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Models for evaluating the anti-inflammatory effects of inhibitors of arachidonic acid metabolism

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Inhibitors of arachidonic acid metabolism were characterized by their ability to modulate slow reacting substance (SRS) and prostaglandin E_2 (PGE_2) release from stimulated mouse peritoneal macrophages in-vitro. Differential effects of cyclo-oxygenase (CO) and lipoxygenase (LO) enzyme inhibitors and compounds which inhibit both enzymes were demonstrated using several animal models of inflammation. Carrageenan-impregnated sponges implanted subcutaneously in rats and immune-complexes injected intraperitoneally in mice produced inflammatory responses characterized respectively by polymorphonuclear (PMN) cell infiltration and by increased vascular permeability. Dual CO/LO inhibitors (eg. BW 755c and timegadine) were capable of suppressing both parameters and reduced SRS and PGE_2 formation in-vivo. In contrast, selective CO inhibitors (e.g. indomethacin, naproxen and R-830) were less active against permeability, and potentiated SRS release. Although selective CO inhibitors reduced PMN migration, this occurred at doses which exceeded those required for inhibition of PGE_2. Compounds possessing LO inhibitory activity suppressed the cellular component of an Arthus type reaction in the rat pleural cavity, but were less active than selective CO inhibitors against carrageenan-induced paw oedema in rats.

Both cyclo-oxygenase (CO) and lipoxygenase (LO) products of arachidonic acid (AA) metabolism are known to be pro-inflammatory and act synergistically (Bray et al 1981). Thus, prostaglandins are associated with vasodilatation (Williams 1979), and leukotrienes and some mono-HETES possess chemotactic (Palmer et al 1980) and vascular permeability-inducing properties (Dahlen et al 1981). However, most established non-steroidal anti-inflammatory drugs (NSAIDs) are selective inhibitors of CO and prevent only the formation of prostaglandins, thromboxanes and endoperoxides (Ferriera & Vane 1979). Consequently, much interest has been focused recently on the development of dual CO/LO inhibitors as a potentially new class of anti-inflammatory agents. Since the conventional screening models of inflammation, such as carrageenan paw oedema and adjuvant arthritis in rats are particularly sensitive to inhibition by NSAIDs (Lombardino et al 1975; Wax et al 1975), it has been necessary to develop models which demonstrate a causal relation between LO products and the inflammatory condition. They should also distinguish between NSAIDs and dual enzyme or selective LO inhibitors. Few models have been reported that satisfy these criteria although the carrageenan sponge model of cell infiltration (Salmon et al 1983) and a model of immune-complex induced vascular permeability in mouse peritoneum (Blackham & Woods 1984) appear to involve LO products. We have evaluated both models for their potential value in detecting novel anti-inflammatory agents based on inhibition of AA metabolism, using a series of compounds capable of preventing the formation of prostaglandins and/or leukotrienes. These have included NSAIDs (e.g. indomethacin and naproxen), inhibitors of CO and LO (e.g. BW 755c and timegadine) and selective inhibitors of LO (e.g. nafazatrom). Other in-vivo models have been studied for purposes of comparison and have included a carrageenan paw oedema test in rats and an immune model of inflammation in rat pleural cavities. Before studies in-vivo, the selected compounds were compared for their abilities to inhibit PGE_2, LTC_4 and lysosomal enzyme release from stimulated mouse peritoneal macrophages in-vitro.

A report of some of these findings has been published in abstract (Blackham et al 1984; Blackham & Woods 1984).

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METHODS

Mouse peritoneal macrophages: in-vitro studies

Female, LACA mice (20–30 g) were killed by asphyxiation with CO_2 and their bodies sterilized by immersion in 70% ethanol. 3 ml ice-cold M199 medium (containing 10% heat-inactivated swine serum, penicillin 20 u ml^{-1}, streptomycin 10 u ml^{-1} and heparin 10 u ml^{-1}) was used to wash out resident peritoneal cells from each mouse. The cell washouts

from 20 mice were pooled, counted in a Coulter counter and aliquots containing 2·5–3 × 10^6 cells transferred to 'Multiwell' plates. Previous experiments suggested that 30–50% of transferred cells eventually adhere to the plates. The cells were cultured for 3 h at 37 °C under air/CO_2 in an incubator; non-adherent cells were then removed by washing 3 times with phosphate buffered saline and the adherent cells overlaid with incubation buffer (prepared as described by Jakschik et al 1977). Drug (5 μl) solution or vehicle control (50% DMSO) were added to triplicate wells and the plates incubated for 10 min (or 48 h for experiments with the steroid). Preformed washed immune complex (8·25 μg ovalbumin + equivalence of rabbit antiserum) (rabbits sensitized to ovalbumin as described by Blackham et al 1974) or an equivalent volume of incubation buffer (0·1 ml) was then added to each well. At the end of a 3 h incubation period the supernatant medium was collected and the adherent cells lysed with 2 ml of 0·1% Triton X-100 in saline containing 0·1% bovine serum albumin. Aliquots of the supernatant medium and cell lysate were taken for β-glucuronidase assay. The remainder of the supernatant medium was pooled and stored at −20 °C until bioassayed for SRS and PG.

β-Glucuronidase assay

0·1 ml of both supernatant and cell lysate fractions were each incubated for 16 h at 37 °C in the presence of 0·5 ml of 0·1 M acetate buffer, pH 4·5 and 0·1 ml 5 nM *p*-dinitrophenyl-β-D-glucuronide. The reaction was stopped by adding 0·3 ml of 0·8 M glycine buffer, pH 10·4 and the optical density of the released dinitrophenol read at 400 nm. Results are expressed in terms of released (supernatant) enzyme as a percentage of total (supernatant + cell lysate) enzyme.

SRS and PG bioassay

Rat isolated fundic strips or guinea-pig longitudinal ileal muscle strips were superfused with Krebs/Hensleit buffer (8 ml min^{-1}) containing mepyramine (1 μg ml^{-1}), methysergide (2 μg ml^{-1}), atropine (1 μg ml^{-1}) and indomethacin (1 μg ml^{-1}) and gassed with 5% CO_2 in O_2. The SRS receptor antagonist FPL 55712 (Augstein et al 1973) was included in the buffer for the PG assays. Isotonic contractions to SRS, present in the supernatant medium were quantified using the ileum preparation, and the area under the response curve was integrated electronically and compared with that produced by authentic LTC_4. Isometric PG contractions were estimated in terms of PGE_2 equivalents using the fundic strip preparations.

The presence of LTC_4 and PGE_2 in the supernatants of immune-complex stimulated peritoneal macrophages was verified using HPLC and radioimmunoassay, respectively.

Intraperitoneal injection of immune complexes in mice

Female, LACA mice (20–30 mg) received 0·25 ml of 0·5% pontamine sky blue in saline, intravenously, immediately before intraperitoneal injection of 0·75 ml of a suspension of washed immune complex (200 μg ovalbumin + equivalence of rabbit antiserum) as described by Blackham & Woods (1985). Groups of 6 mice were killed at 5–10 min and at 30 min and the peritoneal washouts (2 ml buffer: Jakschik et al 1977) were pooled for the determination of SRS by bioassay. Individual animals were examined for dye extravasation by measuring the optical density of the peritoneal exudate supernatant at 630 nm. Drugs were given orally in 0·05% polysorbate 80 in distilled water, 30 min before injection of immune complex.

Carrageenan sponge model

Two polyvinyl sponge discs (20 ± 1 mg) soaked in 1% Viscarin carrageenan in saline were implanted subcutaneously in female, Cobb-Wistar rats (150–200 g) ($n \geq 6$). After 24 h the animals were killed and the sponges removed and immersed in 2 ml heparinized saline containing indomethacin (1 μg ml^{-1}). The cellular infiltrate was recovered from the sponges by squeezing with forceps and total cell counts determined using a Coulter counter. In addition cell-free supernatants from both sponges were pooled and their total PGE_2 content measured by radioimmunoassay. Drugs were administered orally in 0·05% polysorbate in distilled water, 1 h before implantation and 5 h and 21 h after implantation as described by Higgs et al (1980).

Reversed passive Arthus reaction

A reversed passive Arthus reaction (RPAR) was induced in the pleural cavity of female, Cobb-Wistar rats (70–100 g) in groups of 5 or more as described by Blackham et al (1984) using a slight modification of the procedure described originally by Keogh et al (1981). Animals were injected intrapleurally with 0·2 ml rabbit anti-ovalbumin antiserum followed 20 min later by intravenous administration of 0·5 ml of 1% ovalbumin and 0·5% Evans blue in saline. The animals were killed 4 h after antigen challenge and

the pleural exudates were recovered for measurement of volume and for determinations of cell content using a Coulter counter. Drugs were administered orally in 0·05% polysorbate 80 1 h before the intrapleural injection.

Carrageenan paw oedema

The method was based on that described by Winter et al (1962). Briefly, paw oedema was induced in groups of 10 female, Sprague Dawley rats (130–160 g) by the subplantar injection of 0·1 ml of 1% Viscarin carrageenan in saline. Compounds were given orally in 0·05% polysorbate 80, 1 h before carrageenan injection and the effect on foot swelling was measured 4 h after injection using a mercury plethysmograph.

Materials

Carrageenan (Viscarin) was obtained from Marine Colloids, Springfield, New Jersey, USA. Linbro multiwell plates, M199 culture medium, penicillin/streptomycin and swine serum were all purchased from Flow Laboratories, Irvine, Scotland. Heparin was obtained from Evans Medical, Speke, Liverpool. Bovine serum albumin (BSA), Triton X-100, PGE_2, phenidone and *p*-dinitrophenyl-β-D-glucuronide were purchased from Sigma Chemical Co Ltd, Poole, Dorset. LTC_4 was purchased from Miles Research Products Ltd, Slough, Bucks. Radioimmunoassay (RIA) kit for PGE_2 was from New England Nuclear, Boston, USA. Five times crystallized ovalbumin (OA) and polysorbate 80 (Tween 80) were bought from Koch-Light Laboratories Ltd, Colnbrook, Bucks. Polyvinyl sponge discs were purchased from Declon Ltd, Corby, Northants. Cell counts were made using a Coulter Counter Model ZF, Coulter Electronics Ltd, Harpenden, Herts., and cell smears were differentially stained with buffered Wrights stain on an Ames Hema-Tek slide staining machine, Miles Research Products Ltd, Slough, Bucks. Other stains, Evans blue and pontamine sky blue were purchased from Searle Scientific Services, High Wycombe, Bucks.

The following drugs were used (the suppliers are indicated in parentheses): indomethacin and dexamethasone sodium phosphate (Merck, Sharp and Dohme Ltd, Hoddesdon, Herts); naproxen (Syntex Pharmaceuticals Inc., Palo Alto, Calif., USA); benoxaprofen (Lilly Research Centre Ltd, Windlesham, Surrey); 2,6-di-t-butyl-4-(2′-thenoyl) phenol (R830) (Riker Laboratories Inc., St Paul, Minnesota, USA), timegadine (Leo Pharmaceutical Products, Ballerup, Denmark); 3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline (BW 755c) (The Wellcome Research Laboratories, Beckenham, Kent); nafazatrom (Bayer UK Ltd, Haywards Heath, West Sussex); dipyridamole (Ciba-Geigy UK Ltd, Horsham, West Sussex); hydrocortisone (BDH Chemicals Ltd, Poole, Dorset); 7-[3-(4-acetyl-3-hydroxy-2-propylphenyloxy)-2-hydroxypropyloxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylic acid, lysine salt (FPL 55712), (Department of Medicinal Chemistry, Fisons plc, Loughborough, Leics.).

Other chemicals used in the preparation of standard buffers, physiological balanced salt solution and HPLC solvents were supplied by Fisons Scientific Equipment, Loughborough, Leics.

RESULTS

Cultured mouse peritoneal macrophages

Stimulation of mouse peritoneal macrophages (approx. 1×10^6) with immune complexes caused a release of β-glucuronidase (45 ± 3%; n = 15 experiments), PGE_2 (71 ± 14 ng; n = 15) and SRS (170 ± 14 ng; n = 15). Non-challenged macrophages released only about 10% of their total β-glucuronidase and negligible SRS-like activity, but still released PGE_2 (21 ± 8 ng; n = 15). The effects of a variety of compounds representing selective CO inhibitors, LO inhibitors, dual enzyme inhibitors and a steroid tested against these parameters are summarized in Table 1. Indomethacin (IC50 0·2 μM) and R830 (IC50 1·3 μm) were potent inhibitors of PG formation and although a much weaker inhibition

Table 1. Inhibition of lysosomal enzyme (β-glucuronidase) release, SRS release and PGE_2 release from immune complex challenged mouse peritoneal macrophages in-vitro by modulators of arachidonic acid metabolism.

	Inhibition		
Compound	Lysosomal enzyme IC30 (μM)	SRS IC50 (μM)	PGE_2 IC50 (μM)
Indomethacin	>10·0	>10·0*	0·2
Naproxen	>10·0	>10·0*	10·0
R830	>10·0	>10·0*	1·3
Benoxaprofen	>10·0	10·0	4·0
Timegadine	>10·0	15·0	1·5
BW 755c	9·4	9·2	3·4
Phenidone	>10·0	9·5	>10·0†
Nafazatrom	>10·0	11·0	>10·0†
Dipyridamole	>10·0	73% at 10 μM	>10·0†
Hydrocortisone	1·0	—[a]	45% at 0·1 μM[b]

* SRS release enhanced at 0·1–10 μM.
† PGE_2 release enhanced at 0·1–10 μM.
[a] No SRS detected in incubations studied under the conditions used for evaluating hydrocortisone (see Methods).
[b] PGE_2 formation enhanced at 1·0 nM.

was seen with naproxen (IC50 10 μM), all these compounds potentiated SRS release. Benoxaprofen, timegadine and BW 755c behaved as dual enzyme inhibitors but all showed greater potency at inhibiting cyclo-oxygenase. Phenidone, nafazatrom and dipyridamole selectively inhibited the lipoxygenase pathway with similar potencies and potentiated the release of PGE_2. Hydrocortisone at 10^{-7} M inhibited PGE_2 formation but actually enhanced production at 10^{-9} M. SRS-like activity was not detectable in the supernatants of incubates used to demonstrate steroid activity (see Methods). Hydrocortisone (IC30 1 μM) and BW 755c (IC30 9·4 μM) were the only compounds to cause significant dose-related inhibitions of enzyme release.

Intraperitoneally injected immune complexes in mice

Since previous experiments (Blackham & Woods 1985) suggested that SRS formation reaches a maximum at 5–10 min (104 ng LTC_4 equivalents per mouse; n = 8 expts) and dye extravasation is maximal at 30 min after injection of immune complexes, drug effects were evaluated at both these times (Tables 2a, b). Indomethacin inhibited dye extravasation at the earlier time (IC50 1·0 mg kg^{-1}) but potentiated SRS release. A small enhancement of dye leakage occurred at 30 min and this was also accompanied by enhanced SRS release (Tables 2a, b). Naproxen (10 mg kg^{-1}) and R830 (25 mg kg^{-1}) were inactive against dye leakage but potentiated SRS release when studied at 5–10 min post challenge. BW 755c significantly reduced vascular permeability at 5–10 min (IC50 40 mg kg^{-1}) and at 30 min (IC50 30 mg kg^{-1}) post challenge; phenidone also caused dose related reductions of dye extravasation at both times but the inhibitions were not significant. Both compounds exhibited greater inhibitory activity against SRS release than against permeability. Timegadine (30 mg kg^{-1}) demonstrated inhibition of vascular permeability and reduced SRS formation by a moderate extent at 5–10 min post challenge. Although nafazatrom (25 mg kg^{-1}) appeared to reduce SRS release, it failed to achieve an inhibitory effect against permeability.

Dexamethasone, like BW 755c, inhibited dye extravasation at 5–10 min (IC50 3·5 mg kg^{-1}) but this was associated only with a weak inhibition of SRS at both times.

Table 2a. Effects of orally administered drugs on dye extravasation and on SRS release in mouse peritoneal cavity measured at 5–10 min after i.p. injection of immune complexes.

Compound	mg kg^{-1}	% Inhibition of Dye extravasation	% Inhibition of SRS release
Indomethacin	0·3	19	0(+14)
	1·0	49*	0(+62)
	3·0	54*	0(+57)
Naproxen	10·0	10	0(+18)
R830	25·0	0	0(+115)
Timegadine	30·0	33*	33
BW755c	25·0	24	77
	50·0	68*	100
	200·0	77*	100
Phenidone	25·0	22	85
	50·0	27	100
Nafazatrom	25·0	3	35
Dipyridamole	25·0	18	46
Dexamethasone[a]	3·0	47*	33
	10·0	69*	11
	30·0	69*	46

* $P < 0·05$ by Student's *t*-test.

[a] Dexamethasone given orally 6 h before immune complexes.

Results for dye extravasation represent mean values from six animals. Results for SRS release represent a single value from peritoneal exudate supernatants pooled from six animals.

Table 2b. Effects of orally administered drugs on dye extravasation and on SRS release in mouse peritoneal cavity measured at 30 min after i.p. injection of immune complexes.

Compound	mg kg^{-1}	% Inhibition of Dye extravasation	% Inhibition of SRS release
Indomethacin	0·3	0(+13)	0(+23)
	1·0	0(+ 7)	0(+41)
	3·0	0(+19)	15(+41)
BW 755c	25·0	46*	100
	50·0	38	100
	200·0	46*	100
Phenidone	25·0	0	67
	50·0	40	100
Dexamethasone[a]	3·0	32*	10
	10·0	25	15
	30·0	41*	0

* $P < 0·05$ by Student's *t*-test.

[a] Dexamethasone given orally 6 h before immune complexes.

Results for dye extravasation represent mean values from six animals. Results for SRS release represent a single value from peritoneal exudate supernatants pooled from six animals.

Carrageenan sponge implants

Carrageenan sponge implants from control animals attracted on average 65·5 ± 3 × 10^6 cells (predominantly PMNs) per sponge (n = 10 experiments) and each sponge contained 21·6 ± 2 ng PGE_2.

Although the selective CO inhibitors (indomethacin, naproxen and R830) were potent inhibitors of cell infiltration, they also inhibited the

formation of PGE_2 to a much greater extent (Table 3). Benoxaprofen produced a similar response to the

Table 3. Effects of orally administered drugs on cell infiltration and on PGE_2 formation in 1% carrageenan-soaked sponges implanted subcutaneously in rats.

	Inhibition of	
	Cell infiltration IC30	PGE_2 formation IC50
Compound	(mg kg^{-1} × 3 24 h^{-1})	
Indomethacin	2·0	0·04
Naproxen	5·0	0·33
R830	12·0	1·00
Benoxaprofen	12·0	2·4
Timegadine	30·0	21·00
BW 755c	21·0	20·00
Phenidone	33·0	55·00
Nafazatrom	>50·0	>50·00
Dipyridamole	27·0	>50·00[a]
Dexamethasone	<1·0	>1·00[a]

[a] PGE_2 formation is enhanced at 1·0 mg$^-$ kg^{-1} dexamethasone and at 50 mg kg^{-1} dipyridamole.

NSAIDs, but was less active. Timegadine, BW 755c and phenidone demonstrated moderate inhibitory activity against cell infiltration and against PGE_2 formation (Table 3). The activity profile of dipyridamole in-vivo was consistent with its effects in-vitro and enhanced PG formation by 73% at 25 mg kg^{-1}. This was accompanied by moderate inhibitory activity against cell infiltration. Dexamethasone produced similar effects to dipyridamole, but was much more active at reducing cell infiltration (IC30 < 1·0 mg kg^{-1}). At 1 mg kg^{-1}, dexamethasone increased the amount of PGE_2 in the exudate by 94%. Nafazatrom was inactive at doses up to 50 mg kg^{-1}.

Reversed passive Arthus reaction (RPAR)

The total cell count recovered from the pleural cavity 4 h after the induction of a RPAR was 36 ± 6 × 10^6 and the exudate volume was 0·8 ± 0·1 ml (n = 5 experiments) for control animals. A group of 12 animals received antiserum intrapleurally, without antigen challenge, and produced a mean exudate volume of 0·3 ± 0·05 ml accompanied by a total cell count of 3·3 ± 1·0 × 10^6 cells.

Naproxen effectively reduced cell infiltration (IC50 3·4 mg kg^{-1}) whereas indomethacin was inactive up to 3 mg kg^{-1} (Table 4). BW 755c (IC30 8 mg kg^{-1}), timegadine (IC30 27 mg kg^{-1}) and phenidone (IC30 43 mg kg^{-1}) all reduced cell infiltration. Nafazatrom was inactive at doses up to 50 mg kg^{-1}. Dexamethasone potently inhibited

Table 4. Effects of orally administered drugs on cell infiltration and on exudate formation in a reversed passive Arthus reaction in the pleural cavity of rats.

	Inhibition of	
	Cell *infiltration* IC30	Exudate *formation* IC30
Compound	(mg kg^{-1})	
Indomethacin	>3·0	>3·0
Naproxen	3·4	>10·0
Timegadine	27·0	>50·0
BW 755c	8·0	>50·0
Phenidone	43·0	>100·0
Nafazatrom	>50·0	>50·0
Dexamethasone	0·02	<1·0

cellular migration (IC30 0·02 mg kg^{-1}) and was the only compound tested to reduce exudate formation (IC30 < 1·0 mg kg^{-1}).

Carrageenan paw oedema

Indomethacin and naproxen were potent inhibitors of swelling, whereas dual enzyme inhibitors showed slightly less activity in this test (Table 5). Phenidone was weakly active and nafazatrom and dipyridamole were without any activity at doses up to 50 mg kg^{-1}.

Table 5. Effects of orally administered drugs on 1% carrageenan-induced paw oedema in rats.

Compound	Inhibition of oedema IC30 (mg kg^{-1})
Indomethacin	2·1
Naproxen	2·3
R830	13·0
Benoxaprofen	5·0
Timegadine	25·0
BW 755c	9·0
Phenidone	54·0
Nafazatrom	>50·0
Dipyridamole	>50·0

DISCUSSION

The formation of PGE_2 and SRS following stimulation of mouse peritoneal macrophages in-vitro provided a rapid and reproducible system for estimating CO and LO inhibitory activities of the selected compounds. Indeed, our results support previous findings by other groups using the same cell system to study the modulatory effects of anti-inflammatory agents on AA metabolism (Bray & Gordon 1978; Humphray et al 1981; Humes et al 1983). Moreover, with minor exceptions, our results with individual compounds also agree with those obtained using isolated enzymes or other cell systems to measure

LO and CO activity, e.g. R830 (Moore & Swingle 1982); BW 755c (Higgs et al 1979), benoxaprofen and timegadine (Ahnfelt–Ronne & Arrigoni-Martelli 1982) and nafazatrom (Honn & Dunn 1982). As an extension to the in-vitro studies, we have demonstrated SRS release in-vivo following i.p. injection of immune complexes resulting in a model of peritoneal inflammation in mice, characterized by changes in vascular permeability (Blackham & Woods 1984). When dye extravasation was studied at 30 min post challenge, it was found that indomethacin was totally ineffective whereas LO inhibitors and dexamethasone produced significant reductions in the permeability response. However, fundamental differences exist between the steroid and LO inhibitors in terms of their effects on dye extravasation, since dexamethasone achieved its effects at doses which only partially suppressed SRS release and the LO enzyme inhibitors required complete inhibition of SRS. These combined findings suggest that LO products are probably involved in the permeability response, although mediators other than AA metabolites are also responsible. The inhibitory activity of dexamethasone may be explained in part by its ability to cause vasoconstriction (Zweifach et al 1953) or to inhibit directly the endothelial cell contraction induced by phlogistic agents (Tsurufugi et al 1979). The antipermeability activity of timegadine, at a dose which only partially suppressed SRS formation suggests that this compound also possesses some anti-inflammatory activity which is unrelated to its effects on AA metabolism and deserves further evaluation.

The discovery of chemotactic lipids derived from AA (e.g. LTB_4, 5-HETE) (Palmer et al 1980) has led to much interest in animal models of PMN recruitment for use in the development of inhibitors of LO (Higgs et al 1980; Blackham & Norris 1983; Tsurufugi et al 1984). Biochemical support for this approach has come from recent studies demonstrating a correlation between maximum LTB_4 generation (Salmon et al 1982) and the maximal rate of PMN infiltration into subcutaneously implanted sponges in rats (Simmons et al 1983). Although these findings have been confirmed using HPLC analysis of sponge exudates (D. Wilkinson, personal communication) we did not assay for chemotactic lipoxygenase products routinely during these studies. PGE_2 was measured in inflammatory exudates as a marker for drug effects on the CO system. Although the NSAIDs were potent inhibitors of cell infiltration (Table 3), this occurred at doses in excess of those required to inhibit PG synthesis and therefore supports previous findings (Walker et al 1976). It has been demonstrated recently that the antichemotactic activity of NSAIDs is also independent of effects on LTB_4 formation (Salmon et al 1983). Other mechanisms for inhibition of carrageenan-induced cell infiltration have been proposed for indomethacin, including a suppressive effect on extravasation of a chemotactic plasma protein (Lo et al 1984) and this mechanism may be pertinent in the present model. The inhibitory effect of timegadine, BW 755c, phenidone and dipyridamole against cell infiltration occurred at doses similar to or lower than those required to reduce PG formation and could therefore be distinguished clearly from NSAIDs. Their inhibitory activity against cell accumulation may well be related to their ability to inhibit 5-LO. However, in view of the findings with NSAIDs it is reasonable to suppose that chemotactic products other than LO-derived mediators are responsible for cell recruitment in this model. The potent antichemotactic activity of dexamethasone may be related to its inhibitory effect on LTB_4 generation in-vivo (Salmon et al 1983; Tsurufugi et al 1984) or it may be due to other mechanisms, such as its ability to reduce PMN adhesion to vascular endothelium (Skidmore 1981). The latter activity may be more likely in the present circumstances since dexamethasone ($1\ mg\ kg^{-1}$) failed to prevent the formation of CO products (Table 3) which suggests that it failed to induce the synthesis of macrocortin, an inhibitor of phospholipase A_2 (Blackwell et al 1980) whose enzymatic activity releases AA from phospholipids (Flower & Blackwell 1979). To demonstrate that the sponge model of cell infiltration and the peritoneal model of vascular permeability were capable of detecting specifically the LO-inhibitory activity of compounds, the selected enzyme inhibitors were tested in the carrageenan paw oedema model (Winter et al 1962) which is known to be very sensitive to inhibitors of CO enzyme (Lombardino et al 1975). Indeed, a distinction could be made between compounds possessing CO inhibitory activity and the more selective LO inhibitors, which were largely inactive in the test.

A pharmacological evaluation of the role of LO products in an Arthus type reaction based on two observations was also carried out. Firstly it is generally accepted that NSAIDs are relatively inactive in a RPAR (Pflum & Gaeme 1979; Chang & Otterness 1981); secondly, it has been demonstrated recently that LTB_4 is present in synovial fluid of patients with rheumatoid arthritis (Klickstein et al 1980), a disease which has features in common with

an Arthus reaction (Gardner 1972). The unexpected activity of naproxen in the present study may be reconciled with previous findings in which naproxen reduced synovial cellular infiltration more effectively than other NSAIDs (Blackham et al 1979) by a mechanism not yet understood. The activity observed with BW 755c, timegadine, and phenidone suggests the possibility that certain products of the LO pathway act as a recruiting system for PMNs in this model. Indeed, Myers & Siegel (1983) have already demonstrated that PMNs isolated from RPAR in the pleural cavity release LTB_4 in reponse to calcium ionophore. The present findings however are at variance with a report by Bailey & Sturm (1983) who claimed only weak inhibitory activity with BW755c in an RPAR although their studies were carried out in skin. The potent inhibitory activity of dexamethasone against exudate formation and against cell infiltration suggests that this compound is working in part through mechanisms other than inhibition of phospholipase A_2, as described previously. Clearly, further biochemical studies are required to elucidate the role of AA metabolites in this model and its potential use in the development of inhibitors of LO.

As a result of these studies we believe that orally active anti-inflammatory agents that are capable of modulating AA metabolism at both CO and LO pathways may be detected and distinguished clearly from classical NSAIDs using a combination of in-vitro and in-vivo models as described in this paper.

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