Effect of inhaled prostaglandin E2
on methacholine and leukotriene
D4 airway responsiveness in
asthmatic subjects

WHM STEVENS PhD, CHERYL P VANDERHEYDEN,
PAUL M O’BYRNE MB FRCP I FRCP C FCCP
Asthma Research Group, McMaster University, Hamilton, Ontario

Asthma Research Group, McMaster University, Hamilton, Ontario

Previous studies in asthmatics have demonstrated that the endogenous release of inhibitory prostaglandins limits the bronchoconstrictor response to repeated challenges with exercise and histamine, and that inhaled prostaglandin (PG) E2 attenuates allergen-induced asthmatic responses and exercise bronchoconstriction in asthmatics. Inhaled PGE2 does not significantly attenuate methacholine airway responsiveness. These results, taken together, indicate that inhaled PGE2 attenuates the bronchoconstriction caused by stimuli, such as allergen and exercise, that result in bronchoconstriction through cysteinyl leukotriene (LT) release. The purpose of this study was to determine whether inhaled PGE2 could selectively attenuate LTD4-induced bronchoconstriction in seven stable asthmatic subjects. Each subject was studied on four different study days. On two occasions the subjects inhaled 100 mg PGE2, 30 mins before a methacholine, or LTD4 challenge test. On the other two study days, the subjects were pretreated with its diluent. Results were expressed as the provocation concentration causing a 20% fall in forced expiratory volume in 1 s (FEV1) (PC20). PGE2 pretreatment significantly increased the LTD4 PC20, but not the methacholine PC20. The mean LTD4 PC20 increased from 2.00 mg/mL (%SEM 1.65) after diluent pretreatment to 3.01 mg/mL (%SEM 1.64) after PGE2 pretreatment (P=0.008). The mean methacholine PC20 was 1.28 mg/mL (%SEM 1.68) after diluent pretreatment and 1.62 mg/mL (%SEM 1.46) after PGE2 pretreatment (P=0.28). These results suggest that PGE2 partially attenuates LTD4-induced bronchoconstriction; however, the magnitude of the effect is unlikely to account for its attenuation of exercise and allergen-induced bronchoconstriction.

Key Words: Airway responsiveness, Bronchoconstriction, Prostaglandin E2

Effet de la prostaglandine E2 sur l’hyperréactivité bronchique causée par les leucotriènes D4 et la méthacholine chez les sujets asthmatiques

RÉSUMÉ : De précédentes études menées sur des asthmatiques ont démontré que la libération endogène de prostaglandines inhibitrices limite la réponse bronchoconstrictrice aux provocations répétées induites par l’exercice et l’histamine, et que la prostaglandine (PG) E2 en inhalation atténue les réactions asthmatiques induites par des allergènes, et la bronchoconstriction causée par l’exercice chez les asthmatiques. La PGE2 en inhalation n’atténue pas l’hyperréactivité bronchique induite par la méthacholine de manière significative. Globalement, ces résultats démontrent que la PGE2 en inhalation atténue la bronchoconstriction causée par des stimuli tels qu’allergènes et exercice, qui résultent en une bronchoconstriction causée par la libération des cystéinyl-leucotriènes (LT). Le but de la présente étude était de déterminer si la...

Correspondence and reprints: Dr Paul M O’Byrne, Faculty of Health Sciences, Department of Medicine, 1200 Main Street West, Hamilton, Ontario L8N 3Z5. Telephone 905-521-2100 ext 6652, fax 905-521-5053, e-mail o’byrne@fh.s.mcmaster.ca
Previous studies in asthmatic subjects have suggested that the endogenous release of inhibitory prostaglandins (PG) limits the bronchoconstrictor response to repeated challenges with exercise (1), inhaled histamine (2,3) and inhaled leukotriene (LT) D4 (4). This concept has been supported by the demonstration that inhaled PGE2 significantly attenuates exercise bronchoconstriction, but not the bronchoconstriction caused by inhaled methacholine in asthmatics (5). Inhaled PGE1 (6) and PGE2 (6,7) have also been demonstrated to protect subjects with asthma against the early (6,7) and late bronchoconstrictor responses (6) to inhaled allergen. The allergen-induced airway hyperresponsiveness was also inhibited by pretreatment with inhaled PGE2 (7).

Inhaled LTD4 is a potent bronchoconstrictor mediator of human airways (8,9). Exercise- and allergen-induced bronchoconstriction can be largely abolished by pretreatment with LTD4 receptor antagonists (10-12) or synthetase inhibitors (13,14), thereby implicating LTD4 as an important mediator in causing exercise- and allergen-induced bronchoconstriction. The fact that inhaled PGE2 selectively inhibits bronchoconstrictor responses to these stimuli caused by LTD4, but not to methacholine-induced bronchoconstriction, raises the possibility that PGE2 can selectively antagonize LTD4-induced bronchoconstriction. The purpose of this study, therefore, was to evaluate whether inhaled PGE2, administered in doses known to attenuate exercise- and allergen-induced bronchoconstriction, also attenuates LTD4 or methacholine-induced airway responsiveness in stable asthmatic subjects.

**PATIENTS AND METHODS**

**Subjects:** Seven stable asthmatic subjects (five females, two males), aged between 19 and 42 years, were studied when their asthma was controlled by the as-required use of inhaled beta2-agonist alone. The subjects had no exacerbations of asthma for at least eight weeks before the study, and baseline forced expiratory volume in 1 s (FEV1) was 80% predicted normal (15) in all subjects on each study day. Subjects were instructed to withhold use of inhaled bronchodilators at least 8 h before challenges. All subjects were atopic as demonstrated by at least one positive skin test to a battery of 16 common allergens. The project was approved by the Ethics Committee of McMaster University Medical Centre, and each subject gave written informed consent before taking part.

**Study design:** All subjects attended the laboratory for five study periods. The first period was a screening day during which subjects’ characteristics, including methacholine airway responsiveness, were documented. During the next four study periods baseline spirometry was measured and subjects were pretreated with either inhaled PGE2 or its diluent. Spirometry was repeated 5 mins and 30 mins after the diluent or PGE2 pretreatment, followed immediately by an LTD4 or methacholine inhalation test. In an effort to blind the investigator doing the methacholine or LTD4 challenges, a different investigator delivered the PGE2 and diluent pretreatments in a different room from that used for the inhalation challenge procedures. The study used a single blinded, diluent controlled, crossover design. All spirometric measurements were made using a 14 L water spirometer (Warren E Collins Inc, Massachusetts).

**PGE2 or diluent pretreatment:** The PGE2 pretreatment was as previously described (5) by this laboratory. PGE2 stock solution (2 mg/mL) was prepared by diluting dry powder (Sigma, Missouri) in ethanol and stored at −70°C. One millilitre of the stock solution of PGE2 was diluted with 0.2 mL 0.9% saline and delivered using a breath-activated dosimeter (PK Morgan, Gillingham, United Kingdom) set to produce an output of 10 mg (0.006 mL PGE2) per breath. Subjects were instructed to take 10 deep breaths of the aerosolized solution, for a total dose of 100 mg. The diluent was prepared by diluting 1 mL of ethanol in 0.2 mL saline.

**Methacholine inhalation test:** Methacholine inhalation was performed as previously described (16). Doubling concentrations of methacholine were inhaled from a Wright nebulizer (Roxon) beginning with a concentration of 0.03 mg/mL for periods of 2 mins. Following each inhalation period, FEV1 was measured at 30 s, 1.5 mins, 3 mins and then every 2 mins, if necessary, until the lowest value was obtained. Once a fall in FEV1 of 20% or greater occurred, the test was terminated and the concentration of methacholine required to produce a fall in FEV1 of 20% was calculated, and expressed as the provocative concentration causing a 20% fall in FEV1 (methacholine PC20). After the test, two puffs of salbutamol (200 mg) were given to reverse the bronchoconstriction.

**LTD4 inhalation test:** The LTD4 inhalation was done as previously described (4). Subjects inhaled 10 breaths of increasing doubling concentrations of LTD4, from 0.025 to 50 mg/mL, at intervals of 5 mins, from a breath-activated dosimeter (PK Morgan) set to produce an output of 10 mg. Stock solutions of LTD4 diluted in dH2O (1 mg/mL) (Merck...
Frosst) was stored at –70°C and before use was diluted in phosphate buffered saline with benzyl alcohol (pH=7.4) (Bencard) to the appropriate concentrations. The response was measured by FEV1 performed at 30 s, 1.5 mins and 3 mins, and then every 2 mins, if necessary, until the lowest value was obtained. Once a fall in FEV1 of 20% or greater occurred, the test was terminated and the concentration of LTD4 required to produce a fall in FEV1 of 20% was calculated and expressed as the LTD4 PC20. After the test, two puffs of salbutamol (200 mg) were given to reverse bronchoconstriction.

Results: Statistical analyses were performed using the STATISTICA (StatSoft Inc, Oklahoma) computer software program. Data distributions were checked for normality using Kolmogorov-Smirnoff and \( \chi^2 \) analysis. Because PC20 values are log-normally distributed, log transformed methacholine and LTD4 PC20s were used to compare the effect of diluent and PGE2. The results were also evaluated as the maximal fall in FEV1 after the highest inhaled concentration of inhaled LTD4 or methacholine used after diluent pretreatment. FEV1 values were not log transformed. A two-tailed paired \( t \) test was used to determine significance and \( P=0.05 \) was considered significant.

**RESULTS**

Inhaled PGE2 slightly, but significantly, improved LTD4 airway responsiveness. The geometric mean LTD4 PC20 increased from 2.00 mg/mL (%SEM 1.65) after diluent to 3.01 mg/mL (%SEM 1.64) after PGE2 (\( P=0.008 \)) (Figure 1). The LTD4 PC20 increased in six subjects and was unchanged in one. By contrast, inhaled PGE2 did not significantly attenuate methacholine airway responsiveness. The geometric mean methacholine PC20 was 1.28 mg/mL (%SEM 1.68) after diluent and 1.62 mg/mL (%SEM 1.46) after PGE2 (\( P=0.28 \)) (Figure 1).

The results were also evaluated as the maximal fall in the FEV1 after the highest inhaled concentration of LTD4 and methacholine after diluent and the fall in FEV1 after the same concentrations of the agonists after inhaled PGE2. According to this analysis, inhaled PGE2 again slightly, but significantly, reduced the maximal fall in FEV1 after inhaled LTD4 from a mean value of 24.8% (SEM 2.8%) after diluent to 17.3% (SEM 2.8%) after PGE2 (\( P=0.04 \)) but not after inhaled methacholine, which was 27.5% (SEM 3.3%) after diluent and 22.03% (SEM 3.6%) after PGE2 (\( P=0.31 \)).

The initial mean baseline FEV1 values before diluent on the two days on which diluent was inhaled was 2.96 L (SEM 0.08), and before PGE2 on the two days on which PGE2 was inhaled was 3.10 L (SEM 0.19) (\( P=0.43 \)) (Figure 2). The FEV1 significantly decreased by 0.23 L (SEM 0.08) (\( P=0.016 \)) 5 mins after inhaled PGE2 (Figure 2), but was no longer significantly reduced by 30 mins after PGE2. Inhaled diluent had no significant effect on the FEV1 at either 5 or 30 mins after inhalation (Figure 2).

In all subjects, inhaled PGE2 caused transient coughing, lasting 15 to 20 s after beginning inhalation, and most subjects complained of retrosternal soreness, lasting 1 to 2 mins after beginning inhalation.

**DISCUSSION**

This study has demonstrated that pretreatment with inhaled PGE2 significantly attenuates airway responsiveness to inhaled LTD4, but not to methacholine, in asthmatic subjects. These results suggest that inhaled PGE2 selectively attenuates LTD4-induced bronchoconstrictor responses. However, the lack of effect of inhaled PGE2 on inhaled methacholine may have resulted from the small sample size in the study.
The result is, however, consistent with another study from our laboratory, which demonstrated no significant effect of inhaled PGE$_2$ on methacholine airway hyperresponsiveness (5).

Inhaled PGE$_2$ caused slight, but significant, bronchoconstriction measured 5 mins after PGE$_2$ inhalation. This bronchoconstriction had resolved by 30 mins after the PGE$_2$ inhalation, immediately before the LTD$_4$ or methacholine inhalation. Inhaled PGE$_2$ has been previously shown to cause transient bronchoconstriction, even in normal subjects, lasting up to 5 mins (17). Also, as in other studies using inhaled PGE$_2$ in human subjects (17,18), we found that PGE$_2$ caused cough in all subjects, which was very transient, and retrosternal soreness in most subjects.

Previous studies have reported that inhaled PGE$_2$ markedly attenuates allergen-induced early responses by more than 90% and the late bronchoconstrictor response by more than 50% (7). Also, Melillo et al (5) have shown that the same dose of inhaled PGE$_2$ given 30 mins before exercise significantly attenuates exercise-induced bronchoconstriction by 66%. Pretreatment with LTD$_4$ receptor antagonists has been shown to attenuate allergen-induced asthmatic responses (19) and exercise-induced bronchoconstrictor responses (10,12) by an almost identical magnitude. Manning et al (4) have shown that exercise refractoriness is, at least in part, caused by LTD$_4$-induced inhibitory prostaglandin release in asthmatic airways. Taken together, these studies raise the possibility that the protective effect of inhaled PGE$_2$ occurs through a specific effect on LTD$_4$ receptors, resulting in receptor antagonism. The absence of a significant effect of inhaled PGE$_2$ on the methacholine PC$_{20}$ is consistent with the previous findings of Melillo et al (5), and is also consistent with the fact that no tachyphylaxis to repeated challenges performed 1 h apart occurs to the cholinergic agonists methacholine (20) and acetylcholine (3) in asthmatic subjects.

Although this study has demonstrated that PGE$_2$ attenuates LTD$_4$-induced bronchoconstriction, the magnitude of this effect shows that this mechanism is unlikely to account entirely for PGE$_2$-induced attenuation of exercise and allergen-induced bronchoconstriction. If the results are analyzed as the maximal fall in FEV$_1$ after the highest inhaled concentration of LTD$_4$, the magnitude of protection achieved by PGE$_2$ was small and only significant because the effect occurred in all subjects. This is in marked contrast to the major degree of protection by this dose of inhaled PGE$_2$ against exercise and allergen challenge. One other possible mechanism for the differences between inhaled LTD$_4$ and exercise or allergen challenge is that the site of action in the airway tree of inhaled LTD$_4$ and endogenous LTD$_4$ is different. This possibility cannot be discounted; however, it is unlikely to explain the lack of marked effect of inhaled PGE$_2$ on inhaled LTD$_4$ bronchoconstrictor responses, because these media tors were delivered by the same nebulizer into the same subjects.

In conclusion, our results suggest that inhaled PGE$_2$ partially attenuates LTD$_4$-induced bronchoconstriction, an effect not seen in this or another study (5) with inhaled methacholine; however, another inhibitory effect is likely to explain its attenuation of exercise and allergen-induced bronchoconstriction.

ACKNOWLEDGEMENTS: This study was supported by Respiratory Health Network of Centres of Excellence, and the Medical Research Council of Canada. PM O’Byrne is a Medical Research Council of Canada Senior Scientist. The authors thank Dr A Ford-Hutchinson, Merck-Frosst, Canada, for supplying the LTD$_4$.

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