Br. J. Pharmac. (1986), **89**, 431–438

The inflammatory response of rabbit skin to topical arachidonic acid and its pharmacological modulation

D. Aked, S.J. Foster[1], A. Howarth, M.E. McCormick & H.C. Potts

Imperial Chemical Industries plc, Pharmaceuticals Division, Bioscience I Department, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

1 The inflammatory reaction induced by the intradermal injection of arachidonic acid into the rabbit dermis has been investigated. Plasma extravasation was measured by the leakage of ^{125}I-albumin into the tissues and polymorphonuclear leukocyte (PMNL) accumulation was assessed histologically.

2 Arachidonic, 5,8,11,14,17-eicosapentaenoic and 8,11,14-eicosatrienoic acids, but not oleic, linoleic or linolenic acids, caused a concentration-related plasma extravasation following their intra-dermal injection. The plasma extravasation induced by arachidonic acid was dependent on PMNLs.

3 PMNL infiltration and plasma extravasation into arachidonic acid-injected skin sites was inhibited by the mixed cyclo-oxygenase-lipoxygenase inhibitor, BW755C.

4 Arachidonic acid-induced plasma extravasation was inhibited by cyclo-oxygenase and 5-lipoxygenase inhibitors but not by the Paf antagonist, kadsurenone.

5 The inflammation induced by arachidonic acid in the rabbit dermis may be a useful model for evaluating 5-lipoxygenase inhibitors which could be potentially useful anti-inflammatory agents for the treatment of psoriasis and other inflammatory diseases.

Introduction

Oxidative metabolism of arachidonic acid leads to the formation of a variety of mediators of inflammation. Products of the cyclo-oxygenase pathway such as prostaglandin E_2 (PGE_2) and prostacyclin contribute to inflammatory erythema, oedema and hyperalgesia (for review see Higgs *et al.*, 1980). Indeed the therapeutic efficacy of the non-steroidal anti-inflammatory drugs (NSAIDs) is generally accepted to be due to inhibition of the biosynthesis of these products (Ferreira & Vane, 1979).

Recent attention has focussed on products of the 5-lipoxygenase pathway which include the peptido-leukotrienes and leukotriene B_4 (LTB_4) (for review see Samuelsson, 1983). Peptido-leukotrienes contribute to oedema in some species by increasing vascular permeability (Ueno *et al.*, 1981), an effect potentiated by vasodilator prostaglandins (Williams & Piper, 1980; Peck *et al.*, 1981). LTB_4 is potently chemotactic towards PMNLs in rabbits (Bray *et al.*, 1981a; Higgs *et al.*, 1981) and man (Camp *et al.*, 1983a, 1984; Soter *et al.*, 1983) and when combined with vasodilator prostaglandins increases vascular permeability (Higgs *et al.*, 1981; Bray *et al.*, 1981b) by a mechanism which depends on the presence of polymorphonuclear leukocytes (PMNL) (Wedmore & Williams, 1981). Based on such observations it has been proposed that agents which inhibit leukotriene synthesis or antagonize their receptors might represent a new class of anti-inflammatory drugs.

Elevated levels of non-esterified arachidonic acid (Hammarström *et al.*, 1975), lipoxygenase products of arachidonic acid oxidation (Grabbe *et al.*, 1984; Camp *et al.*, 1983b; Brain *et al.*, 1984) and epidermal phospholipase A_2 activity (Forster *et al.*, 1983) have been reported in the skin of psoriasis patients. Recently, Cunningham *et al.* (1985) have demonstrated that arachidonic acid applied topically to human forearm skin under occlusion induces an erythematous reaction. High concentrations of arachidonic acid applied to the ears of mice (Young *et al.*, 1984; Opas *et al.*, 1985) or injected intradermally in rabbits (Higgs *et al.*, 1982) also cause inflammatory oedema. It is possible that the inflammation induced by arachidonic acid challenge and that associated with psoriasis may, in part, be a consequence of the metabolism of arachidonic acid to pro-inflammatory eicosanoids. We have therefore investigated the induction of inflammation by arachidonic acid in rabbit skin as a potential model for evaluating the anti-inflammatory effects of 5-lipoxygenase inhibitors.

[1]Author for correspondence.

Methods

Plasma extravasation

Plasma extravasation in rabbit skin was measured as described by Williams (1979). The backs of female New Zealand White rabbits (3.0–4.0 kg: Ranch Rabbits, Crawley Down, Sussex) were shaved and marked with a felt-tipped pen into a fixed pattern 2 h before each experiment. Each rabbit was given an intravenous injection of 5 μCi ^{125}I-human serum albumin in a 0.5% solution of Evans blue in physiological saline. Arachidonic acid or other test substances in 0.1 ml of pyrogen-free saline (Steriflex, the Boots Co. PLC, Nottingham) were injected intradermally into triplicate sites with a 26G1/2 hypodermic needle; each animal received up to 12 different treatments and 3 or 4 rabbits were used per experiment. After various times the animals were killed with a lethal intravenous dose of Euthatal (May & Baker Ltd., Dagenham) and a blood sample was taken by cardiac puncture into heparinized tubes. The skin was removed and the injection sites separated with a 15 mm diameter metal punch. Radioactivity in the skin sites and aliquots of plasma was determined by use of an LKB ultragamma counter and the plasma volume at each injection site was calculated in μl.

Leukocyte analysis

Leukocyte infiltration into skin sites was estimated histologically. Following histological processing, sections of skin (5 μm thick) were cut at several different points through each site. The sections were stained with haemotoxylin and eosin and the total number of leukocytes observed microscopically in 5 high-power fields arranged vertically through the dermis were determined. The total leukocyte content of blood was measured with a Coulter counter and the percentage of PMNLs determined on blood smears stained with Giemsa's stain.

Neutropenia

Neutropenia was induced in rabbits by intravenous injection of 1.75 mg kg^{-1} nitrogen mustard (mustine hydrochloride BP, The Boots Company PLC, Nottingham, UK). Three days following such treatment the PMNL content of blood was $< 3\%$ of normal.

Drugs

^{125}I-human serum albumin (2.5 μCi mg^{-1} protein) was purchased from Amersham International PLC, Amersham. The following unsaturated fatty acids were obtained from Sigma Chemical Company Ltd. (Poole, Dorset); arachidonic acid (5,8,11,14-eicosatetraenoic acid), EPA (5,8,11,14,17-eicosapentaenoic acid), ETA (8,11,14-eicosatrienoic acid), oleic acid (9-octadecenoic acid), linoleic (9,12-octadecadienoic acid) and linolenic acid (9,12,15-octadecatrienoic acid). These unsaturated fatty acids were stored under nitrogen and suspended to the desired concentrations in pyrogen-free physiological saline immediately prior to their intradermal injection.

Prostaglandin E_2, phenidone (1-phenyl-3-pyrazolidone), NDGA (nordihydroguaiaretic acid) and quercetin, were also obtained from Sigma. Platelet activating factor (Paf, 1-0-octadecyl-2-acetyl-Sn-glycero-3-phosphorylcholine) was obtained from Bachem AG (Bubendorf, Switzerland). BW755C (3-amino-1-[*m*-trifluoromethyl) phenyl]-2-pyrazoline HCl) was synthesized in the Chemistry Department at ICI Pharmaceuticals Division. Synthetic LTB_4 was prepared by Dr Y.K. Yee (Stuart Pharmaceuticals, Wilmington, Delaware) by a modification of the procedure of Corey *et al.* (1981). Flurbiprofen, REV5901 (2- [3'- (1''-hydroxyhexyl) -phenoxymethylquinone, AA861 (2 - (12 - hydroxydodeca - 5, 10-diynyl) - 3, 5, 6 - tri-methyl - 1, 4 benzoquinone) and kadsurenone were generously provided by The Boots Company PLC, Revlon Health Care Group, Takeda Chemical Industries and Merck, Sharp and Dohme respectively.

Drugs were dissolved in dimethylsulphoxide (DMSO), diluted with pyrogen-free saline then mixed with arachidonic acid suspension immediately prior to intradermal injection. The final DMSO concentration was 1% (v/v).

Statistics

The effects of drugs on plasma extravasation was calculated as the percentage difference from control for each animal (4 per experiment) and the statistical significance was assessed by use of a one sample Student's *t* test unless stated otherwise.

Results

Arachidonic acid-induced inflammation

The effect of intradermally injected polyunsaturated fatty acids on vascular permeability in the rabbit dermis is shown in Figure 1. The C20 fatty acids arachidonic acid, EPA and ETA caused a concentration-dependent increase in plasma extravasation. Arachidonic acid was 2 to 3 fold more effective than the two related fatty acids. In contrast the C18 fatty acids, oleic, linoleic and linolenic acids did not induce plasma extravasation at concentrations ranging from 25 to 200 μg per injection site. Since the C18 fatty acids are not metabolized directly to prostaglandins or leuk-

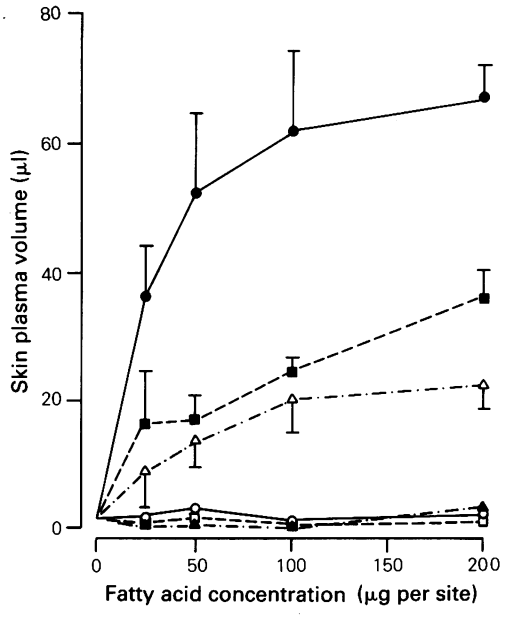

Figure 1 The concentration-dependent effect of polyunsaturated fatty acids on plasma extravasation in rabbit skin. Plasma extravasation was assessed 60 min following the intradermal injection of various concentrations of fatty acids as shown: (●) arachidonic acid; (■) 5,8,11,14,17-eicosapentaenoic acid; (△) 8,11,14-eicosatrienoic acid; (▲) linoleic acid; (○) oleic acid; (□) linolenic acid. Each value is the mean of three rabbits and the bars represent s.e.mean. For clarity, no error bars are shown for oleic, linoleic and linolenic acid where s.e.mean was < 30% of the mean values.

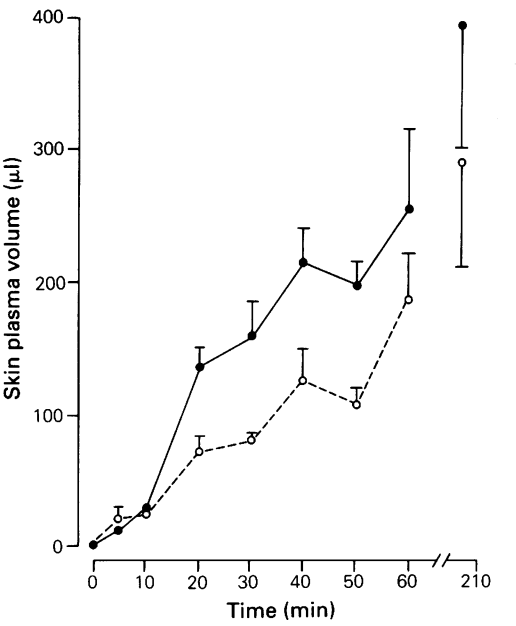

Figure 2 The time course of plasma extravasation in rabbit skin following the intradermal injection of eicosanoids. At various times following an intravenous injection of ^{125}I-human serum albumin in Evans blue (see Methods), arachidonic acid (100 μg) (○) or leukotriene B_4 (100 ng) combined with prostaglandin E_2 (200 ng) (●) were injected into separate skin sites. The rabbits were killed 210 min after injection of the radiolabelled albumin and the plasma extravasation into the skin sites, for the various time intervals, were determined. Each value is the mean of four rabbits and the bars represent s.e.mean.

otrienes the induction of plasma extravasation appears to be selective for fatty acids which are precursors of these eicosanoids. In fact, the time course of plasma extravasation induced by intradermal injections of arachidonic acid or LTB_4 combined with PGE_2 are very similar as shown in Figure 2.

Histological examination of arachidonic acid-injected skin sites 2 h after injection revealed an infiltration of PMNL which was related to the concentration of arachidonic acid (Figure 3). This figure also shows that the PMNL content of the arachidonic acid injected skin sites paralleled the plasma extravasation. The role of the PMNL in arachidonic acid-induced plasma extravasation was therefore investigated. As shown in Figure 4 rabbits that were depleted of PMNL by treatment with nitrogen mustard did not show an increase in plasma extravasation when injected intradermally with arachidonic acid. These animals did, however, increase plasma extravasation in response to an intradermal injection of bradykinin, an inflammatory mediator which increases vascular permeability probably by acting directly on the microvasculature and independent of PMNL (Wedmore & Williams, 1981).

Pharmacological modulation

The mixed cyclo-oxygenase-lipoxygenase inhibitors BW755C and phenidone caused a concentration-dependent inhibition of plasma extravasation induced by arachidonic acid as shown in Figure 5. In addition to its effect on plasma extravasation BW755C also inhibited PMNL infiltration into arachidonic acid injected skin sites but had no effect on these inflammatory parameters in skin sites co-injected with LTB_4 and PGE_2 as shown in Figure 6. Several other inhibitors of arachidonic acid metabolism were evaluated as shown in Table 1. The cyclo-oxygenase inhibitors, indomethacin and flurbiprofen, inhibited arachidonic acid-induced plasma extravasation but less effectively than the 5-lipoxygenase inhibitors, quercetin and NDGA. AA861 and REV5901 which

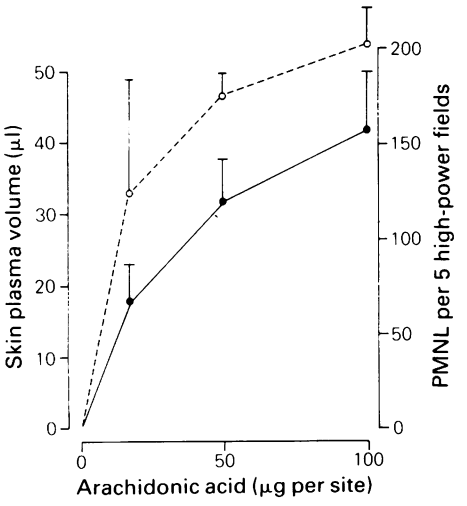

Figure 3 The concentration-dependent effect of arachidonic acid on PMNL infiltration and oedema in rabbit skin: 2 h following intradermal injections of arachidonic acid the excised injected skin sites were placed in formol-saline fixative and gamma counted to determine plasma exudation (●). The skin sites were then processed histologically for an assessment of their PMNL content (○). Values are the means of 4 animals and the bars represent s.e.mean.

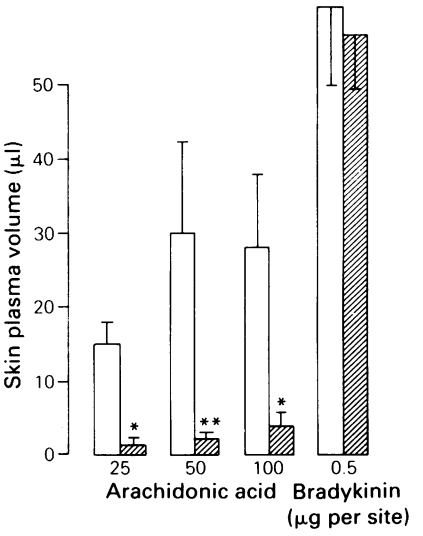

Figure 4 The effect of neutropenia on inflammation induced by arachidonic acid or bradykinin in rabbit skin. Five control (open columns) and six neutropenic animals (hatched columns) were injected intradermally with various concentrations of arachidonic acid or bradykinin as shown. Plasma extravasation was assessed 60 min later. Bars show the means ± s.e.mean. Significance of the differences with respect to control animals was assessed by Student's t test. $*P<0.05$; $**P<0.01$.

have been shown to be selective 5-lipoxygenase inhibitors (Ashida *et al.*, 1982; Coutts *et al.*, 1985) were also inhibitory. Although REV5901 caused a concentration-dependent inhibition of arachidonic acid-induced plasma extravasation it had no statistically significant effect on plasma extravasation induced by co-injections of LTB_4 and PGE_2 (Table 2).

The platelet activating factor antagonist, kadsurenone, was inactive against arachidonic acid-induced inflammation when tested at 20 μg per skin site, a concentration that caused 45% inhibition of the plasma extravasation induced by combined injections of Paf with PGE_2 in the same animals (Figure 7).

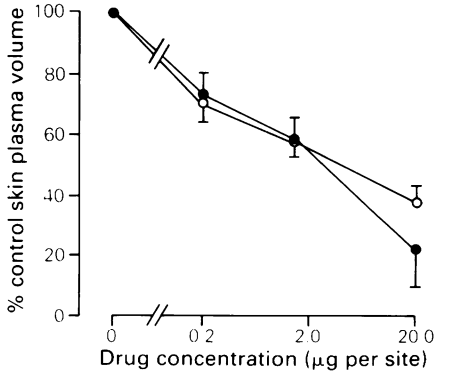

Figure 5 The effect of mixed cyclo-oxygenase and lipoxygenase inhibitors on arachidonic acid-induced inflammation in rabbit skin: phenidone (○); BW755C (●). Arachidonic acid (100 μg) alone or in combination with various concentrations of drug as indicated were injected intradermally and the plasma extravasation was assessed 60 min later. Values are the mean % of control skin plasma volume; bars show s.e.mean, 4 animals per group.

Discussion

The objective of this work was to investigate the utility of arachidonic acid-induced inflammation in rabbit skin as a model for evaluating the potential anti-inflammatory activity of 5-lipoxygenase inhibitors. Such models have so far proved elusive although topical application of high concentrations of arachidonic acid to mouse ears produces an inflammatory oedema which is sensitive to lipoxygenase inhibitors (Young *et al.*, 1984; Carlson *et al.*, 1985). Opas *et al.* (1985) extracted products of the cyclo-oxygenase pathway (PGE_2) and 5-lipoxygenase pathway (LTC_4/D_4), but not LTB_4, from mouse ears treated with arachidonic acid and suggested that these products, by acting synergistically on the microvasculature, are responsible for the oedema. The mechanisms by which arachidonic acid induces plasma extravasation in the

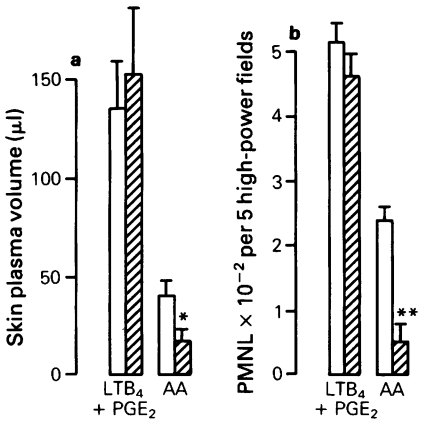

Figure 6 The effect of BW755C on eicosanoid-induced inflammation in rabbit skin. Inflammation was induced by intradermal injection of arachidonic acid (100 μg) or a combination of leukotriene B_4 (LTB_4, 100 ng) plus prostaglandin E_2 (PGE_2, 200 ng) in the presence (hatched columns) or absence (open columns) of 10 μg of BW755C per injection site. Plasma extravasation (a) and PMNL infiltration (b) was assessed 90 min later as described in the legend to Figure 3. Columns show the means of 4 animals with bars indicating s.e.mean. $*P<0.05$; $**P<0.0025$ with respect to non-drug treated control.

rabbit skin cannot be due to the metabolism of arachidonic acid to peptido-leukotrienes since these 5-lipoxygenase products do not induce plasma extravasation in rabbits (Ueno *et al.*, 1981).

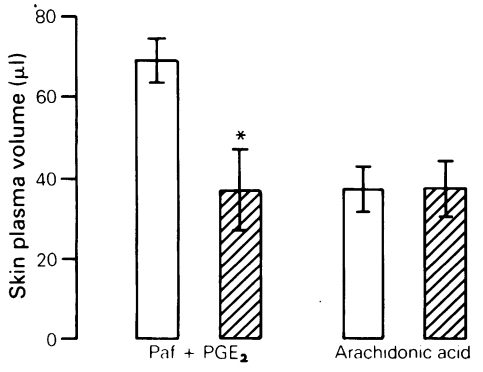

Figure 7 The effect of kadsurenone on Paf and arachidonic acid-induced inflammation in rabbit skin. Arachidonic acid (100 μg) or Paf (500 ng) combined with prostaglandin (PGE_2, 200 ng) were injected intradermally alone (open columns) or in combination with kadsurenone (20 μg) (hatched columns) and the plasma extravasation was assessed 60 min later. Values are the means of 4 animals with bars indicating s.e.mean. $*P<0.05$ with respect to control.

Table 1 The effect of cyclo-oxygenase and lipoxygenase inhibitors on arachidonic acid-induced plasma extravasation in rabbit skin

Compound	*Concentraton* (μg per site)	*% inhibition*
Indomethacin	2	2
	20	34**
Flurbiprofen	2	42*
	20	48**
Quercetin	2	40**
	20	66**
NDGA	2	64**
	20	66**
REV5901	2	18
	20	39*
AA861	2	38**
	20	56**

Arachidonic acid (100 μg) alone (control) or in combination with drug was injected intradermally and the plasma extravasation was assessed 60 min later. Values are the mean % inhibition of control values of 4 animals. $*P<0.01$; $**P<0.001$.

It could be argued that the relatively high concentrations of arachidonic acid used in the experiments described here may cause plasma extravasation by a non-specific mechanism. This is unlikely to be the case since only those C20 unsaturated fatty acids which can be metabolized directly to prostaglandins or leukotrienes increased plasma extravasation, whilst the C18 unsaturated fatty acids were inactive. Furthermore, inhibition of plasma extravasation by the various pharmacological agents tested would be inconsistent with a non-specific mechanism.

It is most likely that the plasma extravasation is caused by products of arachidonic acid oxidation. Higgs *et al.* (1982) demonstrated that when [1 – ^{14}C]-arachidonic acid was injected into rabbit dermis it was metabolized to products which co-chromatograph with 5-HETE and 5,12 di-HETE. The 5,12 di-HETE, LTB_4, is a potent chemotactic agent when injected into the rabbit dermis and, therefore, could account for the numerous PMNL which accumulate in arachidonic acid-injected skin sites. Inhibition of PMNL accumulation by the 5-lipoxygenase inhibitor BW755C supports the view that arachidonic acid is oxidatively metabolized in the dermis to a chemotactic product(s). BW755C did not inhibit plasma extravasation caused by co-injection of the arachidonic acid oxidation products LTB_4 and PGE_2 which demonstrates that the agent is not acting non-specifically. The profiles of the PMNL content and plasma extravasation in skin sites injected with various concentrations of arachidonic acid were parallel (Figure 3)

Table 2 The effect of REV5901 on plasma extravasation induced by intradermal injections of eicosanoids into rabbit skin

Compound	*Concentration* (μg per site)	*Arachidonic acid*	*LTB_4 + PGE_2*
REV5901	30	50**	25
	20	38*	15
	10	32*	−5
	5	0	−1
BW755C	20	54***	−1

Arachidonic acid (100 μg) or leukotriene B_4 (LTB_4, 100 ng) combined with prostaglandin E_2 (PGE_2, 200 ng), alone (controls) or in combination with the various drug concentrations shown, were injected into the dermis of rabbits and the plasma extravasation was assessed 60 min later. BW755C is shown for comparison. Values are the mean % inhibition of control values of 4 animals. $*P<0.025$; $**P<0.01$; $***P<0.005$.

suggesting that these events are coupled. Moreover, arachidonic acid failed to increase plasma extravasation in neutropenic animals which supports a role for PMNL in this inflammatory reaction in a manner analogous to that demonstrated for the combination of chemotactic agents and vasodilator substances (Wedmore & Williams, 1981). The conversion of arachidonic acid to chemotactic products alone, however, is not sufficient to explain the increased plasma extravasation since such an effect, in rabbits, requires the presence of a vasodilator (Williams, 1979; Wedmore & Williams, 1981). Inhibition of arachidonic acid-induced plasma extravasation by the cyclo-oxygenase inhibitors indomethacin and flurbiprofen is consistent with the view that the vasodilator is a cyclo-oxygenase product such as PGE_2 or prostacyclin.

In addition to its oxidative metabolism arachidonic acid can be non-enzymically oxidized to biologically active products. Auto-oxidation of arachidonic acid in air produces a complex mixture of hydroperoxy arachidonic acid derivatives including 5-HPETE (Porter *et al.*, 1979), which is an important intermediate in the biosynthesis of the leukotrienes. Our pharmacological studies demonstrated that several known 5-lipoxygenase inhibitors (BW755C, phenidone, NDGA, quercetin and AA861) reduced arachidonic acid-induced plasma extravasation. Since all of these agents also have anti-oxidant properties it is not possible to deduce the mechanism by which arachidonic acid is oxidized in the rabbit dermis. However, REV5901 which is not an anti-oxidant, has been shown to be a selective 5-lipoxygenase inhibitor (R.M. McMillan & S.J. Foster, unpublished). Therefore the reduction of arachidonic acid-induced plasma extravasation observed with this compound may be a consequence of 5-lipoxygenase inhibition. Since the compound was inactive against plasma extravasation induced by a co-injection of LTB_4 and PGE_2, its anti-inflammatory effect is unlikely to be the result of a non-specific mechanism. However, definitive evidence showing the contribution of 5-lipoxygenase products requires identification and pharmacological modulation of such products in the inflamed skin site.

Because Paf has been shown to increase plasma extravasation (Humphrey *et al.*, 1982a) and PMNL infiltration (Humphrey *et al.*, 1982b) into rabbit skin it may have a role in arachidonic acid-induced plasma extravasation. However, since the Paf antagonist, kadsurenone (Hwang *et al.*, 1985), failed to inhibit arachidonic acid-induced plasma extravasation in the same animals in which it inhibited Paf-induced plasma extravasation, it is unlikely that Paf contributes to the reaction.

Finally, does arachidonic acid-induced inflammation in rabbit skin have any pathophysiological relevance? Although the concentration of unesterified arachidonic acid is usually very low in tissues (Lands & Samuelsson, 1968), markedly elevated levels ($36.3 \pm 16.7\,\mu g\,g^{-1}$) have been measured in the involved epidermis of psoriasis patients (Hammarström *et al.*, 1975). These levels of arachidonic acid are within the range which cause plasma extravasation and PMNL accumulation in the rabbit dermis. PMNL are a prominent feature of psoriatic lesions. It is possible, therefore, that high concentrations of arachidonic acid contribute to the pathophysiology of psoriatic and other inflammatory lesions.

In conclusion, arachidonic acid appears to be oxidized in the rabbit dermis to products which induce a PMNL-dependent inflammatory reaction. Although the precise oxidative mechanism is unknown, inhibition of the inflammatory reaction by REV5901 and NSAIDs suggest that both 5-lipoxygenase and cyclo-oxygenase products contribute to the inflammation. The model therefore has utility for evaluating lipoxygenase inhibitors which may be potentially useful anti-inflammatory agents for the treatment of psoriasis and other inflammatory diseases.

The authors wish to thank Dr R.M. McMillan for useful comments during the preparation of this manuscript and Mrs A. Robson for excellent secretarial assistance.

References

ASHIDA, Y., TERAO, S. & SHIRAISHA, M. (1982). 2,3,5-Trimethyl-6-(12-hydroxy-5, 10-dodecadiynyl)-1, 4-benzoquinone (AA-861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. *Biochim. biophys. Acta*, **713**, 470–473.

BRAIN, S.D., CAMP, R.D.R., CUNNINGHAM, F.D., DOWD, P.M., GREAVES, M.W. & KOBZA BLACK, A. (1984). Leukotriene B_4-like material in scale of psoriatic skin lesions. *Br. J. Pharmac.*, **83**, 313–317.

BRAY, M.A., CUNNINGHAM, F.A., FORD-HUTCHINSON, A.W. & SMITH, M.J.H. (1981a). Leukotriene B_4: an inflammatory mediator in vivo. *Prostaglandins*, **22**, 213–222.

BRAY, M.A., CUNNINGHAM, F.M., FORD-HUTCHINSON, A.W. & SMITH, M.J.H. (1981b). Leukotriene B_4: A mediator of vascular permeability. *Br. J. Pharmac.*, **72**, 483–486.

CAMP, R.D.R., COUTTS, A.A., GREAVES, M.W., KAY, A.B. & WALPORT, M.J. (1983a). Responses of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. *Br. J. Pharmac.*, **80**, 497–502.

CAMP, R.D.R., MALLET, A.I., WOOLLARD, P.M., BRAIN, S.D., KOBZA BLACK, A. & GREAVES, M.W. (1983b). The identification of hydroxy fatty acids in psoriatic skin. *Prostaglandins*, **26**, 431–447.

CAMP, R.D.R., JONES, R., BRAIN, S., WOOLLARD, P. & GREAVES, M.W. (1984). Production of intraepidermal microabscesses by topical application of leukotriene B_4. *J. invest. Derm.*, **82**, 202–204.

CARLSON, R.P., O'NEILL-DAVIES, L., CHANG, J. & LEWIS, A. (1985). Modulation of mouse ear edema by cycloxygenase and lipoxygenase inhibitors and other pharmacological agents. *Agents & Actions*, **17**, 197–204.

COREY, E.J., MARFAT, A., MUNROE, A., KIM, K.S., HOPKINS, P.B. & BRION, F. (1981). A stereo controlled and effective synthesis of leukotriene B (*1*). *Tetrahedron Lett.*, **22**, 1077–1080.

COUTTS, S.M., KHANDWALA, A., VAN INWEGAN, R., CHAKRABORTY, U., MUSSER, J., BRUENS, J., JARIWALA, N., DALLY-MEADE, V., INGRAM, R., PRUSS, T., JONES, H., NEISS, E. & WEINRYB, I. (1985). Arylmethyl phenyl ethers: a new class of specific inhibitors of 5-lipoxygenase. In *Prostaglandins, Leukotrienes and Lipoxins*. ed. Bailey, M.J. pp. 627–637. New York: Plenum Press.

CUNNINGHAM, F.M., WOOLLARD, P.M. & CAMP, R.D.R. (1985). Pro-inflammatory properties of unsaturated fatty acids and their monohydroxy metabolites. *Prostaglandins*, **30**, 497–509.

FERREIRA, S.H. & VANE, J.R. (1979). Mode of action of anti-inflammatory agents which are prostaglandin synthetase inhibitors. In *Anti-inflammatory Drugs*. ed. Vane, J.R. & Ferriera, S.H. pp. 373–389. Berlin: Springer-Verlag.

FORSTER, S., ILDERTON, E., SUMMERBY, R. & YARDLEY, H.J. (1983). Epidermal phospholipase A_2 activity is raised in the uninvolved skin of psoriasis. *Br. J. Derm.*, **109**, Suppl. 25, 30–35.

GRABBE, J., CZARNETZKI, B.M., ROSENBACK, T. & MARDIN, M. (1984). Identification of chemotactic lipoxygenase products of arachidonic acid in psoriatic skin. *J. invest. Derm.*, **82**, 477–479.

HAMMARSTRÖM, S., HAMBERG, M., SAMUELSSON, B., DUELL, E.A., STAWISKI, M. & VORHEES, J.J. (1975). Increased concentrations of non esterified arachidonic acid, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ in epidermis of psoriasis. *Proc. natn. Acad. Sci. (U.S.A.)*, **72**, 5130–5134.

HIGGS, G.A., MONCADA, S. & VANE, J.R. (1980). The mode of action of anti-inflammatory drugs which prevent the peroxidation of arachidonic acid. *Clin. Rheum. Dis.*, **6**, 675–693.

HIGGS, G.A., SALMON, J.A. & SPAYNE, J.A. (1981). The inflammatory effects of hydroperoxy and hydroxy acid products of arachidonate lipoxygenase in rabbit skin. *Br. J. Pharmac.*, **74**, 429–433.

HIGGS, G.A., BAX, C.M.R. & MONCADA, S. (1982). Inflammatory properties of lipoxygenase products and the effect of indomethacin and BW755C on prostaglandin production, leukocyte migration, and plasma exudation in rabbit skin. In *Leukotrienes and other lipoxygenase products*. ed. Samuelsson, B. & Paoletti, R. pp. 331–339. New York: Raven Press.

HUMPHREY, D.M., McMANUS, L.M., SATOUCHI, K., HANAHAN, D.J. & PINCKARD, N.P. (1982a). Vasoactive properties of acetyl glyceryl ether phosphorylcholine and analogues. *Lab. invest.*, **46**, 422–434.

HUMPHREY, D.M., HANAHAN, D.J. & PINCKARD, N.P. (1982b). Induction of leukocytic infiltrates in rabbit skin by acetyl glyceryl ether phosphoryl choline. *Lab. invest.*, **47**, 227–234.

HWANG, S-B, LI, C-L, LAM, M-H & SHEN, T.Y. (1985). Characterisation of cutaneous vascular permeability induced by platelet-activating factor in guinea-pigs and rats and its inhibition by a platelet-activating factor receptor antagonist. *Lab. invest.*, **52**, 617–630.

LANDS, W.E.M. & SAMUELSSON, B. (1968). Phospholipid precursors of prostaglandins. *Biochim. biophys. Acta*, **164**, 426–429.

OPAS, E.E., BONNEY, R.J. & HUMES, J.L. (1985). Prostaglandin and leukotriene synthesis in mouse ears inflamed by arachidonic acid. *J. invest. Derm.*, **84**, 253–256.

PECK, M.J., PIPER, P.J. & WILLIAMS, T.J. (1981). The effect of leukotrienes C_4 and D_4 on the microvasculature of guinea-pig skin. *Prostaglandins*, **21**, 315–321.

PORTER, N.A., WOLF, R.A., YARBRO, E.M. & WEENEN, H. (1979). The autoxidation of arachidonic acid: Formation of the proposed SRS-A intermediate. *Biochem. biophys. Res. Comm.*, **89**, 1058–1064.

SAMUELSSON, B. (1983). Leukotrienes: mediators of hypersensitivity reactions and inflammation. *Science*, **220**, 568–575.

SOTER, N.A., LEWIS, R.A., COREY, E.J. & AUSTEN, K.F. (1983). Local effects of synthetic leukotrienes (LTC_4, LTD_4, LTE_4 and LTB_4) in human skin. *J. invest. Derm.*, **80**, 115–119.

UENO, A., TANAKA, K., KATORI, M., HAYASHI, M. & ARAI, Y. (1981). Species difference in increased vascular permeability by synthetic leukotriene C_4 and D_4. *Prostaglandins.*, **21**, 637–648.

WEDMORE, C.V. & WILLIAMS, T.J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature*, **289**, 646–650.

WILLIAMS, T.J. (1979). Prostaglandin E_2, prostaglandin I_2 and the vascular changes, of inflammation. *Br. J. Pharmac.*, **65**, 517–524.

WILLIAMS, T.J. & PIPER, P.J. (1980). The action of chemically pure SRS-A on the microcirculation in vivo. *Prostaglandins*, **19,** 779–789.

YOUNG, J.M., SPIRES, D.A., BEDORD, C.J., WAGNER, B., BALLARAN, S.J. & DE YOUNG, L.M. (1984). The mouse ear inflammatory response to topical arachidonic acid. *J. invest. Derm.*, **82,** 367–371.

(Received May 8, 1986.
Revised June 2, 1986.
Accepted June 9, 1986.)