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

J A ROBERTS, I W RODGER, N C THOMSON

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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 (V₃₀(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 (PC₃₀V₃₀(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 β₂ adrenoceptor agonists by pressurised aerosol, three were taking inhaled corticosteroids regularly, and two were taking sodium cromoglycate. All β₂ 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 (Fenyves and Gut), a computerised data collection and analysis system⁸ based on the method of DuBois et al⁹ being used. The results were expressed as specific airways conductance (sGaw) (= 1/Raw × TGV). The mean of eight measurements was taken for the sGaw value. The maximum ex-
piratory flow at 70% of expired vital capacity, obtained from a partial flow-volume ($V_{30(p)}$) curve, and the forced expiratory volume in one second ($FEV_1$) were measured automatically (Collingwood Measurement). The flow-volume curves were obtained as follows: 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 $FEV_1$ was calculated. Body plethysmographic measurements preceded flow-volume recordings. Aerosols were generated with a Wright nebuliser by air of 50 lb/in$^2$ (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 $V_{30(p)}$ and $FEV_1$ (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 (PC$_{35}$sGaw), a 30% fall in $V_{30(p)}$ (PC$_{30}V_{30(p)}$), and a 10% fall in $FEV_1$ (PC$_{10}$FEV$_1$).

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 LTD$_4$ 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 Hargrave et al.

The average PC value for the two postsaline LTD$_4$ 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 PC$_{20}$FEV$_1$ 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 FEV$_1$, sGaw, or $V_{30(p)}$ (table 2). All patients developed appreciable bronchoconstriction as assessed by PC$_{10}$FEV$_1$, PC$_{35}$sGaw and PC$_{30}V_{30(p)}$. Pretreatment with verapamil did not modify this response (fig 1). The geometric mean PC$_{10}$FEV$_1$ was 0·35 µg/ml after verapamil compared with 0·47 µg/ml after the control (NS). PC$_{35}$sGaw was 0·69 µg/ml after control and 0·37 µg/ml after verapamil (NS). Mean PC$_{30}V_{30(p)}$ was 0·41 µg/ml after the control and 0·31 µg/ml after verapamil (NS). Sample dose-response curves for

### Table 1 Characteristics of the patients

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (y)</th>
<th>Sex</th>
<th>FEV$_1$ (% pred)</th>
<th>Atopy</th>
<th>Methacholine PC$_{20}$FEV$_1$ (mg/ml)</th>
<th>Current treatment</th>
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<tr>
<td>1</td>
<td>28</td>
<td>M</td>
<td>2·96</td>
<td>86</td>
<td>+</td>
<td>2·6</td>
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<tr>
<td>2</td>
<td>49</td>
<td>F</td>
<td>3·43</td>
<td>139</td>
<td>+</td>
<td>1·3</td>
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<tr>
<td>3</td>
<td>25</td>
<td>F</td>
<td>2·39</td>
<td>83</td>
<td>+</td>
<td>0·95</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>M</td>
<td>3·60</td>
<td>86</td>
<td>+</td>
<td>0·7</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>F</td>
<td>3·49</td>
<td>103</td>
<td>+</td>
<td>0·23</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>M</td>
<td>4·57</td>
<td>113</td>
<td>+</td>
<td>0·90</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>F</td>
<td>4·21</td>
<td>121</td>
<td>+</td>
<td>0·81</td>
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</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>FEV$_1$ (l)</th>
<th>sGaw (s$^{-1}$ kPa$^{-1}$)</th>
<th>$V_{30(p)}$ (l s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After treatment</td>
<td>Baseline</td>
</tr>
<tr>
<td>Control</td>
<td>2·59 (0·36)</td>
<td>2·60 (0·36)</td>
<td>1·13 (0·16)</td>
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<tr>
<td>Verapamil</td>
<td>2·38 (0·51)</td>
<td>2·37 (0·54)</td>
<td>1·39 (0·49)</td>
</tr>
</tbody>
</table>

*No significant differences in baseline and post-treatment values of FEV$_1$, specific airways conductance, and partial flow-volume curve ($V_{30(p)}$) within or between treatments.

Conversion: SI to traditional units—sGaw: 1 s$^{-1}$ kPa$^{-1}$ = 1 s$^{-1}$ mm H$_2$O$^{-1}$. 

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Fig 1  Effect of pretreatment with verapamil (2.5 mg/ml) or control (phosphate buffered saline) on airway responsiveness to leukotriene D₄ (LTD₄). Results are expressed as the provocation concentration (PC) producing a 10% decrease in FEV₁ (PC₁₀FEV₁), a 35% decrease in specific airways conductance (sGaw—PC₃₅sGaw), and a 30% fall in the partial flow-volume curve (V₃₀(p)—PC₃₀V₃₀(p)). Mean values of PC₁₀FEV₁, PC₃₅sGaw, and PC₃₀V₃₀(p) are shown as horizontal bars.

sGaw against log concentration LTD₄ (two after placebo and one after verapamil) are shown in figure 2.

Sodium cromoglycate did not significantly alter baseline FEV₁ sGaw or V₃₀(p) (table 3) and did not alter responsiveness to LTD₄ (fig 3). The geometric mean PC₁₀ FEV₁ was 0.22 µg/ml after control and 0.24 µg/ml after sodium cromoglycate (NS). PC₃₅sGaw was 0.21 µg/ml after control and 0.19 µg/ml after sodium cromoglycate. PC₃₀V₃₀(p) was 0.21 µg/ml after control and 0.19 µg/ml after sodium cromoglycate. Sample dose-response curves for sGaw against log concentration LTD₄ (after placebo and cromoglycate) are shown (fig 4). All patients developed chest tightness but none coughed after LTD₄.

Fig 2  Graph of percentage change in specific airways conductance (sGaw) against log concentration of inhaled leukotriene D₄ (LTD₄) after placebo 1 (○—○), placebo 2 (●—●), and verapamil (▲—▲).
Table 3  Effect of sodium cromoglycate on baseline airway function* (means with standard errors in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>FEV₁ (l)</th>
<th>sGaw (s⁻¹ kPa⁻¹)</th>
<th>V₃₀,p (l s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After treatment</td>
<td>Baseline</td>
</tr>
<tr>
<td>Control</td>
<td>2.35 (0.2)</td>
<td>2.46 (0.21)</td>
<td>1.20 (0.9)</td>
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<tr>
<td>Sodium cromoglycate</td>
<td>2.42 (0.18)</td>
<td>2.51 (0.21)</td>
<td>1.2 (0.8)</td>
</tr>
<tr>
<td></td>
<td>1.00 (0.43)</td>
<td>1.07 (0.2)</td>
<td>0.99 (0.25)</td>
</tr>
</tbody>
</table>

*No significant differences in baseline and after treatment values of FEV₁, specific airways conductance (sGaw), and partial flow-volume curve (V₃₀,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 subjects the calcium channel blocker significantly reduced the constrictor response to LTD₄.

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. 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
SRS-A antagonist FPL55712 inhibits only the low dose part of LTD₄ induced contraction.¹² Since the non-asthmatic subjects inhalled 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 bronchoconstriction⁷ 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. ² 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.

References
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Thorax 1986 41: 753-758
doi: 10.1136/thx.41.10.753

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