

## LEUKOTRIENE B<sub>4</sub>: A MEDIATOR OF VASCULAR PERMEABILITY

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- 1 Intradermal injection of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) or prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), 1 to 100 ng per skin site produced little or no change in plasma exudation in the rabbit, guinea-pig and rat.
- 2 Intradermal injection of LTB<sub>4</sub> together with PGE<sub>2</sub> produced a significant increase in plasma exudation in the rabbit, guinea-pig and rat.
- 3 Intradermal injection of LTB<sub>4</sub> or PGE<sub>2</sub> together with bradykinin (500 ng) resulted in a significant potentiation of the plasma exudation produced by bradykinin alone in the rabbit and guinea-pig.
- 4 LTB<sub>4</sub> (1 to 10 ng) had no effect on blood flow in the rabbit skin, in contrast to PGE<sub>2</sub> which was a potent vasodilator in this species.
- 5 It is concluded that LTB<sub>4</sub> is a mediator of vascular permeability and that this effect can only be observed in the presence of a vasodilator such as PGE<sub>2</sub>.

### Introduction

Leukotriene B<sub>4</sub> (LTB<sub>4</sub>) is a metabolite of arachidonic acid (AA) formed via a lipoxygenase enzyme (Samuelsson & Hammerström, 1980). LTB<sub>4</sub> is released by polymorphonuclear leucocytes (PMNs) (Borgeat & Samuelsson, 1979a; Stenson & Parker, 1979), eosinophils (Goetzl, Weller & Sun, 1980) and macrophages (Doig & Ford-Hutchinson) when these cells are exposed to the calcium ionophore A23187 and is also present in perfusates obtained from antigen-challenged lung (Morris, Taylor, Piper & Tippins, 1979). LTB<sub>4</sub> is a potent chemokinetic and chemotactic agent for PMNs and macrophages *in vitro* and *in vivo* (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Smith, Ford-Hutchinson & Bray, 1980) and has been proposed as a potential mediator of the cellular aspects of inflammation (Smith *et al.*, 1980). The present work was designed to investigate a possible role for this compound in the vascular aspects of inflammation.

### Methods

Animals used were New Zealand white rabbits (female, 2.5 to 3 kg) (Buxted Rabbits, Buxted, Sussex), Dunkin-Hartley guinea-pigs (female, 250 to 300 g) (Redfern Animal Breeders) and Wistar rats (female, 250 to 300 g) (Olac (1976) Ltd., Oxfordshire).

#### Preparation of leukotriene B<sub>4</sub>

LTB<sub>4</sub>, corresponding to the enzymatically produced

isomer III (Rådmark, Malmsten, Samuelsson, Clark, Goto, Marfat & Corey, 1980), was prepared from rat peritoneal PMNs exposed to the calcium ionophore A23187 and was purified by ether extraction, silicic acid chromatography and reverse phase high pressure liquid chromatography as previously described (Ford-Hutchinson *et al.*, 1980). The concentration of LTB<sub>4</sub> was determined using an  $\epsilon_{281}^{MeOH}$  of 39,500 (Borgeat & Samuelsson, 1979b).

#### Vascular permeability changes

Vascular permeability changes were monitored in the rat, guinea-pig and rabbit by the extravasation of [<sup>125</sup>I]-human serum albumin (Radiochemical Centre, Amersham) (Williams, 1979). Briefly [<sup>125</sup>I]-albumin (15  $\mu$ Ci/kg) in Evans Blue solutions (2.5% w/v in saline) was administered intravenously immediately prior to intradermal injections of LTB<sub>4</sub>, prostaglandin E (PGE) or bradykinin in 0.1 ml Tyrode solution. Thirty min after the last intradermal injection, blood samples were taken and the animals killed. Skin sites were punched out with a 18 mm diameter punch and were counted in a  $\gamma$  counter. Aliquots of blood were counted in order to estimate  $ct\ min^{-1}\ \mu l^{-1}$  in the plasma and results have been expressed as  $\mu$ l of plasma exudate per skin site. In man, 100 ng or 10 ng of LTB<sub>4</sub> in 0.1 ml of saline was injected into the forearm and the skin observed at intervals for erythema and induration.

*Blood flow changes*

The effects of LTB<sub>4</sub> on blood flow were measured in separate experiments in rabbits using the <sup>133</sup>Xe clearance technique described by Williams (1979). For comparison PGE<sub>2</sub> was used as an example of a known vasodilator and noradrenaline as an example of a vasoconstrictor.

*Drugs*

The following were used: calcium ionophore A23187 (Calbiochem); PGE<sub>1</sub>, PGE<sub>2</sub> (Dr J.E. Pike, Upjohn); bradykinin triacetate (Sigma).

**Results**

The effects of LTB<sub>4</sub> and PGE<sub>2</sub> on vascular permeability changes in rabbit, guinea-pig and rat skin are shown in Table 1. Neither LTB<sub>4</sub> nor PGE<sub>2</sub> alone produced any significant change in vascular permeability, except at the highest dose of each compound in rabbit skin where a small, statistically significant effect was observed. In all three species, plasma exudation was observed when LTB<sub>4</sub> was administered together with 10 ng of PGE<sub>2</sub>, statistically significant effects being observed with doses of LTB<sub>4</sub> of 1, 10 and 100 ng in the rabbit, 10 and 100 ng in the guinea-pig and 100 ng in the rat. No further effects were seen in the rabbit when the dose of PGE<sub>2</sub> was raised to 100 ng as used by other workers (Wedmore & Williams, 1980). PGE<sub>1</sub> has been reported (Williams & Morley, 1973) to be a more effective potentiator than PGE<sub>2</sub> in the guinea-pig. No difference was observed between the effects of LTB<sub>4</sub> on vascular permeability in the presence of 100 ng of PGE<sub>1</sub> rather than 10 ng of PGE<sub>2</sub>.

Both LTB<sub>4</sub> and PGE<sub>2</sub> potentiated the vascular permeability changes produced by bradykinin in the rabbit and guinea-pig (Table 2). In contrast, in the rat, no potentiation of the response to 500 ng of bradykinin was observed with either agent. LTB<sub>4</sub> produced no changes in blood flow in the rabbit skin over the dose range 1 to 100 ng per skin site (Figure 1). In contrast PGE<sub>2</sub>, (0.1 to 10 ng per skin site) produced a dose-related increase in blood flow and 1 ng of noradrenaline produced a significant decrease in blood flow. When injected into the human forearm, LTB<sub>4</sub> (10 or 100 ng) produced no visible erythema or induration and no pain responses were observed (M.A. Bray, personal experience).

**Discussion**

The results of the present work show that either LTB<sub>4</sub>

**Table 1** Effects of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and the E type prostaglandins (PGE) on plasma exudation in the rabbit, guinea-pig and rat skin

LTB <sub>4</sub> concentration (ng/0.1 ml)	Rabbit		Guinea-pig		Rat	
	Tyrodine alone	PGE <sub>2</sub> (10 ng/0.1 ml)	Tyrodine alone	PGE <sub>2</sub> (10 ng/0.1 ml)	Tyrodine alone	PGE <sub>2</sub> (10 ng/0.1 ml)
0	4.6 ± 2.4	9.2 ± 2.8	12.1 ± 2.4	10.5 ± 2.9	10.7 ± 2.4	25.5 ± 6.5
1	26.0 ± 10.0	19.9 ± 4.9**	12.6 ± 1.9	16.3 ± 5.7	10.1 ± 3.3	35.9 ± 2.2
10	7.4 ± 3.6	77.8 ± 13.0**	13.1 ± 5.8	19.1 ± 5.3**	17.1 ± 2.0	35.1 ± 8.6
100	29.2 ± 7.8*	166.0 ± 13.0**	21.0 ± 6.1	38.8 ± 2.2**	25.5 ± 6.5	88.6 ± 7.8**

Results are expressed as  $\mu$ l of plasma exudate and are shown as means  $\pm$  s.e. mean. \*  $P < 0.05$  when compared to treatment with Tyrodine alone; \*\*  $P < 0.05$  when compared with corresponding treatment in the absence of LTB<sub>4</sub> (Student's *t* test),  $n = 5-10$ .

**Table 2** Effects of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) on the plasma exudation response to intradermal bradykinin (500 ng/0.1 ml) in the rabbit and guinea-pig

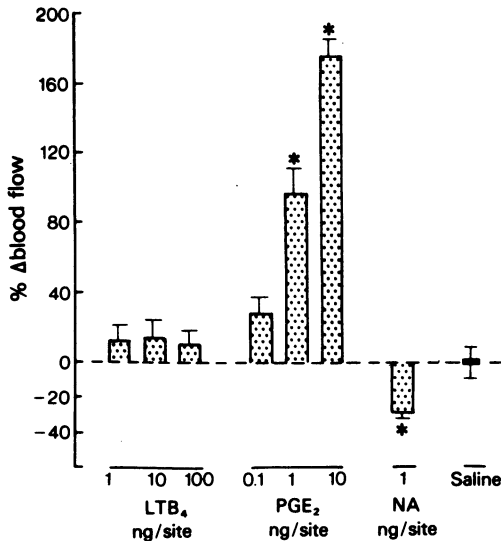
LTB <sub>4</sub> or PGE <sub>2</sub> (ng/0.1 ml)	Rabbit		Guinea-pig	
	+ LTB <sub>4</sub>	+ PGE <sub>2</sub>	+ LTB <sub>4</sub>	+ PGE <sub>2</sub>
0	77.2 ± 9.4		196.2 ± 13.3	
1	104.8 ± 53.7*	95.4 ± 5.5*	219.3 ± 22.5	212.1 ± 13.7
10	128.6 ± 24.1*	178.8 ± 13.0*	228.1 ± 11.7	245.1 ± 17.6*
100	185.8 ± 13.0*	334.2 ± 36.8*	257.3 ± 17.7*	298.5 ± 20.1*

Results are expressed as µl of plasma exudate and are shown as means ± s.e. mean. \* *P* < 0.05 when compared with treatment with bradykinin alone (500 ng 0.1 ml<sup>-1</sup>) (Student's *t*-test). *n* = 5 for rabbit and 10 for guinea-pig.

or PGE<sub>2</sub>, when injected into rabbit, guinea-pig or rat skin, cause little or no change in vascular permeability (Table 1). When the compounds are administered together a significant increase in plasma exudation occurs (Table 1) and in the rabbit and guinea-pig, both LTB<sub>4</sub> and PGE<sub>2</sub> potentiate the vascular permeability response to bradykinin (Table 2). LTB<sub>4</sub> has no effect on blood flow in the rabbit (Figure 1) in contrast to PGE<sub>2</sub>, a potent vasodilator in this species (Williams, 1979). These results support the general hypothesis, advanced by Wedmore & Williams, that chemotactic agents, including the complement-

derived peptide C5a and the synthetic peptide f-met-leu-phe, are mediators of vascular permeability (Wedmore & Williams, 1980). The increase in plasma exudation, due to LTB<sub>4</sub> or other chemotactic mediators, can only be observed in the presence of a vasodilator such as PGE<sub>2</sub> and we suggest that this increase may result from PMN accumulation at the site of injection. Potentiation of the bradykinin response by PGE<sub>2</sub> and LTB<sub>4</sub>, therefore, occurs through different mechanisms. PGE<sub>2</sub> potentiation of this response is a result of a vasodilator action of PGE<sub>2</sub> potentiating the vascular permeability response of bradykinin. The effects of LTB<sub>4</sub> on the bradykinin response, however, is a result of the vasodilator actions of bradykinin (Williams & Peck, 1977) potentiating the vascular permeability response to LTB<sub>4</sub>. Finally, increased plasma exudation induced by a combination of PGE<sub>2</sub> and LTB<sub>4</sub> is caused by potentiation of the vascular permeability effects of LTB<sub>4</sub> by the vasodilator actions of PGE<sub>2</sub>. The lack of vasodilator activity shown by LTB<sub>4</sub> may explain the failure of this compound to potentiate carrageenan-induced paw oedema in the rat (Smith, Ford-Hutchinson & Bray, 1980). In contrast, substances with known vasodilator actions such as PGI<sub>2</sub>, PGE<sub>1</sub> and PGE<sub>2</sub> cause a considerable enhancement of this reaction (Ford-Hutchinson, Walker, Davidson & Smith, 1978).

These results clearly demonstrate that LTB<sub>4</sub> affects vascular aspects of inflammation as well as cellular aspects. The mechanism of action of LTB<sub>4</sub> is different from that of the prostaglandins and the combined effects of vasodilators such as prostaglandins with LTB<sub>4</sub> may be an important aspect in the initiation and development of acute inflammatory reactions.



**Figure 1** The effects of leukotriene B<sub>4</sub> (LTB<sub>4</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and noradrenaline (NA) on normal blood flow in rabbit skin measured over a 20 min period. Columns represent means (*n* = 6); vertical lines show s.e. mean. \**P* < 0.05 compared to treatment with saline alone (Student's *t* test).

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