Effects of inhaled steroids on methacholine-induced bronchoconstriction and gas trapping in mild asthma


ABSTRACT: According to a recent hypothesis, airway smooth muscle regulates airway calibre mostly at high lung volume, whereas the mucosa and adventitia dimensions dominate at low lung volumes. It was thought that if inhaled steroids decrease the thickness of airway wall in asthma, then forced vital capacity (FVC), which reflects the functional changes at low lung volume, should decrease less during induced bronchoconstriction than flow at high volume.

The study was conducted in 31 mild asthmatics under control conditions and during a methacholine challenge before and after 4-weeks treatment with inhaled fluticasone dipropionate (1.5 mg daily, 16 patients) or placebo (15 patients).

After fluticasone dipropionate treatment, control forced expiratory volume in one second (FEV1), and maximal flow at 50% of control FVC during forced expiration after a maximal (Vmax,50) and a partial inspiration (Vp,50) significantly increased. During methacholine challenge, FVC decreased less than did FEV1 or Vmax,50, and so did inspiratory vital capacity compared to Vp,50. Both the provocative dose of methacholine causing a 20% fall in FEV1 and the bronchodilator effect of deep inhalation significantly increased. The latter was assessed by means of the regression coefficient of all Vmax,50 plotted against Vp,50. No significant changes in these parameters occurred after placebo.

These data show that inhaled steroids remarkably blunt the occurrence of gas trapping during induced bronchoconstriction in mild bronchial asthma, possibly due to their effect on airway wall remodelling.

Committee and written consent was obtained from each subject before enrolment.

Study design

This was a double-blind randomized study parallel consisting of 1-months treatment with inhaled fluticasone dipropionate (1,500 μg daily) or placebo. Lung function tests and bronchial challenge with inhaled MCh were performed before and after treatment.

Lung function measurements

A Vmax 22 system (SensorMedics Corporation, Yorba Linda, CA, USA) was used. Flow was measured at the mouth through a mass flow sensor, and volume obtained by numerical integration of the flow signal.

Baseline FEV1 and FVC were calculated from three reproducible maximal forced expiratory manoeuvres [9]. Then, the subjects were asked to perform three sets of reproducible manoeuvres, each one consisting of a forced expiration from ~70% of FVC to residual volume (RV) (partial expiratory flow/volume curve (PEFV)) and a forced expiration from total lung capacity (TLC) (maximal expiratory flow/volume curve (MEFV)). The inspiratory vital capacity (IVC) manoeuvre preceding the MEFV was fast and no breath hold was allowed at full inflation. A single set of PEFV and MEFV was obtained during the bronchial challenge after each dose of MCh. All forced expiratory manoeuvres were preceded by ≥2 min of quiet tidal breathing.

MCh inhalation challenge

Dry powder MCh (Laboratorio Farmaceutico Lofarma, Milan, Italy) was dissolved in distilled water and aerosolized using a breath-activated dosimeter system (ME-FAR, Brescia, Italy) driven by compressed air (1.5 kg·m⁻²). The system was set to deliver 5 μL solution-actuation. The dose of MCh was varied by changing the number of breaths, the concentration of MCh or both. Subjects were requested to maintain their spontaneous tidal breathing during aero-sol inhalation and to refrain from taking deep breaths.

After control inhalation of saline, MCh was adminis-

tered in doubling doses starting from 50 μg. The test was terminated when a decrement in FEV1 of >40% of control was attained, or upon the subject’s request due to respira-

tory discomfort or dyspnoea.

At the end of the challenge, the subjects were given salbutamol (200 μg, by metered dose inhaler) and left the laboratory after their FEV1 had returned to within 10% of the control value. No complications occurred during the challenges.

Data analysis

Expiratory flow was measured at an absolute lung volume corresponding to 50% of control FVC for both PEFV (Vₚ₅₀) and MEFV (Vₚ₅₀). This lung volume was determined by superimposing MEFV and PEFV at TLC, which was assumed to be constant throughout the study. IVC was calculated as the difference between the RV achieved after the PEFV and the lung volume at which the MEFV was started. Any decrement in FVC or IVC was taken as indicative of an increment in RV (gas trapping).

In order to compare the protective effects of fluticasone dipropionate against the decrease in FVC and FEV1, linear regression analysis was performed on the FVCs recorded at each step of the challenge against the corresponding FEV1 and Vₚ₅₀. The same analysis was applied using IVCs and Vₚ₅₀, which are indexes independent of the effects of deep inhalation [10]. A low regression coefficient (slope) and a high intercept indicate the occurrence of airway narrowing with a small or no increment in RV, and vice versa.

The effect of deep inhalation (DI) on airway calibre was inferred from the slope (MPSlope) and intercept (MPint) of the linear regression of all Vₚ₅₀ and Vₚ₅₀ measured during the challenge [10]. An MPSlope of 0 indicates that DI fully reverses induced airway narrowing, whereas an MPslope of 1 indicates that it has no effect.

The degree of airway responsiveness was assessed by means of PD20. This was determined by interpolation between two appropriate points on the dose (log)/response curve.

Statistical analysis

Group characteristics and baseline lung function data were compared using Student’s two-tailed paired t-test or the Chi-squared test, as appropriate. Two-factor repeated-measure analysis of variance with Duncan’s post hoc test was used to compare the effect of treatment between groups. Correlations were assessed by means of Pearson’s test. A p-value of <0.05 was considered statistically significant. All values are reported as mean±sd.

Results

Before treatment

Baseline pulmonary function data (table 1) were not significantly different between groups. Airway responsiveness to MCh (PD20) and maximal doses of inhaled MCh were also similar in the two groups. The maximum decrements in FEV1 and FVC after MCh were 39±10% (range 20–57%) and 29±12% (range 11–51%) in the fluticasone group, respectively, and 40±7% (range 30–53%) and 27±10% (range 12–50%) in the placebo group, respectively. The mean expiratory times of the forced expiratory manoeuvres were similar between the fluticasone dipropionate and placebo groups (7.6±1.7 and 7.8±2.1 s, respectively, for the MEFV manoeuvre, and 6.4±2.5 and 6.6±1.5 s, respectively, for the PEFV manoeuvre).

Table 1. – Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Fluticasone group</th>
<th>Placebo group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects n</td>
<td>16</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>8/8</td>
<td>8/7</td>
<td>NS</td>
</tr>
<tr>
<td>Age yrs</td>
<td>31±9</td>
<td>31±12</td>
<td>NS</td>
</tr>
<tr>
<td>Height cm</td>
<td>170±12</td>
<td>170±11</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking n</td>
<td>6</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Atopy n</td>
<td>15</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1 %</td>
<td>98±15</td>
<td>102±14</td>
<td>NS</td>
</tr>
<tr>
<td>FVC % pred</td>
<td>110±12</td>
<td>112±12</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as mean±sd. M: male; F: female; FEV1, forced expiratory volume in one second; FVC, forced vital capacity.
The regression lines of FVC versus FEV1 as well as those of FVC versus \( V_{\text{max,50}} \) and of IVC versus \( V_{\text{p,50}} \) were not significantly different (both in slope and intercept) between groups (table 2, fig. 1), suggesting similar effects of MCh on airway narrowing and gas trapping.

The regression line of \( V_{\text{max,50}} \) versus \( V_{\text{p,50}} \) was similar in both groups, with mean MPint values close to 1, suggesting a similar inability of the airways to dilate after DI (table 2, fig. 2) [10].

### After treatment

In the fluticasone dipropionate group, but not in the placebo group, control FEV1, \( V_{\text{max,50}} \), and \( V_{\text{p,50}} \) were significantly increased compared to pretreatment values (table 2).

After MCh challenge, the maximum decrements in FEV1 and FVC were 34±9% (range 20–49%) and 19±11% (range 5–37%), respectively, in the fluticasone dipropionate group and 39±5% (range 29–45%) and 25±6% (range 11–35%), respectively, in the placebo group. The maximal dose of MCh administered was significantly greater than that prior to treatment in the fluticasone dipropionate group but not in the placebo group. Airway responsiveness to MCh was significantly decreased and the intercepts increased in the fluticasone dipropionate group after inhaled steroid treatment, suggesting an improved ability of DI to reverse induced bronchoconstriction (table 2, fig. 2).

The correlation coefficients of the linear regressions between relevant variables before and after fluticasone dipropionate and placebo treatment are shown in table 3.

### Discussion

The results of this study show that inhaled fluticasone dipropionate improved the lung function of mild asthmatic subjects both under control conditions and during induced bronchoconstriction.

Particularly remarkable was the greater protection afforded by fluticasone dipropionate against the increase in RV (gas trapping) than against the reduction in forced expiratory flow during the bronchial challenge.

Only subjects with mild asthma were included, which may limit the clinical relevance of the study. This was, however, done purposely in order to minimize spontaneous fluctuations as well as marked effects of fluticasone dipropionate on baseline lung function, which would have made responses to MCh hardly comparable. In addition, a dose of fluticasone dipropionate higher than those recommended for these patients [6] was used in order to minimize the effects of possible low adherence to therapy [11], thus increasing the likelihood of observing significant effects over a relatively short period of time. Therefore, no inference about the clinical use and dosage of inhaled steroids for regular treatment of mild asthma should be made on the basis of the recent study.

Because of nonuniform lung emptying and thoracic gas compression, expiratory flow may not decrease linearly with lung volume, especially during bronchoconstriction.

### Table 2. – Functional variables before and after treatment

<table>
<thead>
<tr>
<th></th>
<th>Fluticasone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>FEV1 L</td>
<td>3.51±0.76</td>
<td>3.81±0.76</td>
</tr>
<tr>
<td>FVC L</td>
<td>4.65±1.03</td>
<td>4.76±0.95</td>
</tr>
<tr>
<td>IVC L</td>
<td>4.38±0.90</td>
<td>4.55±0.93</td>
</tr>
<tr>
<td>( V_{\text{max,50}} ) ( \text{L}\cdot\text{s}^{-1} )</td>
<td>3.52±1.09</td>
<td>4.16±1.23 *</td>
</tr>
<tr>
<td>( V_{\text{p,50}} ) ( \text{L}\cdot\text{s}^{-1} )</td>
<td>3.24±0.80</td>
<td>4.06±1.31 *</td>
</tr>
<tr>
<td>log max dose of MCh mg</td>
<td>6.12±1.54</td>
<td>7.51±1.52 *</td>
</tr>
<tr>
<td>log PD20 mg</td>
<td>4.31±1.63</td>
<td>6.35±1.64 *</td>
</tr>
<tr>
<td>FVC versus FEV1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.50±1.11</td>
<td>2.39±1.13 *</td>
</tr>
<tr>
<td>Slope</td>
<td>0.94±0.28</td>
<td>0.66±0.27 *</td>
</tr>
<tr>
<td>FVC versus ( V_{\text{max,50}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>3.04±1.07</td>
<td>3.70±0.99 *</td>
</tr>
<tr>
<td>Slope</td>
<td>0.59±0.31</td>
<td>0.32±0.17 *</td>
</tr>
<tr>
<td>IVC versus ( V_{\text{p,50}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.86±0.97</td>
<td>3.42±0.95 *</td>
</tr>
<tr>
<td>Slope</td>
<td>0.59±0.29</td>
<td>0.37±0.19 *</td>
</tr>
<tr>
<td>( V_{\text{max,50}} ) versus ( V_{\text{p,50}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.43±0.39</td>
<td>0.85±0.60 *</td>
</tr>
<tr>
<td>Slope</td>
<td>0.99±0.28</td>
<td>0.84±0.15</td>
</tr>
</tbody>
</table>

The Intercept and Slope are those of the linear regression analysis between the variables. \*: \( p<0.05 \); \*: \( p<0.01 \); \*: \( p<0.001 \) versus before (Duncan test. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; \( V_{\text{max,50}} \) and \( V_{\text{p,50}} \): maximal and partial forced expiratory flows at 50% of control FVC, respectively; MCh: methacholine; PD20: provocative dose of methacholine causing a 20% fall in FEV1.)
High correlation coefficients between FVC and FEV1 or \( V'_{\text{max},50} \) and between IVC and \( V'_{p,50} \) (table 3) suggest, however, that such nonlinearities do not invalidate this approach to assessing changes in the relationship between changes in flow and volume during induced bronchoconstriction.

Isovolumic measurements of \( V'_{\text{max},50} \) and \( V'_{p,50} \) were taken by superimposing MEFVs at full inflation, thus assuming that TLC remained constant throughout the study, i.e. during both bronchial challenges and the treatment period. To the author’s knowledge, TLC remains fairly constant during bronchial challenge [12, 13], and also after a 4-week course of inhaled steroids, as suggested by unpublished data on similar subjects from the authors’ laboratory.

The bronchoconstrictor stimulus was MCh, which is probably the most direct stimulus to airway smooth muscle, although it may also affect mucosal blood flow [14]. It cannot be excluded, therefore, that the different effects of MCh before and after fluticasone dipropionate might have been mediated, in part, by changes in mucosal blood flow.

Finally, some of the subjects included in this study were smokers. As the effects of smoking may represent a confounding factor, the data from nonsmokers were reanalysed separately. All significant differences between the fluticasone dipropionate and placebo groups were still present.

Although airway smooth muscle contraction seems to be a key event in the response to bronchoconstrictor stimuli, the magnitude of airway narrowing in vivo is modulated by several factors, including mechanical load on airway smooth muscle and airway wall geometry [5, 15–17]. Major sources of mechanical load are airway wall elastic elements (internal load) and lung elastic recoil (external load). The latter is probably the most efficient mechanism opposing airway narrowing, as the response to a bronchoconstrictor stimulus is greatly reduced at increased lung volume, i.e. when lung elastic recoil is greater. The effectiveness of this mechanism in preventing airway narrowing depends on lung elastic recoil and alveolar attachments to the external airway wall [5, 15]. Airway wall thickness seems to be the most critical geometric factor modulating
Table 3. – Correlation coefficients of the linear regressions between variables before and after fluticasone dipropionate and placebo treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fluticasone Before</th>
<th>Fluticasone After</th>
<th>Placebo Before</th>
<th>Placebo After</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC versus FEV1</td>
<td>0.98±0.02</td>
<td>0.97±0.02</td>
<td>0.97±0.03</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td>FVC versus V'_max,50</td>
<td>0.96±0.04</td>
<td>0.95±0.04</td>
<td>0.95±0.04</td>
<td>0.96±0.04</td>
</tr>
<tr>
<td>IVC versus V'_p,50</td>
<td>0.95±0.04</td>
<td>0.93±0.04</td>
<td>0.95±0.05</td>
<td>0.94±0.05</td>
</tr>
<tr>
<td>V'_max,50 versus V'_p,50</td>
<td>0.97±0.03</td>
<td>0.94±0.02</td>
<td>0.94±0.02</td>
<td>0.97±0.02</td>
</tr>
</tbody>
</table>

FVC: forced vital capacity; FEV1: forced expiratory volume in one second; V'_max,50: maximal forced expiratory flow at 50% of control FVC; IVC: inspiratory vital capacity; V'_p,50: partial forced expiratory flow at 50% of control FVC.

Several studies have examined the effects of glucocorticoids on cytokine production by airway smooth muscle cells. This and other cell types, previously considered non-secretory in nature, may be important targets for the anti-inflammatory effects of inhaled steroids [28]. Moreover, inhaled corticosteroids may directly modulate airway smooth muscle contractility. Even though differences in airway smooth muscle contractility have never been demonstrated between normal and asthmatic airways [29], in vitro studies have shown that allergic inflammation may alter the magnitude and velocity of airway smooth muscle shortening [30] as well as the autonomic control of airway smooth muscle cells [33]. Alternatively, the anti-proliferative effect of glucocorticoids on airway smooth muscle raises the possibility that the mass of airway smooth muscle was reduced by fluticasone dipropionate treatment [28]. These direct effects of corticosteroids on airway smooth muscle cannot explain the different effects on flow and volume as they should have affected airway mechanics similarly at low and high lung volumes.

Theoretical models have shown that changing airway wall thickness or the load on airway smooth muscle markedly affects the response to bronchoconstrictor agents with only modest functional changes under control conditions [15]. This interpretation is supported by the finding that the bronchodilator effect of DI during the MCh challenge was enhanced after fluticasone dipropionate treatment, as indicated by the increase in MPst in the present study, which in turn confirms previous data by Bruij et al. [34]. Corticosteroids may have improved the ability of DI to dilate constricted airways by reducing peri-bronchial oedema, thus restoring the forces of interdependence between the airways and the lung parenchyma, and/or by decreasing mucosal thickness, thus increasing the compliance of the wall with mechanical stretching. It should be noted, however, that the bronchodilator effect of DI during the bronchial challenge was small and remained far less than that in normal subjects [13], despite the remarkable improvement in lung function and the reduction in airway hyperresponsiveness.

In conclusion, this study sheds new light on the way in which a 4-week course of inhaled fluticasone dipropionate improves lung function in mildly asthmatic subjects. In particular, the findings show that inhaled steroids...
remarkably blunt the occurrence of gas trapping when airways narrow, probably by interfering with those structural changes in the airway wall that are a consequence of chronic inflammation. These data suggest that changes in forced vital capacity in response to bronchoconstrictor stimuli should be considered when the long-term effects of anti-inflammatory treatments are to be monitored.

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References