mice. Male and female CFW strain mice (Tuck) were used.

Rabbits. New Zealand white rabbits from a closed colony at Dista Products Ltd were used.

Compounds

Compounds for administration by mouth were homogenized suspensions in 0·5% sodium carboxymethylcellulose with 0·05% Tween 80 (suspension vehicle). Benoxaprofen in a soluble form, was as the sodium salt (LRCL 4197) in 0·9% NaCl solution. Phenylbutazone was kindly supplied by Geigy (U.K.) Ltd. Hydrocortisone suspensions were prepared from 20 mg hydrocortisone tablets (Merck, Sharp and Dohme Ltd). Aspirin, paracetamol and codeine were B.P. standard. The carrageenan used was Gelozone ST1 (Whiffen and Sons).

Anti-inflammatory efficacy testing

(i) *Carrageenan-induced oedema of the rat paw.* The method is based on that of Winter, Risley & Nuss (1962). Groups of four rats (150–170 g) were dosed orally with the test compound 1 h before injection of 0·1 ml of a 1% suspension of carrageenan in 0·9% NaCl solution into the right hind paw. Controls (n = 10) had suspension vehicle. 2·5 and 5 h later

paw volumes were measured and the results expressed as the percent reduction of the control oedema and ED30 values were calculated by interpolation.

(ii) *Cellulose pellet induced granuloma in rats.* The method is based on that of Meier, Schuler & Desaulles (1950). Groups of six rats (female, 140–150 g) anaesthetized with methohexitone sodium (50 mg kg^{-1}, i.p.) had a small mid-line incision made dorsally and two sterile pellets (7 mm diam. 15–17 mg), of cellulose sponge cloth (Wettex) were implanted subcutaneously. Benoxaprofen was administered in seven daily oral doses starting on the day before implantation. Controls had suspension vehicle and positive controls were treated with hydrocortisone (25 mg kg^{-1}).

The rats were killed 7 days after implantation and the pellets were freed from extraneous tissue, dried overnight at 60°, weighed and the mean gain in weight in the pellets in each group calculated. Thymus and adrenal glands from each animal were also weighed.

The dried pellets from each group were then bulked, boiled in de-ionized water for 5–10 min and after centrifugation the supernatant was analysed by flame photometry for K^+.

(iii) *Adjuvant arthritis in rats.* We have carried out two types of experiment: the prophylactic effect of benoxaprofen was shown by dosing (a) immediately before and during the development of the arthritis (Newbould, 1963) and (b) the therapeutic effect was shown by dosing after the development of the arthritis (Newbould, 1969).

(a) *Effect on developing adjuvant arthritis.* Female rats (150–170 g) in groups of three had the arthritic syndrome induced by subcutaneous injection into the plantar surface of the right hind foot of 0·25 mg dead tubercle bacilli (human strains PN, DT, and C) homogenized in 0·05 ml liquid paraffin. The volume of each injected foot was measured initially and then every two or three days; non-injected hind feet were measured initially and then at two day intervals from the tenth day onwards. The areas under the curves for the course of inflammation for days 0 to 8 (primary lesion) and for days 10 to 18 (secondary lesions) were compared. Joint involvement was assessed by measuring the angle through which the hind paws could be moved easily.

Benoxaprofen was administered in 16 daily oral doses starting on the day before adjuvant injection at 1, 3, 10 or 33 mg kg^{-1} day^{-1}. Phenylbutazone at 10 and 33 mg kg^{-1} day^{-1}, was given as a positive control.

(b) *Effect on established adjuvant arthritis.* Adjuvant arthritis was induced as in (a) above and the rats left untreated until the 14th day. Those with no clear secondary lesions were discarded and the remainder in groups of six were given drug treatment daily, beginning on day 14, and terminating on day 25. Foot volumes were measured every two or three days and the progress of the inflammation was plotted graphically. In some rats, treatment reduced the swelling below the initial value. Joint mobility was also assessed. Benoxaprofen and phenylbutazone were given as in (a).

Analgesic efficacy testing

(i) *Mouse writhing test.* (Koster, Anderson & de Beer, 1959). Groups of ten mice, 19–23 g, were dosed orally with 0·5 ml of solution of sodium benoxaprofen, 3 h before intraperitoneal injection of 0·25 ml of 0·75% v/v acetic acid and individually housed in Perspex compartments. The number of writhes (or abdominal stretching movements) seen in the following 15 min was recorded. 20 mice were dosed with vehicle and other test groups received codeine 45 min before acetic acid. Mice were observed in groups of five. The mean number of writhes in each group was used to calculate percent reductions attributable to drug treatment.

(ii) *Tail immersion test in mice.* The analgesic activity of sodium benoxaprofen was further investigated by a method based on that of Jusykiewiez-Donsbach & Levy (1962). Groups of ten mice (CFW), 19 to 23 g, were dosed orally with benoxaprofen 3 h before their tails were placed in water at 49° and the withdrawal time measured. A control group ($n = 20$) was tested concurrently and a further group ($n = 10$) was given codeine and tested 45 min later.

Antipyretic efficacy testing

(i) *Yeast-induced pyrexia in rats.* A test similar to that of Loux, de Palma & Yanksell (1972) was used. Male rats, 200 to 250 g, injected subcutaneously with 2·5 ml of a 20% aqueous suspension of baker's yeast had rectal temperatures recorded initially and at 18 h. Rats developing a satisfactory pyrexia were divided into groups of five and 30 min after the 18 h reading, were dosed orally with 10 or 33 mg kg^{-1} of benoxaprofen, 33 or 100 mg kg^{-1} of aspirin, or suspension vehicle. Temperatures were recorded at 0·5 h and hourly up to 7·5 h, after dosing. The mean

temperatures after treatment were compared with those at 18 h and expressed as a 'temperature index' which was the sum of the mean changes (Winter & Nuss, 1963).

(ii) *'E' pyrogen induced pyrexia in rabbits.* (Cashin & Heading, 1968). Rabbits (3–6 kg) fasted overnight had rectal temperatures recorded at 30 min intervals for 6 h. 15 min after the first reading, 'E' pyrogen* (0·01 μg kg^{-1}, i.v.) was injected and 30 min later, between the second and third temperature readings, groups of three rabbits were dosed orally with benoxaprofen, paracetamol 125 mg kg^{-1}, or vehicle alone.

Ulcerogenic activity testing

(i) *Gastric ulceration.* Food was withheld from groups of five female rats (130–140 g) for 16 h and the test compounds then administered orally. 3 h after dosing, the rats were killed, the stomachs removed, washed with saline, and opened along the lesser curvature. Ulceration of the mucosa was scored according to an arbitrary system: 0 = no lesions, 0·5 = hyperaemia, 1 = one or two slight lesions present, 1·5 = more than two slight lesions present, 2 = severe lesions, 3 = very severe lesions, 4 = lesions involving the whole mucosa.

A control group was dosed with the suspension vehicle; another group was treated with phenylbutazone.

(ii) *Effect on the gastric mucosal membrane of rats* (cf. Davenport, 1969).Male rats, (235–250 g) given 2 ml 0·1 N HCl by gavage and fasted for 24 h, were anaesthetized (pentobarbitone) and the oesophageal and duodenal ends of the stomach cannulated for perfusion. The stomach was then washed for 1 h with 0·1 N HCl. Perfusion was subsequently carried out for 5 and 1 h periods by recirculating 15 ml of control solution (0·1 N HCl, pH 1) during the first, second, fourth and fifth periods and the drug solution (at a pH near to its pK) during the third period. The drugs used were: aspirin (pK 3·5); 12·5 mM at pH 3·5: phenylbutazone (pK 4·5); 12·5 mM at pH 6·5, suspension: benoxaprofen (pK 5·62); 12·5 mM and 2·5 mM at pH 5·62, suspension.

Phenylbutazone was dissolved in dilute tris buffer (0·1 M) and when brought to pH 6·5 with hydrochloric acid gave an opalescent solution.

Eight rats were used for each drug at each dose. Na^+ and K^+ concentrations in the perfusate were determined by atomic absorption. Their net loss during the second perfusion period was compared with that during the fifth perfusion period. The incidence of bleeding was also noted.

Effect on prostaglandin synthetase

(i) *Biochemical estimation.* A particulate fraction of frozen ram seminal vesicles was prepared by blade homogenization in phosphate buffer (pH 7·4, 0·1 M) and centrifuged at 1200 *g* for 10 min. The pellet was resuspended in buffer and centrifuged at 900 *g* for 2 h. The final pellet was suspended in 0·1 M phosphate buffer at pH 8 and freeze-dried. The incubation mixture consisted of 6 mg particulate enzyme preparation, 200 mg glutathione and 0·2 M tris buffer (pH 8) to a final volume of 2 ml. It was gently shaken at 37°. Benoxaprofen or indomethacin was added to the incubation mixture 20 min before [^{14}C] arachidonic acid (68000 d min^{-1}, Radiochemical Centre, Amersham) at 0·175, 0·5 or 2 μg ml^{-1}. After a further 20 min incubation, the reaction was stopped by adjusting to pH 3 with 0·2 M citric acid. The reaction products were extracted with 2 × 5 ml volumes of ethyl acetate, evaporated almost to dryness and separated by t.l.c. in the A1 system of Greén & Samuelsson (1964). Standard prostaglandins E_2, $F_{2\alpha}$ and D_2 were run at the same time. The plate was scanned and then scraped in 1 cm bands, which were counted in a toluene based scintillation fluid using a Packard Tricarb scintillation spectrometer. The biological activity eluted from duplicate plates of both test and standard material was assayed using guinea-pig ileum and rat stomach strip preparations.

(ii) *Pharmacological estimation.* Standard contractions were induced in a strip of the fundus of rat stomach (Vane, 1957) by arachidonic acid, 5 μg ml^{-1} and prostaglandin E_2 (PGE_2), 10 ng ml^{-1}. Benoxaprofen or indomethacin in Tyrode solution was added to the bath to give approximately 50–60% of the maximal response. Control responses, in normal Tyrode, were interpolated between each concentration of drug. A dose was given every 30 min and left in contact for 3 min.

RESULTS

Carrageenan-induced paw oedema

Benoxaprofen caused a dose-related inhibition of oedema at 2·5 and 5 h (Table 1) and was effective at lower doses then phenylbutazone at both times. There was no significant decrease in the activity of benoxaprofen in adrenalectomized rats.

* 'E' pyrogen (Organon) is derived from *Proteus vulgaris.*

Table 1. *Effect of graded doses of benoxaprofen and phenylbutazone on carrageenan oedema in rats.*

No. rats	Dose mg kg^{-1} orally	% reduction of oedema (mean ± s.e.) 2·5 h	5 h
Benoxaprofen			
20	10	36·8 ± 5·2	27·7 ± 4·2
20	33	49·1 ± 4·4	36·0 ± 3·7
20	100	57·0 ± 3·2	30·8 ± 4·8
Phenylbutazone			
12	10	0	0
12	33	35·2 ± 7·2	18·6 ± 5·2
12	100	52·7 ± 5·5	44·7 ± 5·5

Cellulose pellet induced granuloma in rats

Benoxaprofen and hydrocortisone were equally effective in this test (Table 2). The reduction in K^+ content of the implant at the higher doses of both compounds indicates a reduction in the number of cells entering the granuloma. The implantation

Table 2. *Effect of benoxaprofen and hydrocortisone on cellulose pellet granuloma in groups of 6 rats.*

Dose mg kg^{-1} day^{-1} orally	Granuloma: Weight (mg) mean ± s.e.	% reduction mean ± s.e.	K^+ content (% of controls)
Control			
—	13·2 ± 0·5	—	—
Benoxaprofen			
3	12·7 ± 0·5	3·8 ± 5·3	85
10	10·0 ± 0·6	24·2 ± 5·4	59
Hydrocortisone			
3	12·3 ± 0·6	6·8 ± 5·8	75
10	10·3 ± 0·4	22·0 ± 4·2	59

caused a small reduction in thymus weight in untreated animals 375 ± 12 mg/6 rats, s.e.m., n = 3 to 338 ± 12 mg, n = 11, but adrenal weight was not modified (51 ± 3·5 mg per rat n = 24). Treatment with hydrocortisone whilst reducing the size of the granuloma, markedly reduced thymus weight 273 ± 18 and 130 ± 10 mg/6 rats at 10 and 25 mg kg^{-1} day^{-1}, but had no significant effect on adrenal weight. Benoxaprofen produced no significant changes in either adrenal weight (50 ± 2 and 48 ± 2 mg/rat) or thymus weight (341 ± 16 and 315 ± 18 at 10 and 33 mg kg^{-1} day^{-1}), suggesting that its mode of action does not involve adrenal stimulation.

Adjuvant arthritis

Benoxaprofen inhibited both the primary and secondary lesions in developing adjuvant induced arthritis and was about three times as effective as phenylbutazone (Table 3). Both compounds had a beneficial effect over the 18 days of the test, although dosing stopped on day 14. Joint mobility showed wide variability, but there was significant benefit from benoxaprofen at 10 and 33 mg kg^{-1} and from phenylbutazone at 33 mg kg^{-1}.

Table 3. *Effect of benoxaprofen and phenylbutazone on adjuvant arthritis in groups of 6 rats.*

Dose mg kg^{-1} day^{-1} orally	Assessment of anti-inflammatory effect: Primary lesion (% reduction ± s.e.)	Secondary lesions (% reduction ± s.e.) Injected foot	Non-injected foot	Joint mobility (% improvement) Injected foot	Non-injected foot
Benoxaprofen					
1	14 ± 15	20 ± 24	36 ± 28	2·5	8·8
3	24 ± 10	60 ± 12	43 ± 26	5·1	8·8
10	25 ± 11	70 ± 10	75 ± 12	27·8	52·6
33	21 ± 12	83 ± 5	89 ± 4	25·3	59·6
Phenylbutazone					
10	26 ± 10	70 ± 7	52 ± 5	8·6	11·3
33	38 ± 5	87 ± 7	65 ± 5	24·1	36·5

Benoxaprofen was significantly active in the established adjuvant arthritic test, at oral doses of 3 mg kg^{-1} day^{-1} and still retained some activity at 1 mg kg^{-1} day^{-1}. Phenylbutazone was marginally active at 10 mg kg^{-1} day^{-1} (Table 4).

Table 4. *Effect of benoxaprofen and phenylbutazone on established adjuvant arthritis in groups of 12 rats.*

Dose mg kg^{-1} day^{-1} orally	Assessment of anti-inflammatory effect: Hind foot oedema (% reduction ± s.e.) Injected foot	Non-injected foot	Joint mobility (% improvement) Injected foot	Non-injected foot
Benoxaprofen				
1	43 ± 24	49 ± 34	16	21
3	77 ± 21	87 ± 30	24	36
10	144 ± 21	113 ± 26	45	56
33	136 ± 26	106 ± 29	43	49
Phenylbutazone				
3	0	0	2	0
10	78 ± 92	13 ± 27	4	0
33	104 ± 24	120 ± 32	0	0

Analgesic activity

(i) *Acetic acid-induced writhing in mice.* Benoxaprofen in oral doses of 30–120 mg kg^{-1} caused a dose related reduction in the number of writhes, and had activity similar to codeine (Table 5).

(ii) *Tail immersion test in mice.* Benoxaprofen caused a small increase (41%, $P < 0.01$) in tail withdrawal time at 120 mg kg^{-1} but was much less active than codeine which at 40 mg kg^{-1} had 112% increase over the control value ($P < 0.001$).

Table 5. *Effect of benoxaprofen, sodium salt and codeine on acetic acid-induced writhing in groups of 10 mice.*

Dose mg kg^{-1} orally	Time given before testing (h)	Writhing: Mean no. in 15 min (± s.e.)	Writhing: % reduction from control	*P* (difference from control)
Control				
—	—	26·7 ± 2·3	—	—
Benoxaprofen sodium salt				
30	3	20·9 ± 4·8	22	NS
60	3	6·2 ± 2·6	77	< 0·001
120	3	4·4 ± 0·8	84	< 0·001
Codeine*				
40	0·75	11·4 ± 3·3	57	< 0·01

* Administered as codeine phosphate.

Antipyretic activity

Benoxaprofen given orally at 10 or 33 mg kg^{-1}, reduced the increase in body temperature induced by subcutaneous injection of yeast (Table 6) and was three times more potent than aspirin. Benoxaprofen also reduced 'E' pyrogen-induced pyrexia in rabbits. It was four times more potent than paracetamol and exerted its effect over at least 6 h (Fig. 1).

Table 6. *Effect of benoxaprofen and aspirin on yeast-induced pyrexia in groups of 6 rats.*

Dose (mg kg^{-1} orally)	Mean temperatures (°C) after hours shown: 0	1	2	3	4	5	6	7	TI
Control									
—	38·7	38·8	38·9	38·8	38·6	38·5	38·5	38·3	−0·5
Benoxaprofen									
10	38·7	38·6	38·1	38·3	38·0	38·0	38·0	38·0	−3·9
33	38·7	38·3	38·0	37·9	37·8	37·7	37·7	37·7	−6·5
Aspirin									
33	38·7	38·1	38·0	38·2	38·3	38·2	38·2	38·3	−3·6
100	38·8	38·2	37·9	37·9	37·9	38·0	38·1	38·2	−5·4

TI. Temperature index is the sum of the mean temperature changes from the initial: time '0' is 18 h after yeast injection, compounds were given 30 min after 0.

Ulcerogenic activity

Similar degrees of hyperaemia and small foci of haemorrhage were produced in fasted female rats given oral doses of phenylbutazone, or benoxaprofen 100 mg kg^{-1} (Table 7). No frank ulceration was seen.

There is thus at least a 20-fold difference between the dose of benoxaprofen required for effective control of established adjuvant arthritis and the potentially ulcerogenic dose.

The more sensitive test in which the compounds were perfused through the stomach of rats under light anaesthesia provided evidence of mucosal damage when aspirin or phenylbutazone 12·5 mM was used, but none with benoxaprofen 12·5 mM. Whilst there

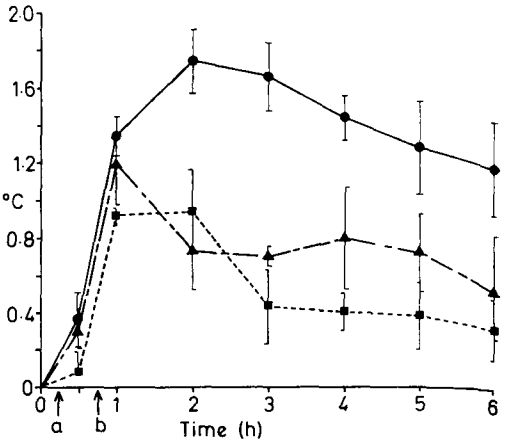


FIG. 1, Reduction of pyrexia induced by 'E' pyrogen in rabbits. The mean increase in temperature (°C) ± s.e.m. for rabbits per group receiving 0·01 μg kg^{-1} 'E' pyrogen intravenously followed after 30 min by no drug ●—●, paracetamol 125 mg kg^{-1}, orally ▲— - —▲, or benoxaprofen 33 mg kg^{-1}, orally ■ - - - ■. Arrows a–'E' pyrogen, b–drug.

Table 7. *Ulcerogenic activity of benoxaprofen, phenylbutazone and aspirin in groups of 5 rats.*

Dose mg kg^{-1} oral	Ulcer score Mean ± s.e. mean	*P* (Comparison with controls)
Controls		
—	0·1 ± 0·18	—
Benoxaprofen		
50	0·30 ± 0·20	N.S.
100	0·90 ± 0·37	N.S.
200	0·90 ± 0·24	< 0·02
Phenylbutazone		
50	0 ± 0	N.S.
100	0·80 ± 0·25	< 0·05
200	1·10 ± 0·29	< 0·02

P, calculated by Student's *t* test.

was no significant increase in Na^+, with phenylbutazone the K^+ efflux increased from 0·25 ± 0·03 mM to 1·12 ± 0·13, 0·90 ± 0·06 and 0·44 ± 0·08 in periods 2–5 respectively. For aspirin the figures were: 0·21 ± 0·07, 0·37 ± 0·02, 0·25 ± 0·05, 0·58 ± 0·20 mM ± s.e.m.

Inhibition of PG synthesis

(i) *Biochemical estimation.* Benoxaprofen caused a small reduction of PG synthetase activity (Table 8). The effect was not dose-related and the maximum concentration (500 μg ml^{-1}) was below the IC50 (50% inhibition). It was not possible to calculate the concentration required to give 50% inhibition (IC50) as increased concentrations of the compound did not increase the degree of inhibition seen at 10 μg ml^{-1}. The IC50 for indomethacin was in the

Table 8. *Effect of benoxaprofen, sodium salt and indomethacin on total prostaglandin synthesis in sheep seminal vesical enzyme preparations. Each result is the mean of four replicates.*

	Reduction in total prostaglandin synthesis (%) at arachidonic acid concentrations of:		
Concn. $\mu g\ ml^{-1}$	0·125 $\mu g\ ml^{-1}$	0·5 $\mu g\ ml^{-1}$	2·0 $\mu g\ ml^{-1}$
Benoxaprofen, sodium salt			
0·1	0	1·4	0
1·0	0·5	0	0
10·0	24·9	35·1***	23·3*
Indomethacin			
0·1	37·5***	19·7**	34·1***
1·0	75·4***	63·3***	60·2***

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$. Using Student's *t*-test.

range 0·2–1 $\mu g\ ml^{-1}$ which agrees well with Vane's (1971) value of 0·17 $\mu g\ ml^{-1}$.

(ii) *Pharmacological estimation.* From the responses induced in rat stomach strips by arachidonic acid, indomethacin was seen to have a marked inhibitory effect with an IC50 about 0·02 $\mu g\ ml^{-1}$, whilst the IC50 of benoxaprofen was about 1 $\mu g\ ml^{-1}$ (Fig. 2). At much higher concentrations, both compounds inhibited the responses to PGE_2.

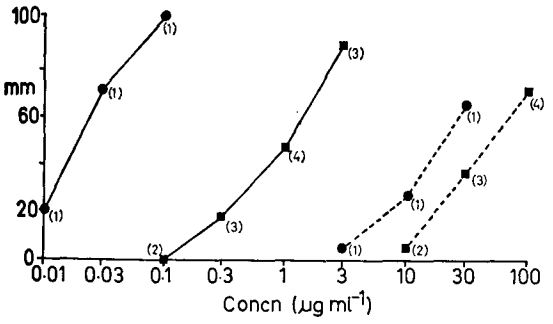


FIG. 2, The effect of benoxaprofen (■) or indomethacin (●) on the contractions (mm) of the rat stomach strip produced by arachidonate 5 $\mu g\ ml^{-1}$ (■——■, ●——●) or prostaglandin E_2 10 ng ml^{-1} (■ - - - ■ or ● - - - ●). Number of experiments shown in parentheses. Abscissa —Bathing fluid concentration ($\mu g\ ml^{-1}$).

DISCUSSION

Benoxaprofen is a potent acidic anti-inflammatory compound with considerable antipyretic activity and some analgesic activity in the tests reported. Its low ulcerogenic potential may be related to its poor ability to inhibit prostaglandin synthetase.

The drug reduces carrageenan-induced oedema, and both developing and established adjuvant arthritis in the rat, being three to five times more potent than phenylbutazone and acting for a longer time. There is reduction in the permeability changes caused by acute inflammation and the decrease in joint mobility seen in more chronic arthritic lesions. The activity of benoxaprofen does not appear to involve the adrenals, as it was equipotent in normal and adrenalectomized rats injected with carrageenan and had minimal effects on adrenal and thymus weight in the subcutaneous cellulose pellet granuloma test. In our hands most acidic anti-inflammatory drugs have little activity in the granuloma test, but benoxaprofen was equipotent with hydrocortisone in reducing granuloma weight. This may be attributable to a reduction in the influx of cells.

Benoxaprofen was active in both the yeast-produced and pyrogen-induced pyrexia tests, in which it was more potent than aspirin or paracetamol.

The drug's analgesic activity in the acetic acid writhing test was similar to that of codeine, but in the tail withdrawal test was poor by comparison.

In view of its striking anti-inflammatory activity, it is surprising that benoxaprofen showed relatively little ability to inhibit PG synthetase. Vane's hypothesis (1971) that acidic anti-inflammatory compounds owe their activity to inhibition of this enzyme, has received wide acceptance and is compatible with data from a range of chemical compounds. However, Brocklehurst & Dawson (1974) reported that acidic anti-inflammatory substances do not always inhibit synthetase enzyme preparations, particularly when only small amounts of substrate are present. They suggested that although Vane's data showed correlation between anti-inflammatory potency and ability to prevent prostaglandin synthesis *in vitro*, this might not apply *in vivo*. Also, steroids show that inflammation can be controlled by other means. Inhibition of PG synthesis results in poor uterine function during parturition and may also underlie the pre-ulceration changes in the gastric mucosa, so that possession of good anti-inflammatory activity not involving this type of action would appear to be an advantage.

The reduction in the connective tissue granuloma surrounding a foreign body can be the consequence of a reduction in the circulating leucocytes, as caused by glucocorticosteroids, or perhaps of the reduction or inhibition of leucotactic agents at the site of the stimulus. The reversal of established adjuvant arthritis shows that damage generated by immuno-

Table 9. *Summary of data. Approx. equivalent doses of benoxaprofen and test drugs for stated percentage change in measured parameters (mg kg^{-1}).*

Test	Effect	Benoxaprofen	Comparator drugs	
Carrageenan oedema	ED30% (2½ h)	4·5	Phenylbutazone	24
Adj. arthritis (Developing) secondary reaction	ED50%	4	Phenylbutazone	8
Adj. arthritis (established) secondary reaction	ED50%	2	Phenylbutazone	15
Cellulose pellet granuloma	ED20%	8	Hydrocortisone	8
Yeast-induced pyrexia	Test Index 2–7 h	10	Aspirin	33
'E' Pyrogen pyrexia	ED50% (2–6 h)	33	Paracetamol	125
Mouse writhing	ED50%	40	Codeine phosphate	36
Tail immersion	ED50%	140	Codeine phosphate	20
Inhibition PG synthetase—Microsomal enzyme	ED30% (μg ml^{-1})	10	Indomethacin	0·1

logical mechanisms is controlled by benoxaprofen. This suggests that the effect on developing adjuvant arthritis is not the result of interference with the development of the hypersensitive state, but rather upon the delayed hypersensitivity reaction or its sequelae. Abatement of the accumulation or activation of leucocytes at the site of inflammation would be compatible with data from both the adjuvant arthritis and subcutaneous foreign body experiments, but the mode of action of benoxaprofen is not yet clear.

Table 9 summarizes the properties of benoxaprofen and shows that it is a potent anti-inflammatory substance of somewhat unusual pharmacological profile. It has low toxicity and long duration of action (Chatfield & Green, 1977), and is considered to be a promising therapeutic agent.

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