

Mannitol as an indirect bronchoprovocation test for the 21st century

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Objectives: To review mannitol challenge data and advocate the approval of this testing modality in the United States.

Data Sources: A literature review was performed using the MEDLINE database for English-language articles published between January 1, 1993, and July 31, 2009, using the following keywords: *mannitol bronchoprovocation test*, *inhaled mannitol*, *inhaled mannitol and asthma*, and *inhaled mannitol and exercise-induced asthma*.

Study Selection: Trials were selected that established the effect of mannitol as a bronchoprovocation challenge, explored mannitol's mechanism of action, and compared mannitol to other accepted bronchoprovocation challenges.

Results: Mannitol has demonstrated the ability to detect airway hyperreactivity in individuals. The mechanism of action is through the release of mast cell mediators. The sensitivity and specificity compare well with other indirect challenge testing methods.

Conclusion: Mannitol is a polyol sugar that can be converted to a powdered form and encapsulated. Once encapsulated it can be inhaled and causes narrowing of the airways in susceptible individuals. Mannitol likely triggers the release of inflammatory and/or bronchospasm mediators, causing the smooth muscle of the airway to contract and resulting in airway narrowing. The magnitude of decrease in forced expiratory volume in 1 second and the dose of mannitol needed to provoke the airway response provide a readily measurable and clinically useful assessment of airway hyperreactivity. Mannitol challenge is an accepted testing method in Australia, Europe, and Korea. Acceptance of the mannitol challenge in the United States would complement existing methods for assessing bronchial hyperreactivity and likely improve patient care.

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INTRODUCTION

Asthma is an inflammatory disease characterized by abnormal airway constriction in response to a variety of stimuli, including irritants, cold air, exercise, hyperventilation, and histamine. The susceptibility to bronchoconstriction is a characteristic of asthma and is termed *airway hyperreactivity*. The reduction in airflow resulting from airway constriction is a major contributor to the cardinal symptoms of asthma: shortness of breath, chest tightness, cough, and wheezing. Airway hyperreactivity is usually detectable in asthma patients even

when baseline lung function is normal. Some experts argue that following airway hyperreactivity may be a useful strategy in asthma management because hyperreactivity may be abnormal even in patients with mild asthma and normal lung function. Airway hyperreactivity may better reflect airway inflammation and risk for deterioration than does lung function or asthma symptoms because susceptibility can be quantified before changes in airflow limitation.¹

Airway hyperresponsiveness may be assessed with a variety of provoking agents or maneuvers. Hyperresponsiveness is most commonly measured in the laboratory or clinic after challenge of the test patient with inhaled methacholine or histamine. These agents act *directly* on the smooth muscle of the airway, causing contraction. This direct method of testing has limitations, including imprecision in measuring the dose administered and limited specificity of a positive response.²

Additional tests were developed to overcome some of these limitations during the last 30 years. These tests are termed *indirect* because the airway narrowing results from the challenge substance stimulating cells to release bronchoconstricting mediators rather than the challenge substance directly constricting airway muscle. Testing with indirectly acting agents may be more specific for asthma because the results reflect characteristics of the airway in addition to the responsiveness of the airway smooth muscle. Among the indirect tests are exercise challenge, eucapnic hyperventilation, inha-

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Table 1. Comparison of Major Characteristics of Direct and Indirect Challenge Tests

Characteristic	Indirect challenges	Direct challenges
Mechanism of action	Inflammatory mediators	Muscle contraction
Effect of airway size on data	Minor effect	Potentially large effect
Cost	Highly variable	Relatively inexpensive
Used for EIB diagnosis	Yes	No
Current accepted use in asthma diagnosis	Asthma rule in	Asthma rule out

Abbreviation: EIB, exercise-induced bronchoconstriction.

lation of hypertonic saline, cold air challenge, and inhalation of mannitol.³

The challenge methods present a variety of barriers that potentially limit their use. For many years the gold standard for indirect testing was the exercise challenge test, with the US Army using exercise as a screening assessment for new recruits.⁴ However, the limitations of exercise testing (eg, a lack of consensus with respect to the testing protocol, requirement of trained technicians to operate specialized exercise equipment, inability of some patients to exercise, and reduced sensitivity and reproducibility when used in clinical settings) prompted the search for an alternative.⁵ Also, the exercise challenge test was developed when the duration and intensity of exercise were thought to be the primary factors in determining the severity of the exercise-induced asthma. Later studies show bronchoconstriction occurs due to evaporative water loss and/or cooling of the airway during exercise. The physical effects of airway dehydration and/or cooling result in the release of mediators such as histamine, prostaglandin D₂ (PGD₂), and leukotrienes. These mediators act on the smooth muscle of the airway to cause the narrowing associated with exercise-induced bronchoconstriction.⁶ Table 1 highlights the differences between direct and indirect testing modalities.^{3,5,7,8}

The remainder of this review focuses on issues related to mannitol. A literature review was performed using the MEDLINE database for English-language articles published between January 1, 1993, and July 31, 2009, using the following keywords: *mannitol bronchoprovocation test*, *inhaled mannitol*, *inhaled mannitol and asthma*, and *inhaled mannitol and exercise-induced asthma*. We selected trials that established the effect of mannitol as a bronchoprovocation challenge, explored mannitol's mechanism of action, and compared mannitol to other accepted bronchoprovocation challenges. The goal of this article is to review evidence supporting mannitol being used as a testing method in the United States. While this article was being revised, mannitol was approved for use within the United States.

STRUCTURE OF MANNITOL

Mannitol (182.17 Da) is a sugar alcohol that is a stereoisomer of sorbitol and was originally isolated from the secretions of

the flowering ash (*Fraxinus ornus*). Mannitol has been used as a food additive for more than 60 years, is nonhygroscopic, and is not metabolized after ingestion or carcinogenic.⁹

MECHANISM OF ACTION

The mechanism by which mannitol stimulates mediator release is likely a physical effect of osmolarity.¹⁰ Inhalation of powders such as mannitol may make the extracellular airway fluid transiently hyperosmolar, which provokes mediator release similar to the hypertonicity that results from exercise.¹¹ A 2003 study measured prostaglandin and leukotriene metabolites in the urine of patients with asthma before and after mannitol challenge. An increase of such metabolites after mannitol challenge supports the hypothesis that the mannitol response is associated with mast cell activation. The urinary concentrations of leukotriene E₄ (LTE₄) and 9α11BPF₂ (a PGD₂ metabolite) were measured in 12 asthmatic patients and 9 healthy controls before and after challenge with mannitol. After mannitol challenge, the forced expiratory volume in 1 second (FEV₁) was reduced by a mean of 34.5% in asthmatic patients and 1.0% in nonasthmatic controls.¹² The urinary concentrations of the inflammatory markers both before and after mannitol challenge are given in Table 2. The increase of leukotriene and prostaglandin metabolites in the urine of nonasthmatic controls after mannitol challenge, as noted in Table 2, was an unanticipated finding. The authors explain this observation as resulting from the nonasthmatic controls receiving doses of mannitol several-fold greater than did the asthmatic patients. These data suggest that the inhalation of mannitol causes mast cells to release mediators in both asthmatic and nonasthmatic airways but results in bronchoconstriction only in those with asthma.

Additional support of mannitol's effect on mast cells and other airway inflammatory cells is provided by 2 studies examining the effects of anti-inflammatory therapy on mannitol challenge.^{13,14} In the first study, patients were challenged with mannitol at their initial visit to establish a positive response. Within the next 3 weeks, each patient was twice rechallenged after double-blind treatment with inhaled

Table 2. Urinary Concentrations of LTE₄ and 9α11BPF₂ Before and After Mannitol Challenge in Asthmatic and Nonasthmatic Patients

	Urinary concentration, ng/mmol of creatinine, mean (SD)		P value
	Before challenge	After challenge	
Asthmatic patients			
LTE ₄	32.9 (4.7)	51.3 (7.5)	<.05
9α11BPF ₂	66.9 (6.6)	91.9 (8.2)	<.001
Nonasthmatic patients			
LTE ₄	43.0 (3.3)	58.5 (5.3)	<.05
9α11BPF ₂	56.0 (5.8)	68.4 (6.9)	<.05

Abbreviations: LTE₄, leukotriene E₄; 9α11BPF₂, prostaglandin D₂ metabolite.

nedocromil sodium, 8 mg, or a placebo immediately before mannitol challenge. The average mannitol dose required to provoke a positive challenge was 109 mg on the control day, 156 mg when pretreated with a placebo, and 409 mg when pretreated with nedocromil sodium. Fifty percent of those given nedocromil sodium had a negative mannitol challenge result as defined by less than a 15% decrease in FEV₁ compared with baseline. The 50% of individuals with a positive mannitol challenge result required more than a 3-fold increase in total dose, 83.4 to 308 mg. The amount of mannitol required for a positive challenge test result in the placebo group was not significantly different from the control day, with values of 109 vs 156 mg.¹³

The second study investigated the effect of disodium cromoglycate or formoterol fumarate on the response to a mannitol challenge.¹⁴ Fourteen asthmatic patients underwent mannitol challenge to establish the mannitol dose required to decrease FEV₁ 25% or more from baseline. On 3 different occasions each patient was rechallenged with the same dose of mannitol after double-blinded pretreatment with formoterol fumarate, disodium cromoglycate, or placebo. The study drugs were administered as a dry powder inhalation. The potential, protective effect of either formoterol fumarate or nedocromil sodium was calculated by dividing the FEV₁ difference between the placebo and active drug before treatment by the decrease in FEV₁ with the placebo before treatment, and the results were expressed as percentage of protection. The urinary concentration of LTE₄ and 9 α 11BPF₂ was measured before and after challenge using enzyme immunoassays. The average mannitol dose needed to cause an average 29% decrease in FEV₁ from baseline was 252 mg on the control day. When the patients were given the placebo, the same dose of mannitol caused a decrease in FEV₁ of 32%, not statistically different from the initial challenge. Formoterol fumarate and disodium cromoglycate provided statistically significant ($P < .001$) bronchoprotection percentages of 95% and 63%, respectively. A statistically significant increase in urinary LTE₄ (19 to 31 ng/mmol of creatinine) and 9 α 11BPF₂ (61 to 92 ng/mmol of creatinine) followed the placebo pretreatment. Pretreatment with either formoterol fumarate or disodium cromoglycate inhibited urinary 9 α 11BPF₂ but did not affect urinary LTE₄.¹⁴ The results are not easily explained but suggest 2 possibilities. The first is that prostaglandin mediators released after mannitol challenge are more readily suppressed than leukotriene mediators because both the long-acting β -agonist and a mast cell stabilizer inhibited 9 α 11BPF₂. The second possibility is that both formoterol fumarate and disodium cromoglycate are not sufficiently powerful to suppress mast cell degranulation after mannitol challenge or cells other than mast cells are affected by mannitol.

Additional evidence that mannitol affects mast cell mediator release is provided by an allergic rhinitis study and in vitro work with mast cells. The use of histamine and leukotriene receptor antagonist has been shown to reduce the response to a nasal mannitol challenge in patients with aller-

gic rhinitis, suggesting mast cell mediators are important in the mannitol response.¹⁵ Finally, an in vitro experiment using umbilical cord-derived human mast cells showed that mannitol incubation with mast cells is associated with mediator release. PGD₂ and leukotriene C₄ increased after mannitol was added to the culture media.¹⁰

COMPARISON OF MANNITOL CHALLENGE TO OTHER BRONCHOPROVOCATION TESTS

Anderson et al¹⁶ published data in 1997 using a dry mannitol preparation as a bronchoprovocation test. In this landmark study, 43 asthmatic patients, whose FEV₁ decreased 20% or more when challenged with nebulized 4.5% hypertonic saline, and 7 nonasthmatic controls inhaled mannitol with monitoring of lung function. The FEV₁ of each patient was measured before challenge twice at 10-minute intervals to establish the consistency of the value. After a placebo challenge each patient inhaled mannitol delivered by an inhaler with sequential dosing of 5, 10, 20, 40, 80, and 160 mg. The final 160-mg dose was repeated 2 additional times for a maximum possible total dose of 635 mg. FEV₁ values were obtained in triplicate 60 seconds after each dose of mannitol or placebo with the highest value being recorded. The study was terminated if FEV₁ decreased more than 15% from the FEV₁ obtained after the placebo challenge. The cumulative dose resulting in the 15% or greater FEV₁ decrease was recorded as the provoking dose. Test patients were labeled nonresponsive if they received 635 mg of mannitol without experiencing a 15% decrease in FEV₁. Patients without asthma did not react to mannitol. Asthmatic patients required on average 64 mg of mannitol to reduce their FEV₁ by 15% or greater. The investigators repeated the mannitol challenge in 23 of the patients with asthma within a median of 7 days to establish reproducibility, and no significant difference occurred on the 2 testing days. Twenty-five of the patients with asthma were also challenged on a separate day with methacholine to compare the 2 challenge results. All 25 had positive responses. The dose of methacholine required for a positive response correlated with the dose of mannitol, with a correlation coefficient of 0.54.¹⁶ These observations suggest that the susceptibility to mannitol reflects disease severity, similar to methacholine. Although the study suggests that mannitol could be used as a bronchoprovocation test, the data do not resolve the issues of sensitivity and specificity or how mannitol compares with other indirect tests.

Another study assessed whether there was a relationship between airway responsiveness to mannitol or methacholine and markers of airway inflammation in asthmatic patients. Fifty-three asthmatic patients were studied on 2 occasions, with at least 2 but not more than 14 days between challenges. Thirty-four patients (64%) had a positive response to methacholine with a 20% or greater decrease in FEV₁ (maximum dose, 8 μ mol or 2 mg; mean, 1.36 μ mol), and 34 patients (64%) had a positive challenge test result to mannitol with 15% or greater decrease in FEV₁ (maximum dose, 635 mg; mean, 119 mg). Eighty-two percent of patients with a positive

mannitol challenge had an exhaled nitric oxide level of 20 ppb or higher compared with 15% of those with a negative challenge result. Eighty-eight percent of patients with a positive methacholine challenge had a nitric oxide level of 20 ppb or greater. Blood eosinophil counts were greater than 1% of total white blood cells in 70% of patients with a positive mannitol challenge result and 79% of patients with a positive methacholine test result. Blood eosinophil counts were less than 1% of total white blood cells in all patients with a negative mannitol challenge result or a negative methacholine challenge result. Mannitol response correlated significantly with exhaled nitric oxide (Pearson coefficient [r_p], -0.63), degree of eosinophilia (r_p , -0.52), peak flow variability (r_p , -0.55), and quality-of-life questionnaire responses (r_p , 0.35). A positive methacholine challenge result correlated only with exhaled nitric oxide (r_p , -0.43) and peak flow variation (r_p , -0.42).¹⁷ This study demonstrates that mannitol challenge has a stronger relationship to potential airway inflammation or asthma control markers than methacholine challenge.

A double-blind study compared mannitol, cold air, and histamine as provocateurs of bronchospasm in patients with difficult to diagnose asthma.¹⁸ Thirty-seven patients with newly diagnosed asthma and 10 normal controls were challenged with mannitol, cold air, and histamine (a direct test) in random order, with at least 2 but less than 14 days between tests. Fifty-one percent of the asthmatic patients had a positive response to mannitol (maximum dose of <635 mg and decrease of $\geq 15\%$ of FEV₁), 24% a positive response to cold air (chilled air inhaled for 4 minutes and decrease of $\geq 9\%$ of FEV₁), 81% a positive response to high-dose histamine (maximum dose of 1 mg and decrease of $\geq 15\%$ FEV₁), and 49% a positive response to low-dose histamine (maximum dose of 0.4 mg and decrease of $\geq 15\%$ FEV₁). The healthy controls did not respond to any of the challenges.¹⁸ This study demonstrates that mannitol challenge is more sensitive than cold air challenge in provoking airway bronchospasm and that mannitol challenge (maximum dose of 635 mg) is comparable in sensitivity to histamine challenge when the maximum histamine dose is limited to 0.4 mg. The research team justified using the 0.4 mg of histamine as their comparison value with the argument that investigators consider the 0.4-mg maximal histamine dose to be preferable for the diagnosis of asthma because false-positive results occur with higher doses.¹⁹

A phase 3, multicenter, operator-masked, crossover design, randomized study was performed comparing the safety and efficacy of bronchoprovocation with mannitol and hypertonic saline.²⁰ Four hundred eighty-seven asthmatic patients and 105 controls were studied. Two hundred ninety-six patients (50%) had a positive mannitol challenge result, and 322 (54%) had a positive hypertonic saline challenge result. The mean maximum decrease in FEV₁ was 21.0% for the mannitol group and 21.3% for the hypertonic saline group. In those with a positive mannitol response, the mean (SD) dose was 239 (185) mg, and the mean (SD) dose in those with a positive response to 4% hypertonic saline was 12.9 (11.3)

mL. Nonasthmatic patients had a mean maximum decrease in FEV₁ of 5.5% for mannitol, with a mean (SD) dose of 635 (0.9) mg, and a mean maximum decrease in FEV₁ of 5.4% for hypertonic saline, with a mean (SD) dose of 32.4 (6.3) mL. The investigators hypothesized that the large percentage (75%) of the asthmatic test patients using inhaled corticosteroids (ICSs) contributed to the higher than expected number of negative challenge results. The hypothesis was that the ICSs reduced the airway inflammation and therefore reduced the response to mannitol. When the data were evaluated by analyzing patients with a clinical diagnosis of asthma, but without ICSs, the sensitivity and specificity of mannitol challenge increased to 70% and 95%, respectively, comparable with the results for hypertonic saline as noted in Table 3.²⁰ A similar reevaluation of the hypertonic saline data was not provided in the study. The most common adverse effect associated with inhaled mannitol is cough, occurring in 85%. Other adverse effects include headaches (8.3%), nausea (2.4%), and throat discomfort (3.5%).²⁰

Several studies have examined the relationship of bronchoprovocation tests and ICS therapy. These data show that the dose of mannitol required for bronchospasm decreases and measured hyperreactivity increases as the dose of ICS decreases.^{18,21–24}

A 2009 study compared the ability of mannitol and methacholine challenge to predict exercise-induced bronchoconstriction and the clinical diagnosis of asthma.²⁵ Five hundred nine people, 6 to 50 years of age, without a diagnosis of asthma but with symptoms suggestive of asthma were screened for this study. After exclusion criteria were applied, 430 people were enrolled and 375 completed the study. One hundred sixty-three patients had a positive exercise challenge test result defined as a 10% or greater decrease in FEV₁. One hundred sixty-eight patients had a positive mannitol challenge test result defined as a decrease in FEV₁ of 15% or greater from baseline or a decrease in FEV₁ of 10% or greater between consecutive doses of mannitol. A positive methacholine test result was defined as a decrease in FEV₁ of 20% or more from the baseline value. At the final study visit a physician made a clinical diagnosis regarding asthma based on the exercise challenges, history, examination, skin test

Table 3. Sensitivity and Specificity of Response to Mannitol Compared With Hypertonic Saline and Clinical Assessment of Asthma^a

	Sensitivity, %	Specificity, %
Mannitol vs hypertonic saline	80.7	86.7
Mannitol vs clinical diagnosis	59.8	94.5
Hypertonic saline vs clinical diagnosis	65.1	95.2
Mannitol vs clinical diagnosis when excluding patients taking ICSs	70.0	95.0

Abbreviation: ICSs, inhaled corticosteroids.

^a Table reproduced with consent of author, from *Respir Res*. 2005;6:144.

Table 4. Comparison of Bronchial Provocation Challenge Tests

	Equipment	Cost	Convenience	Safety	Testing time	Sensitivity	Specificity
Cold air	—	—	—	+/-	—	+/-	+/-
Eucapnic hyperventilation	—	—	—	+/-	+/-	+/-	+/-
Exercise	—	—	—	—	—	+/-	+/-
Histamine	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Mannitol	+	+	+/-	+	+	+/-	+/-
Methacholine	+/-	+/-	+/-	—	+/-	+	+/-

Symbols: +, superior; +/-, intermediate; —, inferior.

results, and FEV₁ reversibility. This physician did not have access to the mannitol and methacholine challenge test results. When compared with those who had a 10% decrease in their FEV₁ during an exercise challenge, mannitol had a specificity of 65.2% and a sensitivity of 58.6%, whereas methacholine had a specificity of 68.9% and sensitivity of 55.2%. The prevalence of bronchial hyperreactivity was found to be similar for all 3 challenges: exercise, 43.5%; mannitol, 44.8%; and methacholine, 41.6%. The degree of hyperreactivity was mild, with a mean (SD) FEV₁ decrease of 19% (9.2%) for exercise challenge, 27.7% (8.2%) for methacholine, and 16.1% (5.6%) for mannitol. For a clinical diagnosis of asthma the sensitivity of mannitol was 56% and the specificity was 73%, whereas the sensitivity of methacholine was 51% and the specificity 75%.²⁵ This study shows that in patients with mild asthma the sensitivity and specificity of the mannitol and methacholine challenges are similar. The most common adverse effect of the mannitol challenge was cough, with 93% of the patients having a cough on at least 1 occasion. Although not the major aim of the investigation, 2 advantages of the mannitol test were demonstrated. First, the mean (SE) time to administer a mannitol challenge was much shorter than the time to administer a methacholine challenge: 19.9 (0.9) vs 44.5 (1.4) minutes. Second, the mannitol challenge was a more consistent test as evidenced by the statistically greater log odds ratio comparing the sensitivity of methacholine and mannitol among the various testing centers.²⁵

2010 APPROVAL STATUS

The mannitol challenge test is established in the literature as a bronchoprovocation test to assess airway hyperresponsiveness. Korea, the European Union, and Australia recognize inhaled mannitol challenge as an accepted testing method for the diagnosis of asthma and exercise-induced asthma.²⁶

The increasing reports of performance-enhancing drug use by athletes and the subsequent negative publicity have resulted in the governing bodies of most sports to reassess their drug testing guidelines. Such concerns prompted the International Olympic Committee (IOC) to review the use of inhaled β -agonists. The use of inhaled β -agonists increased from 3.7% in the Atlanta games of 1996 to 5.7% in the Sydney games of 2000.²⁷ The estimated prevalence of asthma in the United States during these 2 periods was 5.5%²⁸ and 7.2%, respectively.²⁹ A 2008 consensus conference concluded that the increased prevalence of asthma among Olympic athletes

was the result of unconfirmed diagnoses based solely on history. The IOC conference report recommended the clinical diagnosis of asthma be confirmed by a 12% increase in FEV₁ in response to bronchodilator treatment or a positive bronchoprovocation test result.²⁷ The IOC committee concluded that (1) the safety and efficacy of mannitol in detecting airway hyperreactivity are established and (2) mannitol challenge is recognized as 1 of the 4 indirect tests, including eucapnic voluntary hyperpnea, exercise test, hyperosmolar sodium chloride, and dry powdered mannitol, able to diagnose asthma.³⁰

While this article was in revision for publication, the US Food and Drug Administration approved mannitol for use within the United States on October 6, 2010.³¹

CONCLUSION

Multiple studies have shown mannitol to be equal or superior to comparable testing methods for diagnosing patients with asthma or exercise-induced asthma. Indirect bronchoprovocation tests compared with direct bronchoprovocation tests better reflect the complex effects of inflammation as opposed to only identifying smooth muscle hyperreactivity. The attributes of the various bronchoprovocation tests are summarized in Table 4. The mannitol challenge test has the following advantages over other indirect bronchoprovocation tests: a standardized test kit, minimal required equipment, relative low cost, ease of administration, a consistent dose response, enhanced safety, and ease of performance.¹¹ A positive mannitol test result indicates active asthma and the potential for exercise-induced asthma.¹² The major adverse effect of the mannitol challenge is cough.^{21,32,33} The availability of mannitol challenge testing will complement existing methods for assessing bronchial hyperreactivity and likely improve patient care.

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