Effect of inhaled leukotriene B₄ alone and in combination with prostaglandin D₂ on bronchial responsiveness to histamine in normal subjects

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ABSTRACT The effect of intradermal injection of leukotriene B₄ alone and in combination with prostaglandin D₂ and E₂ and the effect of inhaled leukotriene B₄ in combination with prostaglandin D₂ were studied in six non-asthmatic men. The intradermal injection of leukotriene B₄ (1 µg) alone caused no immediate or late response in five of the six subjects but greatly potentiated the flare response to intradermal prostaglandin D₂ (0.5 µg) and E₂ (0.5 µg) in all subjects. In contrast, inhaled prostaglandin D₂ (6 µg) alone and in combination with inhaled leukotriene B₄ (12 µg) caused no change in the response to inhaled histamine, measured 30 minutes and three and six hours after the inhalation. These findings provide no support for the suggestion that leukotriene B₄ has an important role in causing bronchial hyperresponsiveness. The possibility that higher doses of inhaled leukotriene B₄ may alter bronchial responsiveness cannot, however, be ruled out.

Introduction

Bronchial hyperresponsiveness is a characteristic feature of asthma.¹ The basis of bronchial hyperresponsiveness is not fully understood, although it is increased, often for days afterwards, when the inhalation of antigen is followed by a late response.² The late response is associated with inflammatory cell infiltration and it has been suggested that mediators released by these cells are responsible for the increase in bronchial responsiveness.³ Cellular infiltration is presumed to follow the release of chemotactic agents during allergen challenge. The arachidonic acid metabolite leukotriene B₄ (LTB₄) is a potent chemotaxin for polymorphonuclear leucocytes.⁴ LTB₄ release has been detected from both human lung mast cells⁵ and human alveolar macrophages⁶ stimulated with anti-IgE and it has been detected in perfusates of isolated guinea pig lung⁷ and in human nasal secretions⁸ after allergen challenge. In anaesthetised dogs O'Byrne *et al*⁹ found that airway responsiveness

to inhaled acetylcholine was increased after inhalation of LTB₄. The increase in responsiveness was maximal three hours after inhalation (and was present at 24 hours); it was associated with a substantial increase in the number of neutrophils in bronchoalveolar lavage fluid. Instillation of LTB₄ (1.6 µg) into a subsegmental bronchus in normal human volunteers led to a considerable increase in the number of neutrophils in lavage fluid.¹⁰ Thus formation of LTB₄ during the immediate response to allergen could lead to cellular recruitment and account, in part at least, for the inflammation associated with the late response and the resulting increase in bronchial responsiveness.

We investigated this hypothesis and studied the effects of inhaled LTB₄ on bronchial reactivity to histamine in man. After preliminary studies in two subjects, in whom LTB₄ alone had no effect on airway conductance or the subsequent response to histamine, we elected to study the effect of LTB₄ in combination with the mast cell derived prostaglandin (PG) D₂. The combination of this mediator with LTB₄ has previously been shown to enhance the cutaneous effects of LTB₄¹¹ and, as both could be released after airway antigen exposure, the interaction could occur *in vivo*. To test the biological activity of our batches of LTB₄ and PGD₂, we studied the cutaneous effects of LTB₄ in combination with PGE₂ and PGD₂.

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Methods

We studied six men aged 23–35 years. No subject had asthma, though three were atopic (positive skin test responses to more than two common inhalant antigens); none was taking any medication. In each subject the airway response to inhaled histamine was within the normal range. The study was approved by the ethics committee of the Hammersmith and Queen Charlotte's Hospitals Special Health Authority. All the subjects gave written informed consent.

PGD₂ was supplied by Glaxo (Ware, Herts), PGE₂ by Upjohn (Crawley, Sussex), and LTB₄ by Cascade Biochem (Reading).

INTRADERMAL CHALLENGE

Intradermal injections were made into the volar surface of the forearm in a volume of 50 μ l with a 27 gauge needle. Each individual received six injections: vehicle (1% ethanol in saline), 1 μ g LTB₄, 0.5 μ g PGE₂, 0.5 μ g PGD₂, 1 μ g LTB₄ with 0.5 μ g PGE₂ and 1 μ g LTB₄ with 0.5 μ g PGD₂. The area of the flare was measured by tracing the contour on to a clear sheet of cellophane placed over the skin. The sizes of the flares were recorded at five, 10, and 20 minutes and one, two, four, and six hours (four subjects) after injection. The areas of the tracings were calculated by computerised planimetry. Results are expressed as arithmetic means (with standard errors in parentheses).

INHALED CHALLENGE

Subjects were studied on three days, at least a week apart. Each visit started at the same time of day. Measurements of specific airways conductance (sGaw) were made with a computerised body plethysmograph.¹² On each occasion baseline measurements of sGaw were followed by an inhaled histamine challenge. The histamine was delivered from a nebuliser, controlled by a dosimeter (Mefar, Brescia, Italy) with an output of 0.024 ml/breath and mass median particle size of 4 μ m. Each dose was given during five slow breaths to vital capacity. After inhalation of a control solution, doubling concentrations of histamine were inhaled in a cumulative fashion every two minutes until a greater than 35% fall in sGaw was achieved. Seventy five minutes later the subjects inhaled aerosols of either PGD₂ (6 μ g), PGE₂ (6 μ g) combined with LTB₄ (12 μ g), or a control solution (5% ethanol in saline), delivered as five breaths from the nebuliser. The aerosols were administered in a double blind fashion in randomised order. Further histamine challenges were performed 30 minutes and three and six hours later. These challenges were conducted in the same fashion as the initial histamine challenge. In a pilot study conducted at an earlier date histamine responsiveness was measured in two subjects, before

and after inhalation of LTB₄ alone; otherwise the study design was as described above. The provocative dose of histamine that caused a 35% fall in sGaw (PD₃₅) was calculated by linear interpolation from the last two doses on the histamine dose-response curve.

ANALYSIS

Results are calculated as geometric means (95% confidence intervals in parentheses). The PD₃₅ values and flare responses were compared by multifactor analysis of variance, and differences were considered significant if $p < 0.05$.

Fig 1 Effect of leukotriene (LT) B₄ (1 μ g) on the flare response to intradermal prostaglandin in six subjects: (a) PGE₂, 0.5 μ g; (b) PGD₂, 0.5 μ g. The flare areas (mean and SEM) for PGE₂ and PGD₂ are shown as open columns and those for PGE₂ and PGD₂ in combination with LTB₄ as hatched columns.

Results

INTRADERMAL CHALLENGE

The intradermal injection of LTB₄ alone had no effect in five subjects. In one atopic subject LTB₄ produced a flare that was maximal at 20 minutes and had resolved at 1 hour but returned at 2 hours and was still evident at 6 hours. This late response was associated with induration of the skin. Both PGD₂ and PGE₂ produced a flare that was observed in the first 10 minutes after injection and was maximal in the first hour. In some subjects the flare had resolved by 4 hours but in others it was still present at 6 hours (fig 1, table). PGE₂ caused greater flare formation than did PGD₂. With both PGD₂ and PGE₂ there was hyperalgesia at the site of injection. At all time points LTB₄ led to potentiation ($p < 0.05$) of the response to PGE₂ (fig 1a) and PGD₂ (fig 1b). This effect was significantly greater at 4 and 6 hours than at earlier time points ($p < 0.05$).

INHALED CHALLENGE

In the pilot study of two subjects we did not observe an increase in bronchial responsiveness after inhalation of LTB₄ alone. For the first subject the PD₃₅ for histamine was 4.3 μ mol before and 5.4 μ mol 6 hours after LTB₄, whereas for the second subject PD₃₅ was 0.38 μ mol before LTB₄ and 0.46 μ mol 6 hours later. For the six subjects studied with inhaled LTB₄ and prostaglandin D₂, baseline histamine PD₃₅ ranged from 7 to 59 μ mol. Cough was noted with the two aerosols that contained PGD₂. There was no significant change in mean PD₃₅ for histamine from baseline values at 30 minutes, 3 hours, or 6 hours for any of the three treatments (fig 2, table). Geometric

Fig 2 Effect of prostaglandin (PG) D₂ and leukotriene (LT) B₄ on the provocative dose of histamine causing a 35% fall in specific airways conductance before and 30 minutes, three hours, and six hours after inhalation of vehicle (saline containing 5% ethanol, open boxes), PGD₂ alone (left semi hatched boxes), and PGD₂ together with LTB₄ (right semi hatched boxes). Geometric mean values and standard errors for six subjects are shown.

mean PD₃₅ at 6 hours was 33.4 (18.2–55.0) μ mol after inhalation of the vehicle, 17.5 (9.1–27.5) μ mol after inhalation of PGD₂ alone, and 26.7 (14.4–43.6) μ mol after inhalation of PGD₂ with LTB₄.

Discussion

Leukotriene B₄ has been shown to potentiate the response to intradermal PGD₂ and PGE₂ as early as 5 minutes and up to 6 hours after injection. The skin studies were undertaken to show that both LTB₄ and

Effect of prostaglandin (PG) D₂ and E₂ and leukotriene (LT) B₄ on flare area and reactivity to histamine (PD₃₅)*

Time (min)	Flare area (cm ²)				Geometric mean histamine PD ₃₅ (μmol) (log mean histamine PD ₃₅ (log SEM))		
	PGE ₂	PGE ₂ /LTB ₄	PGD ₂	PGD ₂ /LTB ₄	Saline	PGD ₂	PGD ₂ /LTB ₄
0					37 [1.57 (0.09)]	23 1.35 (0.11)	19 1.28 (0.13)]
5	7.8 (1.7)	11.3 (1.6)	4.0 (0.9)	5.5 (1.1)			
10	7.6 (1.6)	10.8 (1.6)	4.3 (1.1)	6.2 (1.3)			
20	7.4 (1.2)	10.4 (1.5)	4.1 (0.8)	6.1 (1.6)			
30					39 [1.59 (0.13)]	36 1.56 (0.10)	31 1.49 (0.14)]
60	6.1 (0.8)	8.5 (1.6)	4.9 (1.1)	4.9 (1.1)			
120	4.1 (1.0)	6.1 (0.8)	3.2 (1.5)	3.2 (1.5)			
180					49 [1.69 (0.14)]	39 1.60 (0.16)	23 1.36 (0.11)]
240	0.6 (0.3)	3.8 (0.8)	2.8 (0.1)	1.9 (0.6)			
360	0.2 (0.1)	2.5 (0.3)	0.3 (0.2)	1.7 (0.3)	33 [1.52 (0.08)]	18 1.24 (0.10)	27 1.43 (0.06)]

*The flare area (cm²) following intradermal injection of either PGE₂ (0.5 μ g) or PGD₂ (0.5 μ g) is significantly potentiated ($p < 0.05$) by LTB₄ (1 μ g) at all time points. The degree of potentiation is also significantly higher at 4 and 6 hours than at earlier time points ($p < 0.05$). In contrast, there was no significant effect on histamine PD₃₅ from PGD₂ (6 μ g) with or without LTB₄ (12 μ g). PD₃₅—provocative dose of histamine causing a 35% fall in specific airways conductance.

PGD₂ were biologically active and to determine whether there was an interaction in vivo before we studied their effects on bronchial responsiveness. Our data for skin are consistent with the report from Soter and colleagues,¹¹ who found substantial neutrophil infiltration at the site of injection after 6 hours. At the early stages of flare development (<10 min), the interaction that we observed between LTB₄ and the two prostanoids (PGD₂ and PGE₂) is presumed to be a direct effect and unlikely to be mediated through neutrophils as LTB₄ induced cellular infiltration would be minimal.¹³ Neutrophil accumulation will, however, have occurred by 4–6 hours, when potentiation was greatest.

PGD₂ is a mast cell derived mediator, released in asthma after antigen challenge.¹⁴ It was studied in preference to PGE₂, although PGE₂ produced a greater degree of erythema in the skin. We have shown previously that PGD₂ potentiated the response to histamine if they were inhaled together, though not when histamine was inhaled 30 minutes after the PGD₂.¹⁵ We have shown here that LTB₄ (at a dose of 12 µg), either alone or in combination with PGD₂, did not lead to an increase in bronchial reactivity. This is in contrast to the effect in anaesthetised dogs,⁹ where an increase in bronchial responsiveness was observed after inhalation of LTB₄ in comparable doses. Our subjects inhaled 12 µg of LTB₄, of which about 5% would be expected to reach the lower airways.¹⁶ In the study in dogs 10 µg of LTB₄ was nebulised through an endotracheal tube. This divergence in results may reflect a species difference in the response to neutrophil influx into the lung, for Martin and colleagues¹⁰ found neutrophil accumulation in human lung after direct administration of a dose of LTB₄ comparable to those used in our study.

Inhalation of platelet activating factor has been shown to increase bronchial responsiveness in man,¹⁷ though in molar terms the dose of platelet activating factor administered was much greater than the dose of LTB₄ given in our study. Although both platelet activating factor and LTB₄ are potent chemotactic agents, LTB₄ unlike platelet activating factor, is not very effective in causing neutrophil degranulation.¹⁸ In addition, platelet activating factor, unlike LTB₄, is a potent chemotactic agent for eosinophils and it is also a potent activator of these cells.¹⁹ Increased bronchial responsiveness may be related to airway infiltration by eosinophils^{3,20} and LTB₄, by selectively attracting neutrophils into the airways, may not lead to an increase in bronchial responsiveness in man.

In summary, our study does not provide support for a role for LTB₄ in inducing bronchial hyperresponsiveness. Nevertheless, we cannot preclude a contribution of LTB₄ to the late asthmatic response or to the associated bronchial hyperresponsiveness, either

through an interaction with other mediators or cells or, if LTB₄ is biologically active, at higher concentrations than we could give by inhalation.

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