



Refractoriness to inhaled mannitol 3 hours after allergen challenge

Daniel O. Amakye, BSc; Beth E. Davis, BSc, PhD; Alexandra L. Martin, BSc; Gregory E. Peters, MD, FRCP(C); and Donald W. Cockcroft, BSc, MD, FRCP(C)

Division of Respiriology, Critical Care and Sleep Medicine, Department of Medicine, University of Saskatchewan, Royal University Hospital, Saskatoon, Saskatchewan, Canada

ARTICLE INFO

Article history:

Received for publication May 31, 2013.

Received in revised form June 11, 2013.

Accepted for publication June 11, 2013.

ABSTRACT

Background: A positive response to indirect airway challenges (eg, mannitol) that cause release of mast cell mediators causes refractoriness to a repeat exposure to the same or different indirect stimulus that lasts for at least several hours. Allergen challenge causes increased response to methacholine measured at 3 hours. **Objective:** To compare allergen-induced changes in airway response to methacholine and mannitol 3 hours after completion of an allergen challenge.

Methods: Ten atopic patients with asthma completed a randomized clinical trial. The provocation concentration of methacholine causing a 20% decrease in forced expiratory volume in 1 second (FEV₁) was measured 24 hours before and 3 hours after a standard allergen challenge. The provocation dose of mannitol causing a 15% decrease in FEV₁ was also measured 24 hours before and 3 hours after allergen challenge. The allergen challenges were separated by 7 to 14 days.

Results: The allergen-induced early responses, expressed as the maximum (SD) percent decrease in FEV₁, were 29.7% (11.1%) and 27.8% (7.6%) on the methacholine and mannitol days, respectively. Airway response to methacholine increased significantly after allergen challenge ($P = .02$). By contrast, the airway response to mannitol was reduced by almost a doubling dose ($P = .02$) after allergen.

Conclusion: Three hours after allergen challenge at a time when the airways are more responsive to methacholine, there is a significant refractoriness to the indirect stimulus mannitol.

Trial Registration: clinicaltrials.gov Identifier: NCT01699594

© 2013 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Nonallergic indirect stimuli cause airway smooth muscle contraction via release of mast cell mediators.¹ For several hours after a positive indirect challenge result, there is refractoriness to a second challenge.^{2–5} Although less well studied, there is evidence for cross-refractoriness among different indirect stimuli.⁶ The early asthmatic response to allergen also involves mast cell mediator release and, consequently, can be regarded as a type of indirect challenge, albeit a bit more involved, largely because of the clinically important late sequelae, including the late asthmatic response (LAR),⁷ allergen-induced increase in airway responsiveness,⁸ and allergen-induced eosinophilia.⁹ In a preliminary study that compared allergen-induced increase in airway responsiveness to methacholine and mannitol, we identified significant refractoriness to the response to inhaled mannitol 3 hours after a positive allergen challenge result.

Methods

Patients

Ten young, atopic patients with asthma (Table 1) completed this randomized but unblinded study. Patients were required to have a forced expiratory volume in 1 second (FEV₁) greater than 70% of predicted, a methacholine provocation concentration causing a 20% decrease in FEV₁ (PC₂₀) of less than 16 mg/mL, no treatment other than infrequent inhaled salbutamol, and no allergen exposure or respiratory tract infection for at least 4 weeks. Pregnant and lactating females were excluded. The study was approved by the University of Saskatchewan Ethics Committee, and signed informed consent was obtained.

Study Design

The study consisted of 5 visits at the same time of day. The first visit was a screening visit to obtain spirometry, methacholine challenge, and allergen skin testing to ensure eligibility. The remaining 4 days consisted of two 2-consecutive day periods in random order separated by 14 days or more (mean, 17.7 days). Patients underwent a methacholine or mannitol challenge on 1 day and 24 hours later had an allergen challenge. Three hours after the completion of the allergen challenge, the methacholine or

Reprints: Donald W. Cockcroft, BSc, MD, FRCP(C), Division of Respiriology, Critical Care and Sleep Medicine, Royal University Hospital, 103 Hospital Drive, Ellis Hall, Fifth Floor, Saskatoon, Saskatchewan, Canada; E-mail: don.cockcroft@usask.ca.

Disclosures: Authors have nothing to disclose.

Table 1
Characteristics of the study participants

Participant No.	Sex	Age, y	Baseline FEV ₁ , L	Baseline FEV ₁ , %	Height, in	Weight, lb	Allergen details		
							Allergen ^a	Skin test end point	Concentration inhaled
1	F	22	3.41	100	65	150	DP	1:4,096	1:256
2	M	36	3.69	86	70	181	Cat	1:4,096	1:32
3	F	21	3.16	85	68	160	Cat	1:1,024	1:64
4	F	21	3.52	96	68	145	Cat	1:512	1:16
5	M	25	3.99	83	72	228	Cat	1:1,024	1:16
6	F	26	3.00	89	65	99	DP	1:128	1:1,024
7	F	24	3.06	102	61	120	Horse	1:2,048	1:32
8	F	36	2.69	93	62	117	DP	1:512	1:256
9	F	21	3.10	89	66	125	Cat	1:256	1:32
10	M	27	4.96	97	75	190	Cat	1:256	1:2

Abbreviations: DP, *Dermatophagoides pteronyssinus*; FEV₁, forced expiratory volume in 1 second.

^aAllergen stock solutions: cat, 10,000 BAU/mL; DP, 30,000 AU/mL; and horse, 1:20 (wt:vol) (purchased from Omega Laboratories, Montreal, Quebec, Canada).

mannitol challenge was repeated. At least 14 days later, investigations were repeated using the alternate nonselective challenge. After completion of all investigations on the allergen challenge days, 500 µg of inhaled fluticasone was administered to prevent any potential LAR.

Allergen Challenge

Allergen challenges were performed as previously documented.⁷ Doubling concentrations of allergen were inhaled for 2 minutes of tidal breathing via a Wright nebulizer calibrated to deliver an aerosol output of 0.13 mL/min. FEV₁ was initially measured in triplicate and was repeated twice 10 minutes after each allergen concentration. Allergen inhalations were continued until a 20% or greater decrease in FEV₁ was achieved.

Methacholine Inhalation

Methacholine challenges were performed with the standardized 2-minute tidal breathing technique.¹⁰ Doubling concentrations of methacholine were inhaled from a Bennett Twin jet nebulizer calibrated to run at 0.13 mL/min. FEV₁ was measured in triplicate and then repeated 30 and 90 seconds after each inhalation.

Methacholine concentrations were doubled at 5-minute intervals (start to start), and the challenge was continued until a 17% or greater decrease in FEV₁ was obtained. The PC₂₀ was interpolated¹¹ or extrapolated¹² from the log concentration vs response curve.

Mannitol Challenge

Mannitol inhalation was performed by the standardized method.¹³ Increasing doses of dry powder mannitol were inhaled at as close to 2-minute intervals as possible. FEV₁ was measured initially in triplicate and then repeated twice 1 minute after each mannitol inhalation. The challenge was continued until a 15% or greater decrease in FEV₁ was achieved or until the top cumulative dose of 635 mg had been administered. The provocation dose (cumulative) of mannitol causing a 15% FEV₁ decrease (PD₁₅) was calculated from the log dose vs response curve.

Statistical Analysis

The PC₂₀ and PD₁₅ values were log transformed, and preallergen and postallergen values were compared with the paired *t* test (Statistix for Windows; Analytical Software, Tallahassee, Florida).

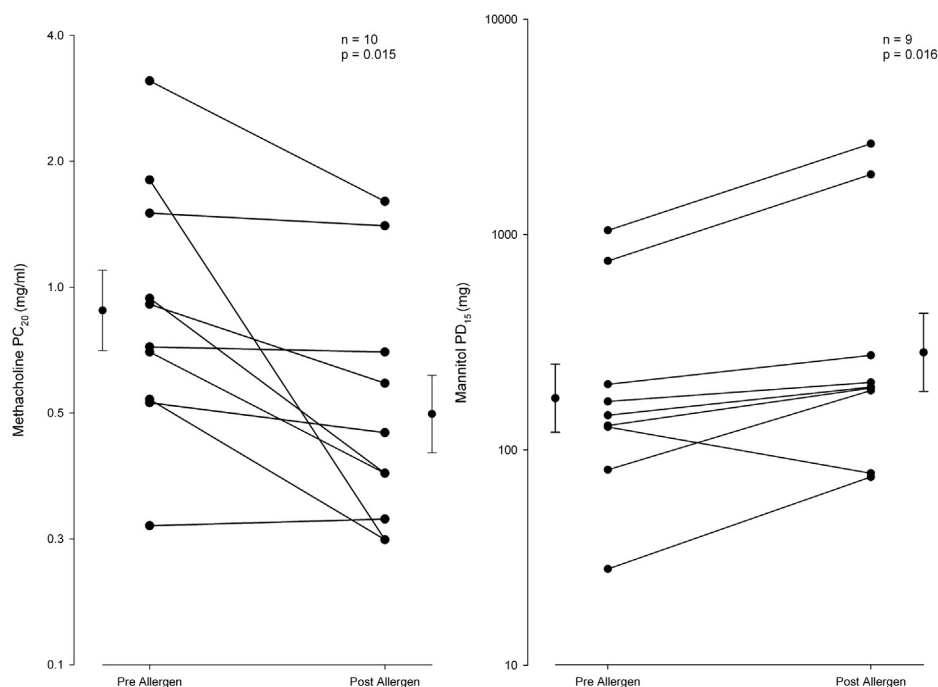


Figure 1. Individual values for methacholine provocation concentration causing a 20% decreased in forced expiratory volume in 1 second (FEV₁) (PC₂₀) (left) and mannitol provocation dose causing a 15% decrease in FEV₁ (PD₁₅) (right) 24 hours before and 3 hours after allergen challenge. The vertical axis is a log scale.

Results

Ten patients completed the investigations without adverse effects. The mean (SD) percentage decrease in FEV₁ after the allergen challenge was 29.7% (11.1%) on the methacholine day and 27.8% (7.6%) on the mannitol day ($P = .59$).

Methacholine and mannitol results are shown in Figure 1. Three hours after allergen challenge, there was a significant increase in airway responsiveness to methacholine. The geometric mean methacholine PC₂₀ decreased from 0.88 to 0.50 mg/mL ($P = .02$). At the same time after allergen challenge, a significant reduction occurred in airway response to mannitol, with the geometric mean PD₁₅ increasing from 174 to 284 mg ($P = .02$) ($n = 9$; 1 patient was nonresponsive to mannitol both before and after allergen challenge).

Discussion

We found that 3 hours after allergen challenge, in atopic patients with asthma not selected to have an LAR, airway responsiveness to the direct stimulus methacholine is increased by almost one doubling concentration. At the same time, airway responsiveness to mannitol was reduced by almost one doubling dose.

Allergen-induced increase in airway responsiveness to direct stimuli, histamine, and methacholine was first identified in the laboratory in 1977.⁸ Airway responsiveness was measured 7 and 30 hours after the allergen challenge. Follow-up studies identified that an increase in airway responsiveness was associated with the LAR¹⁴ and with an allergen-induced increase in airway inflammation.⁹ Studies looking at the time course have identified that airway response has not increased 2 hours after allergen challenge¹⁵ but is increased by 3 hours¹⁶ after allergen challenge. Despite a relationship between allergen-induced airway eosinophilia and allergen-induced airway responsiveness, the 2 do not appear to be causally related.¹⁷ Indirect airway responsiveness is more closely associated with airway inflammation and changes more with both proinflammatory and anti-inflammatory events.^{18,19} Our original hypothesis was that airway responsiveness to mannitol might increase more than airway responsiveness to methacholine. We still consider this a valid hypothesis; it will be necessary to study this 24 hours after allergen challenge.

There was considerable heterogeneity in the allergen-induced increase in methacholine airway hyperresponsiveness. This is always an issue in our LAR studies; however, in this preliminary study we did not select for patients with an LAR. We suspect this may have introduced even more inhomogeneity. Nevertheless, all patients' postallergen methacholine PC₂₀ values changed in the same direction, and the results were statistically significant. The number of patients was too small to attempt to stratify the population, and we did not capture the LAR; in fact, we inhibited any potential LAR with a single dose of fluticasone.

Refractoriness, even cross-refractoriness, is well recognized after indirect challenges.^{2–6} Early studies documented that airway responsiveness to methacholine⁵ and histamine²⁰ were preserved at a time when airways were refractory to a repeat indirect challenge. This led to speculation that the mechanism might be due to depletion of mast cell mediators. However, subsequent studies have clearly documented that this is not the case.^{21,22} Although the mechanism of the refractoriness is not completely understood, hypotheses have focused on the release of inhibitory prostaglandins^{21,22} and on the desensitization of cysteinyl leukotriene₁ receptors.^{22,23}

In summary, we have confirmed that allergen-induced increase in airway responsiveness to methacholine is present 3 hours after

an allergen challenge, even in asthmatic patients who did not have an LAR. At the same time, the airways have become refractory to mannitol challenge.

Acknowledgments

We thank Jacquie Bramley for preparation of the manuscript and Pharmaxis Ltd for supplying the mannitol.

References

- [1] Pauwels R, Joos G, Van Der Straeten M. Bronchial hyperresponsiveness is not bronchial hyperresponsiveness is not bronchial asthma. *Clin Allergy*. 1988;18:317–321.
- [2] Schoeffel RE, Anderson SD, Gillam I, Lindsay DA. Multiple exercise and histamine challenge in asthmatic patients. *Thorax*. 1980;35:164–170.
- [3] Rakotosihanaka F, Melaman F, d'Athis P, Florentin D, Dessanges JF, Lockhart A. Refractoriness after hyperventilation-induced asthma. *Bull Eur Physiopathol Respir*. 1986;22:581–587.
- [4] Daxun Z, Rafferty P, Richards R, Summerell S, Holgate ST. Airway refractoriness to adenosine 5'-monophosphate after repeated inhalation. *J Allergy Clin Immunol*. 1989;83:152–158.
- [5] Suh DI, Lee JK, Kim JT, Koh YY. Airway refractoriness to inhaled mannitol after repeated challenge. *Pediatr Pulmonol*. 2011;46:1007–1014.
- [6] Belcher NG, Rees PJ, Clark TJ, Lee TH. A comparison of the refractory periods induced by hypertonic airway challenge and exercise in bronchial asthma. *Am Rev Respir Dis*. 1987;135:822–825.
- [7] O'Byrne PM, Dolovich J, Hargreave FE. State of the art: late asthmatic responses. *Am Rev Respir Dis*. 1987;136:740–751.
- [8] Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy*. 1977;7:503–513.
- [9] Pin I, Freitag AP, O'Byrne PM, et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. *Am Rev Respir Dis*. 1992;145:1265–1269.
- [10] Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy*. 1977;7:235–243.
- [11] Cockcroft DW, Murdock KY, Mink JT. Determination of histamine PC₂₀: comparison of linear and logarithmic interpolation. *Chest*. 1983;84:505–506.
- [12] Jokic R, Davis EE, Cockcroft DW. Extrapolation of methacholine PC₂₀. *Chest*. 1998;103:1796–1797.
- [13] Anderson SD, Brannan J, Spring J, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med*. 1997;156:758–765.
- [14] Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway calibre. *J Allergy Clin Immunol*. 1982;70:170–177.
- [15] Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. *Thorax*. 1987;42:302–308.
- [16] Durham SR, Craddock CF, Cookson WO, Benson MK. Increases in airway responsiveness to histamine precede allergen-induced late asthmatic responses. *J Allergy Clin Immunol*. 1988;82:764–770.
- [17] Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet*. 2000;356:2144–2148.
- [18] van den Berge M, Kerstjens HA, Meijer RJ, et al. Corticosteroid-induced improvement in the PC₂₀ of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC₂₀ of methacholine. *Am J Respir Crit Care Med*. 2001;164:1127–1132.
- [19] van Velzen E, van den Bos JW, Benckhuijsen JA, van Essel T, de Bruijn R, Aalbers R. Effect of allergen avoidance at high altitude on direct and indirect bronchial hyperresponsiveness and markers of inflammation in children with allergic asthma. *Thorax*. 1996;51:582–584.
- [20] Hahn AG, Nogrady SG, Tumilty DM, Lawrence SR, Morton AR. Histamine reactivity during the refractory period after exercise induced asthma. *Thorax*. 1984;39:919–923.
- [21] Manning PJ, Watson RM, O'Byrne PM. Exercise-induced refractoriness in asthmatic subjects involves leukotriene and prostaglandin interdependent mechanisms. *Am Rev Respir Dis*. 1993;148:950–954.
- [22] Larsson J, Perry CP, Anderson SD, Brannan JD, Dahlén SE, Dahlén B. The occurrence of refractoriness and mast cell mediator release following mannitol-induced bronchoconstriction. *J Appl Physiol*. 2011;110:1029–1035.
- [23] Larsson J, Anderson SD, Dahlén SE, Dahlén B. Refractoriness to exercise challenge: a review of the mechanisms old and new [published online March 29, 2013]. *Immunol Allergy Clin N Am*. doi: 10.1016/j.iac.2013.02.004.