

Original article

Relationship between airway hyperresponsiveness to mannitol and adenosine monophosphate

Background: Assessment of airway hyperresponsiveness (AHR) to indirect bronchoconstrictor stimuli is a useful noninvasive tool in the evaluation of asthma and its treatment. We investigated the putative relationship in AHR between inhaled adenosine monophosphate and mannitol.

Methods: Fifteen mild-to-moderate atopic asthmatics were evaluated. On two separate screening days, the threshold AMP concentration and threshold mannitol dose to provoke a given fall in FEV₁ were measured.

Results: For AMP PC₂₀ vs. mannitol PD₁₅, the Pearson's correlation coefficient was 0.80, $P < 0.001$. For AMP PC₁₅ vs. mannitol PD₁₅ and AMP PC₁₀ vs. mannitol PD₁₀ corresponding values were 0.83, $P < 0.001$ and 0.68, $P = 0.005$.

Conclusions: There was a highly significant association between the threshold concentration of AMP and dose of mannitol causing a given fall in FEV₁. Further studies are required to evaluate the relationship between inhaled mannitol and other surrogate inflammatory markers.

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Asthma is characterized by airway hyperresponsiveness (AHR), which results in episodic bronchoconstriction. In the laboratory, AHR is most commonly assessed using direct bronchoconstrictor stimuli such as methacholine or histamine, while it can also be assessed using indirect bronchoconstrictor stimuli such as adenosine monophosphate (AMP) (1). AMP-induced bronchoconstriction occurs following stimulation of mast cells and neuronal cells, suggesting it to be of greater relevance in the evaluation of asthma than directly acting agents (2, 3). Indeed, the provocative concentration of AMP required to cause a 20% fall in forced expiratory volume in 1 s (FEV₁; PC₂₀), is more closely correlated to the extent of airway eosinophilia and response to steroids than AHR to methacholine (4–6).

The inhalation of dry powder mannitol has been developed as a novel indirect stimulus for assessing AHR in asthmatics (7). From a practical point of view, it does not require a nebulizer and can be performed outside the laboratory environment using a simple hand held inhaler device. Moreover, when compared to another indirect challenge, for example, exercise, the use of mannitol results in a more controlled reduction of lung function. This is due to the carefully observed sequential fall in FEV₁ after each inhalation, allowing the procedure to be stopped immediately after the desired reduction. This, in turn, reduces the chance of excessive airway narrowing.

Inhaled mannitol causes a rise in the osmolarity, resulting in the release of a variety of inflammatory

mediators from cells on the respiratory epithelial surface (8). These in turn cause airway narrowing. Furthermore, there are similarities in the mechanism of action of mannitol and AMP in that bronchoconstriction ultimately results from the effects of mediators released from inflammatory cells.

The relationship between the sensitivity to inhaled mannitol and AMP (a more widely used tool to measure AHR) has not previously been investigated. The object, therefore, of this study was to evaluate the putative association between these two different indirectly acting stimuli in terms of their ability to provoke a reduction in FEV₁. We elected to compare the relationship between different doses of mannitol and concentrations of AMP to provoke a given fall in FEV₁ as other work has evaluated, for example, the relationship between the mannitol PD₁₀ and provocation with eucapnic voluntary hyperpnea (9). Moreover, for this latter challenge, a fall in FEV₁ of 10% or more is regarded as an abnormal response (10).

Materials and methods

Study design

Mild-to-moderate persistent atopic asthmatics were enrolled at random from our database of asthma volunteers into a double blind randomized placebo controlled crossover trial (11). The first two occasions served as screening days to assess eligibility and determine AHR to AMP and mannitol.

Both screening visits to the department occurred at the same time of day (8 AM to 12 noon), within a 2 h window for each subject, and within 2 weeks of one another. A minimum of 3 days was allowed between either screening bronchial challenges, which were performed in random sequence. On both mornings, inhaled corticosteroids were withheld and short acting β_2 -agonists withheld for 8 h. The inhaled corticosteroid dose remained unchanged prior to each visit and any other second line anti-asthma medication was not allowed.

Subjects

All subjects were required to have asthma according to American Thoracic Society criteria, and have an FEV₁ of >60% predicted prior to performing a bronchial challenge test for safety reasons (12). A skin prick test required to be positive to at least one common aeroallergen. For 3 months before screening, all patients were using a short-acting β_2 -agonists alone, or maintained on a constant dose of inhaled corticosteroid, had no history of respiratory tract infection and no oral corticosteroid use. Subjects gave written consent and the Tayside committee on medical research ethics gave approval.

Spirometry

Spirometry was carried out according to American Thoracic Society criteria (13). This was performed using a vitalograph compact spirometer (Vitalograph Ltd., Buckinghamshire, UK) with a pneumotachograph head, which was calibrated daily. On both screening days, all patients were required to have a baseline FEV₁ within 5% of one another.

Adenosine monophosphate bronchial challenge

Fresh solutions of AMP (Sigma, Poole, UK) were made up in distilled water in a range of doubling concentrations from 0.09 to 800 mg/ml on a daily basis. Bronchial challenge testing was carried out using a Nebiheck nebulizer controller (PK Morgan Ltd., Rainham, Kent, UK) with a system 22 Acorn nebulizer (Medicaid Ltd., Pagham, West Sussex, UK) with a driving pressure of 20 psi (138 kPa). Subjects inhaled five breaths of distilled water to act as a baseline and AMP was subsequently administered in doubling concentrations. Spirometry was then performed in triplicate, beginning 90 s after the last inhalation of distilled water or AMP. The inhalation of AMP was continued at 90 s intervals until a fall in FEV₁ greater than or equal to 20% of the distilled water baseline was recorded. The AMP threshold concentration to cause a 20, 15 and 10% fall in FEV₁ (PC₂₀, PC₁₅ and PC₁₀, respectively) was calculated by linear interpolation of the log dose-response curve. In other words, AHR to AMP was expressed in a noncumulative fashion.

Mannitol bronchial challenge

Mannitol was obtained preprepared after spray drying (Buchi 190 Mini Spray Dryer, Flawil, Switzerland) a solution of 15 mg/ml. The particle size of mannitol was measured using a multi-stage liquid impinger (Astra Pharmaceuticals, Lund, Sweden). The mannitol powder used had 66% of the particles by mass under 7 μ m in diameter. An Inhalator device (Boehringer Ingelheim Pty, Ltd, Ingelheim, Germany) was used to deliver the powered mannitol.

Spirometry was initially measured in triplicate to establish a baseline value. Patients were then asked to inhale sequential doses

of 0 (empty capsule acting as placebo), 5, 10, 20, 40, 80, 160, 160 and 160 mg of mannitol via the inhalator. After each inhalation, patients were required to hold their breath for 5 s. At 60 s intervals after completion of inhalation, spirometry was measured in triplicate and the highest FEV₁ was recorded. If patients had a 10% or greater fall in FEV₁ from the previously measured, the same dose of mannitol was given. Once a 20% fall in FEV₁ from the empty capsule was reached or a cumulative dose of 635 mg had been given, the bronchial challenge ceased. The mannitol cumulative dose to cause a 15 and 10% fall in FEV₁ (PD₁₅ and PD₁₀, respectively) was calculated by linear interpolation of the log dose-response curve.

Statistical analysis

All data were analysed using SPSS for Windows (Statistical Products and Service Solutions Inc., Chicago, IL). Prior to performing a Pearsons correlation, AMP and mannitol data were logarithmically transformed. An Altman Bland plot was performed to assess the repeatability of both challenges on screening and placebo days (14).

Results

The demographic data of the 15 subjects fulfilling eligibility criteria are shown in Table 1.

For AMP PC₂₀ vs. mannitol PD₁₅ (the most commonly used ways of expressing AMP and mannitol data), Pearsons correlation coefficient was 0.80, $P < 0.001$ (Fig. 1A). For AMP PC₁₅ vs. mannitol PD₁₅ and AMP PC₁₀ vs. mannitol PD₁₀, the corresponding values were 0.83, $P < 0.001$ and 0.68, $P = 0.005$ (Fig. 1B and C).

Figure 2 illustrates the repeatability on screening and placebo days of the AMP PC₂₀ and mannitol PD₁₅.

Discussion

Bronchial provocation with indirect stimuli is becoming more common and assessment of AHR to AMP is widely used in Europe for this purpose. Similarly, hypertonic stimuli such as 4.5% saline can be used, and more recently, inhaled dry powder mannitol has been introduced. Our results have demonstrated that there is a highly significant association, evident throughout several doses and concentrations required to provoke a particular fall in FEV₁, between the sensitivity to inhaled mannitol and AMP in mild-to-moderate atopic asthmatics. The Altman Bland plot (Fig. 2) also demonstrated a close agreement between performing the two tests on separate days.

AMP and mannitol act by different mechanisms to release inflammatory mediators; however, the airway narrowing results appear to follow a similar time course (1, 15). AMP causes the release of mediators through the activation of A2 receptors on airway mast cells (3). In contrast, mannitol does not act on a specific receptor but is thought to cause mediator release from mast cells

Table 1. Demographic data at screening

Gender	Age (years)	FEV ₁ (% predicted)	FEF _{25–75} (% predicted)	ICS	ICS dose (µg)	SPT	AMP PC ₂₀ (mg/ml)	Mannitol PD ₁₅ (mg)
F	44	80	39	BDP	400	3	170	155
F	21	98	73	BDP	500	4	8.9	29
F	36	79	61	BUD	400	4	0.19	0.2
F	46	75	40	None		7	25	49
M	32	65	38	BDP	400	5	32	7
F	25	98	72	None		1	137	155
F	21	75	44	BDP	500	1	7	45
M	55	66	48	BDP	400	2	20	13
F	44	104	66	BDP	400	3	69	18
M	25	85	52	BDP	800	1	200	122
M	66	70	54	None		2	46	209
F	49	92	51	BDP	200	4	30	103
M	41	81	50	BDP	200	6	109	122
F	26	82	37	BDP	800	4	20	100
F	35	89	86	None		6	93	25
38 (3)		83 (3)	54 (4)				30 (14)	37 (18)

Summary statistics are shown in bold; values are shown as means (SE) except AMP PC₂₀ and mannitol PD₁₅, which are shown as geometric means (geometric SE). SPT, number of positive skin prick tests; ICS, inhaled corticosteroid daily dose; BUD, budesonide; BDP, beclomethasone dipropionate.

by the intracellular events relating to cell volume regulation in response to an increase in osmolarity (7). AMP and mannitol cause mediator release *in vitro* from human lung mast cells (6, 16, 17), while *in vivo*, drugs that prevent mediator release from mast cells such as nedocromil sodium inhibit the airway response to both AMP and mannitol (18, 19). Furthermore, montelukast (a leukotriene receptor antagonist), while having a minimal inhibitory effect on airway sensitivity to these stimuli, causes a more rapid recovery from airway narrowing to mannitol (15) and AMP (11). This in turn suggests that leukotrienes sustain the response to both AMP and mannitol. The inhibitory effects of nedocromil and montelukast documented with these indirect stimuli are not found with agents such as histamine and methacholine (20, 21).

AHR to various stimuli is an integral component in asthma of all severities, and often coexists despite normal or near normal lung function (22–24). Since indirect bronchoconstrictor stimuli cause the release of inflammatory mediators, the response is, dependent upon the presence of inflammatory cells and tends to be a better indicator of inflammatory status. The use of indirect bronchoconstrictor stimuli to assess AHR is, therefore, considered to have greater clinical relevance in everyday life than direct ones. For example, when used to assess changes in AHR following anti-inflammatory therapy, changes in the AMP PC₂₀ are more sensitive than changes in methacholine PC₂₀ (4, 5). In the dose-ranging study by Wilson et al. effects of inhaled budesonide were greater with AMP than with methacholine by approximately 1 doubling dilution (5). Furthermore, when also evaluating effects of inhaled corticosteroids, Van den Berge et al. demonstrated that reductions in AMP responsiveness were more closely related to decreases in

airway eosinophils than with methacholine (6). In terms of sensitivity between direct and indirect bronchial challenge, Holzer et al. evaluated the effects of broncho-provocation with both methacholine and eucapnic voluntary hyperpnea with dry air (25). In 42 patients with one or more respiratory symptoms in the previous year, nine of these had a positive methacholine challenge, while in addition to these nine subjects, a further 16 had a positive response to eucapnic voluntary hyperpnea. Thus, methacholine bronchoprovocation had only a 36% sensitivity towards the identification of exercise induced bronchoconstriction.

What is the everyday clinical relevance of assessing AHR? Sont et al. highlighted the usefulness of monitoring the degree of AHR to methacholine as a tool with which to guide treatment. In a study involving 75 patients, titrating inhaled corticosteroid dose in accordance to AHR plus symptoms and spirometry resulted in superior control than if the latter alone were used (26). There is also an increasing need for clinicians to have a rapid and easy-to-perform method of confirming or refuting the diagnosis of active asthma, especially when symptoms are equivocal and lung function normal. Thus, assessment of AHR may be a more useful tool than measures of airway calibre alone when the diagnosis is uncertain (27, 28).

In a study by Green et al. an asthma management strategy targeted against another surrogate inflammatory marker (sputum eosinophils) led to significantly fewer severe asthma exacerbations than using standard guidelines alone (29). However, the practicality of performing sputum induction in everyday clinical practice, especially in terms of obtaining instant feedback of the result for the patient in the clinic is questionable. Assessment of AHR may be a more practical tool and in this respect,

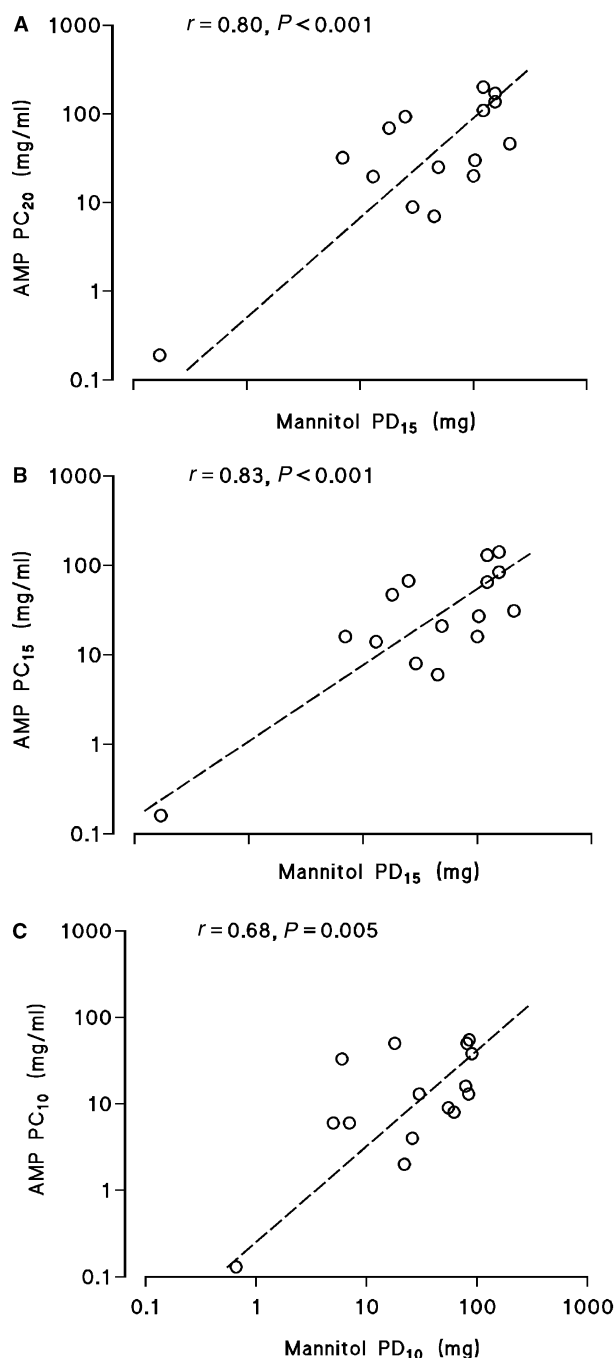


Figure 1. Correlations between (A) AMP PC₂₀ vs mannitol PD₁₅; (B) AMP PC₁₅ vs mannitol PD₁₅ and (C) AMP PC₁₀ vs mannitol PD₁₀.

knowledge of patients' AHR to mannitol has been shown to be useful in guiding the reduction of inhaled corticosteroid doses (30).

In conclusion, inhaled mannitol is a useful, practical means of assessing AHR and correlates closely to the

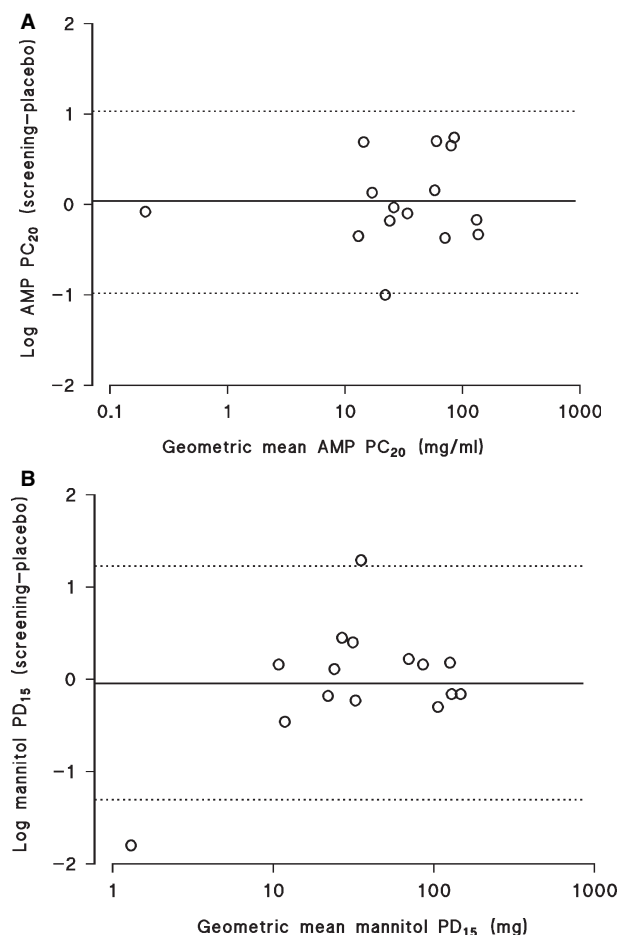


Figure 2. Altman Bland plots showing the repeatability between (A) AMP and (B) mannitol on screening and placebo days. The solid line indicates the point of no difference between days and the dashed lines illustrates the 95% CI.

airway response following inhaled AMP. Further studies are required to determine whether AHR to inhaled dry powder mannitol in the outpatient clinic, in conjunction with conventional markers of asthma control, leads to clinically relevant reductions in asthma exacerbations, than use of the latter alone.

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The use/application for mannitol described in this study is covered by US patent No 5 817 028 and internationally by PCT/AU95000086. The patent is owned by the Central Sydney Area Health Service and licensed to Pharmaxis Pty Ltd, Canberra, ACT, Australia.

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