

Effect of a 5-lipoxygenase inhibitor, ZM 230487, on cutaneous allergic inflammation in the guinea-pig

¹Mauro M. Teixeira & Paul G. Hellewell

Department of Applied Pharmacology, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY

1 Leukotrienes have potent biological effects *in vitro* and *in vivo* and are found in tissue and in biological fluids in various pathological conditions including allergic diseases. Leukotriene B₄ (LTB₄) is a potent stimulus for eosinophil accumulation and activation and there is much interest in determining its importance in mediating the accumulation of eosinophils at sites of allergic inflammation *in vivo*. In this study, we investigated the effects of a potent 5-lipoxygenase inhibitor, ZM 230487, on the accumulation of eosinophils and on local oedema formation in cutaneous inflammation in the guinea-pig.

2 The i.d. injection of increasing concentrations of arachidonic acid (AA) led to a dose-dependent accumulation of ¹¹¹In-eosinophils but oedema formation was only significant at the top dose of AA tested (3×10^{-8} mol per site). Co-injection of ZM 230487 with AA inhibited ¹¹¹In-eosinophil accumulation up to 99% but the small oedema response to AA was only partially inhibited. AA-induced oedema formation was only effectively inhibited when a combination of a PAF antagonist, an antihistamine and ZM 230487 was used.

3 Local administration of the cyclo-oxygenase inhibitor, ibuprofen, partially inhibited AA-induced oedema formation suggesting that vasodilator prostaglandins may be released following i.d. injection of AA. AA-induced ¹¹¹In-eosinophil accumulation was also partially inhibited by ibuprofen.

4 PAF-induced ¹¹¹In-eosinophil accumulation was partially suppressed by local administration of ZM 230487. In contrast, LTB₄-induced ¹¹¹In-eosinophil accumulation was enhanced by ZM 230487. These data suggest that locally-released leukotrienes may modulate mediator-induced eosinophil accumulation. ZM 230487 had no effect on PAF-or LTB₄-induced oedema formation.

5 ZM 230487 significantly inhibited the accumulation of ¹¹¹In-eosinophils, but did not affect local oedema formation, in a passive cutaneous anaphylaxis (PCA) reaction. However, the PAF antagonist WEB 2086 either alone or in combination with ZM 230487 had no effect on ¹¹¹In-eosinophil accumulation or oedema formation in the PCA reaction.

6 In conclusion, it appears that a product of 5-lipoxygenase, probably LTB₄, is important for the accumulation of ¹¹¹In-eosinophils, but not local oedema formation, in the PCA reaction in guinea-pig skin. These data support a major role for LTB₄ in allergic inflammation in the guinea-pig and make this animal (and the PCA model) suitable for studying the effects of inhibitors of leukotriene synthesis or action *in vivo*.

Keywords: Eosinophils; leukotrienes; 5-lipoxygenase inhibitors; oedema formation; passive cutaneous anaphylaxis; allergy

Introduction

Leukotrienes are oxidation products of arachidonic acid synthesized via the 5-lipoxygenase pathway (Piacentini & Kaliner, 1991). These eicosanoids not only have potent inflammatory effects in man and experimental animals but also affect bronchial and vascular smooth muscle tone (Bray, 1986; Batt, 1992). Elevated levels of leukotrienes or their metabolites have been detected in tissue or fluids of different pathological conditions (Bray, 1986; Busse & Gaddy, 1991; Salmon & Garland, 1991). For example, the urinary excretion of leukotriene E₄ (LTE₄, a metabolite of LTD₄) and bronchoalveolar lavage fluid levels of LTC₄ was enhanced after allergen challenge in allergic human subjects (Taylor *et al.*, 1989; Wenzel *et al.*, 1990). These levels of leukotrienes appeared to correlate with the severity of the bronchoconstrictor response (Wenzel *et al.*, 1990; Salmon & Garland, 1991). 20-OH-LTB₄, a metabolite of LTB₄, has been found in sputum of patients with asthma (Lam *et al.*, 1988) and synovial fluid from patients with rheumatoid arthritis (Davidson *et al.*, 1983). In addition, biologically active levels of LTB₄-like material were found in antigen-treated skin of allergic subjects (Barr *et al.*, 1984). Because of the widespread presence of leukotrienes in pathological conditions and their potent biological effects, great efforts have been made to identify drugs which can inhibit leukotriene synthesis (5-

lipoxygenase inhibitors) or drugs which may interfere with their action (LTD₄ and LTB₄ antagonists) (McMillan & Walker, 1992). It is hoped that these drugs will be a major improvement in the treatment of chronic inflammatory human diseases.

In asthma and other related diseases, it has been suggested that eosinophils and eosinophil products play an important role in the lesion of the airways and development of bronchial hyperresponsiveness (Djukanovic *et al.*, 1990). Leukotrienes, specially LTB₄, have been shown to be potent eosinophil chemoattractants both *in vivo* (Faccioli *et al.*, 1991) and *in vitro* (Czarnetzki & Mertensmeier, 1985; Sehmi *et al.*, 1991). Interestingly, human eosinophils also release LTC₄ which may contribute to bronchoconstriction in airway disease (Kauffman *et al.*, 1987).

In the guinea-pig, the intradermal injection of different chemotactic agents leads to accumulation of eosinophils in skin sites (Faccioli *et al.*, 1991; Collins *et al.*, 1993; Teixeira *et al.*, 1993a). In this model, LTB₄ is potent in inducing the accumulation of ¹¹¹In-eosinophils but causes very little increase in microvascular permeability and subsequent oedema formation (Faccioli *et al.*, 1991). The injection of antigen in sites previously sensitized with an IgG₁-rich sera also leads to accumulation of ¹¹¹In-eosinophils and to oedema formation in a passive cutaneous anaphylaxis (PCA) reaction (Weg *et al.*, 1992; Teixeira *et al.*, 1993a,b). Oedema formation in a PCA reaction is due to the release and action of histamine

¹ Author for correspondence.

and to newly synthesized LTD₄ and PAF (Weg *et al.*, 1991). However, the mediators responsible for the accumulation of ¹¹¹In-eosinophils in the same reaction do not seem to depend on PAF (as assessed by use of the PAF antagonist WEB 2086) or leukotrienes (as assessed by use of the weak leukotriene synthesis inhibitor PF 5901) (Weg *et al.*, 1992). While the endogenous mediators responsible for inducing ¹¹¹In-eosinophil accumulation in the PCA reaction are unknown, it has recently been established that the mechanism of their accumulation involves the leukocyte cell adhesion molecules CD18 and VLA-4 (Weg *et al.*, 1993; Teixeira *et al.*, 1994a). In view of the potent action of exogenous LTB₄ in inducing ¹¹¹In-eosinophil accumulation in the guinea-pig, we have re-evaluated, by using the potent 5-lipoxygenase inhibitor ZM 230487 (formerly ICI 230487), the role of this mediator in contributing to the accumulation of ¹¹¹In-eosinophils in a PCA reaction in guinea-pig skin.

Methods

Preparation of zymosan-activated plasma

Zymosan-activated plasma (ZAP) was used as a source of guinea-pig C5a des-Arg. ZAP was prepared by incubating heparinized (10 iu ml⁻¹) plasma obtained from naive guinea-pigs (Harlan Porcellus, Oxon, 350–400 g) with zymosan (5 mg ml⁻¹) for 30 min at 37°C. Zymosan was then removed by centrifugation (2 × 10 min at 3000 g). The activated plasma was desalted with a PD-10 Sephadex G-25M column and stored in aliquots at –20°C.

Preparation of passive cutaneous anaphylaxis sera and reactions

Details of the preparation of IgG₁-rich sera are described elsewhere (Weg *et al.*, 1991). Briefly, male guinea-pigs (Harlan Porcellus, 350–400 g) were immunized with bovine gamma-globulin (BGG) in Freund's complete adjuvant (0.2 mg BGG 0.2 ml⁻¹ of adjuvant s.c.). These animals received a boost of antigen in Freund's incomplete adjuvant on day 21 and the serum prepared on day 30. Recipient animals received an injection of 50 µl of a 1/50 dilution of the anti-serum i.d., followed 16–20 h later by the i.d. injection of antigen (BGG, 0.1 or 1.0 µg per site).

Measurement of local oedema formation and ¹¹¹In-eosinophil accumulation in guinea-pig skin

Radiolabelled eosinophil infiltration and oedema formation were measured simultaneously at sites of cutaneous inflammation. ¹²⁵I-labelled human serum albumin (¹²⁵I-HSA, ~5 µCi) was added to ¹¹¹In-labelled eosinophils (purified and radiolabelled as previously described by Faccioli *et al.*, 1991; Teixeira *et al.*, 1993a) and injected i.v. (5 × 10⁶ cells per animal) into recipient guinea-pigs (350–400 g) anaesthetized with Hypnorm (0.15 ml, i.m.). After 5 min, inflammatory stimuli or antigen were injected i.d. in 0.1 ml volumes into the dorsal shaved skin. ZM 230487 or ibuprofen were mixed with the stimuli or saline before the i.d. injections. Each animal received a duplicate of each treatment following a randomized injection plan and the inflammatory response (¹¹¹In-labelled eosinophil accumulation and oedema formation) was assessed after 1 h. This time point was chosen because previous experiments showed that the majority of oedema formation induced by a local injection of arachidonic acid and the greatest portion of the ¹¹¹In-eosinophil accumulation in a PCA reaction occur during the first 60 min after i.d. injection of antigen (Weg *et al.*, 1992; Teixeira *et al.*, unpublished observations). At this time, a blood sample was obtained by cardiac puncture, the animals were killed by an overdose of sodium pentobarbitone, the dorsal skin was removed, cleaned free of excess blood and the sites punched

out with a 17 mm punch. The samples were counted in an automatic 5-head gamma-counter (Canberra Packard Ltd, Pangbourne, Berks) and the counts were cross-channel corrected for the two isotopes.

Eosinophil numbers in the skin sites were expressed as the number of ¹¹¹In-eosinophil per skin site and oedema formation as the ratio of ¹²⁵I counts of the skin sample divided by the ¹²⁵I counts in 1 µl of plasma.

Reagents

The following compounds were purchased from Sigma Chemical Company (Poole, Dorset): arachidonic acid (AA), bovine gamma globulin (BGG), dimethyl sulphoxide (DMSO), sodium nitroprusside (SNP) and zymosan. Hanks solutions, HEPES buffer and horse serum were purchased from Life Technologies Ltd (Paisley, Scotland). Percoll was purchased from Pharmacia (Milton Keynes, Bucks). PAF and calcitonin-gene related peptide (CGRP) from Bachem (Saffron Walden, Essex) and LTB₄ from Cascade Biochem Ltd (Reading, Berks). ¹²⁵I-HSA and ¹¹¹InCl₃ were obtained from Amersham International plc (Amersham, Bucks). ZM 230487 (1-ethyl-6-[fluoro-5-(4-methoxy-3, 4, 5, 6-tetrahydro-2H-pyran-4-yl) phenoxy] methyl-quinol-2-one) was a kind gift from Zeneca Pharmaceuticals (Macclesfield, Cheshire) and ibuprofen a gift from Boots Chemical Company plc (Nottingham, Notts). WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thienol[3,2-β][1,-2,4]-triazolo-[4,3,a][1,4]-diazepin-2-yl]-1-(4-morpholymyl)-1-propanone) was donated by Boehringer-Ingelheim KG (Ingelheim am Rhein, Germany). ZM 230487 was dissolved in 100% DMSO and further diluted in saline. The final concentration of DMSO in the injection fluid was never greater than 1%. All other drugs were dissolved in saline.

Statistics

Data were analysed by two-way analysis of variance on normally distributed data. *P* values were assigned using the Newman-Keuls procedure and values of *P* < 0.05 were considered statistically significant. Percentage inhibition was calculated after subtracting background values.

Results

Effect of ZM 230487 on inflammation induced by arachidonic acid

In the guinea-pig skin, i.d. injection of AA induced a dose-dependent accumulation of ¹¹¹In-eosinophils (Figure 1a). Significant oedema formation was induced only at the top dose of AA tested (3 × 10⁻⁸ mol per site, Figure 1b). Whereas ¹¹¹In-eosinophil accumulation was markedly greater than saline (Figure 1a), oedema formation was only around 1.5 times greater (Figure 1b). Figure 2 shows the inhibitory effects of ZM 230487 on the ¹¹¹In-eosinophil accumulation and oedema formation induced by AA (3 × 10⁻⁸ mol per site). AA-induced ¹¹¹In-eosinophil accumulation was abolished by ZM 230487 with an IC₅₀ of approximately 2 × 10⁻¹⁰ mol per site (Figure 2a). The small level of oedema formation induced by AA was slightly enhanced at the lower doses of ZM 230487 but inhibited at doses greater than 10⁻¹⁰ mol per site although the degree of inhibition was never greater than 40% (Figure 2b). Since inhibition of AA-induced inflammation by ZM 230487 was maximum at 10⁻⁸ mol per site, this dose was chosen to be used in further experiments. ZM 230487 possessed no significant inflammatory properties itself.

The effects of ibuprofen, a cyclo-oxygenase inhibitor, on AA-induced inflammation are shown in Figure 3, for comparison with ZM 230487. Ibuprofen (10⁻⁷ mol per site) partially inhibited ¹¹¹In-eosinophil accumulation and oedema formation induced by AA (3 × 10⁻⁸ mol per site). In the

same experiments, ZM 230487 virtually abolished the accumulation of ^{111}In -eosinophils (Figure 3a) but did not significantly reduce oedema formation induced by AA (Figure 3b).

The mechanisms of AA-induced local oedema formation were examined in further experiments. We tested the effects of a PAF antagonist (WEB 2086) and an anti-histamine (mepyramine) used alone or in combination with ZM 230487 against AA-induced oedema formation. Administration of ZM 230487 or mepyramine (2.5×10^{-8} mol per site) partially inhibited AA-induced oedema formation while WEB 2086 (10^{-7} mol per site) was without effect (Figure 4). These doses of WEB 2086 and mepyramine effectively inhibited oedema formation induced by PAF (10^{-9} mol per site) and histamine (2.5×10^{-8} mol per site), respectively (data not shown). However, the combination of ZM 230487, WEB 2086 and mepyramine effectively suppressed AA-induced oedema formation by 76% (Figure 4) while the combination of any two of the antagonists/inhibitor did not show similar inhibitory effects (data not shown).

Effect of ZM 230487 on other inflammatory mediators

ZM 230487 was tested against ^{111}In -eosinophil accumulation and oedema formation induced by PAF (10^{-9} mol per site), LTB_4 (5×10^{-10} mol per site) and ZAP (30% in saline). PAF-induced ^{111}In -eosinophil accumulation was slightly but significantly inhibited by ZM 230487 while LTB_4 -induced ^{111}In -eosinophil accumulation was significantly enhanced by the compound (Figure 5a). ZM 230487 had no effect on ZAP-induced responses (Figure 5). In the same experiments, AA-induced ^{111}In -eosinophil accumulation was abrogated and oedema formation partially suppressed by ZM 230487

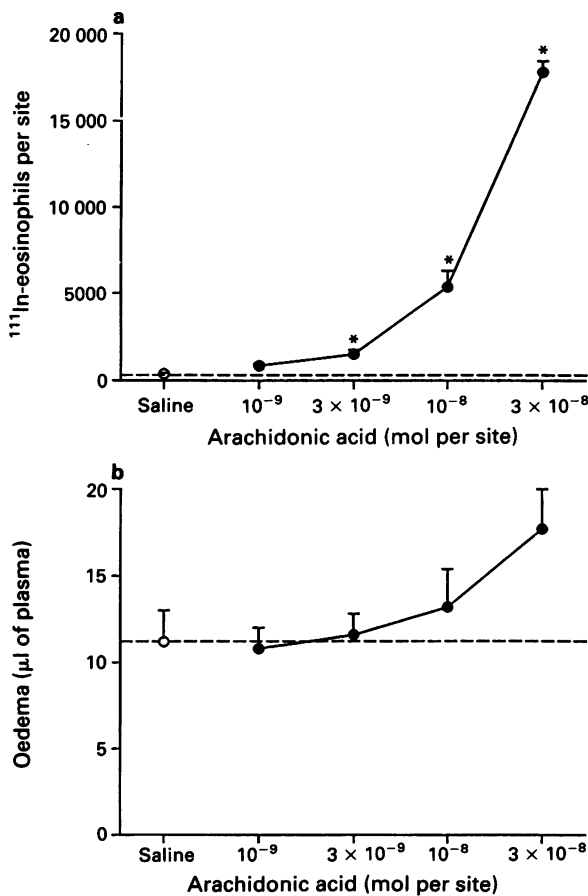


Figure 1 Local inflammation induced by i.d. injection of arachidonic acid in guinea-pig skin. ^{111}In -eosinophil accumulation (a) and oedema formation (b) were measured over 1 h. The dashed line across the graphs represents background values in sites injected with saline. Results are mean \pm s.e. mean of 4 guinea-pigs.

(Figure 5). As shown in Figure 5b, ZM 230487 had no effect on oedema formation induced by PAF or LTB_4 .

Effect of ZM 230487 on local inflammation in a passive cutaneous anaphylaxis reaction

The injection of antigen (BGG) in sites previously sensitized with a IgG_1 -rich anti-serum (anti-BGG) leads to a dose-dependent ^{111}In -eosinophil accumulation and oedema formation in a PCA reaction (Weg *et al.*, 1992; Teixeira *et al.*, 1993a) (Figure 6). ZM 230487, when co-injected with 0.1 μg and 1.0 μg of antigen per site, significantly inhibited the accumulation of ^{111}In -eosinophils by 72% and 44%, respectively (Figure 6a). Oedema formation measured in the same sites was not affected (Figure 6b). Again in these experiments, ZM 230487 abrogated AA-induced ^{111}In -eosinophil accumulation (Figure 6a) but had little effect on AA-induced oedema formation (Figure 6b).

Weg *et al.* (1992) have shown that WEB 2086, when tested alone, had no effect on oedema formation or ^{111}In -eosinophil accumulation in a PCA reaction. In the present study, we also examined the effect of WEB 2086. As shown in Figure 7,

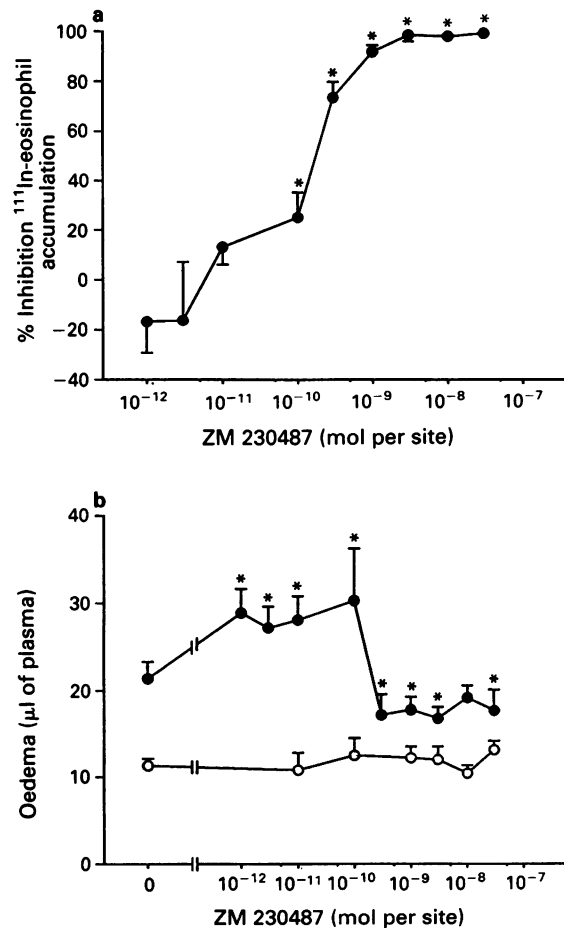


Figure 2 Effect of ZM 230487 on arachidonic acid-induced ^{111}In -eosinophil accumulation (a) and oedema formation (b) in guinea-pig skin. Arachidonic acid was used at a dose of 3×10^{-8} mol per site and ^{111}In -eosinophil accumulation and oedema formation were measured over 1 h. Percentage inhibition of ^{111}In -eosinophil accumulation was calculated by subtracting values in the presence of ZM 230487 alone. There were 11880 ± 2331 , 305 ± 47 and 273 ± 41 ^{111}In -eosinophils accumulating per skin site after injection of arachidonic acid, saline and ZM 230487 (10^{-8} mol per site), respectively. Local oedema formation was measured in sites injected with ZM 230487 alone (\circ) or with arachidonic acid (\bullet). Results are mean \pm s.e. mean of 8 guinea-pigs. $*P < 0.01$ when compared to injection of AA alone.

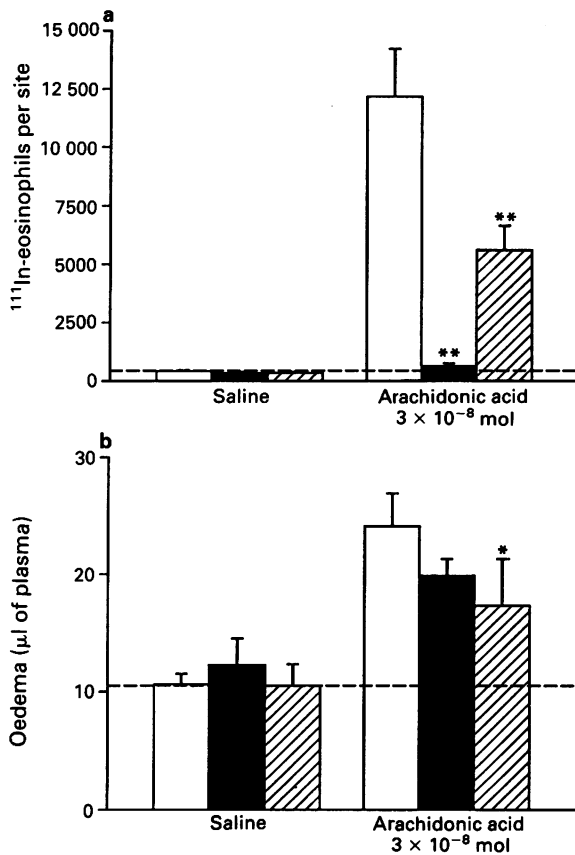


Figure 3 Effect of ZM 230487 and ibuprofen on arachidonic acid-induced ¹¹¹In-eosinophil accumulation (a) and oedema formation (b) in guinea-pig skin. Saline or arachidonic acid (3×10^{-8} mol per site) were injected alone (open columns) or with ZM 230487 at 10^{-8} mol per site (solid columns) or ibuprofen at 10^{-7} mol per site (hatched columns). ¹¹¹In-eosinophil accumulation and oedema formation were measured over 1 h. The dashed line across the graphs represents the background values in sites injected with saline. Results are mean \pm s.e.mean of 8 guinea-pigs. * $P < 0.05$; ** $P < 0.01$.

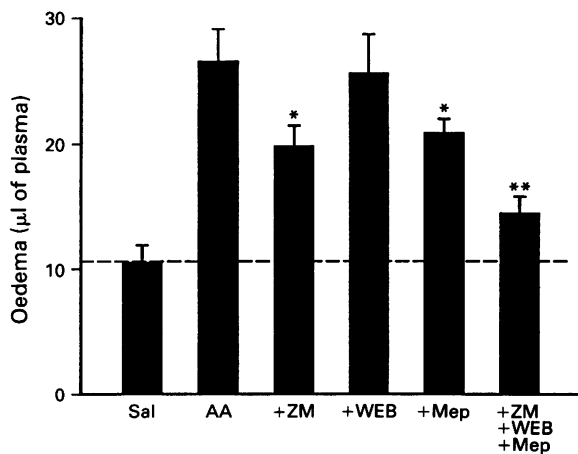


Figure 4 Effect of ZM 230487, WEB 2086 and mepyramine on arachidonic acid-induced oedema formation in guinea-pig skin. Arachidonic acid (AA) was used at a dose of 3×10^{-8} mol per site, ZM 230487 (ZM) at 10^{-8} mol per site, WEB 2086 (WEB) at 10^{-7} mol per site and mepyramine (Mep) at 2.5×10^{-8} mol per site. Drugs were mixed prior to i.d. injection and oedema formation measured over 1 h. The dashed line across the graph represents the background values in sites injected with saline. Results are mean \pm s.e.mean of 4 guinea-pigs. * $P < 0.01$ when compared to AA-induced oedema formation and ** $P < 0.01$ when compared to AA plus any other treatment.

WEB 2086 abrogated PAF-induced ¹¹¹In-eosinophil accumulation but had no effect on the PCA reaction when tested alone or when tested in combination with ZM 230487.

Discussion

The increased prevalence of asthma and other inflammatory diseases even with the anti-inflammatory therapy available at present warrants a search for more effective treatment (Piacentini & Kaliner, 1991; Salmon & Garland, 1991). The potent biological effects of leukotrienes together with their widespread presence in different pathological conditions make these mediators of considerable interest. The protective effects of LTD₄ antagonists against allergen-induced bronchoconstriction in man (O'Shaughnessy *et al.*, 1993) and in exercise-induced asthma (Makker *et al.*, 1993) further support the importance of leukotrienes in the pathogenesis of asthma (Cheng, 1992). It is hoped that the development of drugs that inhibit 5-lipoxygenase and thus reduce the synthesis not only of peptidoleukotrienes (LTD₄, LTC₄) but also of LTB₄ will add substantially to the protective effects of LTD₄ antagonists since 5-lipoxygenase inhibitors may also have anti-inflammatory effects. Special interest has been given to a series of non-redox inhibitors of 5-lipoxygenase because of their efficacy both *in vitro* and *in vivo* and the

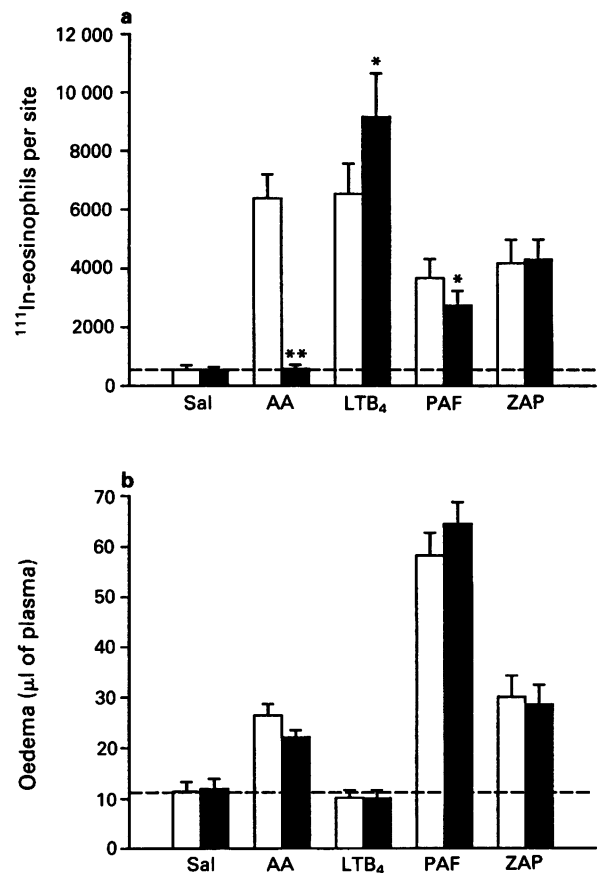


Figure 5 Effect of ZM 230487 on ¹¹¹In-eosinophil accumulation (a) and oedema formation (b) induced by arachidonic acid (AA), PAF, leukotriene B₄ (LTB₄) and zymosan-activated plasma (ZAP) in guinea-pig skin. AA (3×10^{-8} mol per site), PAF (10^{-9} mol per site), LTB₄ (5×10^{-10} mol per site) and ZAP (30% in saline) were injected alone (open columns) or with ZM 230487 (10^{-8} mol per site, solid columns). ¹¹¹In-eosinophil accumulation and oedema formation were measured over 1 h. The dashed line across the graphs represents the background values in sites injected with saline. Results are mean \pm s.e.mean of 8 guinea-pigs. * $P < 0.05$; ** $P < 0.01$.

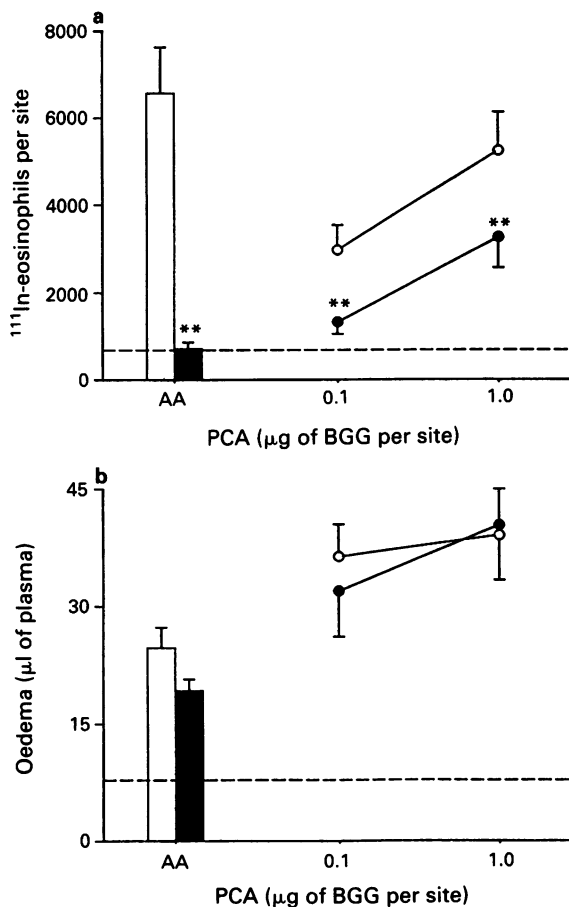


Figure 6 Effect of ZM 230487 on ¹¹¹In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylaxis (PCA) reaction. Arachidonic acid (AA, 3×10^{-8} mol per site) and bovine gamma-globulin (BGG, 0.1 and 1 µg per site in sites previously sensitized with anti-BGG) were injected alone (open symbols and columns) or with ZM 230487 (10^{-8} mol per site, solid symbols and column). ¹¹¹In-eosinophil accumulation and oedema formation were measured over 1 h. The dashed line across the graphs represents the background values in sites injected with saline. Results are mean \pm s.e.mean of 8 guinea-pigs. ** $P < 0.01$.

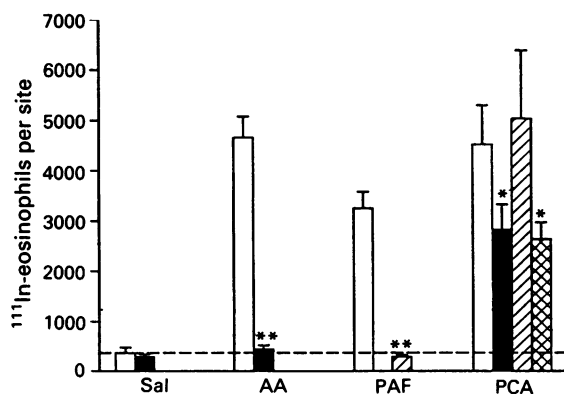


Figure 7 Effect of ZM 230487 alone or in combination with WEB 2086 on the ¹¹¹In-eosinophil accumulation in a passive cutaneous anaphylaxis (PCA) reaction. Arachidonic acid (AA, 3×10^{-8} mol per site), PAF (10^{-9} mol per site) and bovine gamma-globulin (BGG 1.0 µg per site in sites previously sensitized with anti-BGG) were injected alone (open columns), with ZM 230487 (10^{-8} mol per site, solid columns), with WEB 2086 (10^{-7} mol per site, hatched columns) or with ZM 230487 and WEB 2086 (cross-hatched column). ¹¹¹In-eosinophil accumulation was measured over 1 h. The dashed line across the graph represents background values in sites injected with saline. Results are mean \pm s.e.mean of 4 guinea-pigs. * $P < 0.05$; ** $P < 0.01$.

stereospecificity observed with methoxyalkyl thiazole analogues (McMillan *et al.*, 1991; McMillan & Walker, 1992). A related series, methoxy tetrahydropyrans, includes ZD 2138 (formerly ICI D2138; McMillan *et al.*, 1992) which is undergoing clinical evaluation and a close achiral analogue, ZM 230487 (Crawley *et al.*, 1993), which was employed in these studies.

In rabbit skin, intradermal injection of high doses of AA ($> 10^{-8}$ mol per site) causes an inflammatory response (oedema formation and neutrophil accumulation) which is dependent on the formation of LTB_4 and PGE_2 (Aked & Foster, 1987) and can be modulated pharmacologically with 5-lipoxygenase inhibitors (Aked *et al.*, 1986; McMillan *et al.*, 1992). In the guinea-pig skin, i.d. injection of AA caused a dose-dependent accumulation of ¹¹¹In-eosinophils. In contrast to the rabbit skin (Aked *et al.*, 1986), oedema formation was only of mild intensity and only significant at the top dose of AA tested (3×10^{-8} mol per site). Ibuprofen partially inhibited oedema formation suggesting that vasodilator prostaglandins may be important in this reaction (Williams & Peck, 1977). The effects of ZM 230487 were more complicated since they partially inhibited oedema formation at higher doses ($> 10^{-10}$ mol per site) but potentiated at lower doses. Indeed the interaction between leukotrienes in guinea-pig skin is complicated since they can have vasoconstrictor effects in addition to permeability-increasing effects in the cutaneous microcirculation (Hellewell & Williams, 1988). In this species, LTD_4 causes potent increase in microvascular permeability (Ueno *et al.*, 1981) whereas LTC_4 is a more potent vasoconstrictor (Hellewell & Williams, 1988). It is possible that at lower doses, ZM 230487 is interfering with the action of any LTC_4 (vasoconstrictor) generated. Vasoconstrictors or drugs which inhibit vasodilatation are known to inhibit inflammation in rabbit and guinea-pig skin (Brain *et al.*, 1989; Teixeira *et al.*, 1993b). It was surprising that AA-induced oedema formation was not abolished by ZM 230487 since in the same skin sites ¹¹¹In-eosinophil accumulation was completely blocked. We found that AA-induced oedema formation could be effectively inhibited when a combination of a PAF antagonist, an anti-histamine and ZM 230487 was used (Figure 4). This observation is identical to that found in earlier studies in our laboratory in which a combination of similar mediator antagonists was also necessary to abrogate oedema formation in a PCA reaction in guinea-pig skin (Weg *et al.*, 1991).

Accumulation of ¹¹¹In-eosinophils was abrogated by ZM 230487 suggesting that endogenous leukotrienes are important for AA-induced ¹¹¹In-eosinophil accumulation. Some workers have described chemoattractant properties for peptidoleukotrienes (in particular LTD_4) in guinea-pig conjunctiva (Spada *et al.*, 1986) and lung (Crean *et al.*, 1989) but studies in our department (Weg, unpublished observations) could not detect any ¹¹¹In-eosinophil accumulation in response to i.d. injection of doses of LTD_4 similar to or higher than those of LTB_4 in guinea-pig skin. In contrast, LTB_4 is a potent agonist for ¹¹¹In-eosinophil accumulation in the model (Faccioli *et al.*, 1991). Thus, it is probable that LTB_4 is the main leukotriene responsible for ¹¹¹In-eosinophil accumulation in guinea-pig skin induced by AA but further experiments using a specific LTB_4 antagonist are necessary to confirm this idea. We have used the LTB_4 antagonist LY 255,883 but it did not produce consistent inhibition of LTB_4 -induced ¹¹¹In-eosinophil accumulation in guinea-pig skin (data not shown). The possible cellular sources of leukotrienes in our system have not been investigated but they could be the mast cell or even migrating neutrophils. Guinea-pig eosinophils also release LTB_4 as their main leukotriene product (Hirata *et al.*, 1990).

Ibuprofen, a cyclo-oxygenase inhibitor, partially inhibited ¹¹¹In-eosinophil accumulation induced by AA. This inhibition was not reversed by PGE_1 suggesting that suppression of vasodilator prostaglandins was not the mechanism by which ibuprofen inhibited AA-induced ¹¹¹In-eosinophil accumula-

tion (data not shown). However, the demonstration that PGE₁ alone may inhibit ¹¹¹In-eosinophil accumulation induced by different stimuli when injected in guinea-pig skin (Teixeira *et al.*, 1993a) complicates interpretation of these experiments. Nevertheless other vasodilators (CGRP and SNP) that do not share the inhibitory effect of PGE₁ on ¹¹¹In-eosinophil accumulation (Teixeira *et al.*, 1993a) were unable to reverse the effects of ibuprofen on AA-induced ¹¹¹In-eosinophil accumulation, despite all three vasodilators greatly potentiating AA-induced oedema formation even in the presence of ibuprofen (data not shown). It is possible that ibuprofen is partially inhibiting 5-lipoxygenase (5-LO) in guinea-pig skin. For example it has an IC₅₀ of 69.5 μM on LTB₄ generation by rat leukocytes (Bray, 1986). Alternatively, it may be having some non-specific inhibitory effects on leukocyte function, as previously described (Nielson & Webster, 1987), which inhibits their accumulation. Another interesting possibility is that ibuprofen may be inhibiting local production of prostaglandin D₂ (PGD₂). Indeed, PGD₂ is able to elicit accumulation of eosinophils in dog trachea (Emery *et al.*, 1989). However, in guinea-pig skin, we have no evidence for the release of PGD₂ after i.d. injection of AA and this mediator (PGD₂) only weakly induced the accumulation of ¹¹¹In-eosinophils (data not shown).

PAF is a potent eosinophil chemoattractant and activator (Wardlaw *et al.*, 1986; Kroegel *et al.*, 1991). Nevertheless, guinea-pig eosinophils seem to be less responsive to PAF than to LTB₄ (Coeffier *et al.*, 1991; Faccioli *et al.*, 1991) (See Figure 5). Interestingly, PAF-induced ¹¹¹In-eosinophil accumulation was partially inhibited by ZM 230487. Indeed, PAF is capable of releasing leukotrienes in different experimental conditions and this may account for some of its effects (Voelkel *et al.*, 1982; Anderson & Fennessy, 1988; Kidney *et al.*, 1993). Another interesting finding was the small but consistent enhancement of LTB₄-induced ¹¹¹In-eosinophil accumulation by ZM 230487. We have not addressed this finding in greater detail but it is possible that ZM 230487 is inhibiting the action of vasoconstrictor leukotrienes (LTC₄ potently vasoconstricts in guinea-pig skin; Hellewell & Williams, 1988). If this is true, locally produced LTC₄ may be modulating the inflammatory effects of cutaneously-injected LTB₄.

The migration of inflammatory cells into lungs of sen-

sitized subjects challenged with antigen may be important in the development of late phase response (Pradaliere, 1993). Even though the microcirculation of the skin and bronchial tree may be different, the study of the mechanisms involved in this migration and its pharmacological modulation may give insights into pathological events in the lung. In a PCA reaction in guinea-pig skin, ZM 230487 significantly inhibited ¹¹¹In-eosinophil accumulation but had no effect on oedema formation. This suggests an important role for leukotrienes as agonists of ¹¹¹In-eosinophil accumulation in this reaction. The addition of a potent PAF antagonist had no further inhibitory effect on the accumulation of ¹¹¹In-eosinophils. Future studies will be aimed at characterizing the other mediator(s) generated in the PCA reaction that may lead to ¹¹¹In-eosinophil accumulation, including the possibility of mast cell-derived cytokines.

Our group has previously shown that the 5-LO inhibitor, PF 5901, was ineffective on inflammation induced in a guinea-pig PCA reaction (Weg *et al.*, 1992). The lesser potency of PF 5901 compared with ZM 230487 may explain these findings, suggesting that almost complete inhibition of 5-LO is necessary to be able to measure an inhibitory effect in the PCA reaction. A similar conclusion has been drawn from clinical studies with 5-LO inhibitors and LTD₄ antagonists in man (see Hui & Barnes, 1993 for review). Efficacy in acute bronchospasm was only apparent when inhibition of leukotriene synthesis or action was complete. Alternatively, extensive metabolism of PF 5901 *in vivo* and its high capacity for binding to proteins may prevent it from reaching its site of action in sufficient concentration in guinea-pig skin (Musser & Kreft, 1992).

Thus it seems that a product of 5-LO, probably LTB₄, is important for the accumulation of ¹¹¹In-eosinophils in a PCA reaction in guinea-pig. This, taken together with the results of others (Richards *et al.*, 1991; Sehmi *et al.*, 1991; Hidi *et al.*, 1992; Ishida *et al.*, 1993), support a major role for LTB₄ in the guinea-pig and makes this animal (and this PCA model) suitable for studying the effects of inhibitors of leukotriene synthesis or action *in vivo*.

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