

Absence of Deep Inspiration–induced Bronchoprotection against Inhaled Allergen

George Pyrgos, Trisevgeni Kapsali, Solbert Permutt, and Alkis Togias

Divisions of Clinical Immunology and Respiratory and Critical Care Medicine, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; and Division of Allergy, Hellenic Army Veteran Hospital, Athens, Greece

Deep inspiration–induced bronchoprotection appears to be a major mechanism through which airway obstruction by spasmogens is avoided. Loss of bronchoprotection is associated with airway hyperresponsiveness. Individuals with allergic rhinitis and no airway hyperresponsiveness develop obstruction after allergen inhalation. To test the hypothesis that deep inspiration–induced bronchoprotection is not active against allergic reactions, we performed four single-dose bronchial challenges, two with methacholine and two with allergen, on 10 subjects with allergic rhinitis. Without deep inspirations, the methacholine-induced reduction in FEV₁ from baseline was $36.9 \pm 3.6\%$ (mean $\pm$ SEM); this was attenuated to $15.0 \pm 2.0\%$ when five deep inspirations preceded methacholine inhalation ($p = 0.0001$). When allergen was inhaled, the reduction in FEV₁ was $24.7 \pm 2.9\%$ and $28.8 \pm 6.4\%$ without and with deep inspirations, respectively. We conclude that bronchoprotection by deep inspirations is absent against allergic reactions. Understanding the cause of this phenomenon may shed light into the pathogenesis of airway hyperresponsiveness in allergic asthma.

Keywords: bronchial provocation tests; airway hyperresponsiveness; allergic rhinitis; asthma; bronchial hyperreactivity

We and others have demonstrated the beneficial effects that deep inspirations have against bronchoconstriction induced by methacholine (1–3). We have further defined these effects as bronchoprotection, the effect of deep inspirations taken before the administration of methacholine, and bronchodilation, the effect of deep inspirations taken after the administration of methacholine (4, 5). Whereas bronchodilation seems to be universally present in healthy individuals, in individuals having rhinitis with hyperresponsiveness, and in individuals with mild asthma, bronchoprotection is only present in healthy humans and is absent in most individuals with airway hyperresponsiveness, regardless of whether they have rhinitis alone or asthma. We have postulated that the loss of the bronchoprotective effect of deep inspirations can account for a substantial proportion of the phenomenon of airway hyperresponsiveness (6).

The mechanism accounting for the loss of bronchoprotection by deep inspiration in subjects with airway hyper-

responsiveness is unknown. This study was designed to test the hypothesis that, in the presence of an allergic reaction, the bronchoprotective effect of deep inspiration is absent. One can assume that the airways of individuals with allergic asthma are almost continuously exposed to allergen and, therefore, allergic reactions constantly occur. It is reasonable to hypothesize that these allergic reactions inhibit the bronchoprotective effects of deep inspiration and, through this mechanism, they induce airway hyperresponsiveness. This becomes even more reasonable knowing that patients with allergic rhinitis who are completely nonresponsive to methacholine do develop bronchoconstriction when they inhale allergen (7). If an allergic reaction did not influence the bronchoprotective effect of deep inspiration, these individuals would have not developed bronchoconstriction with inhaled allergen.

To test our hypothesis we had to use a population that on one hand had intact deep inspiration–induced bronchoprotection against methacholine and, on the other hand, was capable of reacting to inhaled allergen. Individuals with allergic rhinitis and no airway hyperresponsiveness to methacholine were chosen for this purpose.

METHODS

Subjects

We originally enrolled 18 individuals. Inclusion criteria were a history of rhinitis with at least one clinically relevant positive skin test to ragweed, Timothy grass, *Dermatophagoides farinae* or cat, baseline FEV₁ more than 80% predicted, a negative methacholine challenge ($< 20\%$ reduction in FEV₁ at 75 mg/ml), and no history of asthma. The study was approved by the Johns Hopkins Bayview Medical Center IRB, and informed, written consent was obtained from every subject.

Study Design

Phase 1: screening. This included a respiratory questionnaire, skin prick testing to a panel of 10 common aeroallergens, and a routine methacholine challenge (4).

Phase 2: multiple-dose allergen bronchoprovocation in the absence of deep inspirations. The allergen was chosen on the basis of relevancy to the volunteer's symptoms and skin test sensitivity. An intradermal skin test titration was performed to establish the lowest allergen dose to start the bronchoprovocation (8). Bronchoprovocation was performed with doubling doses in 10-minute dosing intervals and was monitored with partial spirometry, as described previously (1). The challenge was discontinued if the ratio of the partial FEV₁ over FVC dropped to ~ 0.5 to 0.55 or if uncomfortable chest symptoms developed.

Phase 3: single-dose allergen bronchoprovocations. The single dose of allergen capable of inducing at least a 15% reduction in FEV₁ in the absence of deep inspirations was determined using methodology described previously for methacholine (5) (Figure 1). The first dose used was the last dose of the multiple-dose allergen bronchoprovocation of Phase 2. If that dose did not induce the targeted bronchoconstriction, the challenge was repeated at least 2 weeks later with a double dose of allergen, and so on, up to a maximal dose.

Phase 4: routine methacholine bronchoprovocation. This was a

(Received in original form January 22, 2003; accepted in final form March 27, 2003)

Supported by the National Institutes of Health grant RO1-HL-61277. G.P. and T.K. are recipients of the George Behrakis Hellenic Fellowship in Respiratory Allergy at the Johns Hopkins Asthma and Allergy Center. T.K. has also received the American Academy of Allergy, Asthma and Clinical Immunology Zeneca Asthma Research Award.

Correspondence and requests for reprints should be addressed to George Pyrgos, M.D., 5501 Hopkins Bayview Circle, Baltimore, MD 21224. E-mail: gpyrgos@jhmi.edu

Am J Respir Crit Care Med Vol 167, pp 1660–1663, 2003

DOI: 10.1164/rccm.2201048

Internet address: www.atsjournals.org

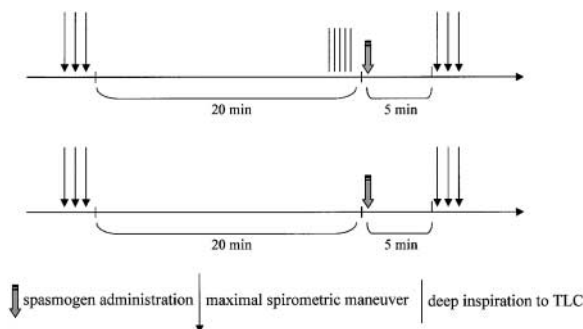


Figure 1. Time diagram of the method used to assess deep inspiration-induced bronchoprotection. On one occasion, after routine spirometry (three reproducible maneuvers), each subject was asked to refrain from taking deep inspirations for 20 minutes. Methacholine or allergen was then delivered with five tidal inhalations. Five minutes later, routine spirometry was performed. On the other occasion, five deep inspirations were taken immediately before the administration of the challenge material.

safety element in the study and was performed at least 2 weeks after the last single-dose allergen challenge. In the event a provocative concentration causing 20% reduction from the post-saline baseline in FEV₁ (PC₂₀) was obtainable, (PC₂₀ < 75 mg/ml), this subject would be excluded from the study. However, no subject was excluded for this reason.

Phase 5: single-dose methacholine bronchoprovocation without deep inspirations. Challenges similar to the single-dose allergen were performed using doubling doses of methacholine (starting at 10 mg/ml) to determine the single dose capable of inducing a greater than 15% reduction in FEV₁ (5).

Phase 6: main study. This consisted of four single-dose challenges, two with allergen and two with methacholine, in a randomized order. For each stimulus, one challenge was performed in the absence of deep inspirations and the other with five consecutive slow deep breaths to total lung capacity without breath holding, immediately before the spasmogen administration (Figure 1). The dose of allergen or methacholine was the one previously determined by Phases 3 and 5, respectively. Challenges were separated from each other by at least 2 weeks.

Data Analysis

The primary outcomes of this study were the allergen- or methacholine-induced changes from baseline in FEV₁ and FVC. A bronchoprotection index was calculated by subtracting the FEV₁ reduction in the protocol devoid of deep inspirations from that in the protocol with deep inspirations and normalizing to the former (4). Comparisons were made by analysis of variance and two-tailed paired *t* tests (*p* values of ≤ 0.05 were considered significant).

RESULTS

Eighteen individuals with allergic rhinitis and no airway hyper-responsiveness were recruited from the screening phase of the

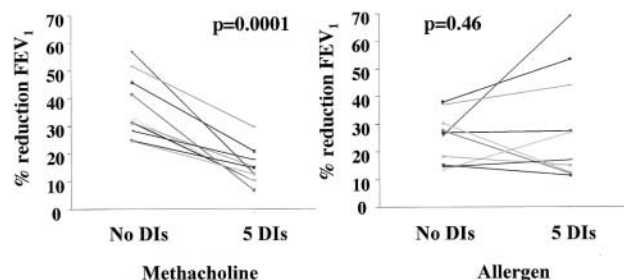


Figure 2. Changes in FEV₁ after the administration of single-dose methacholine (left panel) and allergen (right panel), with or without deep inspirations (DI). *p* Values refer to the comparison of the reductions of FEV₁ in the absence versus the presence of deep inspirations.

study (Phase 1) into the second phase. After completing Phase 2, one individual did not wish to receive any further allergen challenges. Seventeen individuals continued onto Phase 3. These individuals received 1 to 5 single-dose allergen challenges to determine the single dose of allergen capable of inducing at least a 15% reduction in their FEV₁. The median single dose of allergen delivered during this phase was 1,250 allergen units (AU) or protein nitrogen units (PNU), in breath units (25th, 75th percentiles: 322, 2,500; one breath unit = 1 breath of a 1 PNU (or AU)/ml solution). Two individuals had an inadequate reduction in their FEV₁ even with the highest single allergen dose (5,000 PNU/ml ragweed and 1,250 AU/ml Timothy grass, respectively) and were excluded from the rest of the study. Three individuals did not wish to continue with the next phase of the study. Also, one individual relocated. The remaining 12 subjects were recruited into Phase 4 and continued to show no hyper-responsiveness to a routine methacholine challenge. They were then recruited into Phase 5, and the single methacholine dose capable of inducing at least a 15% reduction in their FEV₁ in the absence of deep inspirations was determined (median single dose used: 20 mg/ml, 25th, 75th percentiles: 10, 20). All these individuals entered Phase 6, the main phase of the study. Two individuals could not demonstrate an adequate reduction in their FEV₁ with the single-dose allergen challenge even though they had done so during the third phase of the study. These subjects were excluded from this phase. The characteristics of the 10 subjects who completed all phases of the study are shown in Table 1. During the entire study, all subjects were asked to abstain from taking antihistamines at least 1 week before any visit (48 hours for shorter-acting antihistamines). No subject was using nasal or oral glucocorticosteroids. None of the subjects had symptoms compatible with asthma, and none was receiving any asthma medications or leukotriene modifiers.

Figure 2 depicts the individual findings for FEV₁ after all four

TABLE 1. DEMOGRAPHIC CHARACTERISTICS, BASELINE LUNG FUNCTION OUTCOMES, AND SINGLE DOSE OF ALLERGEN EMPLOYED IN THE 10 SUBJECTS THAT COMPLETED THE STUDY

Subject	Age	Sex	FEV ₁ % Pred	% Pred FVC	Allergen	Single Allergen Dose Concentration	Single Methacholine Dose Concentration
1	19	M	104.1	111.0	Timothy grass	625 BAU/ml	10 mg/ml
2	39	F	87.7	86.3	<i>Dermatophagoides farinae</i>	80 AU/ml	20 mg/ml
3	36	F	103.7	103.8	Ragweed	5,000 PNU/ml	20 mg/ml
4	50	F	104.4	108.6	<i>Dermatophagoides farinae</i>	1,250 AU/ml	20 mg/ml
5	21	M	113.3	127.3	Cat	2,500 BAU/ml	10 mg/ml
6	44	M	106.49	105.5	Ragweed	2,500 PNU/ml	40 mg/ml
7	25	M	85.1	89.0	Timothy grass	625 BAU/ml	20 mg/ml
8	26	F	96.4	102.2	Timothy grass	312.5 BAU/ml	10 mg/ml
9	36	F	91.0	91.6	Timothy grass	312.5 BAU/ml	20 mg/ml
10	31	F	90.7	91.8	Timothy grass	2,500 BAU/ml	20 mg/ml
Mean ± SD	32.7 ± 10		98.3 ± 9.4	101.7 ± 12.5		Median, 937.5	Median, 20 mg/ml

Definition of abbreviations: %Pred = percent predicted; AU = allergen units; BAU = biologic allergy units; PNU = protein nitrogen units.

methacholine and allergen single-dose challenges of the final phase of the study. There was a 36.9 ± 3.6 (mean $\pm$ SEM) reduction in the FEV₁ with the single-dose methacholine challenge, in the absence of deep inspirations. However, when five deep inspirations were taken before the spasmogen administration, a significant attenuation of the methacholine effect was observed ($15.0 \pm 2.1\%$, $p = 0.0001$). This was not the case for allergen. The single-dose allergen challenge induced a $24.7 \pm 2.9\%$ reduction in FEV₁ in the absence of deep inspirations, which was not altered when five deep inspirations preceded the challenge ($28.8 \pm 6.45\%$, $p = 0.47$). In addition to *t* tests, a two-factor repeated measures analysis of variance was performed with the type of inhalation challenge (allergen vs. methacholine) and the presence/absence of deep breaths as main and interactive effects. We found a highly significant interaction between these two factors ($p = 0.0029$), which confirms the *t* test results.

Identical results are obtained when FVC was assessed. These results are shown in Figure 3. There was a $27.5 \pm 3.3\%$ reduction in the absence of deep inspirations with methacholine, and this was reduced to $5.9 \pm 2.7\%$ when five deep inspirations preceded the challenge ($p = 0.0001$). As with FEV₁, deep inspirations had no effect on the allergen-induced reductions in FVC (18.9 ± 3.2 with no deep inspirations vs. 22 ± 6.3 with five deep inspirations, $p = 0.56$). Repeated measures, two-factor analysis of variance was also performed for FVC, as with FEV₁. Here again, the type of inhalation challenge showed a significant interaction with the presence/absence of deep inspirations ($p = 0.005$).

We also calculated the bronchoprotection index, as described previously by Scichilone and coworkers (6). This is a measure of the influence of deep inspirations taken before the administration of a spasmogen, in relation to the effect of the same dose of spasmogen, in the absence of deep inspirations. In Figure 4, the bronchoprotective indices for FEV₁ are shown; the calculated index was $57 \pm 4.9\%$ for methacholine and $-18.5 \pm 20.9\%$ for allergen ($p = 0.01$).

Because two individuals were disqualified secondary to inadequate response to allergen in Phase 6, we addressed the issue of single-dose allergen challenge reproducibility. Bland-Altman analysis (9) was performed (1) between the allergen challenge in Phase 3 and the challenge in Phase 6 that used the same allergen dose and (2) between the methacholine challenge in Phase 5 and the challenge in Phase 6 that used the same methacholine dose. This analysis showed that the single-dose allergen challenges were, if anything, more reproducible than those of methacholine (data not shown).

DISCUSSION

Our group is involved in the examination of the effect of lung inflation during bronchoprovocation (4–6, 10, 11). Because of the striking differences that are observed between healthy subjects and subjects with asthma in the ability of deep inspiration to protect the airways from a methacholine challenge, we have speculated that the phenomenon of airway hyperresponsiveness may be due, to a large extent, to the loss of the beneficial effects of deep inspiration, particularly in bronchoprotection (1, 6). The question that arises is how this loss may occur. The results of the present study propose that allergic reactions are involved in this process. Individuals who display bronchoprotection to methacholine by deep inspiration do not have this ability when allergen is inhaled.

One can raise several hypotheses to explain the absence of deep inspiration-induced bronchoprotection against allergen. Allergen challenge causes the release of a cascade of mediators such as histamine, leukotrienes, prostaglandins, or bradykinin

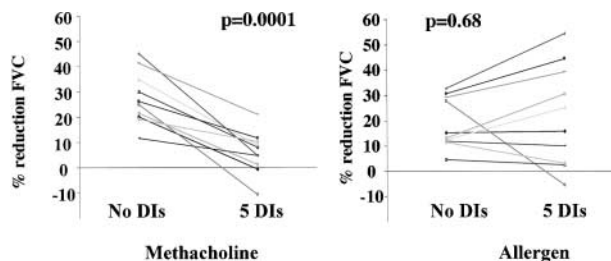


Figure 3. Changes in FVC after the administration of single-dose methacholine (left panel) and allergen (right panel) with or without deep inspirations (DI). *p* Values refer to the comparison of the reductions of FVC in the absence versus the presence of deep inspirations.

(12–14). These substances may not have just a pure bronchoconstrictive effect but also other functional effects on the airways, particularly on airway smooth muscle, which could influence the outcome of bronchoprovocation. Preliminary data suggest that healthy individuals display bronchoprotection to histamine in a manner similar to methacholine, making histamine an unlikely mediator capable of hindering deep inspiration-induced bronchoprotection (15). Leukotrienes should be seriously considered. Inhaled leukotrienes can cause bronchoconstriction in both individuals with asthma and healthy individuals even in the presence of deep inspirations (16). Bradykinin may also be responsible for the loss of the bronchoprotective effect of deep inspirations. We have recently observed that the bronchoconstriction induced by bradykinin does not seem to be modified by deep inspirations (17).

If one or more products of the acute allergic reaction are responsible for the absence of bronchoprotection by deep inspiration, the next question is how would this effect materialize. One possible mechanism is the edema that can be produced by all the mediators of the acute allergic reaction (18). It has been speculated that airway wall edema may uncouple the smooth muscle from the parenchyma leading to unopposed muscle contraction (19). It is worth mentioning, however, that administration of bradykinin through a wedged bronchoscope into a subsegmental bronchus results in equal amounts of plasma extravasation in healthy individuals and in individuals with asthma, but it increases peripheral airway resistance only in the latter group (20). Another possibility is that one or more products of the acute allergic reaction act as functional antagonists to a biochemical event that mediates deep inspiration-induced bronchoprotection. We have recently proposed that nitric oxide, possibly deriving

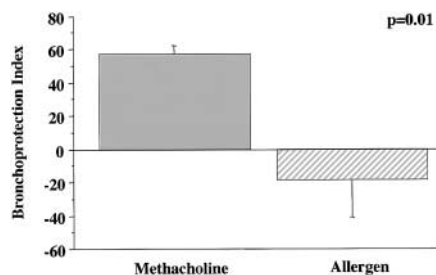


Figure 4. Bronchoprotection indices for methacholine and allergen. This index is calculated as the difference between the % reduction in FEV₁ from baseline when deep inspirations are not taken and when they are taken before the administration of the spasmogen, divided by the reduction in FEV₁ induced when no deep inspirations are taken before spasmogen administration, and multiplied by 100. *p* Value refers to the comparison of the bronchoprotection indices obtained from the methacholine versus the allergen challenges.

from the nonadrenergic, noncholinergic bronchodilation nervous system, may be released by airway stretch and may act on the airway smooth muscle as a mediator of deep inspiration-induced bronchoprotection (21). A role of nitric oxide as a bronchoprotector has been suggested by animal studies and, most recently, by the work of Ricciardolo and coworkers (22). These investigators demonstrated that administration of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine in a group of individuals with asthma leads to increased airway responsiveness to bradykinin. This finding indicates that nitric oxide may have a protective role against bronchoconstrictive stimuli.

This study was not designed to examine the effect of allergen bronchoprovocation on the other well-described property of deep inspiration, bronchodilation. A blunting effect of allergen bronchoprovocation on deep inspiration-induced bronchodilation has been reported in early (23) or early and late phase (24) in allergic reactions. By performing an analysis on the outcomes of the three consecutive spirometric maneuvers that our subjects took 5 minutes after allergen or methacholine were administered, we confirmed the presence of a bronchodilatory effect of deep inspiration against methacholine (analysis of variance, $p < 0.0001$) but failed to observe significant improvement in FEV₁ with repeated spirometry after allergen (analysis of variance, $p = 0.4$) administration. Therefore, in agreement with Bel and colleagues (24) we found evidence that allergen bronchoprovocation also suppresses deep inspiration-induced bronchodilation.

One limitation of our study is that despite the effort to match the reductions in lung function between the allergen and methacholine challenges, it was not possible to accomplish this in all cases. When we compared the induced reduction in FEV₁ in the absence of deep inspirations by methacholine and allergen, a significant difference was found with allergen having a lesser effect ($p = 0.02$). We have demonstrated previously that the beneficial effects of deep inspirations become apparent when the induced reduction in lung function, in the absence of deep inspiration, is greater than 20% (4). It is, therefore, possible that the subjects who did not reach this level of airway obstruction could have demonstrated a bronchoprotective effect of deep inspirations against allergen if the reduction in lung function in the absence of deep inspirations was more profound. However, when we examined the six individuals who did have a greater than 20% reduction in FEV₁ with allergen in the absence of deep inspirations, we still failed to demonstrate any bronchoprotective effect of deep inspirations against allergen administration. Another issue that could be raised is that the lack of bronchoprotection against allergen may in part be due to poor reproducibility of the single-dose allergen challenge. The Bland-Altman analysis that we performed to compare the reproducibility of the single-dose allergen versus the single-dose methacholine challenges indicated that allergen challenge was more reproducible than methacholine. Thus, the lack of bronchoprotection during allergen challenge cannot be attributed to reproducibility issues.

In conclusion, we have found that deep inspiration-induced bronchoprotection is absent in the face of experimental allergen exposure. This observation is of importance because it provides us with a model to explore the cause of the loss of deep inspiration-induced bronchoprotection in individuals with airway hyperresponsiveness. Consequently, it may allow us to shed light on the mechanisms of this pivotal characteristic of asthma.

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