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Leukotriene B_4 and prostaglandin E_2 mediate the inflammatory response of rabbit skin to intradermal arachidonic acid

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1 Acute inflammation was induced in rabbit skin by intradermal injection of arachidonic acid.

2 Inflammation was assessed by the local accumulation of intravenously-injected ^{125}I-serum albumin (plasma extravasation) and histologically (polymorphonuclear leucocyte, PMNL infiltration).

3 Leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) were extracted from skin and fractionated using C_{18} mini-columns. They were quantitated by specific radioimmunoassays and authenticated by reversed-phase high performance liquid chromatography analysis.

4 Maximally elevated levels of LTB_4 and PGE_2 were detected in skin 5 min after arachidonic acid injection. At subsequent times the eicosanoid content of the skin decreased.

5 The decrease in the eicosanoid content of the skin was due to rapid clearance ($t_{\frac{1}{2}}$ approximately 5 min) via the microvasculature and not a consequence of metabolism.

6 The concentration of LTB_4 and PGE_2 measured in inflamed skin was sufficient to account for the plasma extravasation and PMNL infiltration induced by arachidonic acid. The model therefore is useful for evaluating the anti-inflammatory efficacy of inhibitors of arachidonic acid metabolism including 5-lipoxygenase inhibitors.

7 The consequences of the rapid clearance of LTB_4 from inflammatory sites are discussed with respect to its measurement in inflammatory disease and its role as an acute inflammatory mediator.

Introduction

Extravasation of polymorphonuclear leukocytes (PMNL) and plasma are key features of an acute inflammatory response. Recent attention has focused upon the role of leukotriene B_4 (LTB_4), a product of the 5-lipoxygenase pathway, on this response. LTB_4 is potently chemotactic towards PMNL in rabbits (Bray *et al.*, 1981a; Higgs *et al.*, 1981) and man (Camp *et al.*, 1983; 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 circulating 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. This has prompted widespread interest in developing an animal model of inflammation in which LTB_4 contributes to the extravasation of PMNL and plasma associated with the inflammatory process. Such a model would help to strengthen a role for LTB_4 in acute inflammation and allow evaluation of the potential anti-inflammatory efficacy of 5-lipoxygenase inhibitors. Such models have so far proved elusive although topical application of high concentrations of arachidonic acid to the mouse ear 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 both the cyclooxygenase (prostaglandin E_2, PGE_2) and 5-lipoxygenase (LTC_4/D_4) pathway 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. Arachidonic acid also produces an inflammatory response when injected into the rabbit dermis, a response that depends on circulating PMNL (Aked *et al.*, 1986). Pharmacological modulation of this inflammatory response by inhibitors of arachidonic acid

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metabolism suggest that both cyclo-oxygenase and 5-lipoxygenase products contribute to the inflammation. The object of this study was to investigate whether PGE_2 and LTB_4, which are products of these pathways, do indeed contribute to the extravasation of PMNL and plasma induced by the injection of arachidonic acid into rabbit skin.

A brief account of part of this work has been presented to the British Pharmacological Society (Aked & Foster, 1987a).

Methods

Inflammatory parameters

Female New Zealand white rabbits (Ranch Rabbits, Crawley Down, Sussex) weighing between 3.0 and 4.0 kg were used in all experiments. Plasma extravasation and leukocyte recruitment into skin sites were measured as previously described (Aked *et al.*, 1986).

Extraction of eicosanoids from rabbit skin

Fur was removed from the backs of rabbits with Oster clippers (Professional Products, Milwaukee, USA) 24 h before each experiment. Arachidonic acid or other test substances in 0.1 ml of pyrogen-free saline (Steriflex, The Boots Co PLC, Nottingham) were injected, at various times, into the dermis of rabbits. Animals were killed with a lethal intravenous dose of Euthatal (May and Baker Ltd, Dagenham) followed by cervical dislocation. The skin was rapidly removed and immersed in liquid nitrogen prior to storage at −20°C. Injection sites (15 mm discs) were punched out, snap frozen and pulverized between liquid nitrogen cooled tongs and transferred to a tube containing 3 ml of methanol on ice. After 30 min the methanol concentration was adjusted to 50% with 0.1 M sodium acetate pH 4.2, followed by homogenization with a Polytron (Kinematica GmbH, Kriens, Luzern, Switzerland) for 1 min. The homogenate was then centrifuged at 12,000 *g* for 10 min at 4°C and the supernatant fraction adjusted to 15% methanol with 0.1 M sodium acetate, pH 4.2. The di-HETEs (dihydroxy-eicosatetraenoic acids) and prostaglandins in the supernatant fraction were extracted on 1 ml C_{18} mini-columns (Bond Elut, Analytichem International, Technicol, Stockport) by a modification of the procedure described by Powell (1980). Briefly, columns were pretreated sequentially with 1 ml aliquots of ethyl acetate, methanol and methanol:water (15:85). The supernatant fraction (approximately 20 ml) was passed through the column followed sequentially by 1 ml methanol:water (15:85) and 2 × 1 ml hexane:diethyl ether (90:10); hexane:diethyl ether (80:20) and methanol:water (90:10). The methanol:water (90:10) eluate, which contained di-HETEs and prostaglandins was evaporated to dryness using a Speed Vac concentrator (Uniscience, Cambridge, U.K.). The overall recoveries of LTB_4 and PGE_2 in the methanol:water (90:10) fraction were approximately 40 and 70% respectively. Samples were reconstituted in buffer prior to specific radioimmunoassay for LTB_4 and PGE_2

Extraction of leukotriene B_4 from plasma

Plasma obtained from heparinized (100 iu ml^{-1}) blood by centrifugation for 1 min in an Eppendorf 3200 centrifuge was extracted twice with 6 volumes of methylformate. The methylformate was removed by rotary evaporation and the residue resuspended in methanol before reversed-phase h.p.l.c. analysis. The recovery of LTB_4 by this procedure was approximately 90% determined with a [^{3}H]-LTB_4 standard.

Reversed-phase h.p.l.c

Reversed-phase h.p.l.c. was carried out using a 5 μ spherisorb ODS2 column (Hichrom, Reading, U.K.). Prostaglandins were separated isocratically using a mobile phase of 32.5% (v/v) acetonitrile in aqueous phosphoric acid pH 2.0, as described by Haworth & Carey (1986). Di-HETEs were separated by use of a gradient ranging from 60–95% methanol in 0.1% aqueous acetic acid pH 5.6. In both procedures the column flow rate was 1.5 ml per min and 0.3 min fractions were collected for 30 min. Fractions were evaporated to dryness using a Speed Vac concentrator and reconstituted with buffer prior to radioimmunoassay. The column was calibrated by use of radiolabelled eicosanoid standards (see Chemicals).

Radioimmunoassay of eicosanoids

Prostaglandin E_2 and leukotriene B_4 in rabbit skin extracts were measured by specific radioimmunoassays as described previously by Forder & Carey (1984) and Carey & Forder (1986).

Chemicals

Chemicals used were as previously described by Aked *et al.* (1986). The radiochemicals [^{3}H]-LTB_4 (222 Ci $mmol^{-1}$), [^{3}H]-PGE_2 (160 Ci $mmol^{-1}$), [^{3}H]-6-keto $PGF_{1\alpha}$ (150 Ci $mmol^{-1}$), [^{14}C]-$PGF_{2\alpha}$ (59.6 mCi $mmol^{-1}$) and [^{14}C]-arachidonic acid (58 mCi $mmol^{-1}$) were purchased from Amersham International (Amersham, UK). [^{3}H]-12-HETE (40 Ci $mmol^{-1}$) and [^{3}H]-thromboxane B_2 ([^{3}H]-TxB_2) (140 Ci $mmol^{-1}$) were purchased from NEN (Boston, Massachusetts). [^{14}C]-5-HETE was made biosynthetically using an RBL-1 5-lipoxygenase preparation by D. Masters, ICI Phar-

maceuticals Division. Radiolabelled omega-oxidation products of LTB_4 were prepared biosynthetically by Dr R.M. McMillan (ICI Pharmaceuticals Division) by incubating [^{3}H]-LTB_4 with human PMNL according to the method of Powell (1984). All solvents used were of Analar or h.p.l.c. grade standard.

Statistics

Results are expressed as mean ± s.e.mean and compared for statistical significance using a one-sample Student's *t*-test on the logarithm of the mean treated/control values.

Results

Eicosanoid content of arachidonic acid inflamed skin

Injection of arachidonic acid (100 μg) into the rabbit dermis causes a local extravasation of plasma which can be detected within 5 min of the injection (Aked *et al.*, 1986). Table 1 shows the immunoreactive LTB_4 and PGE_2 content of rabbit skin 5 min after the intradermal injection of arachidonic acid or pyrogen-free saline (hereafter referred to as saline). The LTB_4 and PGE_2 content of the arachidonic acid-injected skin sites were elevated significantly over saline-injected control sites. Figure 1 shows the immunoreactive LTB_4 and PGE_2 content of rabbit skin at various times after the intradermal injection of arachidonic acid or saline. Maximal levels of immunoreactive LTB_4 and PGE_2 were detected 5 min after arachidonic acid injection, the earliest practical time point at which measurements could be made. The immunoreactive LTB_4 content decreased approximately 50% during the next 60 min whilst the immunoreactive PGE_2 content fell almost to control levels. Skin sites injected with saline contained 3.5 ± 0.6 and 10.6 ± 0.4 ng of immunoreactive LTB_4 and PGE_2 respectively 5 min after injection and the levels were similar 60 min later.

The immunoreactive LTB_4 and PGE_2 measured in extracts of arachidonic acid inflamed skin sites was authenticated by reversed-phase h.p.l.c. analysis. As shown in Figure 2, single major peaks of immunoreactive LTB_4 (a) and PGE_2 (b), which co-eluted with authentic standards were identified. Extracts of saline-injected or uninjected skin when subjected to reversed-phase h.p.l.c. analysis revealed no major peaks of immunoreactivity.

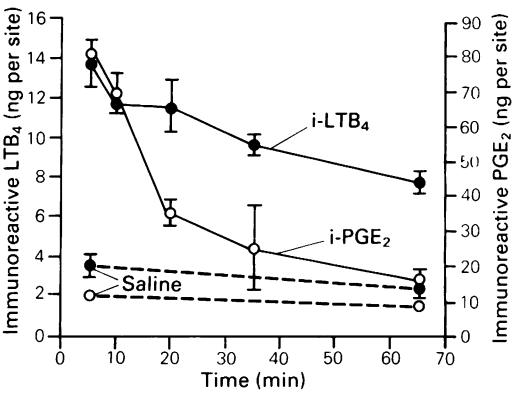

Figure 1 The immunoreactive eicosanoid content of rabbit skin at various times after the intradermal injection of arachidonic acid or saline. Arachidonic acid (100 μg) in 0.1 ml saline or saline alone were injected intradermally into separate sites at various times. Rabbits were killed 65 min after the first injection and the leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) content of the injection sites was determined. Each point is the mean of 4 separate skin sites and the bars represent s.e.mean. The experiment is typical of 3.

Clearance of eicosanoids from skin

The immunoreactive LTB_4 and PGE_2 content of skin at various times following the intradermal injection of arachidonic acid suggests that these eicosanoids are rapidly synthesized and metabolized or rapidly cleared

Table 1 Immunoreactive eicosanoid content of arachidonic acid-treated rabbit skin

Eicosanoid	*Saline controls (ng/site)*	*Arachidonic acid-treated (ng/site)*	P
LTB_4	3.1 ± 0.4	10.1 ± 1.8	<0.0005
PGE_2	5.4 ± 1.1	61.7 ± 6.6	<0.0001

Arachidonic acid (100 μg) in 0.1 ml saline or saline alone were injected intradermally into 4 separate sites of the rabbit dermis. Three min later the animals were killed, the skin removed and plunged into liquid nitrogen 2 min later. Eicosanoids were extracted from the injected skin sites and measured by specific radioimmunoassays for prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4). Values are the mean ± s.e.mean of 7 separate experiments. Significant differences in the PGE_2 and LTB_4 content between the saline and arachidonic acid injected skin sites were determined by a one-sample Student's *t* test on the log of the mean arachidonic acid/saline values.

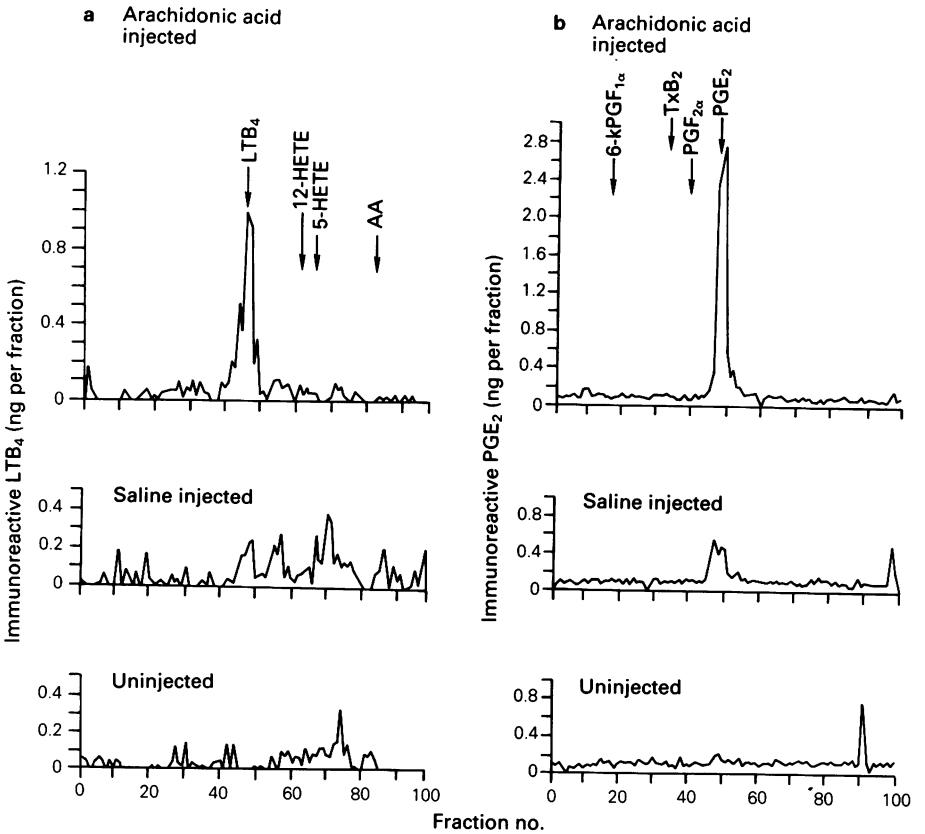

Figure 2 Immunoreactive profiles of leukotriene B_4 (LTB_4, a) and prostaglandin E_2 (PGE_2, b) following fractionation of extracts of arachidonic acid (AA) or saline injected or uninjected rabbit skin by reversed-phase h.p.l.c. analysis. Arachidonic acid (100 μg) in 0.1 ml saline or saline alone were injected intradermally into separate sites. Three minutes later the animals were killed, the skin was removed and plunged into liquid nitrogen 2 min later. The injection sites and areas of uninjected skin were punched out and eicosanoids were extracted.

The diHETE/prostaglandin fractions from the C_{18} mini-columns obtained from 6 skin sites for each condition were pooled, divided equally and evaporated to dryness. One half of the residue was dissolved in 0.15 ml methanol and the other in 0.15 ml acetonitrile; 0.05 ml of the methanol and 0.03 ml of the acetonitrile solutions were fractionated by reversed-phase h.p.l.c. using conditions which separated diHETEs and prostaglandins respectively (see Methods). The h.p.l.c. column was calibrated using the appropriate conditions with radiolabelled standards as indicated by the arrows. The collected fractions were evaported to dryness (Speed Vac Concentrator) and reconstituted with buffer prior to radioimmunoassay for LTB_4 and PGE_2.

from the skin. When authentic LTB_4 and PGE_2 were injected intradermally there occurred a rapid decrease in the immunoreactive eicosanoid content of the dermis with a half-life value for both eicosanoids of approximately 5 min as shown in Figure 3. Although this experiment does not rule out LTB_4 metabolism as a contributing factor to its decay we reasoned that rapid uptake into the bloodstream was the most likely mechanism. Experiments were carried out to substantiate such a mechanism. Figure 4 shows an experiment in which the recovery of [^{3}H]-LTB_4 was monitored at various times following its co-injection with arachidonic acid into rabbit dermis or into the dermis of freshly removed skin. In skin, *in situ*, [^{3}H]-LTB_4 was rapidly cleared with a half-life of <10 min whilst in the freshly removed skin the [^{3}H]-LTB_4 levels remained unchanged through 60 min. Similarly [^{3}H]-PGE_2 was also rapidly cleared from skin *in situ* but not from freshly removed skin. In addition no omega-oxidation metabolites were detected by reversed-phase h.p.l.c. analysis in extracts of the *in situ* or freshly removed skin at 5 or 60 min after injection of arachidonic acid together with [^{3}H]-LTB_4 (data not shown).

As shown in Figure 5 reversed-phase h.p.l.c.

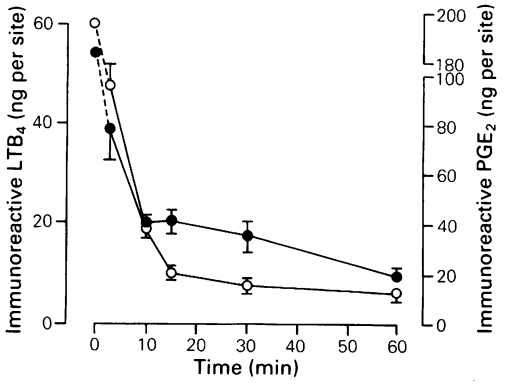

Figure 3 Time course showing the loss of immunoreactive eicosanoids from rabbit skin following a single combined injection of leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2). LTB_4 (●) together with PGE_2 (○) in 0.1 ml saline was injected intradermally into separate skin sites at various times. The zero time point represents the amount of immunoreactive LTB_4 and PGE_2 injected. The rabbit was killed 60 min after the first injection and the LTB_4 and PGE_2 content of the injection sites was determined. Each point is the mean of 4 separate skin sites and the bars represent s.e.mean.

analysis revealed that following the co-injection of [^{3}H]-LTB_4 with arachidonic acid into the rabbit dermis, [^{3}H]-LTB_4 was detected in the plasma of the animals shortly after injection. Moreover, no omega-

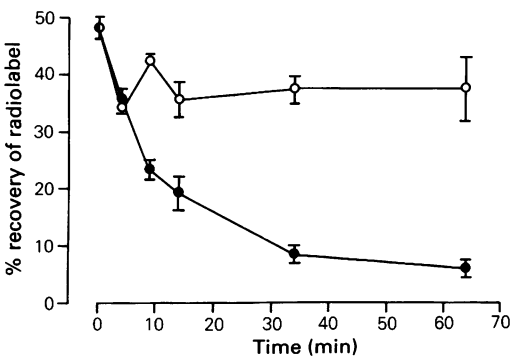

Figure 4 Recovery of radioactivity from rabbit skin *in situ* (●) or freshly removed skin (○) at various times after a combined injection of [^{3}H]-leukotriene B_4 ([^{3}H]-LTB_4) and arachidonic acid. Arachidonic acid (100 μg) together with [^{3}H]-LTB_4 (0.05 μCi) in 0.1 ml saline was injected intradermally at various times into separate sites of rabbit skin (*in situ*) or into the dermis of freshly removed rabbit skin. Rabbits were killed 65 min after the first injections and the injection sites from skin *in situ* and freshly removed skin were extracted and the radioactivity in the di-HETE fractions from the C_{18} mini columns determined. Each point is the mean of 4 separate skin sites and the bars represent s.e.mean. The experiment is typical of 3.

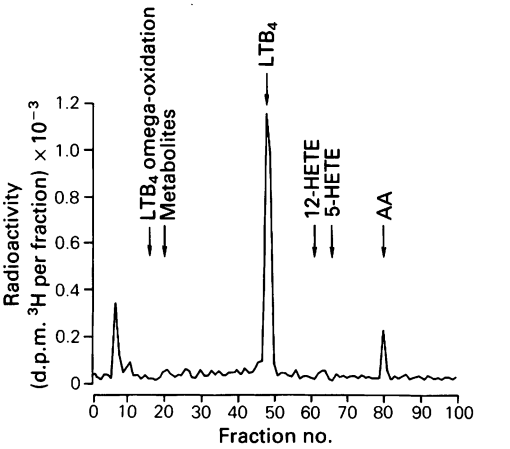

Figure 5 Identification of radiolabelled leukotriene B_4 (LTB_4) in plasma, by reversed-phase h.p.l.c., shortly after the intradermal injection of [^{3}H]-LTB_4 and arachidonic acid. Arachidonic acid (100 μg) together with [^{3}H]-LTB_4 (0.83 μCi) in 0.1 ml saline was injected intradermally into 4 separate sites. Seven minutes later, blood (10 ml) was withdrawn from a marginal ear vein into heparin (100 iu ml^{-1}). [^{3}H]-LTB_4 was extracted from 4 ml of plasma into methylformate (see Methods). The residue was dissolved in 0.4 ml methanol and 40 μl of this was fractionated by reversed-phase h.p.l.c. as described in the Methods for di-HETEs. Radioactivity in the fractions was determined using an LKB 1218 Rackbeta scintillation spectrophotometer. The h.p.l.c. column was calibrated using radiolabelled standards as indicated by the arrows.

oxidation metabolites were evident from the h.p.l.c. profile. The small peak of radioactive material in fraction 80 which co-eluted with authentic arachidonic acid standard is probably [^{3}H]-arachidonic acid derived from ^{3}H exchange with [^{3}H]-LTB_4. The small radioactive peak at fraction 8 corresponding to the solvent front has not been characterized but may be ^3H_2O, a previously reported β-oxidation metabolite of [^{3}H]-LTB_4 in the monkey and rabbit (Serafin *et al.*, 1984).

The effect of eicosanoids on plasma and PMNL extravasation

The objective of the following experiments was to establish whether the concentrations of LTB_4 and PGE_2 measured in arachidonic acid inflamed skin (Table 1) could account for the extravasation of plasma and PMNL into these sites. Figure 6 shows that intradermal injection of LTB_4 (10 ng) or PGE_2 (50 ng) alone caused only a small increase in plasma extravasation over saline-injected control sites. However, when LTB_4 (5 or 10 ng) was co-injected

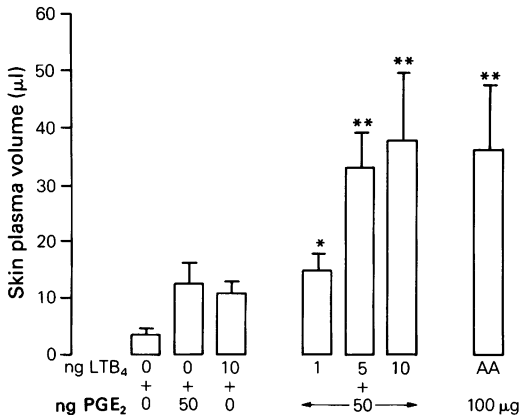

Figure 6 The effect of eicosanoids on plasma extravasation in rabbit skin. Saline, leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2) and arachidonic acid (AA) in 0.1 ml saline or LTB_4 together with PGE_2 were injected intradermally at the indicated concentrations. The rabbits were killed 60 min later and the plasma extravasation into the skin sites was determined. Each point is the mean result from four rabbits and the bars represent s.e.mean. $*P<0.05$; $**P<0.01$.

intradermally with PGE_2 (50 ng) they acted synergistically to increase plasma extravasation. The degree of plasma extravasation was similar to that achieved following the intradermal injection of arachidonic acid (100 μg).

Injections of LTB_4 (1–100 ng) into rabbit dermis caused a concentration and time-dependent infiltration of PMNL as shown in Figure 7a and b respectively. As little as 1–10 ng LTB_4 caused a linear infiltration of PMNL of at least 3 h duration.

Discussion

The objective of this work was to obtain direct evidence as to whether LTB_4 and PGE_2 contribute to the extravasation of plasma and PMNL in arachidonic acid inflamed rabbit skin. The results obtained by radioimmunoassay and reversed-phase h.p.l.c. analysis clearly demonstrate that elevated levels of LTB_4 and PGE_2 are generated in rabbit skin following injection of arachidonic acid into the dermis. When concentrations of LTB_4 and PGE_2 equal to those measured in arachidonic acid-inflamed skin sites were co-injected into rabbit dermis they caused the same degree of plasma extravasation as that induced by arachidonic acid (Figure 6). In addition the same concentrations of LTB_4 injected into the dermis caused PMNL infiltration. Moreover, agents which inhibit LTB_4 and PGE_2 biosynthesis also inhibited plasma extravasation in arachidonic acid inflamed skin (Aked *et al.*, 1986). It is likely, therefore, that these eicosanoids contribute to the plasma extravasation induced by arachidonic acid in rabbit skin and that LTB_4 is a chemotactic mediator of this PMNL-dependent inflammatory reaction.

The experiments described here also raise several issues relating to the kinetics of eicosanoid generation

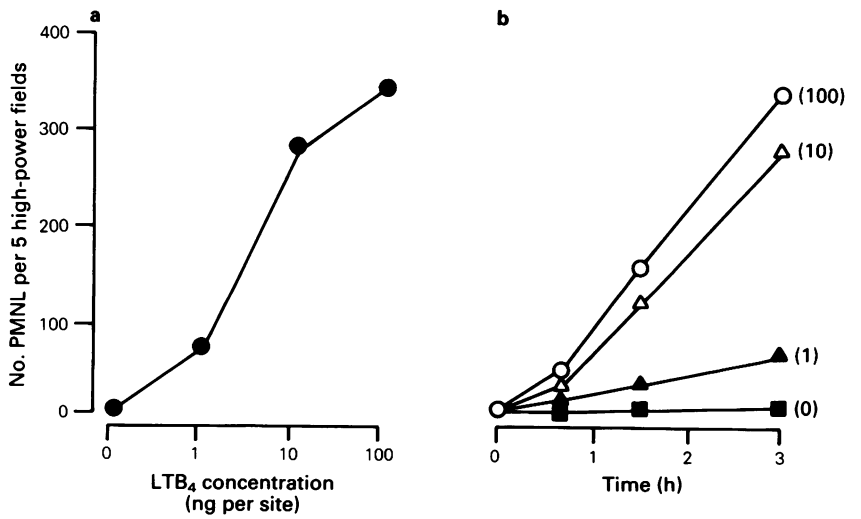

Figure 7 The concentration-dependent (a) and time-dependent (b) effect of leukotriene B_4 (LTB_4) on PMNL infiltration into the rabbit dermis. LTB_4 in 0.1 ml saline or saline alone was injected intradermally into duplicate sites at the concentrations and times indicated. Rabbits were killed 3 h after the first injection, the skin was removed and the injection sites were processed histologically for an assessment of their PMNL content. (a) Shows the PMNL content 3 h after injection. The LTB_4 concentrations (ng) in (b) are indicated in parentheses. Values are the means of 2 animals.

and the role of LTB_4 in the acute inflammatory response. Elevated levels of LTB_4 and PGE_2 were detected in skin shortly after injection of arachidonic acid into the dermis, demonstrating that both eicosanoids are rapidly synthesized. At subsequent times the LTB_4 and PGE_2 content in the skin decreased at different rates, LTB_4 being the slower (Figure 1). However, when authentic LTB_4 and PGE_2 were injected into the dermis they were cleared from the skin at approximately the same rate (Figure 3). Therefore the difference in the rate of decay of the eicosanoid content of the skin suggests that LTB_4 is synthesized for a longer period of time than PGE_2 which may be a consequence of the cellular source of the eicosanoids. Although the cellular source is unknown, LTB_4 may be derived from the infiltrating PMNL, a cell type which has been demonstrated to generate LTB_4 (Borgeat & Samuelsson, 1979; Ford-Hutchinson *et al.*, 1980) whilst PGE_2 may be generated by cells resident at the inflamed site. Whichever cells are responsible for generating these eicosanoids they must have a short-lived capacity for biosynthesis.

It is an interesting observation that despite its rapid clearance from the skin ($t_{\frac{1}{2}}$ approx 5 min) a single intradermal injection of 1 ng LTB_4, which is just above the threshold concentration for chemotaxis (Figure 7a), caused a linear infiltration of PMNL into skin for at least 3 h. We have previously shown, in the rabbit, that LTB_4 can be detected in inflammatory exudates prior to PMNL infiltration (Aked & Foster, 1987b). Moreover, its presence in inflammatory exudates is transient, returning almost to background levels at a time when PMNL continue to infiltrate. It appears, therefore, that LTB_4 does not have to be present in inflammatory exudates or tissues at chemotactic concentrations for the duration of the inflammatory reaction. Perhaps LTB_4 triggers the release of other mediators thus amplifying the acute inflammatory reaction and/or modulates the local microvasculature such that prolonged PMNL extravasation occurs.

Loss of LTB_4 from inflamed rabbit skin appears to be due to its rapid clearance via the bloodstream and not metabolism, based on the following observations: (1), the immunoreactive LTB_4 content of skin sites injected with authentic LTB_4 decreased rapidly with time; (2), reversed phase h.p.l.c. analysis of skin sites injected with [^{3}H]-LTB_4 revealed no ^{3}H omega-oxidation metabolites; (3), the radioactive content of rabbit skin decreased rapidly following injection of [^{3}H]-LTB_4 into the dermis whereas in freshly removed skin the radioactive content remained constant throughout 60 min and (4), shortly after injection of [^{3}H]-LTB_4 into the dermis, [^{3}H]-LTB_4 but not ^{3}H omega-oxidation metabolites were detected in plasma.

Rapid clearance of LTB_4 from inflamed sites may have implications in inflammatory diseases such as psoriasis and inflammatory arthritis. The concentration of LTB_4 measured in synovial fluid of patients with gout or rheumatoid arthritis is very low (Rae *et al.*, 1982). Moreover, these low levels are not a consequence of LTB_4 metabolism (McMillan *et al.*, 1987). One interpretation of this is that LTB_4 may not play a role in the pathology of inflammatory arthritis. However, by an analogous mechanism, reported here for inflamed skin, LTB_4 may be synthesized and rapidly cleared from synovial fluid by the highly vascularized inflamed synovial tissues of the joint. Thus, measurements of LTB_4 in synovial fluid and other inflammatory exudates may underestimate the amount of LTB_4 generated. In support of this we have demonstrated, using reversed-phase h.p.l.c. analysis, that following intra-articular injection of [^{3}H]-LTB_4 into the rabbit knee joint, unchanged [^{3}H]-LTB_4 appears rapidly in the bloodstream (unpublished data, Aked & Foster, 1986).

The rapid clearance of LTB_4 from inflamed sites raises the question of its role as an inflammatory mediator. The major role of LTB_4 may not be chemotaxis but rather to promote adherence of PMNL to the microvascular endothelium (Dahlen *et al.*, 1981; Lindbom *et al.*, 1982), an event which is a prerequisite to PMNL extravasation induced by a variety of naturally occurring chemotactic agents including Paf (platelet activating factor), C_{5a} and formylated bacterial peptides. Thus, by virtue of its rapid synthesis and clearance via the microvessels within the inflamed tissues, LTB_4 might serve to amplify the acute inflammatory response.

In conclusion, arachidonic acid induces a PMNL-dependent inflammatory reaction in rabbit skin in which the local generation of eicosanoids contribute to plasma and PMNL extravasation. This model therefore is useful for evaluating the anti-inflammatory efficacy of inhibitors of arachidonic acid metabolism including 5-lipoxygenase inhibitors. The rapid clearance of LTB_4 from arachidonic acid-inflamed skin sites suggests that the concentrations of LTB_4 measured at inflamed sites in diseases such as rheumatoid arthritis might be an underestimate of LTB_4 synthesis.

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