Effect of inhaled prostaglandin E_2 on methacholine and leukotriene D_4 airway responsiveness in asthmatic subjects

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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) E_2 attenuates allergen-induced asthmatic responses and exercise bronchoconstriction in asthmatics. Inhaled PGE_2 does not significantly attenuate methacholine airway responsiveness. These results, taken together, indicate that inhaled PGE_2 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 PGE_2 could selectively attenuate LTD_4-induced bronchoconstriction in seven stable asthmatic subjects. Each subject was studied on four different study days. On two occasions the subjects inhaled 100 mg PGE_2, 30 mins before a methacholine, or LTD_4 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 (FEV_1) (PC_{20}). PGE_2 pretreatment significantly increased the LTD_4 PC_{20}, but not the methacholine PC_{20}. The mean LTD_4 PC_{20} increased from 2.00 mg/mL (%SEM 1.65) after diluent pretreatment to 3.01 mg/mL (%SEM 1.64) after PGE_2 pretreatment (P=0.008). The mean methacholine PC_{20} was 1.28 mg/mL (%SEM 1.68) after diluent pretreatment and 1.62 mg/mL (%SEM 1.46) after PGE_2 pretreatment (P=0.28). These results suggest that PGE_2 partially attenuates LTD_4-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 E_2*

Effet de la prostaglandine E_2 sur l'hyperréactivité bronchique causée par les leucotriènes D_4 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 bronchoconstrictive aux provocations répétées induites par l'exercice et l'histamine, et que la prostaglandine (PG) E_2 en inhalation atténue les réactions asthmatiques induites par des allergènes, et la bronchoconstriction causée par l'exercice chez les asthmatiques. La PGE_2 en inhalation n'atténue pas l'hyperréactivité bronchique induite par la méthacholine de manière significative. Globalement, ces résultats démontrent que la PGE_2 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

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PGE_2 en inhalation pouvait de façon sélective atténuer la bronchoconstriction induite par les LTD_4 chez sept sujets asthmatiques stables. Chaque sujet a été étudié lors de quatre jours d'étude différents. À deux occasions, ils ont inhalé 100 mg de PGE_2, 30 minutes avant de subir un test de provocation à la méthacholine, ou aux LTD_4. Pendant les deux autres jours d'étude, les sujets ont été prétraités avec le diluant de la PGE_2. Les résultats ont été exprimés en terme de concentrations de la solution utilisée pour la provocation induisant une chute de 20 % du VEMS (CP_{20}). La PGE_2 utilisée en prétraitement a augmenté sensiblement la CP_{20} LTD_4 mais pas la CP_{20} méthacholine. La CP_{20} LTD_4 moyenne est passée de 2,00 mg/mL (% erreur type de la moyenne 1,65) après le prétraitement avec le diluant, à 3,01 mg/mL (% erreur type de la moyenne 1,64) après le prétraitement à la PGE_2 (P=0,008). La CP_{20} moyenne de la méthacholine était de 1,28 mg/mL (% erreur type de la moyenne 1,68) après le prétraitement avec le diluant et de 1,62 mg/mL (% erreur type de la moyenne 1,46) après le prétraitement à la PGE_2 (P=0,28). Ces résultats laissent croire que la PGE_2 atténue partiellement la bronchoconstriction induite par les LTD_4 ; cependant, l'ampleur de l'effet, vraisemblablement n'explique pas son atténuation de la bronchoconstriction provoquée par l'exercice et les allergènes.

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) D_4 (4). This concept has been supported by the demonstration that inhaled PGE_2 significantly attenuates exercise bronchoconstriction, but not the bronchoconstriction caused by inhaled methacholine in asthmatics (5). Inhaled PGE_1 (6) and PGE_2 (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 PGE_2 (7).

Inhaled LTD_4 is a potent bronchoconstrictor mediator of human airways (8,9). Exercise- and allergen-induced bronchoconstriction can be largely abolished by pretreatment with LTD_4 receptor antagonists (10-12) or synthetase inhibitors (13,14), thereby implicating LTD_4 as an important mediator in causing exercise- and allergen-induced bronchoconstriction. The fact that inhaled PGE_2 selectively inhibits bronchoconstrictor responses to these stimuli caused by LTD_4, but not to methacholine-induced bronchoconstriction, raises the possibility that PGE_2 can selectively antagonize LTD_4-induced bronchoconstriction. The purpose of this study, therefore, was to evaluate whether inhaled PGE_2, administered in doses known to attenuate exercise- and allergen-induced bronchoconstriction, also attenuates LTD_4- 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 $beta_2$-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 (FEV_1) 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 PGE_2 or its diluent. Spirometry was repeated 5 mins and 30 mins after the diluent or PGE_2 pretreatment, followed immediately by an LTD_4 or methacholine inhalation test. In an effort to blind the investigator doing the methacholine or LTD_4 challenges, a different investigator delivered the PGE_2 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).

PGE_2 or diluent pretreatment: The PGE_2 pretreatment was as previously described (5) by this laboratory. PGE_2 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 PGE_2 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 PGE_2) 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, FEV_1 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 FEV_1 of 20% or greater occurred, the test was terminated and the concentration of methacholine required to produce a fall in FEV_1 of 20% was calculated, and expressed as the provocative concentration causing a 20% fall in FEV_1 (methacholine PC_{20}). After the test, two puffs of salbutamol (200 mg) were given to reverse the bronchoconstriction.

LTD_4 inhalation test: The LTD_4 inhalation was done as previously described (4). Subjects inhaled 10 breaths of increasing doubling concentrations of LTD_4, 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 LTD_4 diluted in dH_2O (1 mg/mL) (Merck

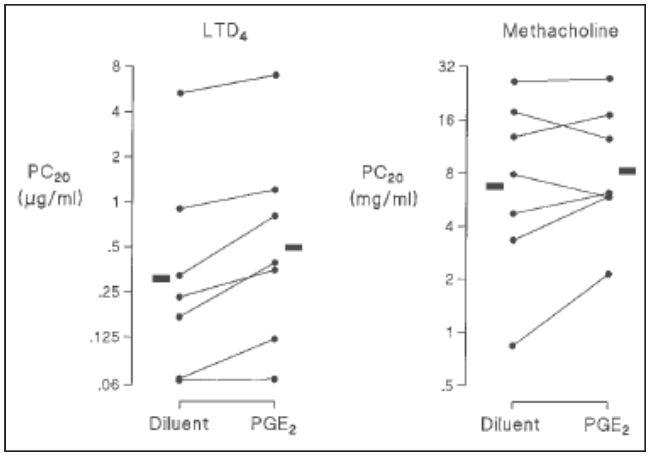

Figure 1) *Effect of inhaled prostaglandin (PG)E_2 or its diluent on leukotriene (LT)D_4 and methacholine airway responsiveness, expressed as the provocation concentration causing a 20% fall in forced expiratory volume in 1 s (PC_{20}). Pretreatment with PGE_2 caused a slight but significant increased in the LTD_4 PC_{20} (P=0.008), but not the methacholine PC_{20} (P=0.28)*

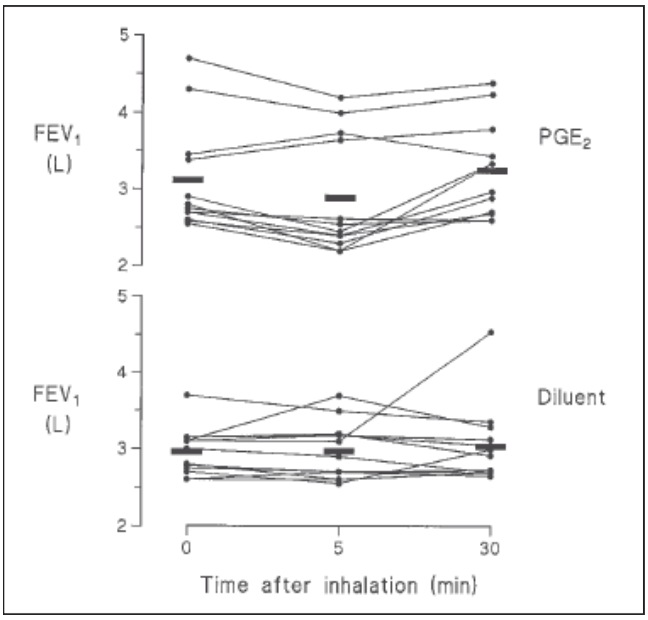

Figure 2) *Effect of inhaled diluent and prostaglandin (PG)E_2 on the baseline forced expiratory volume in 1 s (FEV_1). PGE_2 caused a significant decrease in the FEV_1 5 mins after PGE_2 pretreatment (P=0.016), which was no longer significantly reduced 30 mins after the PGE_2. The FEV_1 was not significantly altered after inhaled diluent at 5 mins and 30 mins*

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 FEV_1 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 FEV_1 of 20% or greater occurred, the test was terminated and the concentration of LTD_4 required to produce a fall in FEV_1 of 20% was calculated and expressed as the LTD_4 PC_{20}. After the test, two puffs of salbutamol (200 mg) were given to reverse bronchoconstriction.

Analysis: Statistical analyses were performed using the STATISTICA (StatSoft Inc, Oklahoma) computer software program. Data distributions were checked for normality using Kolmogorov-Smirnoff and χ^2 analysis. Because PC_{20} values are log-normally distributed, log transformed methacholine and LTD_4 PC_{20}s were used to compare the effect of diluent and PGE_2. The results were also evaluated as the maximal fall in FEV_1 after the highest inhaled concentration of inhaled LTD_4 or methacholine used after diluent preteatment. FEV_1 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 PGE_2 slightly, but significantly, improved LTD_4 airway responsiveness. The geometric mean LTD_4 PC_{20} increased from 2.00 mg/mL (%SEM 1.65) after diluent to 3.01 mg/mL (%SEM 1.64) after PGE_2 (P=0.008) (Figure 1). The LTD_4 PC_{20} increased in six subjects and was unchanged in one. By contrast, inhaled PGE_2 did not significantly attenuate methacholine airway responsiveness. The geometric mean methacholine PC_{20} was 1.28 mg/mL (%SEM 1.68) after diluent and 1.62 mg/mL (%SEM 1.46) after PGE_2 (P=0.28) (Figure 1).

The results were also evaluated as the maximal fall in the FEV_1 after the highest inhaled concentration of LTD_4 and methacholine after diluent and the fall in FEV_1 after the same concentrations of the agonists after inhaled PGE_2. According to this analysis, inhaled PGE_2 again slightly, but significantly, reduced the maximal fall in FEV_1 after inhaled LTD_4 from a mean value of 24.8% (SEM 2.8%) after diluent to 17.3% (SEM 2.8%) after PGE_2 (P=0.04) but not after inhaled methacholine, which was 27.5% (SEM 3.3%) after diluent and 22.03% (SEM 3.6%) after PGE_2 (P=0.31).

The initial mean baseline FEV_1 values before diluent on the two days on which diluent was inhaled was 2.96 L (SEM 0.08), and before PGE_2 on the two days on which PGE_2 was inhaled it was 3.10 L (SEM 0.19) (P=0.43) (Figure 2). The FEV_1 significantly decreased by 0.23 L (SEM 0.08) (P=0.016) 5 mins after inhaled PGE_2 (Figure 2), but was no longer significantly reduced by 30 mins after PGE_2. Inhaled diluent had no significant effect on the FEV_1 at either 5 or 30 mins after inhalation (Figure 2).

In all subjects, inhaled PGE_2 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 PGE_2 significantly attenuates airway responsiveness to inhaled LTD_4, but not to methacholine, in asthmatic subjects. These results suggest that inhaled PGE_2 selectively attenuates LTD_4-induced bronchoconstrictor responses. However, the lack of effect of inhaled PGE_2 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 mediators 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.

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