

The bronchial response to mannitol is attenuated by a previous methacholine test: but not vice versa

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Summary

Aim To examine the airway response to inhaled mannitol performed before or after a methacholine challenge test in a group of asthmatics with different levels of disease.

Methods A total of 48 asthmatics, 18–73 years of age, were included in the study. Two pairs of challenges were performed in a random order on two separate days ≥ 24 h apart: either with mannitol performed first on day one, followed ≥ 1 h by methacholine, and methacholine as the first on day two, followed ≥ 1 h by mannitol or vice versa. A questionnaire-based interview was performed and lung function, exhaled nitric oxide, skin prick test, and blood eosinophil count were measured.

Results A total of 44% of the asthmatics used inhaled corticosteroids and 48% were atopic. The airway response to mannitol was attenuated when mannitol was given after methacholine, compared with the response to mannitol when it was given first [log response dose ratio (RDR): 1.42 vs. 1.60 ($P = 0.004$)], whereas the response to methacholine was unchanged in the opposite test order [log RDR: 0.81 vs. 0.96 ($P = 0.102$)].

Conclusion Bronchial challenges with inhaled mannitol and methacholine may be performed on the same day but provocation with mannitol should be performed before methacholine.

Keywords asthma, bronchial provocation tests, mannitol, methacholine, serial challenging

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Introduction

Airway hyperresponsiveness (AHR) is a common feature of asthma, and measurement of airway responsiveness can be performed using both direct and indirect stimuli. The direct challenges such as methacholine have a high sensitivity for the diagnosis of asthma and will consequently identify most patients with asthma, even mild cases. Therefore, this test is often used in uncharacteristic cases whereas a negative response to methacholine, in general, rules out asthma. On the other hand, indirect tests such as mannitol will often be negative in mild asthma, whereas subjects with airway inflammation often will be positive [1]. A positive response to an indirect test may therefore be used to confirm a diagnosis of asthma, and furthermore, to support a decision to start regular anti-inflammatory treatment. It can therefore be useful to conduct both challenges on a single day in order to strengthen the confidence in the diagnosis of asthma, in a hospital setting, in a specialist out-patient clinic, and in epidemiological studies. If one of the two tests is used on

its own, the sensitivity or the specificity will be moderate. Therefore, use of both tests at the same visit can save time and reduce the need for extra visits for re-evaluation.

The direct and indirect tests are named according to the chief mechanisms and pathways by which they induce airway limitation. Limited available data suggest that the bronchial response to an indirect stimulus provides a better indication of the presence of current airway inflammation – active asthma, when compared with a direct test such as methacholine. Methacholine seems to reflect smooth muscle dysfunction independent of the presence of airway inflammation [1–6]. More work needs to be carried out before this suggestion can either be trusted or discharged. The effect of the two pathways' responses on one another can be described by two competing hypotheses. One states that the two challenges will not affect one another, because of their different modes of action [6], whereas the response to two different indirect agents diminishes the response to the second test, possibly owing to mediator depletion as the cause of refractoriness [7–9]. The second hypothesis states that, besides binding to

receptors on the smooth muscle, direct challenges might bring about the release of inflammatory mediators as well. If this assumption is correct, mediator depletion after the first challenge can have an effect on the next challenge, independent of different physiological modes of action.

Few previous studies have compared the bronchial response to direct and indirect challenges performed on the same day because most indirect tests elicit an all-or-none, response often with a large decrease in the forced expiratory volume in 1 s (FEV₁). Hurwitz et al. [10] investigated the bronchial response to eucapnic voluntary hyperpnoea (EVH) and methacholine in a group of 12 asthmatics and found that challenge with methacholine to some degree diminished the subsequent response to EVH, whereas EVH only affected the bronchial response to methacholine in those with severe AHR. The study somewhat interfered with the hypothesis of the pathways, but it could be explained by the fact that the response of especially those with severe AHR was altered due to a severe decline in FEV₁ after EVH. The aim of the present study was to examine the airway response to the mannitol test, i.e. an indirect dose-response test, performed before or after a methacholine challenge test, in a group of asthmatics with different levels of disease.

Methods

Design

The two pairs of challenges (methacholine following mannitol and mannitol following methacholine) were performed on two separate days, reversing the sequence of the test series on the second day. The two challenges were performed 1-h apart or earlier if FEV₁ was $\geq 95\%$ of the baseline value. During the interval between the two tests, a skin prick test (SPT) was performed and blood samples were taken for measurement of the total eosinophil count. At their first visit, the patients were interviewed about respiratory symptoms by a physician and at both visits exhaled nitric oxide (eNO) was measured (Table 1). The second visit was scheduled ≥ 24 h, but no later than 1 week after the first. The patients were randomized through blinded block randomization with 10 patients in each block, eight in the last block.

Study population

Sixty subjects with symptoms of asthma were recruited through an advertisement during 2007 placed in a local newspaper in Copenhagen, Denmark. Inclusion criteria were an FEV₁ $> 70\%$ of predicted normal, plus claims of recurrent respiratory symptoms suggestive of asthma, i.e. cough, wheezing, chest tightness, and/or shortness of breath. Asthma was defined as respiratory symptoms and a positive challenge test, or a doctor's diagnosis of asthma

Table 1. Summary of the experimental protocol

	Randomization group 1		Randomization group 2	
	Day 1	Day 2	Day 1	Day 2
Mannitol challenge	X	X	X	X
Methacholine challenge	X	X	X	X
Reversibility test	X	X	X	X
eNO	X	X	X	X
FEV ₁ baseline	X	X	X	X
Blood eosinophils	X		X	
Skin prick test	X		X	
Interview	X		X	

Randomization group 1 had the methacholine challenge first on day 1 and the mannitol challenge first on day 2.

Randomization group 2 had the mannitol challenge first on day 1 and the methacholine challenge first on day 2.

eNO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s.

treated with inhaled steroid. Exclusion criteria were smoking within the preceding 12 months, a chronic pulmonary disorder other than asthma, and/or a chest infection within the preceding 4 weeks. All subjects were asked not to take antihistamines for 3 days before testing, or β_2 -agonists, and/or inhaled corticosteroids for 24 h before testing. Of the 60 subjects screened, 48 (80%) had asthma and completed both visits. The Ethics Committee of Copenhagen approved the study, and all subjects gave their informed written consent before enrolment (journal nr. KF01262754).

Lung function tests

Spirometric measurements of FEV₁ and forced vital capacity (FVC) were taken using a dry Vitalograph spirometer (Vitalograph Ltd, Maids Moreton, Buckingham, England). The ratios of the pre-provocation values of FEV₁ and FVC were used to calculate the degree of airway obstruction.

Methacholine challenge

The method of Yan et al. [11] was used to measure airway responsiveness to inhaled methacholine. A Spira nebulizer (Spira[®], Spira, Respiratory Care Unit Ltd, Hämeenlinna, Finland) generated the aerosols in the test solution. Each aerosol was inhaled through the mouth, starting with a dose of isotonic saline. Thereafter, five successively increasing doses of methacholine ranging from 0.06 to 8 μ mol were delivered until a cumulated dose of 8 μ mol or a 20% decrease in FEV₁ was reached. According to the standard procedure, the provocation was performed at half the dose if a 10% decrease in FEV₁ occurred. The response was measured by determining FEV₁ 1 min after each inhalation. Subjects with a cumulative dose of methacholine of ≤ 8 μ mol and a decrease in FEV₁ $\geq 20\%$ were diagnosed as having a positive test (AHR).

Mannitol challenge

Dry powder of mannitol (AridolTM) was supplied in a kit form (Pharmaxis Ltd, Frenchs Forest, NSW, Australia). The mannitol was encapsulated and delivered through an OsmohalorTM (Pharmaxis Ltd, Frenchs Forest, NSW, Australia) at doses of 0, 5, 10, 20, 40, 80, 160, 160, and 160 mg to a cumulated dose of 635 mg. The 80 and 160 mg were given in multiple doses of 40 mg capsules. FEV₁ was measured at least twice 60 s after each dose, and the highest FEV₁ was used in the calculations. The FEV₁ taken after the 0 mg mannitol capsule was taken as pre-challenge FEV₁ and used to calculate the percentage decline in FEV₁. In the case of a 10% decrease in FEV₁ from baseline, the former dose was repeated. The test continued until FEV₁ had declined 15% or the maximum cumulative dose of 635 mg was reached. Subjects with a provocation concentration of ≤ 635 mg and a decrease in FEV₁ of 15% or more were diagnosed as having a positive test [12].

Bronchial response

The response dose ratio (RDR) was defined as the percentage decline in FEV₁ divided by the dose of the last inhaled agent ($\Delta\%FEV_1/\mu\text{mol}$ or mg). The final dose of mannitol (mg) and methacholine (μmol) was estimated as the cumulated dose at the end of provocation. To eliminate negative and zero values, a constant of 7.5% was added to all the results before the logarithm of the RDR was calculated. Example 1 (methacholine test): $RDR = (\Delta\%FEV_1 + 7.5\%)/\mu\text{mol}$. Example 2 (mannitol test): $RDR = (\Delta\%FEV_1 + 7.5\%)/\text{mg} \times 1000$ (Fig. 1).

Skin prick test

SPTs were carried out with SoluprickTM (ALK Abelló, Hoersholm, Denmark) standardized allergens against the following allergens: birch, timothy grass, mugwort, horse, dog, cat, house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), Cladosporium, and Alternaria. Reactions were read after 15 min. Atopy was defined as at least one positive reaction with a mean diameter of at least 3 mm.

Exhaled nitric oxide measurement

eNO was measured with a chemiluminescence analyser (NIOX: Aerocrine AB; Solna, Sweden). Measurements were taken for 10 s at a constant flow rate of 50 mL/s during each exhalation procedure. All measurements were taken in triplicate and the mean concentration was recorded. A level of ≥ 20 p.p.b. was considered elevated.

GINA score and definition of asthma

Asthma was defined as current respiratory symptoms, i.e. cough, wheezing, chest tightness, and shortness of breath,

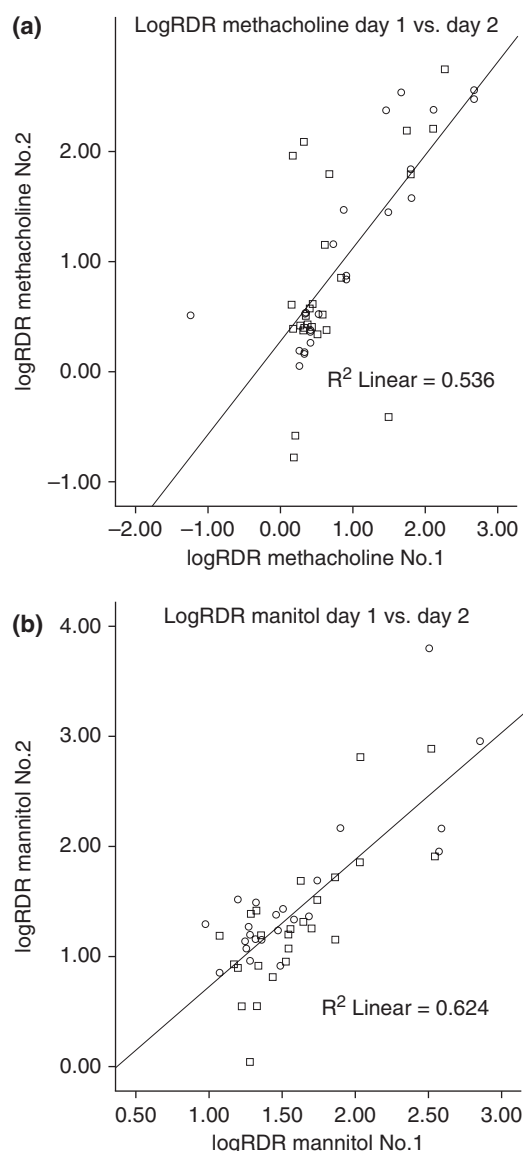


Fig. 1. (a) Response to methacholine is expressed as log response dose ratio (RDR). The round dots show the responsiveness to methacholine when taken as the first challenge and the square dots show when taken as the second. (b) Response to mannitol is expressed as log RDR. The round dots show the responsiveness to mannitol when taken as the first challenge and the square dots show the responsiveness when taken as the second.

during the preceding 12 months and at least one positive bronchial provocation test on day 1. Some of the participants were included as asthmatics based on their general practitioner's diagnosis of asthma. They were already on inhaled steroid treatment when included in the study and were therefore only having a few respiratory symptoms and reduced hyperresponsiveness.

The severity of asthma was diagnosed according to the GINA guidelines on the basis of respiratory symptoms and the FEV₁% of predicted. Current symptoms and severity of asthma and allergy, known triggers of asthma and allergy,

current/former treatment, seasonal variation, frequency of current day and night symptoms, and smoking history, were assessed at the interview.

Statistical analysis

All data were analysed with SPSS (version 15.0). Mean and standard deviations were calculated for the normally distributed data. For the non-normally distributed data, the median and range as well as geometric mean and 95% confidence interval were used. The data for mannitol and methacholine were compared using a *t*-test only on the order in which the challenges were performed, irrespective of whether they were collected on the first or the second day. RDR values were calculated of both the response to mannitol and methacholine, following which, log RDR values were used in the analysis due to knowledge of lognormal distribution.

Results

The characteristics of the study subjects at baseline are given in Table 2. The mean age of the study population was 40.7 years (range 18–73), and 40% were men. A total of 40% had experienced daytime wheezing, 60% shortness of breath, 42% chest tightness, and 51% daytime coughing in 4 weeks before testing. A total of 76% of the subjects had had one or more of these symptoms during the preceding 4 weeks. Cough and breathlessness at night was recorded by 22% and 15%, respectively; 93% had symptoms of rhinitis, 48% were atopic, and 44% were treated with inhaled corticosteroids. The corticosteroid-treated subjects did not respond significantly different to the challenge test compared with the untreated subjects (Table 3). This could indicate that they were not optimally treated, when included in the study.

All except three subjects had an eosinophil count within the normal range ($<0.45 \times 10^9/L$). A total of 41.5% of the subjects had mild intermittent asthma, 37.5% had mild persistent asthma, and 21% had moderate asthma. Of the subjects with moderate asthma, 21 were treated with inhaled steroids. There were no statistically significant differences between the test order groups with respect to the level of AHR, eNO, atopy, FEV₁ and FEV₁/FVC. The average (SEM) time between the two challenge tests on day 1 was 56 (3.4) min when methacholine was the first challenge and 55 (2.7) min when mannitol was the first challenge ($P = 0.83$). No differences in FEV₁ and FVC were found between the 2 days of testing, whereas FEV₁/FVC between the first and second day of visit differed by 1.7%.

The response to the mannitol challenge when it followed the methacholine challenge was smaller than the response when it was given first [log RDR: 1.42 vs. 1.60 ($P = 0.004$)] (Table 4). This represents a reduction in the responsiveness of mannitol of 11% when following a

Table 2. Baseline characteristics of the 48 asthmatic subjects who participated in the study

Characteristics	N = 48
Male sex	19 (40)
Age (years)	40.1 (14.6)
Height (cm)	173 (9.2)
Weight (kg)	75 (12.8)
Ex-smokers	6 (13)
NO (p.p.b.), day 1	46.7 (4.0)
NO (p.p.b.), day 2	46.6 (4.0)
Blood eosinophils ($10^9/L$)	0.20 (0.20)
Atopy	23 (42)
Use of ICS	21 (44)
Untreated subjects	22 (46)
Severity of asthma (GINA)	
Mild intermittent	20 (42)
Mild persistent	18 (37)
Moderate persistent	10 (21)
Lung function	
FEV ₁	3.4 (0.8)
FVC	4.2 (1.0)
FEV ₁ % of predicted	93 (0.8)
FVC% of predicted	98 (0.9)
FEV ₁ /FVC	0.80
Baseline FEV ₁ , mannitol no. 1	3.4 (0.84)
FEV ₁ % of predicted	93 (0.8)
Baseline FEV ₁ , mannitol no. 2	3.1 (0.82)
FEV ₁ % of predicted	86 (0.7)
Baseline FEV ₁ , methacholine no. 1	3.3 (0.82)
FEV ₁ % of predicted	90 (0.8)
Baseline FEV ₁ , methacholine no. 2	3.2 (0.79)
FEV ₁ % of predicted	88 (0.9)

Male subjects, atopy, former smokers, and use of inhaled corticosteroids (ICS) are given in numbers (percentages); all other values are means (SD). NO, nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

methacholine challenge. The effect persisted for at least 56 min and was of a sufficient magnitude to bring about a false-negative result in 13 of the subjects in the subsequent mannitol test. Conversely, the response to a subsequent methacholine challenge was not affected significantly by an initial mannitol challenge [log RDR: 0.81 vs. 0.96 ($P = 0.102$)].

Discussion

The main finding of this study was a significant reduction in airway responsiveness to a mannitol challenge when given 1 h after a methacholine challenge, which indicates that a methacholine challenge attenuates the response to the mannitol challenge test. On the contrary, this was not seen when the methacholine challenge was performed after the mannitol challenge test.

Previous research has indicated that a serial challenge with indirect methods, such as exercise [7] and EVH, can

Table 3. Effect of treatment on the bronchial responsiveness to methacholine and mannitol

Group	Asthmatics untreated with ICS (<i>N</i> = 27)	Asthmatics treated with ICS (<i>N</i> = 21)	SEM	<i>P</i> -value
Methacholine no.1	0.86 (7.24)	0.76 (5.75)	0.13/0.20 (1.40/1.60)	0.673
Methacholine no.2	1.02 (10.47)	0.89 (7.76)	0.16/0.20 (1.40/1.60)	0.641
Mannitol no.1	1.63 (42.66)	1.56 (36.31)	0.08/0.07 (1.20/1.17)	0.577
Mannitol no.2	1.27 (18.62)	1.60 (39.81)	0.09/0.09 (1.23/1.23)	0.094

Values are log RDR (RDR are shown in parentheses) for the methacholine and the mannitol challenge.

RDR, response dose ratio; ICS, inhaled corticosteroids; SEM, standard error of the mean.

Table 4. Bronchial responsiveness to inhaled mannitol and methacholine, performed with either methacholine first or mannitol first

Group	A	B	All	Correlation	SEM	<i>P</i> -value
Methacholine	0.81 (6.46)	0.96 (9.12)	0.89 (7.76)	0.73	0.11/0.13 (1.03/1.35)	0.102
Mannitol	1.42 (26.30)	1.60 (39.81)	1.51 (32.36)	0.79	0.07/0.10 (1.17/1.26)	0.004

Values are log RDR (RDR are shown in brackets) for the methacholine and the mannitol challenge.

SEM, standard error of the mean.

cause refractory periods, possibly owing to mediator depletion [13], whereas repeated direct challenges, such as methacholine in asthmatic subjects, do not cause the same refractory periods [6]. Hahn *et al.* [13] and Rosenthal *et al.* [9] studied testing with both direct and indirect challenges given on the same day. Both studies showed no reduction in airway responsiveness to histamine or to methacholine when asthmatic subjects underwent an exercise test following the histamine challenge test. However, both these studies only performed direct before indirect challenges. These data are consistent with our results for methacholine, where the mean RDR of methacholine was unaffected by a prior mannitol challenge.

Little is known about the effect of methacholine on subsequent indirect challenges and has so far only been studied by Hurwitz *et al.* [10], who found that methacholine blunted the response to subsequent EVH. These data are consistent with ours, which showed a decreased response to mannitol after an initial methacholine challenge. Hahn *et al.* [13] suggested that depletion of mediators is the most reasonable explanation for the refractory period, but this contradicts our results, where a refractory period was observed after methacholine. The mode of action of methacholine is not believed to involve mediators, but it acts by binding directly to the receptors on the smooth muscle. Therefore, our data indicate that mediator depletion cannot be the sole cause of the refractory period, which is consistent with data from other studies [7]. However, mediator depletion may be the cause of the refractory period if our initial hypothesis is true, that a direct challenge synergistically with methacholine connecting with the receptor on the smooth muscle can cause a release of inflammatory mediators and thereby cause mediator depletion secondly. Hurwitz and colleagues speculated that methacholine causes a non-specific chemical effect either by mechanical stress or by neural

reflexes in the constricted airway, which induces refractoriness by altering the Ca^{2+} influx in the smooth muscle or via the release of bronchodilator mediators. Another suggestion is that methacholine causes a vascular leak or increased bronchial blood flow, which induces refractoriness [10].

We initiated the second challenge when FEV_1 was within 95% of the baseline value or 1 h had passed. This may have caused some subjects to be re-challenged while their airway muscle was still refractory, but this cannot be the sole explanation for our observations because the same effect was found when the orders of the tests were reversed. Another issue is that the mean degree of airway obstruction differed on 2 days. The baseline airway calibre has earlier been proposed as one of the important factors in determining the airway response to histamine [14] and could therefore have an impact on our results. Although this may have altered the reactivity of each subject from day 1 to day 2 of the tests, it has probably not affected the final results because both groups were similar and the reduction in FEV_1 was insignificant. The short interval of time between testing (2 days) might have reduced the risk of inconclusive results due to a change in inflammation of the airway, thus strengthening the results of the study.

Existing knowledge on how direct and indirect challenge tests influence each other is limited. This makes it difficult to assess and substantiate whether the observed effect is specific to our test. A minor drawback of this study is the possible difficulties in comparing our data with other studies' findings, because we had to add a value of 7.5% to the RDR due to a huge variation in the achieved FEV_1 . This could hardly have been avoided, due to the fact that we included patients untrained in spirometric tests and the number of tests each test person had to perform. Furthermore, it could have contributed to a better understanding of the achieved results had we collected sputum

samples during the study. eNO and blood eosinophilic count is, however, considered to provide a reasonably good indication of inflammation of the airways. The subjects were entered unselected into the trial and were a mix of treated and untreated asthma patients, which increases the generalizability of our findings. On the other hand, the study was not a completely random sample, which affects the generalizability. Current smokers were excluded, because smoking can induce airway inflammation, which could have influenced our results in unknown directions. Excluding smokers may, however, have reduced the risk of false-positive results due to a diagnostic mix-up with COPD.

In conclusion, our data show that the response to a mannitol challenge test is attenuated when it is preceded by a methacholine challenge but not vice versa. A consequence of this is that mannitol and methacholine challenge testing could be performed on the same day but it should be carried out in the proper order. It is therefore important that mannitol be the first test in serial challenging with methacholine.

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