Inhaled Corticosteroids and the Beneficial Effect of Deep Inspiration in Asthma

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Deep inspiration–induced bronchoprotection and bronchodilation are impaired in asthma. We evaluated the effect of inhaled glucocorticosteroids on these phenomena. Two groups of subjects with asthma, 9 with moderate/severe hyperresponsiveness to methacholine, and 12 with mild/borderline hyperresponsiveness to methacholine, received inhaled fluticasone (880 μg daily) for 12 weeks. Serial bronchoprovocations were performed at Weeks 0, 6, and 12. The impact of deep inspirations on the airway response to methacholine was evaluated on the basis of inspiratory vital capacity and FEV_1. Fluticasone produced a wide spectrum of changes in the beneficial effects of deep inspiration, but the mean changes were not significant. The magnitude of the steroid-induced changes in bronchoprotection by deep inspiration correlated with baseline log PC_{20} (the provocative concentration of methacholine causing a 20% fall in FEV_1; higher log PC_{20} predicted improvement of the deep inspiration effect). The steroid-induced changes led to the emergence of strong positive correlations between the effects of deep inspiration and the methacholine log PC_{20} that did not exist at baseline. We conclude that deep inspiration–induced bronchoprotection can be restored by inhaled glucocorticosteroids only in individuals with mild hyperresponsiveness. After steroid treatment, the beneficial effects of deep inspiration become significant determinants of the magnitude of airway hyperresponsiveness.

Keywords: airway hyperresponsiveness; asthma; deep inspiration; fluticasone; methacholine

We and others (1–6) have shown that deep inspiration exerts two potent beneficial effects on the airways of healthy subjects: the bronchoprotective effect, that is, the ability of deep inspirations performed before the inhalation of a spasmogen to prevent bronchial constriction; and the bronchodilatory effect, where deep inspirations taken immediately after the induction of bronchoconstriction improve airflow. In our previous work (1, 3), we have also found that the bronchoprotective effect of deep inspiration is greatly reduced in subjects with airway hyperresponsiveness, even when it is not accompanied by the diagnosis of asthma, and have suggested that the lack of deep inspiration–induced bronchoprotection is one of the major underlying causes of the hyperresponsive state. On the other hand, the bronchodilatory effect of deep inspiration is less discriminatory between airway hyperresponsiveness and normal responsiveness (3, 4, 6, 7).

Glucocorticosteroids are capable of reducing airway hyperresponsiveness (8), suggesting that this condition is somehow associated with airway inflammation. If so, it is possible that the loss of deep inspiration–induced bronchoprotection is linked to airway inflammation. The current study was designed to indirectly test this hypothesis by assessing the effect of inhaled glucocorticosteroid treatment on deep inspiration–induced bronchoprotection in volunteers with asthma.

We have previously reported that, as a group, individuals with borderline to mild airway hyperresponsiveness to methacholine demonstrate loss of deep inspiration–induced bronchoprotection to the same extent as those with moderate to severe hyperresponsiveness (3). This has raised the possibility that bronchoprotection by deep inspiration is the first normal function to be lost in the development of hyperresponsiveness. If so, and if inhaled glucocorticosteroids were to improve this function, their effect may be more evident in individuals with mild to borderline hyperresponsiveness, as opposed to those with a more advanced condition. To test this hypothesis, the design of this study has included two groups of volunteers with asthma: a group with borderline to mild hyperresponsiveness and a group with moderate to severe hyperresponsiveness.

METHODS

Subjects

We studied 21 subjects with asthma. Inclusion/exclusion criteria were identical to those employed in a previous study (3). All but one subject were skin-test positive to at least one aeroallergen. All subjects had intermittent or mild persistent asthma (9) and none had received inhaled or oral corticosteroids. Short-acting β-agonists were withheld for at least 12 hours before each visit. For subjects who developed respiratory infections, bronchoprovocation sessions were postponed for at least 3 weeks. The study was designed to involve two groups, based on hyperresponsiveness to methacholine: (*1*) moderate to severe hyperresponsiveness (PC_{20} [provocative concentration of methacholine causing a 20% fall in FEV_1] < 1 mg/ml, n = 9) and (*2*) mild to borderline hyperresponsiveness ($1 < PC_{20} < 25$ mg/ml, n = 10). We also entered two subjects with PC_{20} values of 44.7 and 45.4 mg/ml, respectively, but with a clinical history of asthma. The study was approved by the Johns Hopkins Bayview Institutional Review Board, and all subjects gave written, informed consent.

Study Design

The entire study consisted of two phases (Figure 1).

Phase 1. Each subject underwent a routine methacholine bronchoprovocation, using approximately half-log–increasing doses from 0.025 to 75 mg/ml (10), to assess the PC_{20}. Thereafter, and on separate visits, a series of modified, single-dose bronchoprovocations (starting from 0.075 mg/ml) was performed as previously described (2) to determine (*1*) the single dose of methacholine inducing an at least 20% fall in FEV_1 in the absence of deep inspirations (Figure 2a), (*2*) the ability of deep inspiration to reverse bronchoconstriction, expressed as percentage of bronchodilation for inspiratory vital capacity (IVC) and FEV_1 (Figure 2b), and (*3*) the ability of deep inspiration to prevent bronchoconstriction, expressed as percentage of bronchoprotection for IVC and FEV_1 (Figure 2c). This methodology is described in the online supplement.

Phase 2. All subjects received inhaled fluticasone (220 μg, two inhalations twice daily) through a metered-dose inhaler and a spacer (AeroChamber; Trudell Medical International, London, ON, Canada), for

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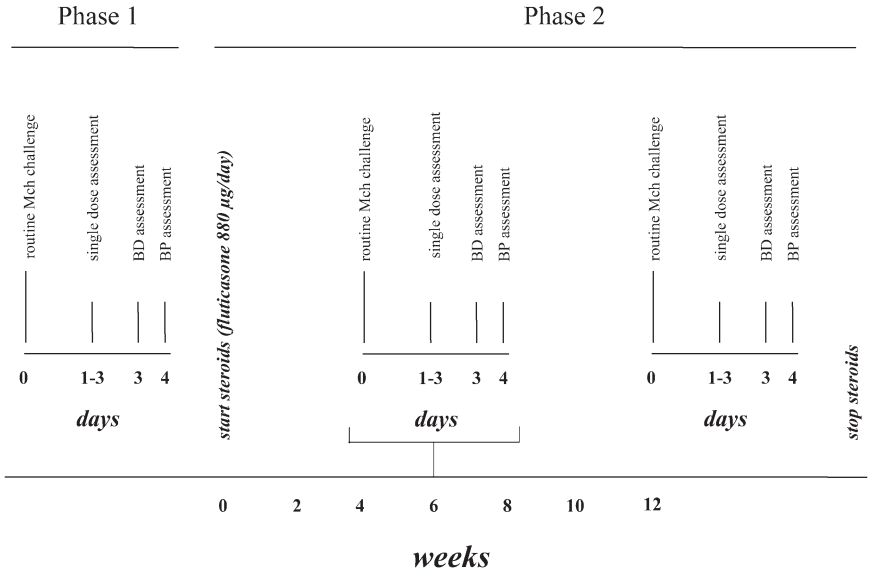

Figure 1. Schematic of the protocol, describing the two phases of the study (*see also* STUDY DESIGN). Phase 1 took place before the initiation of fluticasone treatment. During the course of treatment, subjects visited the laboratory every 2 weeks for routine evaluations. During Week 6 of treatment, as well as after Week 12, the Phase 1 procedures were repeated. Single-dose assessment = determination of the single dose of methacholine that reduces FEV_1 by at least 20%; BD assessment = determination of the bronchodilatory effect of deep inspiration; BP assessment = determination of the bronchoprotective effect of deep inspiration; Mch = methacholine.

12 to 14 weeks. Each subject visited the laboratory every 2 weeks. At 6 weeks, and at the end of 12 weeks, the series of methacholine bronchoprovocations performed in Phase 1 were repeated.

Data Analysis

The log PC_{20}, the percentage of bronchoprotection and the percentage of bronchodilation by deep inspirations, and the log of the single-dose methacholine required to reduce FEV_1 by more than 20% in the absence of deep inspirations represented the outcomes of this study. To calculate bronchoprotection and bronchodilation, we employed the IVC as primary measure. The advantage is that the maneuver to determine IVC (from end-tidal inspiration to residual volume and then to total lung capacity) is free of the potential bronchodilatory effect of the deep inspiration that is involved in the measurement of FEV_1 (11–13). Treatment-induced changes from baseline over time were analyzed by repeated measures one-way analysis of variance (ANOVA) or by Friedman ANOVA, followed by appropriate *post hoc* tests. Linear regressions or the Spearman rank correlation were used to examine the relationship between conventionally measured airway responsiveness (expressed as log PC_{20}) and deep inspiration–induced bronchoprotection and bronchodilation. Nonparametric statistics were used for post-steroid methacholine responsiveness values because, in five subjects who did not reach a PC_{20} after treatment, this parameter was assigned an arbitrary value (225 mg/ml). Significance was assumed for two-tailed values of $p \leq 0.05$.

RESULTS

Phase 1

The demographic and lung function characteristics of the study participants are shown in Table 1. The two subjects who had low responsiveness at baseline were analyzed together with the "mild to borderline hyperresponsiveness" group. The primary analyses were conducted with and without these two subjects, with no qualitative impact on the outcome. The two study groups did not differ in terms of age (unpaired *t* test, $p = 0.70$), FEV_1 % predicted (unpaired *t* test, $p = 0.44$), FVC % predicted (unpaired *t* test, $p = 0.17$), or FEV_1/FVC (unpaired *t* test, $p = 0.74$). Also, the two groups did not differ with respect to the duration of asthma (23 ± 3.9 years in the mild to borderline hyperresponsiveness group vs. 28 ± 2.6 years in the moderate to severe hyperresponsiveness group; $p = 0.33$). By design, the magnitude of airway hyperresponsiveness, expressed as the PC_{20}, was 15-fold different between the two subject groups (0.35 vs. 5.25 mg/ml, geometric mean). Table 2 presents information on the effects of the single-dose methacholine challenges. Notably, we met the target of inducing similar reductions in lung function with single doses of methacholine in the two groups. The single dose of methacholine employed to achieve the targeted change in lung function from baseline in the protocol devoid of deep inspirations was twofold

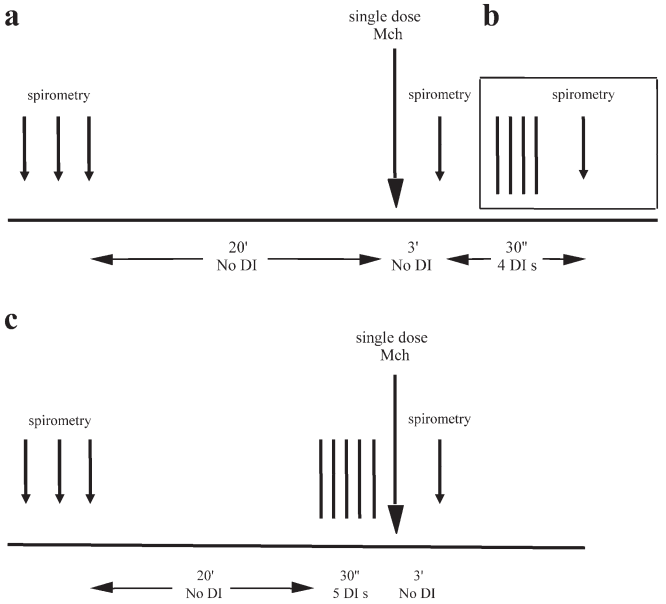

Figure 2. Schematic of the protocol for methacholine bronchoprovocations employed to determine the single dose of spasmogen inducing a 20% reduction in FEV_1 (*a*), the bronchodilatory effect of deep inspiration (*b*), and the bronchoprotective effect of deep inspiration (*a* and *c*). Spirometry in this protocol consists of a partial forced expiration from end-tidal volume to residual volume, followed immediately by deep inspiration to total lung capacity and by another forced expiration to residual volume. This combination maneuver enables us to measure (*1*) the inspiratory vital capacity (IVC) as the difference between the total lung capacity and the preceding residual volume and (*2*) the FEV_1. As opposed to FEV_1, IVC measured in this way has the advantage of not being influenced by a preceding deep inspiration. DI = deep inspiration; Mch = methacholine.

TABLE 1. DEMOGRAPHIC AND FUNCTIONAL CHARACTERISTIC OF THE SUBJECTS PARTICIPATING IN STUDY

Subject No.	Age (*yr*)	Race	Sex	Skin Test Reactivity	FEV_1 (*% pred*)	FVC (*% pred*)	FEV_1/FVC	PC_{20} (*mg/ml*)
Moderate to severe methacholine hyperresponsiveness (PC_{20} < 1 mg/ml)								
1	45	AfrAm	F	+	93	93	0.85	0.43
2	27	Cauc	M	+	112	113	0.85	0.84
5	31	AfrAm	F	+	81	77	0.89	0.56
7	44	AfrAm	F	+	86	87	0.83	0.44
8	30	Cauc	F	+	93	96	0.83	0.11
9	40	AfrAm	F	+	95	98	0.81	0.32
12	26	Cauc	M	+	79	102	0.86	0.2
19	49	Cauc	M	+	72	99	0.61	0.16
20	36	AfrAm	F	+	100	99	0.85	0.86
Mean	36				90.1	96.0	0.82	0.35*
SD	8				12.1	10.0	0.08	
Mild to borderline methacholine hyperresponsiveness (1 mg/ml < PC_{20} < 25 mg/ml)								
3	48	Cauc	M	+	76	88	0.7	3.99
4	41	AfrAm	F	+	83	79	0.87	1.86
6	55	Cauc	M	+	82	98	0.67	1.87
10	28	Cauc	F	+	118	109	0.92	45.4
11	60	AfrAm	F	+	88	85	0.83	44.7
13	43	AfrAm	F	+	71	83	0.71	2.32
14	36	Cauc	M	+	86	85	0.85	7.28
15	31	AfrAm	M	+	77	90	0.73	1.26
16	23	AfrAm	F	+	78	88	0.77	4.96
17	26	Cauc	F	+	96	107	0.77	8.96
18	25	Cauc	F	−	91	85	0.93	8.57
21	55	Cauc	F	+	71	69	0.83	1.93
Mean	39				84.8	88.8	0.80	5.25 *
SD	13				13.0	11.2	0.09	

Definition of abbreviations: AfrAm = African American; Cauc = Caucasian; F = female; M = male.
* Geometric mean.

lower in those subjects with moderate to severe hyperresponsiveness (0.52 mg/ml, geometric mean) than in those with mild to borderline hyperresponsiveness (1.02 mg/ml), the difference not being statistically significant. When all subjects were pooled into a single group, there was a significant correlation between the log PC_{20} to the conventional methacholine bronchoprovocation and the log of the single-dose methacholine required to reduce lung function in the absence of deep inspirations (linear regression, $r^2 = 0.21$, $p = 0.04$).

As shown in Table 2, the bronchoprotective effect of deep inspiration as measured by IVC or FEV_1 did not differ between the two groups. Also, this effect was not significantly different from zero (for IVC: one-sample *t* test, $p = 0.14$ and $p = 0.40$, moderate/severe and mild/borderline hyperresponsiveness, respectively; for FEV_1: $p = 0.07$ and $p = 0.20$), supportive of our previous reports of loss of the effectiveness of deep inspirations to prevent bronchoconstriction in individuals with even borderline airway hyperresponsiveness (1, 3). The bronchoprotective

TABLE 2. MAIN RESULTS OF PHASE 1

	Mild to Borderline AHR Group	Moderate to Severe AHR Group	p Value
Reduction in IVC, %	25.2 ± 2.2	27.7 ± 2.8	0.49
Reduction in FEV_1, %	33.4 ± 1.7	32.2 ± 2.8	0.81
Single-dose MCh, mg/ml	1.02	0.52	0.41
Bronchoprotection by DI in IVC, %	13.7 ± 15.5	18.7 ± 11.4	0.81
Bronchoprotection by DI in FEV_1, %	16.7 ± 12.1	16.9 ± 7.8	0.98
Bronchodilation by DI in IVC, %	52.4 ± 8.5	44.3 ± 12.5	0.59
Bronchodilation by DI in FEV_1, %	47.7 ± 5.7	43.0 ± 12.4	0.71

Definition of abbreviations: AHR = airway hyperresponsiveness; DI = deep inspiration; IVC = inspiratory vital capacity; MCh = methacholine.

The reduction in IVC and in FEV_1 is obtained in the bronchoprovocation devoid of deep inspirations. The single-dose MCh is the provocative single dose of methacholine employed in the no deep inspiration–induced bronchoprovocation to attain the targeted reduction in lung function. The bronchoprotective and bronchodilatory effect of deep inspiration are expressed both in terms of IVC (the primary outcome) and FEV_1. Values are expressed as means ± SEM. The single-dose methacholine is expressed as a geometric mean.

ability of deep inspiration did not correlate with the degree of airway hyperresponsiveness as measured by the log PC_{20} (simple regression, $r^2 = 0.00$, $p = 0.99$).

As we and others have previously described (3, 4, 6, 7), statistically significant bronchodilation by deep inspiration was present in both groups, as shown in Table 2, whether IVC or FEV_1 was considered. No significant difference in the bronchodilatory effect of deep inspiration was found between the two groups. As with deep inspiration–induced bronchoprotection, when we assessed the relationship between the bronchodilatory ability of deep inspiration and the baseline magnitude of airway hyperresponsiveness (methacholine log PC_{20}), no significant correlation was found (simple regression, $r^2 = 0.02$, $p = 0.53$).

Phase 2

Twenty of 21 subjects completed the study. One subject (Subject 19) withdrew at 6 weeks of therapy for reasons not related to the study. During the study, four subjects experienced mild asthma exacerbations, presumably the result of respiratory viral infections; consequently, their next assessment was postponed for 3 weeks while continuing their inhaled corticosteroid use. None of the volunteers reported any adverse events associated with inhalation of the corticosteroids. No adverse events were recorded after any methacholine bronchoprovocation.

Treatment with inhaled fluticasone resulted in a small, but not significant, improvement in FEV_1, and no improvement in FVC, when the two groups were analyzed separately. The increase in FEV_1/FVC reached statistical significance in the mild to borderline hyperresponsiveness group (repeated measures ANOVA, $p = 0.04$). When the groups were combined, post-treatment FEV_1 was significantly higher than the pretreatment value (repeated measures ANOVA, $p = 0.04$).

Fluticasone reduced airway responsiveness in both groups: significant changes in methacholine log PC_{20} occurred at Week 6 and improvement continued between Weeks 6 and 12. Despite the fact that the two groups started treatment with a more than 1-log difference in methacholine PC_{20}, no significant difference was observed at Week 12 (Mann-Whitney U test, $p = 0.28$; Figure 3). Notably, when the conventional bronchoprovocation challenge was performed at the end of the study, five subjects had a less than 20% fall in FEV_1 with the highest concentration of methacholine (75 mg/ml). Two of these five subjects belonged to the group with moderate to severe hyperresponsiveness, which indicates that the improvement in their PC_{20} was on the order of 2 logs. The PC_{20} value for these five subjects was arbitrarily determined as 225 mg/ml, a value that is approximately 0.5 log higher than the highest dose of methacholine administered (75 mg/ml).

Significantly higher single doses of methacholine had to be administered at 12 weeks of treatment to induce the targeted bronchoconstriction in the protocol devoid of deep inspirations in both groups (Figure 4). The change in the log of the single-dose methacholine for the mild to borderline hyperresponsiveness group was 0.79 (Friedman ANOVA, $p = 0.01$); for the moderate to severe hyperresponsiveness group, it was 0.76 (Friedman ANOVA, $p = 0.02$). Remarkably, the steroid-induced change in log PC_{20} from the conventional challenge was in absolute correlation with the change in log of the single-dose methacholine challenge, which was conducted in the absence of deep inspirations ($r^2 = 1.0$).

The reduction in lung function in the single-dose methacholine challenges that were performed in the absence of deep inspirations at Weeks 6 and 12 was similar to that obtained at baseline (for the two subject groups combined, reduction in IVC: 26.3 vs. 27.9 and 27.6%, baseline vs. 6 and 12 weeks, repeated measured ANOVA, $p = 0.81$; reduction in FEV_1: 33.0 vs. 34.5 and 34.8%, repeated measured ANOVA, $p = 0.82$). This was also true for each group analyzed separately, allowing for a direct comparison of the bronchoprotective and the bronchodilatory effects of deep inspiration between baseline and 6 and/or 12 weeks. The effect of fluticasone on bronchoprotection by deep inspirations is depicted in Figure 5 (*top*). We observed some improvement, albeit not statistically significant, in the ability of deep inspirations to protect airways from constriction in the group with mild to borderline hyperresponsiveness. Similar results were obtained when FEV_1 was used as the spirometric outcome to calculate the bronchoprotective effect of deep inspiration. No significant difference was found between the two groups when the fluticasone-induced changes in the percentage of bronchoprotection IVC and FEV_1 were compared.

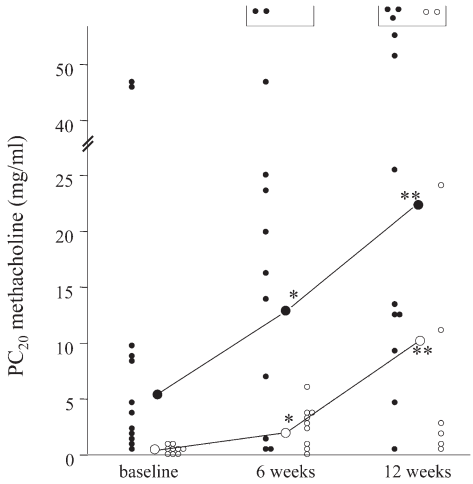

Figure 3. Individual values (*independent circles*) and geometric means (*connected circles*) for methacholine PC_{20} obtained from the routine challenges performed to assess airway responsiveness. These evaluations were obtained at baseline and after 6 and 12 weeks of treatment with inhaled fluticasone. Data are presented separately for each of the two groups of subjects that participated in the study: subjects with mild to borderline hyperresponsiveness (*solid circles*) and subjects with moderate to severe hyperresponsiveness (*open circles*). The *circles* in the *boxes* (*top*) indicate subjects who did not reach a measurable PC_{20} after the highest methacholine concentration was administered. These subjects were attributed an arbitrary value of 225 mg/ml, which is approximately the next to the highest dose (75 mg/ml) half-log incremental concentration of methacholine. Statistical analyses were conducted on the logarithmically transformed data (log PC_{20}). Analysis of variance (ANOVA) showed that significant changes incurred with fluticasone treatment. *Symbols* indicate statistically significant effects of fluticasone treatment in *post hoc* analyses: *6 weeks versus baseline; **12 weeks versus baseline.

Five subjects showed impressive improvement in the bronchoprotective effect of deep inspirations, reaching almost complete bronchoprotection (> 85%). Some of these subjects had dramatically reduced their airway responsiveness, as measured by conventional PC_{20}. Yet we found no overall relationship between the change in log PC_{20} (Δlog PC_{20}, baseline to the end of treatment) and the change in percentage of bronchoprotection IVC induced by inhaled fluticasone (Spearman rank correlation, $r_s = 0.09$, $p = 0.69$). On the other hand, all of these five subjects belonged in the group with mild to borderline hyperresponsiveness. Therefore, we analyzed the relationship between the baseline log PC_{20} and the change in percentage of bronchoprotection. This analysis yielded significant results, whether bronchoprotection by deep inspiration was calculated with IVC or FEV_1 (simple regression, $r^2 = 0.31$, $p = 0.01$, for percentage of bronchoprotection

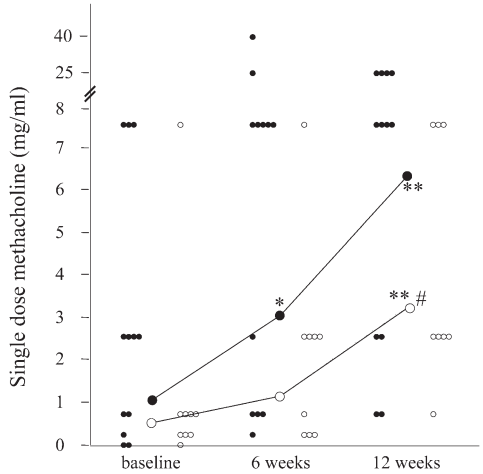

Figure 4. Individual values (*independent circles*) and geometric means (*connected circles*) for the single dose of methacholine administered during the challenges performed in the absence of deep inspirations. These evaluations were obtained at baseline and after 6 and 12 weeks of treatment with inhaled fluticasone. Data are separated for each study group: subjects with mild to borderline hyperresponsiveness (*solid circles*), and subjects with moderate to severe hyperresponsiveness (*open circles*). Statistical analyses were conducted on the logarithmically transformed data (log of single dose). ANOVA showed that significant changes incurred with fluticasone treatment. *Symbols* indicate statistically significant effects of fluticasone treatment in *post hoc* analyses: *6 weeks versus baseline; **12 weeks versus baseline; #12 weeks versus 6 weeks.

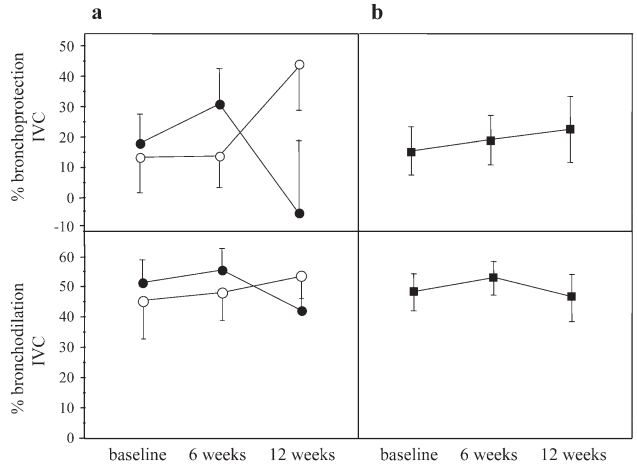

Figure 5. Mean (± SEM) values for deep inspiration–induced bronchoprotection and deep inspiration–induced bronchodilation at baseline and at 6 and 12 weeks of treatment with inhaled fluticasone. (*a*) Data separated for each of the two groups of subjects that participated in the study: subjects with mild to borderline hyperresponsiveness (*solid circles*) and subjects with moderate to severe hyperresponsiveness (*open circles*). (*b*) Data from all subjects pooled into a single group (*solid squares*). The depicted values for percentage of bronchoprotection and bronchodilation by deep inspiration were calculated on the basis of IVC. ANOVA failed to identify any statistically significant effects of fluticasone treatment.

IVC; and $r^2 = 0.36$, p = 0.005, for percentage of bronchoprotection FEV_1; Figure 6a).

Fluticasone treatment did not have any effect on the bronchodilatory function of deep inspiration, as measured by IVC or FEV_1, in either study group, or in the single-group analysis (IVC data in Figure 5, *bottom*). In contrast to our findings with deep inspiration–induced bronchoprotection, no relationship was found between the degree of baseline responsiveness and the changes in deep inspiration–induced bronchodilation after steroid treatment (Figure 6b).

In Phase 1 of the study (presteroid treatment), we found no relationship between the bronchoprotective effect of deep inspiration and airway responsiveness, as measured by the methacholine PC_{20}. We reassessed the relationship between these two outcomes at the 12-week evaluation time point. At the end of the study, the percentage of bronchoprotection IVC correlated significantly with the log PC_{20} when the subject population was treated as a single group (Spearman rank correlation, $r_s = 0.58$, p = 0.01; Figure 7). Analysis within each subgroup yielded a significant relationship only in the mild to borderline hyperresponsiveness group (Spearman rank correlation, $r_s = 0.73$, p = 0.01). Similar findings were obtained when correlation analyses were performed with percent bronchoprotection FEV_1 as the independent variable. We also performed these analyses with the bronchodilatory effect of deep inspiration as the independent outcome. For deep inspiration–induced bronchodilation, the percentage of bronchodilation IVC after steroid treatment correlated significantly with the methacholine log PC_{20} in the single-population analysis after steroid treatment (Spearman rank correlation, $r_s = 0.57$, p = 0.01). The significant baseline relationship between the log PC_{20} of the conventional methacholine challenge and the log of the single-dose methacholine administered in the absence of deep inspirations remained virtually unchanged after fluticasone treatment compared with before treatment ($r_s = 0.58$, p = 0.01, and $r_s = 0.47$, p = 0.04, respectively).

DISCUSSION

The purpose of this study was to examine whether impairment of the beneficial effects of deep inspiration in individuals with asthma can be reversed by inhaled glucocorticosteroid treatment. The study was more focused on the bronchoprotective effect of deep inspiration, because the loss of this effect is strongly linked to airway hyperresponsiveness (3). The study was also designed to assess whether recovery of deep inspiration–induced bronchoprotection by glucocorticosteroids can occur more effectively in individuals with mild hyperresponsiveness, compared with those with moderate or severe hyperresponsiveness.

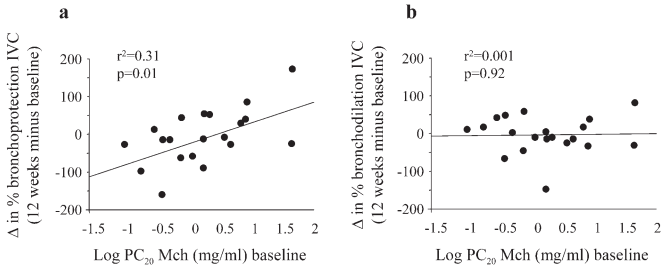

Figure 6. Correlations between baseline airway hyperresponsiveness expressed as the methacholine (Mch) log PC_{20} and changes from baseline in deep inspiration–induced bronchoprotection (*a*) and bronchodilation (*b*) observed at the end of fluticasone treatment, in the entire subject population. The depicted values for percentage of bronchoprotection and bronchodilation by deep inspiration were calculated on the basis of IVC.

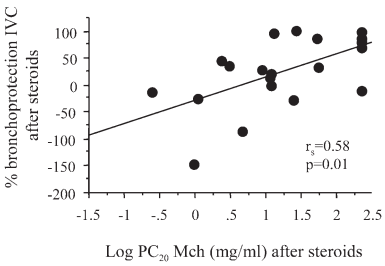

Figure 7. Correlations between deep inspiration–induced bronchoprotection and airway hyperresponsiveness, expressed as the methacholine (Mch) log PC_{20}, measured after treatment with fluticasone in the entire subject population. The depicted values for percentage of bronchoprotection by deep inspiration were calculated on the basis of IVC.

We generated a number of important observations. Twelve weeks of treatment with a moderately high dose of fluticasone did not produce statistically significant improvement in deep inspiration–induced bronchoprotection in either subject group or when the two groups were pooled for analysis (Figure 5). However, a few subjects had a remarkable improvement in bronchoprotection. This was not a random event, because these subjects were among those with the mildest degree of baseline hyperresponsiveness (Figure 6a). Also, this was not a regression to the mean phenomenon, because the relationship between the bronchoprotective effect of deep inspiration at baseline and the fluticasone-induced change in that effect was not statistically significant (data not shown). Equally interesting is the observation that individuals with the highest degree of baseline hyperresponsiveness showed an overall worsening of the protective effect of deep inspiration (Figure 5). Another important finding is that treatment with fluticasone converted an absent effect of deep inspiration–induced bronchoprotection and bronchodilation on airway hyperresponsiveness (as measured by the conventional log PC_{20}) to a statistically significant effect (Figure 7). Finally, we found that inhaled glucocorticosteroid treatment resulted in significant improvement in airway responsiveness, when assessed by the single-dose methacholine bronchoprovocation technique, in the absence of deep inspirations (Figure 4).

It appears that bronchoprotection by deep inspiration is lost at an early stage of the inflammatory airway syndrome to which asthma belongs. We previously reported that this phenomenon is absent in those subjects with asthma with borderline to mild hyperresponsiveness, but also in individuals with allergic rhinitis and airway hyperresponsiveness (3). We are now showing that, at that early stage, the protective effect of deep inspiration can improve to normal levels with antiinflammatory treatment. In contrast, when severe hyperresponsiveness is present, the bronchoprotective effect of deep inspirations is clearly not improved with inhaled glucocorticosteroid treatment. Perhaps the loss of bronchoprotection is a permanent phenomenon at this stage, or perhaps treatment was not given for a long enough period of time. Although we did not measure markers of airway inflammation in this study, the remarkable improvement in the conventional PC_{20}, even in the group of subjects with moderate to severe hyperresponsiveness, offers strong evidence that fluticasone exerted the expected antiinflammatory effects. Overall, these data suggest that the reduced protective effect of deep inspiration is an acquired phenomenon, possibly related to airway inflammation, and does not reflect a preexisting condition, as we had inferred in a previous publication (3).

By improving deep inspiration–induced bronchoprotection in subjects with mild hyperresponsiveness while worsening it in subjects with severe hyperresponsiveness, inhaled glucocorticosteroids led to the emergence of a significant relationship between the log PC_{20} to methacholine and the protective effect of deep inspiration (Figure 6). The absence of such a relationship at baseline was predictable from our previous work, where we have found bronchoprotection by deep inspiration equally lacking in asthmatic subjects with severe and with mild airway hyperresponsiveness (3). In the current study, not only did deep inspiration–induced bronchoprotection at the end of treatment correlate with airway responsiveness at the same time point but it also correlated equally well with airway responsiveness at the beginning of the study. It is reasonable to hypothesize that, at baseline, other active factors, presumably of an inflammatory nature, masked any relationship between the effect of deep inspiration and the log PC_{20}. The impact of these factors may have been reduced or eliminated by the fluticasone treatment. The nature of such factors remains unknown at this point. Of note, our data suggest that, after removing the corticosteroid-sensitive elements of airway inflammation, a better understanding of the role of deep inspiration on airway responsiveness may be attained.

The design of this study allowed for tabulation of the dose of methacholine, which, when administered in the absence of deep inspirations, reduces lung function to a targeted degree. That dose is an outcome that reflects the component of airway responsiveness that is free of the effect of deep inspiration. Perhaps this is a true measure of airway smooth muscle reactivity, although other influencing elements (e.g., thickness of airway wall) cannot be excluded. In the absence of deep breaths, compared with baseline, significantly higher doses of methacholine (3.3- and 4.2-fold for subjects with asthma with moderate to severe hyperresponsiveness and for those with mild to borderline hyperresponsiveness, respectively) were required at the end of glucocorticosteroid treatment to induce the targeted reduction in lung function. Given the fact that glucorticosteroid treatment did not produce major overall improvement in the beneficial effects of deep inspiration, the primary contribution to the steroid-induced improvement in the methacholine PC_{20} can be assigned to this effect. Further dissection of the response to methacholine in the absence of lung inflation may lead to better understanding of the exact mechanism through which inhaled glucocorticosteroids exert their beneficial effects in asthma.

In conclusion, we found that inhaled glucocorticosteroids had a profound effect on the relationship between airway hyperresponsiveness and the beneficial effects of deep inspiration. Our data also suggest that deep inspiration–induced bronchoprotection is lost early in the pathologic process of asthma, at which point it may be recoverable. In more advanced stages, the loss of the beneficial effects of lung inflation is clearly resistant to glucocorticosteroids. Overall, early intervention with inhaled corticosteroids is advisable. Future studies should aim at addressing whether such treatment applied to individuals with airway hyperresponsiveness who do not have clinical asthma would prevent the full development of the disease.

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