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The reproducibility and effect on non-specific airway responsiveness of inhaled prostaglandin D_2 and leukotriene D_4 in asthmatic subjects

RICHARD WOOD-BAKER, G. IAN TOWN, BALDEV BENNING & STEPHEN T. HOLGATE
Medicine 1, Southampton General Hospital, Tremona Road, Southampton, UK

1 Mast cell mediators PGD_2 and LTD_4 may play important roles in asthma pathogenesis. There is little information on the repeatability of inhalation challenge with these agonists in the laboratory.

2 We assessed the repeatability of inhalation challenges using PGD_2 and LTD_4 in two groups of 10 asthmatic volunteers. Non-specific bronchial responsiveness was assessed by histamine inhalation challenges.

3 Using the Bland-Altman method, we found the coefficient of repeatability to be 1.2 doubling doses for LTD_4 and 2.1 for PGD_2 at a 1 week interval. Repeatability for histamine inhalation challenge over the same time period was similar at 1.4 and 2.1 doubling doses respectively.

4 Non-specific bronchial responsiveness following LTD_4 challenge decreased significantly, mean $PD_{20}FEV_1$ increasing from 169 nmol on day 1 to 278 nmol on day 3 ($P = 0.001$), before returning to baseline levels.

5 A progressive decrease in non-specific bronchial responsiveness occurred following PGD_2 challenge. Baseline $PD_{20}FEV_1$ was 195 nmol, increasing to 238 nmol by day 3 (NS) and 313 nmol by day 8 ($P = 0.016$).

6 PGD_2 inhalation challenges performed a week apart are less reproducible than LTD_4 challenges, possibly as a result of significant changes in histamine bronchial responsiveness. Our findings allow accurate power calculations to be made for studies to assess new pharmacological antagonists to these mediators.

Keywords prostaglandin D_2 leukotriene D_4 bronchial responsiveness asthma inhalation challenge

Introduction

The inherent variability of clinical asthma in the community creates difficulties in the study of pathophysiology and treatment. To overcome this laboratory based models of asthma have been developed, largely based on measurement of the bronchoconstrictor response to inhaled provocants. Utilising the response to inhaled allergen, a number of mediators have been identified which are thought to play a role in the early and late phase bronchoconstriction [1]. The mast cell has been implicated as the source of many of these mediators, as evidenced by degranulation during acute attacks [2] and enhanced spontaneous and stimulated mediator secretion [3,4]. In addition to the preformed mediator histamine, contained within the secretory granules, the mast cell is able to produce prostaglandin D_2 (PGD_2) via the cyclooxygenase [5] and leukotriene D_4 (LTD_4) via the lipoxygenase pathways of arachidonic acid metabolism [6]. PGD_2 and LTD_4 have well recognised bronchoconstrictor actions as well as other pro-inflammatory actions which may contribute to the pathophysiology of asthma.

Based on the mediator hypothesis, strategies in asthma research have included developing specific pharmacological antagonists. Assessment of new antagonists often includes their ability to prevent

Correspondence: Dr R. Wood-Baker, Department of Respiratory Medicine, Royal Hobart Hospital, GPO Box 1061 L, Hobart, Tasmania 7001, Australia

mediator-induced bronchoconstriction, measured by inhalation challenge in asthmatic subjects. There is little published data on the repeatability of many types of inhalation challenge or their effect on non-specific bronchial reactivity. This study aimed to assess the repeatability of inhalation challenges with PGD_2 and LTD_4 and their effect on non-specific bronchial reactivity.

Methods

Subjects

Seventeen non-smoking asthmatic volunteers, mean age 39.3 (range 18 to 57) years, participated in the study. Thirteen of the subjects were female and fifteen atopic, defined as a >2 mm wheal response to at least two from a range of common allergens (Bencard, Brentford, UK). All subjects had a minimum two year history of intermittent breathlessness and wheezing, were current non-smokers and had a ≥20% variability in their forced expiratory volume in one second (FEV_1) spontaneously or following bronchodilator administration. The mean FEV_1 at recruitment was 2.55 (s.d. 0.59) l, mean 92 (s.d. 16) % of predicted values in the LTD_4 group and 2.60 (s.d. 0.56) l, mean 84 (s.d. 16) % of predicted values in the PGD_2 group. All subjects had hyperresponsive airways, defined as a provocation concentration of histamine which reduced the FEV_1 by ≥20% from post-saline ($PC_{20}FEV_1$) of <8 mg ml^{-1}. All subjects were taking intermittent inhaled β-adrenoceptor agonist bronchodilator treatment and 10 were taking regular inhaled corticosteroids at doses ranging from 400 to 1500 μg daily. Bronchodilators were withheld for 8 h prior to each study day but inhaled corticosteroids were continued at their usual dose, which remained constant throughout the study period. Subjects gave written informed consent and the study was approved by the Southampton University and Hospitals Ethics Committee.

Study design

The design was that of a two arm (LTD_4 or PGD_2) open study, each involving 10 subjects in three visits to the department over an 8 day period. On day 1, following a 10 min rest period two baseline measurements of FEV_1 were made at 5 min intervals. Providing the measurements did not vary by >10% when subjects were asked to return on a subsequent day, a histamine inhalation challenge was performed. After completing the histamine challenge subjects remained resting in the department until the FEV_1 had spontaneously recovered to within 10% of baseline. An inhalation challenge with LTD_4 or PGD_2 was then performed after which any residual bronchconstriction was reversed using inhaled salbutamol. On day 3 subjects underwent a single histamine inhalation challenge and on day 8 subjects repeated the protocol of day 1, undergoing a histamine inhalation challenge followed by a LTD_4 or PGD_2 inhalation challenge after spontaneous recovery.

Agonist solutions

Histamine acid phosphate (BDH Ltd, Poole, UK) was dissolved in 0.9% sodium chloride solution (saline) buffered to pH 7.4 (Baxter Healthcare Ltd, Thetford, UK) and stored as a stock solution of 64 mg ml^{-1} in 4 ml aliquots at −20° C. On each study day an aliquot of stock solution was thawed immediately prior to use and diluted with saline to produce a range of doubling concentrations between 0.03 and 32 mg ml^{-1}. Leukotriene D_4 (Ultrafine Chemicals, Manchester, UK) was stored at −20° C in 1 ml aliquots dissolved in 20% ethanol in protein buffered saline. On each study day an aliquot of stock solution was thawed immediately prior to use and dissolved in phosphate buffer to produce a range of concentrations between 0.125 and 16 μg ml^{-1} which were stored on ice until use. Prostaglandin D_2 (Ultrafine Chemicals, Manchester, UK) was stored as a stock solution in 1 ml aliquots at −20° C dissolved in absolute ethanol. On each study day an aliquot was diluted in saline immediately prior to use to give a range of concentrations between 1.95 and 250 μg ml^{-1} which were stored on ice until use.

Inhalation challenges

Measurements of FEV_1 were made using a rolling seal spirometer (Morgan Spiroflow, PK Morgan Ltd, Kent, UK) connected via an interface to a desk top computer (Macintosh Plus, Apple Computer UK Ltd, Hertfordshire, UK). For all inhalation challenges a single measurement of FEV_1 was made at each time point unless the effort was felt to be sub-maximal. The lowest FEV_1 at each agonist concentration was subsequently used for analysis. Solutions were administered using an Inspiron Mini-neb nebuliser (CR Bard International, Sunderland, UK) and for all challenges subjects were instructed to take 10 breaths from functional residual capacity to total lung capacity while wearing a nose-clip. The nebulisers were driven by a dosimeter set to deliver compressed air at a pressure of 20 PSI for 0.57 s. The delivery of agonist was determined by weighing the nebuliser pots after inhalation and under the conditions described a mean 88 (s.d. 6.2) μl was delivered to the mouth. The mass median particle diameter of the aerosol generated was 4.7 μm [7].

For histamine challenges subjects initially inhaled saline diluent with FEV_1 measurements at 1 and 3 min. Provided these fell within 10% of baseline measurements subjects immediately proceeded to inhale the lowest concentration of histamine solution. Measurements of FEV_1 were repeated at 1 and 3 min and increasing concentrations of histamine inhaled until a ≥20% fall in FEV_1 had been achieved. For LTD_4 and PGD_2 challenges subjects initially inhaled diluent with FEV_1 measurements at 1, 3 and 5 min. Provided these fell within 10% of the baseline FEV_1 subjects immediately inhaled the lowest concentration

of agonist with further measurements of FEV_1 at 1, 3 and 5 min until a ≥20% fall had been achieved.

Data analyses

The mean of two baseline FEV_1 measurements were used to compare airway calibre between study days by two-way analysis of variance (ANOVA) with subject and study day variables. For all inhalation challenges the successive doses of agonist administered were calculated from the product of the agonist concentration and volume inhaled (88 µl). The lowest FEV_1 measurement at each concentration was used for analysis and expressed as a percentage of the lowest post-diluent measurement. This was plotted on a linear scale against the cumulative dose of agonist administered, expressed on a logarithmic scale. The provocation dose of agonist causing a 20% fall in FEV_1 ($PD_{20}FEV_1$) was derived from the linear portion of the dose-response curve by interpolation.

The $PD_{20}FEV_1$ data expressed in molar terms were subjected to log transformation before statistical analysis. Any relationship between histamine bronchial reactivity and the $PD_{20}FEV_1$ for LTD_4 or PGD_2 was analysed by linear regression of the values from day 1. Between days comparison of challenge procedures was performed using a two-tailed Student's *t*-test. The repeatability of the agonist inhalation challenges was assessed using the Bland-Altman method [8]. The mean $PD_{20}FEV_1$ from the two visits was plotted against the difference between the means, both expressed on a logarithmic (base 2) scale. The standard deviation of the differences from the mean was then calculated from which the coefficient of repeatability was derived.

Results

Baseline measurements

There was no significant difference in baseline FEV_1 measurements between study days, with baseline and post-diluent FEV_1 measurements falling within 10% for all subjects on all visits. The subjects participating in the PGD_2 arm had slightly less reactive airways with a geometric mean baseline histamine PD_{20} FEV_1 of 195 nmol compared with 169 nmol for the LTD_4 group. This difference was not statistically significant ($P = 0.8$).

Leukotriene D_4 arm

All subjects had a dose related fall in FEV_1 folowing inhalation of histamine or LTD_4, allowing calculation of $PD_{20}FEV_1$ values for all challenges (Table 1). On day 1 inhaled LTD_4 was approximately 450 times more potent than histamine as a bronchoconstrictor in asthmatic airways. There was no correlation betwen the $PD_{20}FEV_1$ values for histamine and LTD_4 ($r = 0.47$, $P = 0.17$). All subjects demonstrated a reduction in airway reactivity by day 3, with a significant increase in the geometric mean $PD_{20}FEV_1$ for histamine ($P = 0.001$). By day 8 airway reactivity had returned to baseline levels. There was no significant difference between the $PD_{20}FEV_1$ values for LTD_4 on days 1 and 8 ($P = 0.6$). The calculated coefficient of repeatability for LTD_4 inhalation challenge was 1.2 (Figure 1), similar to the corresponding coefficient for histamine of 1.4.

Prostaglandin D_2 arm

All subjects had a dose related fall in FEV_1 following inhalation of histamine or PGD_2, allowing calculation of $PD_{20}FEV_1$ values for all challenges (Table 1). On day 1 inhaled PGD_2 was approximately 8 times more potent as a bronchoconstrictor than histamine. There was no correlation between the $PD_{20}FEV_1$ values for histamine and PGD_2 ($r = 0.55$, $P = 0.1$). There was a progressive reduction in airway reactivity throughout the study period such that by day 8 histamine $PD_{20}FEV_1$ values were significantly different from day 1 ($P = 0.02$). There was no significant difference between the geometric mean $PD_{20}FEV_1$ for PGD_2 on days 1 and 8 ($P = 0.2$). The coefficient of repeatability for PGD_2 inhalation challenge was 2.1 (Figure 1), identical to that for the corresponding histamine challenges.

Table 1 The $PD_{20}FEV_1$ (nmol) results for inhalation challenges

	LTD_4		Histamine			PDG_2		Histamine		
Subject	*Day 1*	*Day 8*	*Day 1*	*Day 3*	*Day 8*	*Day 1*	*Day 8*	*Day 1*	*Day 3*	*Day 8*
1	6.86	4.12	166	327	349	100.0	64.7	358	297	272
2	0.34	0.49	215	312	228	24.0	20.9	70	115	161
3	0.18	0.24	123	247	72	4.4	7.1	108	48	63
4	0.15	0.09	202	340	336	35.6	91.0	510	1207	1353
5	0.26	0.17	50	92	70	82.6	47.4	854	1351	1011
6	0.13	0.21	98	301	209	20.5	76.6	297	560	976
7	0.09	0.06	90	102	57	4.4	7.8	186	187	273
8	0.74	1.04	530	837	383	5.6	9.6	97	79	154
9	0.37	0.21	101	131	58	212.5	114.4	213	438	599
10	1.25	1.43	901	1058	823	13.5	34.8	58	61	96
GM*	0.38	0.36	169	278	176	23.2	31.4	195	238	313
GSD**	0.003	0.003	2.3	2.2	2.5	3.6	2.7	2.3	3.1	2.7

*GM = geometric mean.
**GSD = geometric standard deviation.

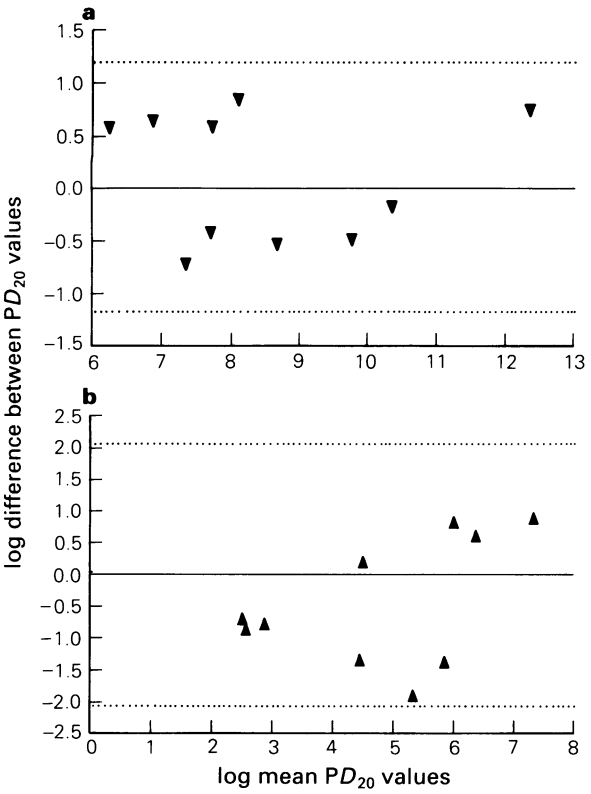

Figure 1 Bland-Altman plots of repeated inhalation challenges with leukotriene D_4 (a) and prostaglandin D_2 (b) one week apart in ten asthmatic subjects.

Discussion

In this study the characteristic bronchoconstrictor response seen in asthmatic subjects was used to construct dose-response curves to histamine, LTD_4 and PGD_2. This allowed accurate assessment of the repeatability of inhalation challenges using the Bland-Altman technique. Calculation of the coefficient of repeatability defines the 95% confidence interval within which repeated tests would be expected to fall and overcomes the difficulties associated with use of correlation coefficients. Transformation of the results to log base 2 allows expression of the coefficient of repeatability in terms of doubling doses of agonist administered, the standard increment for inhalation challenges. The coefficient of repeatability for LTD_4 inhalation challenge in this study was 1.2, similar to that of histamine inhalation challenge. Repeated histamine inhalation challenges would be expected to fall within ± 1.4 doubling doses, similar to that reported by other investigators [9–11]. Following LTD_4 challenge all subjects had an increase in histamine $PD_{20}FEV_1$, consistent with a reduction in non-specific bronchial responsiveness. Although the change in $PD_{20}FEV_1$ lay within the 95% confidence intervals of the test for all but one subject, the consistency of this increase suggests it is unlikely to be due to chance. Bronchial reactivity measured by histamine inhalation challenge remains stable over the time frame of our study [12]. This, together with the overlapping of study period for different subjects make changes due to external environmental influences an unlikely explanation of our findings.

Previous studies of the effect of exogenous leukotrienes have not found any significant effect on non-specific airway responsiveness in non-asthmatics [13]. In asthmatic subjects constrictor doses of leukotrienes increase airways responsiveness in the initial hours following inhalation [14]. This change appears short lived, the increase seen 4 h after leukotriene inhalation was returning towards baseline levels when next assessed at 7 h. Continuation of this late reduction in bronchial responsiveness is consistent with our finding of a significant increase in $PD_{20}FEV_1$ values at 72 h. Reduced non-specific bronchial reactivity following LTD_4 inhalation seems inconsistent with the hypothesis that in asthma sulphidopeptide leukotrienes are involved in generating bronchial hyperresponsiveness. However, their ability to change bronchial responsiveness would explain the disparate study results and the variation seen in clinical asthma and following allergen inhalation. Leukotriene involvement in asthma is supported by elevated concentrations in bronchoalveolar lavage (BAL) of resting asthmatic subjects [15] and from increased BAL [16] and urine [17] concentrations following allergen challenge.

Prostaglandin D_2 inhalation challenge was not as repeatable as LTD_4, with a coefficient of repeatability of 2.1 which was identical to that of histamine in this group of subjects. The progressive reduction in non-specific airway responsiveness also seems inconsistent with PGD_2 contributing to the development of bronchial hyperresponsiveness in asthma. Although a potent bronchoconstrictor in asthmatic airway when administered exogenously [18], the effect on bronchial responsiveness has not been well studied and interaction with other bronchoconstrictor mediators has not been conclusively demonstrated [19, 20]. Interaction between inhaled agonists has been demonstrated for simultaneously administered LTC_4 and histamine, although this was small and significant only for maximum flow at 70% of vital capacity below total lung capacity [21]. Given the spacing between challenges in our study any effect of histamine pre-inhalation on PGD_2 or LTD_4 challenge is likely to be small. In addition a consistent effect would be expected, minimising any influence on the coefficient of repeatability. While tachyphylaxis to hsitamine has been clearly demonstrated *in vitro*, its existence *in vivo* remains controversial. In asthmatic subjects tachyphylaxis may be related to the dose of histamine delivered to the airways [22]. This makes it unlikely to be an important confounder in our study which involved subjects with marked bronchial hyperresponsiveness.

In conclusion, we have shown that the coefficient of repeatability for LTD_4 and PGD_2 inhalation challenge of asthmatic airways is similar to that of inhaled histamine, falling within 1–2 doubling dilutions. Although both agonists caused a reduction in bronchial reactivity we are unable to explain, knowledge of the coefficient of repeatability enables accurate power calculations to be performed and

confident assessment of the protective efficacy of prostanoid and leukotriene receptor antagonists.

References

1 Howarth P, Durham S, Lee T, Kay B, Church M, Holgate S. Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. *Am Rev resp Dis* 1985; **132**: 986–992.

2 Connell J. Asthmatic deaths: role of the mast cell. *J Am med Ass* 1971; **215**: 769–772.

3 Agius R, Godfrey R, Holgate S. Mast cell and histamine content of human bronchoalveolar lavage fluid. *Thorax* 1985; **40**: 760–767.

4 Lebel B, Bousquet J, Chanez P. *et al.* Spontaneous and non-specific release of histamine and PGD_2 by bronchoalveolar lavage cells from asthmatic and normal subjects: effect of nedocromil sodium. *Clinical Allergy* 1988; **18**: 605–613.

5 Lewis R, Soter N, Diamond P, Austen K, Oates J, Roberts L. Prostaglandin D_2 generation after activation of rat and human mast cells with anti-IgE. *J Immunol* 1982; **129**: 1627–1631.

6 MacGlashan D, Schleimer R, Peters S. *et al.* Generation of leukotrienes by purified human lung mast cells. *J clin Invest* 1982; **70**: 747–751.

7 Lewis RA. Therapeutic aerosols. In *Drugs and the lung*, eds Cumming G, Bonsignore C, London: Plenum Publishing, 1984: 63–89.

8 Bland J, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **i**: 307–310.

9 Britton J, Mortagy A, Tattersfield A. Histamine challenge testing: comparison of three methods. *Thorax* 1986; **41**: 128–132.

10 Dehaut P, Rachiele A, Martin R, Malo J. Histamine dose-response curves in asthma: reproducibility and sensitivity of different indices to assess response. *Thorax* 1983; **38**: 516–522.

11 Ryan G, Dolovich M, Roberts R, *et al.* Standardization of inhalation provocation tests: Two techniques of aerosol generation and inhalation compared. *Am Rev resp Dis* 1981; **123**: 195–199.

12 Ruffin R, Alpers J, Crockett A, Hamilton R. Repeated histamine inhalation tests in asthmatic patients. *J Allergy clin Immunol* 1981; **67**: 285–289.

13 Black P, Fuller R, Taylor G, Barnes P, Dollery C. Effect of inhaled leukotriene B_4 alone and in combination with prostaglandin D_2 on bronchial responsiveness to histamine in normal subjects. *Thorax* 1989; **44**: 491–495.

14 O'Hickey S, Hawksworth R, Fong C, Arm J, Spur B, Lee T. Leukotrienes C_4, D_4 and E_4 enhance histamine responsiveness in asthmatic airways. *Am Rev resp Dis* 1991; **144**: 1053–1057.

15 Wardlaw A, Hay H, Cromwell O, Collins J, Kay A. Leukotrienes, LTC_4 and LTB_4, in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. *J Allergy clin Immunol* 1989; **84**: 19–26.

16 Chan-Yeung M, Chan H, Tse K, Salari H, Lam S. Histamine and leukotriene release in bronchoalveolar fluid during plicatic acid-induced bronchoconstriction. *J Allergy clin Immunol* 1989; **84**: 762–768.

17 Manning P, Rokach J, Malo J-L. Urinary leukotriene E_4 levels during early and late asthmatic responses. *J Allergy clin Immunol* 1990; **86**: 211–220.

18 Hardy C, Robinson C, Tattersfield A, Holgate S. The bronchoconstrictor effect of inhaled Prostaglandin D_2 in normal and asthmatic men. *New Engl J Med* 1984; **311**: 209–213.

19 Hardy C, Bradding P, Robinson C, Holgate S. The combined effects of two pairs of mediators, adenosine with methacholine and prostaglandin D_2 with histamine, on airway calibre. *Clin Sci* 1986; **71**: 385–392.

20 Fuller R, Dixon C, Dollery C, Barnes P. Prostaglandin D_2 potentiates airway responsiveness to histamine and methacholine. *Am Rev resp Dis* 1986; **133**: 252–254.

21 Phillips G, Holgate S. Interaction of inhaled LTC_4 with histamine and PGD_2 on airway calibre in asthma. *J appl Physiol* 1989; **66**: 304–312.

22 Polosa R, Finnerty J, Holgate S. Tachyphylaxis to inhaled histamine in asthma: its significance and relationship to basal airway responsiveness. *J Allergy clin Immunol* 1990; **86**: 265–271.

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