LTD₄-induced bronchoconstriction in patients with asthma: lack of a vagal reflex

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The mechanism by which leukotriene D₄ (LTD₄) induces bronchoconstriction in man is unclear. We have investigated this mechanism by examining the effect of nedocromil sodium and ipratropium bromide on LTD₄-induced bronchoconstriction in six mild asthmatic patients. The bronchoconstrictor response to increasing concentrations of inhaled LTD₄ (0.08 to 100 nmol) was assessed by measuring changes in respiratory resistance (Rrs) at 6 Hz by the forced oscillation method. Results were expressed as the provocative dose causing 100% increase in Rrs (PD₁₀₀). Neither nedocromil (4mg) nor ipratropium (0.8mg) showed significant effect on LTD₄-induced bronchoconstriction in asthmatic subjects. These results suggest that in asthmatics LTD₄-induced bronchoconstriction is unlikely to involve vagal reflexes.

Keywords leukotriene D₄ vagal reflex nedocromil ipratropium

Introduction

Leukotriene D₄ (LTD₄) is a potent bronchoconstrictor. In man inhaled LTD₄ is more potent and longer lasting than histamine in causing bronchoconstriction (Barnes *et al.*, 1984). The mechanism of LTD₄-induced bronchoconstriction in asthma has not been established. It is thought that, in asthmatic patients, it may be due to a direct effect on airway smooth muscle (Roberts *et al.*, 1986a,b) although it has been suggested that lipoxygenase products may stimulate sensory nerves or even modulate neural transmission. LTD₄ may therefore have an action in part via a vagal bronchoconstrictor reflex (Stewart *et al.*, 1983; Advenier *et al.*, 1983; Bisgaard & Groth, 1987).

Nedocromil sodium, the disodium salt of paranoquinoline decarboxylic acid, is an anti-asthmatic drug with a profile of action similar to that of sodium cromoglycate (Koning *et al.*, 1987). It has been suggested that nedocromil sodium may act by blocking afferent vagal nerve endings (Dixon *et al.*, 1987). On the other hand ipra-

tropium bromide, an antimuscarinic drug, causes bronchodilation by blocking the motor limb of the neural reflex (Gross, 1975).

The aim of the present study was to investigate whether in asthmatic subjects LTD₄-induced bronchoconstriction could be altered by nedocromil sodium or ipratropium bromide.

Methods

Patients

We studied six non-smoking male patients aged 23 to 38 years with mild asthma. All were taking inhaled β -adrenoceptor agonists by pressurised aerosol, two were taking inhaled corticosteroids regularly. β -adrenoceptor agonists were discontinued for at least 4 h before testing; the use of inhaled steroids was however unaltered. Subjects gave informed consent to take part in this study which had the approval of the hospital Research Ethics Committee.

Airway function

Total respiratory resistance (Rrs) was measured using a forced oscillation technique (Landser *et al.*, 1976). Rrs was measured over a range of frequencies from 2–26 Hz generated in a random order over a 16 s period and the resistance at 6 Hz was recorded as this showed the maximum change. Measurements were made with the subjects sitting relaxed, supporting their cheeks and chin, while breathing tidally in the mouth-piece. The pressure and flow signals generated by each frequency over this period were separated using Fourier analysis by a microprocessor. All measurements are the mean of three consecutive recordings.

LTD₄ challenge

Subjects inhaled a single breath (0.02 ml, mass median diameter 3.5–4 μ) of saline or the different concentrations of LTD₄ from a compressed air driven activated dosimeter (Mefar, Brescia, Italy).

Protocol

Each subject was asked to attend the laboratory at the same time of day on three separate occasions at least 3 days apart. Following baseline

measurements of Rrs at 6 Hz, the subjects inhaled five breaths of saline (0.1 ml) and Rrs was measured at 5 min. They then inhaled increasing doses of LTD₄ (0.08 to 100 nmol) until a 100% increase in Rrs was recorded.

The provocative dose causing a 100% increase in Rrs (PD₁₀₀ Rrs) was calculated from the dose-response curve for LTD₄. Thirty minutes later each subject inhaled two puffs of ipratropium (0.4 mg) or matched placebo, 45 min later 2 puffs of nedocromil (2 mg) or placebo and 60 min later a second LTD₄ challenge was performed. The drugs were given in a randomised double-blind manner. Results were compared by means of analyses of variance and Student's *t*-test for paired observations.

Results

There was no difference in baseline Rrs on the 3 study days and none of the treatment combinations significantly altered baseline Rrs (Table 1). Inhalation of LTD₄ resulted in appreciable bronchoconstriction in all patients by Rrs. All patients developed chest tightness and two of them coughed after LTD₄ challenge. The result in the PD₁₀₀ before and after each treatment (Table 1 and Figure 1) show that neither treatment significantly alter the response to LTD₄.

Table 1 Effect of different treatment on mean (s.d.) baseline Rrs (cm H₂O l⁻¹ s⁻¹) and geometric mean (95% C.I.) PD₁₀₀ (nmol)

	Rrs		PD ₁₀₀	
	Pre	Post	Pre	Post
Placebo	2.4(0.9)	2.7(0.9)	3.3(1.6–6.7)	2.9(1.5–5.6)
Nedocromil	2.5(0.9)	3.3(1.2)	4.7(1.6–13.4)	6.5(2.4–17.8)
Ipratropium	2.6(1.0)	2.4(1.2)	3.6(1.5–8.4)	5.3(0.6–46.5)

Figure 1 Effect of treatment in six patients with placebo (P), nedocromil (N) (4 mg), or ipratropium (I) (0.8 mg). Control = C. Each point represents the value of log PD₁₀₀ Rrs (nmol).

Discussion

These results show that neither nedocromil nor ipratropium alters LTD₄-induced bronchoconstriction in patients with asthma. Although only a small group of patients were studied the use of repeated challenges on the same day allows the probability of a type 2 error to be calculated and this showed that the study would have detected a change of 2.5 fold in the PD₁₀₀ (a two fold increase being the minimum change usually considered significant).

Since these drugs block either the afferent or efferent part of the vagal reflex, it is unlikely LTD₄-induced bronchoconstriction involves vagal reflexes in asthmatic patients although an effect on the axon reflex cannot be ruled out. Our findings would be in agreement with other

authors (Roberts *et al.*, 1986a,b) who suggested that in LTD₄-induced bronchoconstriction in asthmatic patients vagal reflex is less important than in non-asthmatics.

Nedocromil has also been shown to inhibit the release of mast cell derived mediators in some models (Koning *et al.*, 1987). The lack of effect of these drugs on LTD₄-induced bronchoconstriction therefore also implies that LTD₄ does not cause mast cell activation. This study therefore supports the hypothesis that LTD₄ causes bronchoconstriction by a direct effect on airway smooth muscle.

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