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Effect of inhaled leukotriene B_4 alone and in combination with prostaglandin D_2 on bronchial responsiveness to histamine in normal subjects

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ABSTRACT The effect of intradermal injection of leukotriene B_4 alone and in combination with prostaglandin D_2 and E_2 and the effect of inhaled leukotriene B_4 in combination with prostaglandin D_2 were studied in six non-asthmatic men. The intradermal injection of leukotriene B_4 (1 μg) alone caused no immediate or late response in five of the six subjects but greatly potentiated the flare response to intradermal prostaglandin D_2 (0·5 μg) and E_2 (0·5 μg) in all subjects. In contrast, inhaled prostaglandin D_2 (6 μg) alone and in combination with inhaled leukotriene B_4 (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 B_4 has an important role in causing bronchial hyperresponsiveness. The possibility that higher doses of inhaled leukotriene B_4 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 B_4 (LTB_4) is a potent chemotaxin for polymorphonuclear leucocytes.[4] LTB_4 release has been detected from both human lung mast cells[5] and human alveolar macrophages[6] stimulated with anti-IgE and it has been detected in perfusates of isolated guinea pig lung[7] and in human nasal secretions[8] after allergen challenge. In anaesthetised dogs O'Byrne *et al*[9] found that airway responsiveness to inhaled acetylcholine was increased after inhalation of LTB_4. 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 LTB_4 (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 LTB_4 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 LTB_4 on bronchial reactivity to histamine in man. After preliminary studies in two subjects, in whom LTB_4 alone had no effect on airway conductance or the subsequent response to histamine, we elected to study the effect of LTB_4 in combination with the mast cell derived prostaglandin (PG) D_2. The combination of this mediator with LTB_4 has previously been shown to enhance the cutaneous effects of LTB_4[11] 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 LTB_4 and PGD_2, we studied the cutaneous effects of LTB_4 in combination with PGE_2 and PGD_2.

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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.

PGD_2 was supplied by Glaxo (Ware, Herts), PGE_2 by Upjohn (Crawley, Sussex), and LTB_4 by Cascade Biochem (Reading).

INTRADERMAL CHALLENGE

Intradermal injections were made into the volar surface of the forearm in a volume of 50 μl with a 27 gauge needle. Each individual received six injections: vehicle (1% ethanol in saline), 1 μg LTB_4, 0·5 μg PGE_2, 0·5 μg PGD_2, 1 μg LTB_4 with 0·5 μg PGE_2 and 1 μg LTB_4 with 0·5 μg PGD_2. 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 μm. 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 PGD_2 (6 μg), PGD_2 (6 μg) combined with LTB_4 (12 μg), 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 LTB_4 alone; otherwise the study design was as described above. The provocative dose of histamine that caused a 35% fall in sGaw (PD_{35}) 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 PD_{35} values and flare responses were compared by multifactor analysis of variance, and differences were considered significant if $p < 0{\cdot}05$.

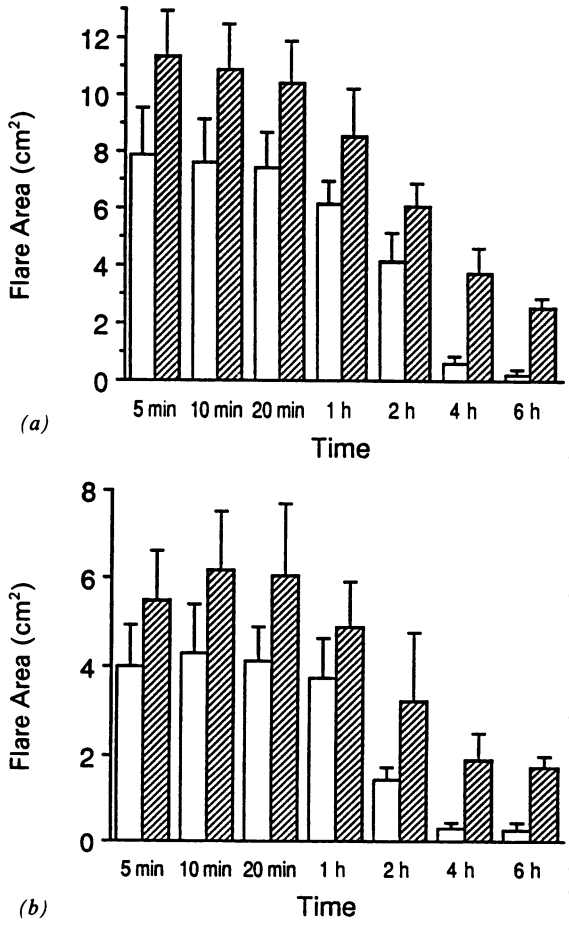

Fig 1 *Effect of leukotriene (LT) B_4 (1 μg) on the flare response to intradermal prostaglandin in six subjects: (a) PGE_2 0·5 μg; (b) PGD_2 0·5 μg. The flare areas (mean and SEM) for PGE_2 and PGD_2 are shown as open columns and those for PGE_2 and PGD_2 in combination with LTB_4 as hatched columns.*

Results

INTRADERMAL CHALLENGE

The intradermal injection of LTB_4 alone had no effect in five subjects. In one atopic subject LTB_4 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 PGD_2 and PGE_2 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). PGE_2 caused greater flare formation than did PGD_2. With both PGD_2 and PGE_2 there was hyperalgesia at the site of injection. At all time points LTB_4 led to potentiation ($p < 0.05$) of the response to PGE_2 (fig 1*a*) and PGD_2 (fig 1*b*). 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 LTB_4 alone. For the first subject the PD_{35} for histamine was 4·3 μmol before and 5·4 μmol 6 hours after LTB_4, whereas for the second subject PD_{35} was 0·38 μmol before LTB_4 and 0·46 μmol 6 hours later.

For the six subjects studied with inhaled LTB_4 and prostaglandin D_2, baseline histamine PD_{35} ranged from 7 to 59 μmol. Cough was noted with the two aerosols that contained PGD_2. There was no significant change in mean PD_{35} for histamine from baseline values at 30 minutes, 3 hours, or 6 hours for any of the three treatments (fig 2, table). Geometric mean PD_{35} at 6 hours was 33·4 (18·2–55·0) μmol after inhalation of the vehicle, 17·5 (9·1–27·5) μmol after inhalation of PGD_2 alone, and 26·7 (14·4–43·6) μmol after inhalation of PGD_2 with LTB_4.

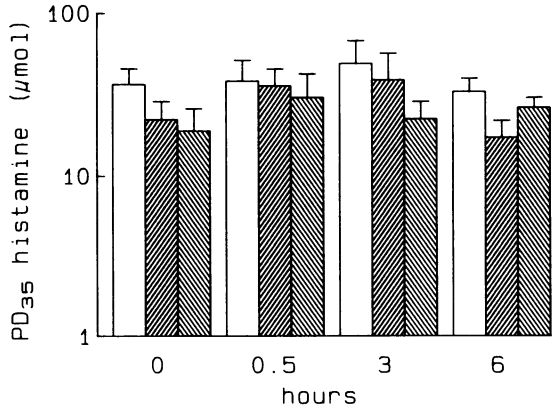

Fig 2 *Effect of prostaglandin (PG) D_2 and leukotriene (LT) B_4 on the provocative dose of histamine causing a 35% fall in specific airways conductance before and 30 minutes, three hours, and six hours after inhalation of vehicle (saline containing 5% ethanol, open boxes), PGD_2 alone (left semi hatched boxes), and PGD_2 together with LTB_4 (right semi hatched boxes). Geometric mean values and standard errors for six subjects are shown.*

Discussion

Leukotriene B_4 has been shown to potentiate the response to intradermal PGD_2 and PGE_2 as early as 5 minutes and up to 6 hours after injection. The skin studies were undertaken to show that both LTB_4 and

*Effect of prostaglandin (PG) D_2 and E_2 and leukotriene (LT) B_4 on flare area and reactivity to histamine (PD_{35})**

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 (cm^2) following intradermal injection of either PGE_2 (0·5 μg) or PGD_2 (0·5 μg) is significantly potentiated ($p < 0.05$) by LTB_4 (1 μg) 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 PD_{35} from PGD_2 (6 μg) with or without LTB_4 (12 μg).
PD_{35}—provocative dose of histamine causing a 35% fall in specific airways conductance.

PGD_2 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 LTB_4 and the two prostanoids (PGD_2 and PGE_2) is presumed to be a direct effect and unlikely to be mediated through neutrophils as LTB_4 induced cellular infiltration would be minimal.[13] Neutrophil accumulation will, however, have occurred by 4–6 hours, when potentiation was greatest.

PGD_2 is a mast cell derived mediator, released in asthma after antigen challenge.[14] It was studied in preference to PGE_2, although PGE_2 produced a greater degree of erythema in the skin. We have shown previously that PGD_2 potentiated the response to histamine if they were inhaled together, though not when histamine was inhaled 30 minutes after the PGD_2.[15] We have shown here that LTB_4 (at a dose of 12 μg), either alone or in combination with PGD_2, 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 LTB_4 in comparable doses. Our subjects inhaled 12 μg of LTB_4, of which about 5% would be expected to reach the lower airways.[16] In the study in dogs 10 μg of LTB_4 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 colleagues[10] found neutrophil accumulation in human lung after direct administration of a dose of LTB_4 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 LTB_4 given in our study. Although both platelet activating factor and LTB_4 are potent chemotactic agents, LTB_4 unlike platelet activating factor, is not very effective in causing neutrophil degranulation.[18] In addition, platelet activating factor, unlike LTB_4, 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 LTB_4, 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 LTB_4 in inducing bronchial hyperresponsiveness. Nevertheless, we cannot preclude a contribution of LTB_4 to the late asthmatic response or to the associated bronchial hyperresponsiveness, either through an interaction with other mediators or cells or, if LTB_4 is biologically active, at higher concentrations than we could give by inhalation.

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