Effect of verapamil and sodium cromoglycate on leukotriene D₄ induced bronchoconstriction in patients with asthma

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ABSTRACT Leukotriene D₄ (LTD₄) may be an important mediator in asthma. The effect of verapamil and sodium cromoglycate on LTD₄ induced bronchoconstriction has been examined in seven patients with asthma. The bronchoconstrictor response to increasing concentrations of inhaled LTD₄ (0.0032–50 µg/ml) was assessed by measuring changes in FEV₁, specific airways conductance, and flow rate at 30% of vital capacity ($\dot{V}_{30(p)}$). Results were expressed as the provocation concentration (PC) producing a 10% fall in FEV₁ (PC₁₀FEV₁), a 35% fall in specific airways conductance (PC₃₅sGaw), and a 30% fall in flow at 30% of vital capacity (PC₃₀ $\dot{V}_{30(p)}$). Neither verapamil nor cromoglycate inhibited LTD₄ induced bronchoconstriction in asthmatic subjects. These results suggest that in asthmatic patients LTD₄ induced bronchoconstriction is not mediated via verapamil or cromoglycate sensitive mechanisms.

The leukotrienes (LTs), including LTD₄, may be important mediators in asthma. LTD₄ is released both in vitro and in vivo after allergen challenge,^{1–3} and is a potent bronchoconstrictor.^{4,5} The mechanism of LTD₄ induced bronchospasm in asthma has not, however, been established. The calcium channel blocker verapamil partially inhibits the bronchoconstrictor response to LTD₄ in vivo but not in vitro in non-asthmatic human bronchi,⁶ and only at very high concentrations in isolated trachea from the guinea pig.⁷ These results suggest that in guinea pigs and non-asthmatic human subjects LTD₄ induced bronchoconstriction occurs as a result both of a direct effect on airway smooth muscle that is insensitive to the inhibitory action of verapamil and of an indirect effect via a verapamil sensitive mechanism. Similarly, in guinea pigs sodium cromoglycate partially inhibits the contractile response to LTD₄ in vivo but not in vitro.⁷ Thus in this species cromoglycate appears capable of inhibiting an indirectly mediated bronchoconstrictor response of LTD₄.

In this study we examined the effect of pretreatment with verapamil and sodium cromoglycate on LTD₄ induced bronchoconstriction to determine

whether in asthma LTD₄ induced airway narrowing depends on a mechanism sensitive to verapamil or to sodium cromoglycate or on both types of mechanism.

Methods

PATIENTS

We studied seven patients with asthma (table 1), four of whom were women. Their ages ranged from 22 to 49 years. All were atopic and were non-smokers. All were taking inhaled β_2 adrenoceptor agonists by pressurised aerosol, three were taking inhaled corticosteroids regularly, and two were taking sodium cromoglycate. All β_2 agonists were discontinued 12 hours before testing and sodium cromoglycate 24 hours before testing. Inhaled corticosteroids were continued. All subjects gave informed consent and the experimental protocol was approved by the Western Infirmary ethical committee.

IN VIVO MEASUREMENTS

Airways resistance (Raw) and thoracic gas volume (TGV) were measured in a constant volume body plethysmograph (Fenyes and Gut), a computerised data collection and analysis system⁸ based on the method of DuBois *et al*⁹ being used. The results were expressed as specific airways conductance (sGaw) (= 1/Raw $\times$ TGV). The mean of eight measurements was taken for the sGaw value. The maximum ex-

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Accepted 12 May 1986

Table 1 Characteristics of the patients

Patient No	Age (y)	Sex	FEV ₁		Atopy	Methacholine PC ₂₀ FEV ₁ (mg/ml)	Current treatment*
			(l)	(% pred)			
1	28	M	2.96	86	+	2.6	S, B
2	49	F	3.43	139	+	1.3	S
3	25	F	2.59	83	+	0.95	S, B
4	42	M	3.00	86	+	0.97	S, B
5	22	F	3.49	103	+	0.23	S, SCG
6	24	M	4.57	113	+	0.90	S
7	24	F	4.21	121	+	0.81	S, SCG

*S—salbutamol inhaler; B—beclomethasone dipropionate inhaler; SCG—sodium cromoglycate.

piratory flow at 70% of expired vital capacity, obtained from a partial flow-volume ($\dot{V}_{30(p)}$) curve, and the forced expiratory volume in one second (FEV₁) were measured automatically (Collingwood Measurement). The flow-volume curves were obtained as follows⁷: after a period of normal tidal breathing, the subject expired maximally from end tidal inspiratory volume to residual volume (RV) to obtain the partial expiratory flow-volume (PEFV) curve. When RV was reached the subject inspired to total lung capacity (TLC) and expired maximally to RV. From this manoeuvre FEV₁ was calculated. Body plethysmographic measurements preceded flow-volume recordings. Aerosols were generated with a Wright nebuliser by air of 50 lb/in² (345 kPa) at a flow rate of 8 l min⁻¹ to achieve an output of 0.15 ml min⁻¹.

DOSE-RESPONSE CURVES

The study was performed in two parts, which were carried out sequentially two months apart. In the first each patient (Nos 1–6) received either verapamil (2.5 mg/ml) or normal saline in a randomised double blind manner on three separate days (two saline). After baseline measurements of sGaw (mean of eight readings) and $\dot{V}_{30(p)}$ and FEV₁ (mean of five readings), the solutions were inhaled for five minutes. After 10 minutes lung function tests were repeated and each subject then inhaled increasing concentrations of leukotriene (0.0032–50 µg/ml). Each concentration was inhaled for two minutes and inhalations were repeated every 15 minutes. Results were expressed as the provocation concentration (PC) producing a 35% fall in sGaw (PC₃₅sGaw), a 30% fall in $\dot{V}_{30(p)}$

(PC₃₀ $\dot{V}_{30(p)}$), and a 10% fall in FEV₁ (PC₁₀FEV₁).

In the second part of the study patients (Nos 1–4, 6, 7) inhaled either sodium cromoglycate (10 mg/ml) or placebo for five minutes in a randomised double blind manner. Measurements were taken before and 10 minutes after each inhalation. A dose-response curve for LTD₄ was then constructed as described above. On a separate day a dose-response curve for methacholine was obtained in a single blind manner according to the protocol described by Hargreave *et al.*¹⁰ The average PC value for the two postsaline LTD₄ dose-response curves was used for the comparison with the results obtained after inhalation of verapamil. Results were compared by means of analysis of variance and Student's *t* test.

Results

The PC₂₀FEV₁ for methacholine ranged from 0.17 to 0.64 mg/ml, confirming that these patients had bronchial responsiveness values in the asthmatic range.^{10 11}

Inhalation of verapamil did not alter baseline FEV₁, sGaw, or $\dot{V}_{30(p)}$ (table 2). All patients developed appreciable bronchoconstriction as assessed by PC₁₀FEV₁, PC₃₅sGaw and PC₃₀ $\dot{V}_{30(p)}$. Pretreatment with verapamil did not modify this response (fig 1). The geometric mean PC₁₀FEV₁ was 0.35 µg/ml after verapamil compared with 0.47 µg/ml after the control (NS). PC₃₅sGaw was 0.69 µg/ml after control and 0.37 µg/ml after verapamil (NS). Mean PC₃₀ $\dot{V}_{30(p)}$ was 0.41 µg/ml after the control and 0.31 µg/ml after verapamil (NS). Sample dose-response curves for

Table 2 Effect of verapamil on baseline airway function* (means with standard errors in parentheses)

	FEV ₁ (l)		sGaw (s ⁻¹ kPa ⁻¹)		$\dot{V}_{30(p)}$ (l s ⁻¹)	
	Baseline	After treatment	Baseline	After treatment	Baseline	After treatment
Control	2.59 (0.36)	2.60 (0.36)	1.13 (0.16)	1.03 (0.13)	1.2 (0.2)	1.36 (0.22)
Verapamil	2.38 (0.51)	2.37 (0.54)	1.39 (0.49)	1.07 (0.28)	1.05 (0.3)	1.02 (0.63)

*No significant differences in baseline and post-treatment values of FEV₁, specific airways conductance, and partial flow-volume curve ($\dot{V}_{30(p)}$) within or between treatments.

Conversion: SI to traditional units—sGaw: 1 s⁻¹ kPa⁻¹ = 1 s⁻¹ mm H₂O⁻¹.

Fig 1 Effect of pretreatment with verapamil (2.5 mg/ml) or control (phosphate buffered saline) on airway responsiveness to leukotriene D_4 (LTD_4). Results are expressed as the provocation concentration (PC) producing a 10% decrease in FEV_1 ($PC_{10}FEV_1$), a 35% decrease in specific airways conductance ($sGaw-PC_{35}sGaw$), and a 30% fall in the partial flow-volume curve ($V_{30(p)}-PC_{30}V_{30(p)}$). Mean values of $PC_{10}FEV_1$, $PC_{35}sGaw$, and $PC_{30}V_{30(p)}$ are shown as horizontal bars.

$sGaw$ against log concentration LTD_4 (two after placebo and one after verapamil) are shown in figure 2.

Sodium cromoglycate did not significantly alter

baseline FEV_1 $sGaw$ or $V_{30(p)}$ (table 3) and did not alter responsiveness to LTD_4 (fig 3). The geometric mean $PC_{10}FEV_1$ was 0.22 $\mu g/ml$ after control and 0.24 $\mu g/ml$ after sodium cromoglycate (NS). $PC_{35}sGaw$ was 0.21 $\mu g/ml$ after control and 0.19 $\mu g/ml$ after sodium cromoglycate. $PC_{30}V_{30(p)}$ was 0.21 $\mu g/ml$ after control and 0.19 $\mu g/ml$ after sodium cromoglycate. Sample dose-response curves for $sGaw$ against log concentration LTD_4 (after placebo and cromoglycate) are shown (fig 4). All patients developed chest tightness but none coughed after LTD_4 .

Fig 2 Graph of percentage change in specific airways conductance ($sGaw$) against log concentration of inhaled leukotriene D_4 (LTD_4) after placebo 1 ($\circ-\circ$), placebo 2 ($\bullet-\bullet$), and verapamil ($\blacktriangle-\blacktriangle$).

Table 3 Effect of sodium cromoglycate on baseline airway function* (means with standard errors in parentheses)

	FEV ₁ (l)		sGaw (s ⁻¹ kPa ⁻¹)		V̇ _{30(p)} (l s ⁻¹)	
	Baseline	After treatment	Baseline	After treatment	Baseline	After treatment
Control	2.35 (0.2)	2.46 (0.21)	1.20 (0.9)	1.14 (0.9)	1.00 (0.43)	1.07 (0.2)
Sodium cromoglycate	2.42 (0.18)	2.51 (0.21)	1.2 (0.8)	1.17 (0.9)	0.99 (0.25)	1.09 (0.29)

*No significant differences in baseline and after treatment values of FEV₁, specific airways conductance (sGaw), and partial flow-volume curve (V̇_{30(p)}) within or between treatments.
Conversion: SI to traditional units—sGaw: 1 s⁻¹ kPa⁻¹ = 1 s⁻¹ mm H₂O⁻¹.

Discussion

These results show that neither the calcium channel blocker verapamil nor sodium cromoglycate modifies LTD₄ induced bronchoconstriction in patients with asthma. In contrast to the finding with verapamil, we recently showed that in non-asthmatic subjects⁶ the calcium channel blocker significantly reduced the constrictor response to LTD₄.⁶

Why should verapamil have a protective effect against LTD₄ induced bronchoconstriction in normal subjects but not in asthmatic patients? It has been suggested that there may be heterogeneity of LTD₄

receptors, and that drugs vary in their ability to block the response to LTD₄ according to the different affinities of their LTD₄ receptors.¹² In support of this hypothesis, the calcium channel blocker diltiazem inhibits the contraction of guinea pig lung parenchymal strips in response to high dose LTD₄ whereas the

Fig 3 Effect of pretreatment with sodium cromoglycate (10 mg/ml) or control (phosphate buffered saline) on airway responsiveness to leukotriene D₄ (LTD₄). Results are expressed in a similar manner to those in figure 1. Sodium cromoglycate did not significantly reduce the response to LTD₄.

Fig 4 Graph of percentage change in specific airways conductance (sGaw) against log concentration of inhaled leukotriene (LTD₄): results after placebo (○—○) and sodium cromoglycate (●—●).

SRS-A antagonist FPL55712 inhibits only the low dose part of LTD₄ induced contraction.¹² Since the non-asthmatic subjects inhaled higher concentrations of LTD₄ than the asthmatic patients,⁶ this may have meant that the low affinity LTD₄ receptors sensitive to calcium channel blockers were stimulated only in the normal subjects. An argument against this mechanism is that verapamil does not significantly inhibit the contractile response of in vitro preparations of human bronchi to different doses of LTD₄, although a slight non-significant reduction of response to the highest concentration of LTD₄ was observed.⁶

An alternative explanation of these findings is that LTD₄ induced bronchoconstriction in asthmatic patients may be due to a direct effect on airway smooth muscle, whereas in non-asthmatic subjects airway narrowing occurs because of a combination of direct action via bronchial smooth muscle receptors and indirect verapamil sensitive mechanisms. This indirect mechanism may have a higher threshold before LTD₄ produces an effect. Possible indirect mechanisms by which LTD₄ might cause bronchoconstriction include reflex vagal bronchoconstriction^{7 13} and release of secondary mediators.¹⁴

A third possibility is that the action of verapamil is dependent on an intact respiratory epithelium. Recently Raeburn *et al*¹⁵ have shown that verapamil inhibits induction of airway smooth muscle contraction by LTD₄ in the rabbit only when the airway mucosa is intact. Since patients with even mild asthma have damaged mucosa,¹⁶ the absence of an inhibitory effect of verapamil on LTD₄ induced bronchoconstriction could be due to the lack of an intact mucosa in those with asthma.

If LTD₄ is confirmed as an important mediator in asthma, it could be predicted from our findings that verapamil would not be an effective drug in asthma. The results of most studies using calcium channel blocking drugs in asthma would support this suggestion. Neither verapamil nor nifedipine significantly alter resting bronchomotor tone and they produce little or no protection against bronchoconstriction induced by allergen.^{17 18} They are moderately effective in inhibiting exercise induced asthma.^{19 20}

Sodium cromoglycate inhibits mast cell degranulation, but may have other modes of action.²¹ Its duration of action at concentrations used in our protocol is two to four hours.²² In guinea pigs in vivo sodium cromoglycate partially inhibits LTD₄ induced bronchoconstriction.⁷ In patients with aspirin induced asthma the response to aspirin challenge can be blocked by sodium cromoglycate.²³ The pathogenesis of aspirin induced asthma is unknown but increased production of lipoxygenase products such as LTD₄ may play a part.²⁴ Possibly sodium cromoglycate acts

as a leukotriene antagonist. Our results show that sodium cromoglycate is not a specific inhibitor of LTD₄ in patients with asthma. This finding confirms and extends the work of Holroyde *et al*,⁵ who showed that in normal subjects sodium cromoglycate had no effect on airway narrowing induced by LTD₄.

In summary, we have shown that neither verapamil nor sodium cromoglycate inhibits LTD₄ induced bronchospasm in patients with asthma. This contrasts with the action of verapamil in non-asthmatic subjects.

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