No impairment of peripheral deposition in novel asthmatics treated with an MDI corticosteroid with spacer

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Abstract  Pulmonary distribution and lung functions were evaluated during a 4-month inhaled corticosteroid treatment period in 10 steroid-naïve novel asthmatics with normal or slightly reduced lung functions. Patients were given a total daily dose of 1000 µg of beclomethasone dipropionate aerosol twice a day via a pressured metered dose inhaler with a large-volume chamber device (Volumatic, GlaxoSmithKline, UK). Gamma lung scintigraphy and lung function tests were performed before and after 2 months and 4 months. Inhaled 99mTc-labelled beclomethasone dipropionate liposomes were used to assess lung deposition patterns during inhaled steroid therapy. Serum eosinophil cationic protein (ECP) concentration was used as a surrogate marker of asthmatic inflammation. Following beclomethasone treatment, all lung functions were enhanced, but only FVC values showed significant improvement. The FEV1/FVC ratio remained slightly reduced in spite of inhaled corticosteroid therapy. However, the association between changes in improved FVC values and reduced ECP levels proved to be statistically significant. In lung scintigraphy, no evidence of changes in pulmonary deposition patterns were seen during the follow-up period. We conclude that inhaled corticosteroid therapy can lead to improvements in lung functions and surrogate markers of airway inflammation in novel asthma without affecting the peripheral deposition pattern of aerosols.

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Keywords  deposition; inhaled corticosteroids; liposome; asthma.

INTRODUCTION

Asthma is regarded as a chronic inflammatory lung disorder in which the inflammatory process is present in the whole respiratory tract. Recent evidence shows that inflammation and other pathophysiological changes in the small airways are important in all stages in asthma (1,2). There is also evidence of the systemic nature of the disease (3).

The use of cortisone as an anti-inflammatory medication is currently accepted as the standard treatment of asthma (4). Early introduction of inhaled steroids is now recommended (5,6) as it may improve lung function (7) and prevent complications, such as irreversible airway damage or exacerbations (8). The view that asthma is a systemic disorder would support the use of systemic therapies (9). The high incidence of severe side effects from oral corticosteroids have directed their usage predominantly to an inhalation route. To achieve suppression of the inflammation throughout the respiratory tract, treatment should be directed at both large and small airways. Unfortunately, reduction of the airway caliber in asthma reduces pulmonary targeting of the inhaled drug and reduces peripheral drug delivery (10–12). This raises the question whether inhaled corticosteroids effectively treat the inflammation present in small airways.

Pulmonary gamma scintigraphy is a functional method of evaluating aerosol deposition in the lower airways, as well as in peripheral parts of the lung. We sought to evaluate lung deposition patterns of the inhaled aerosol during 4 months of inhaled corticosteroid treatment in novel mild asthmatics, while also studying lung function values. Peripheral blood eosinophil activity as
measured by serum eosinophil cationic protein (ECP) concentration was used as a surrogate marker of asthmatic inflammation (13). As a radiolabel we used 99mTc-bound beclomethasone-liposome (DLPC), whose administration via several different nebulizers (14), tolerance (15) and pulmonary deposition in healthy (16) and asthmatic subjects (10) have been demonstrated previously.

MATERIALS AND METHODS

Subjects studied

Ten adult non-smoking steroid-naive patients with novel, mild asthma were included in an open before-and-after trial. All patients had normal or slightly reduced lung functions, baseline FVC and FEV1 measured immediately prior to the study as 94 and 83% of predicted, respectively. The mean age of the subjects was 33 years (range 19–51 years). Asthma diagnoses were based on clinical evaluation by the pulmonary physician and fulfilled the criteria defined by the American Thoracic Society (17), with the addition of an increase in FEV1 >15% following a bronchodilation test (the inhalation of 200 µg of salbutamol). All patients had newly diagnosed asthma with a history of asthma symptoms (cough, wheeze or decreased tolerance to exercise) during the preceding month at least. They were recruited to the study from the outpatient clinic of the Department of Pulmonary Diseases of Tampere University Hospital. An upper viral infection within 4 weeks of the start of the experiment was an exclusion criterion. Moreover, patients with a significant pulmonary or cardiac disease were excluded.

Patients were given 4 months’ inhaled corticosteroid (ICS) treatment of beclomethasone dipropionate (Becotide 250 µg/dose, GlaxoSmithKline, U.K.) with a daily total dose of 1000 µg. Two doses of corticosteroid aerosol were administered twice a day via a pressurized metered dose inhaler (MDI) with a large-volume spacer device (Volumatic, GlaxoSmithKline, U.K.). Spirometric measurements (Vitalograf, Buckingham, U.K.) were performed and serum ECP samples taken always at the same time of day at the beginning (baseline), and after 2 months and 4 months of ICS therapy. In the lung function test, at least three technically correct maneuvers for forced maximal expiratory flow volume curves were performed, and the curve with the greatest sum of FEV1 and FVC was utilized to obtain data.

The study was conducted according to the Declaration of Helsinki. Written, informed consent was obtained from all subjects, and the study protocol was approved by the Ethical Committee of Tampere University Hospital.

Liposome preparation and labelling of Bec-DLPC liposomes with 99mTc

Beclomethasone dipropionate (Bec) and dilauroylphosphatidylcholine (DLPC) liposomes were produced as previously described by Waldrep (18). Briefly, 1 mg Bec and 25 mg DLPC were dissolved in 10-ml t-butanol. After mixing, the Bec-DLPC solution was pipetted into glass vials, rapidly frozen in dry ice–acetone and lyophilized overnight to remove the organic solvent. The liposome suspension was produced by adding ultra-pure water to obtain a final drug concentration of 500 µg/ml. The mixture was stirred for 30 min at 37 °C to allow hydration of the liposomes.

The preformed Bec–DLPC liposomes were labelled with Tc in the presence of SnCl2 as a reducing agent. In the preparation of a stannous chloride solution, it is important to exclude the possibility of oxidation of tin to the non-reactive stannic form. Therefore, before dissolving stannous chloride (67 mg), 100 ml of sterile, pyrogen-free water was bubbled 30 min with nitrogen in order to expel most of the oxygen. One milliliter of Tc pertechnetate in sterile saline — with a radioactivity of approx. 780 MBq (21 mCi) — was then added, and the mixture (total volume 2.5 ml) was shaken vigorously for 1 min and left to react at room temperature for 30 min.

Labelling efficiency was determined by paper chromatography (ITCL-SG, prod. 61885, Gelman Sciences, Ann Arbor, MI, U.S.A.). Free pertechnetate migrates to the top of the paper, while liposomally entrapped material remains at the application point. The labelling yield was expressed as a percentage of the total amount of radioactivity applied in the testing system. Aerosol characteristics as well as assessment of 99mTc attachment to the Bec–DLPC liposome are described in more detail previously by the author (10,19).

Corticosteroid–liposome delivery

The Tc-labelled Bec–DLPC suspension was delivered by Aerotech II nebulizers (CIS-US, Bedford, U.S.A.) connected to an automatic, inhalation-synchronized dosimeter (Spira Elektro 2, Respiratory Care Center, Hameenlinna, Finland). This dosimeter is triggered by a very low inspiratory flow rate with a threshold of < 2 l/min (20,21). The volume of each inhalation is displayed digitally, and the inhalation flow rate is controlled by a flow indicator. A breath-actuated, variable-time circuit regulates air through a solenoid valve to a nebulizer, set at a flow rate of 10 l/min. The volume output of the dosimeter with 0.5 s nebulization periods under these operating conditions is 7 µl/breath ± 0.5. In this study, the dosimeter was set to start nebulization at the beginning of inhalation after the patient had inhaled a volume of 10 ml, with each inhalation lasting approximately 3.0 s.
A total dose of 500 μg Bec within the labelled liposomes (2.5 ml) was placed in the jet nebulizer. Subjects were instructed to place the nebulizer tightly between their lips and inhale deeply. With a noseclip and mouthpiece in place, the subject controlled breathing with a flow indicator (an LED screen) so that the inspiratory flow rate of each breath reached but did not exceed 30 l/min. Inhalation was followed by normal exhalation. Exhaled Bec-liposomes were captured using a Hudson filter. This inspiration procedure was repeated 20 times according to the subject’s own inspiratory cycle with no holding of breath between inhalations. Nebulization was practiced by each subject with saline before the experiment began.

**Gamma camera measurements**

Immediately after inhalation, anterior and posterior views of the lungs and an anterior view of the oropharynx were measured in a supine position by a large field gamma camera (GE, CamStar XR/T, WI U.S.A.) equipped with a low-energy high-resolution parallel collimator. In order to evaluate the retention of the inhaled liposomes, scans were repeated 1, 2, 4 and 24 h after aerosol delivery. In addition, a posterior ventilation scan was obtained after the liposome study by inhaling noble gas $^{133}$Xe with a radioactive dose of 460 MBq (12.5 mCi). All images were stored on a computer (Hermes, Nuclear Diagnostics, Hägersten, Sweden) for subsequent data analysis. $^{133}$Xe posterior images were used when regions of interest (ROI) were manually drawn around central and peripheral (P) lung zones. ROIs were subsequently superimposed upon each liposome aerosol view, enabling the quantity of aerosol dose in each of the zones to be determined. Each image was manually aligned, i.e. each lung view was shifted to adapt to the superimposed ROIs. The lungs were divided into inner and outer regions, with the central zone encompassing 33% (+ 2%) and outer the remaining of the total lung area (22, 23). Lung distribution of the liposome aerosol was described as the ratio between central and peripheral lung areas (C/P ratio). The total lung retention curve was described as a plot of the percentage of initial lung burden vs. time after inhalation.

The number of counts and pixels in each region of interest was measured and saved to a file in the Hermes computer. Subsequently, the data were transferred via a local area network to a personal computer and analyzed with a program specially made for this study. Counts from the anterior and posterior views of the lungs were combined by taking geometric mean values. The camera-to-patient distance was standardized by placing the collimator close to the chest for the anterior view and in contact with the imaging bed for the posterior view. Geometric mean counts were corrected for the room’s background — measured separately from each image — and for radioactive decay.

An approximate tissue absorption correction was carried out by using the method described by Macey and Marshall (24). Briefly, individual transmission images of each subject’s lung region were taken prior to the liposome study using a flat radiation source, keeping the imaging geometry similar both in transmission and ventilation scans. This transmission method was used to correct the individual emission counts recorded with the gamma camera.

**Statistical analyses**

Data are expressed as mean ± SD unless stated otherwise. Lung function variables were measured before and during the treatment. Gamma camera measurements were performed before and during treatment at 0, 1, 2, 4 and 24 h after inhalation. For C/P ratios the area under curve (AUC 0–4 h) values were calculated for baseline, 2 months and 4 months, and were analyzed by using the analysis of variance for repeated measurements. For lung function variables and C/P ratio variables at 0h and 24h the within-patient changes from baseline to 4 months were analyzed using the paired t-test and were described as mean with 95% confidence intervals. Spearman’s rank correlations were calculated to test the association between the changes in the lung functions vs. C/P ratio.

**RESULTS**

All ten patients completed the study. Daily asthma symptoms (wheezing, dyspnea, excess mucus production) were reduced in all patients with no need for additional asthma medication.

The main lung function parameters as well as serum ECP concentrations at the baseline and after each treatment period are shown in Table 1. Baseline forced vital capacity (FVC) and forced expiratory volume in one second (FEV$_1$) were 94% ± 11.4 of predicted and 83% ± 11.5 of predicted, respectively. Lung functions increased gradually during the 4-month treatment period. The mean FVC improved by 0.201 l (95% CI 0.02–0.38) and was considered statistically significant. An increase in FEV$_1$ values (0.24 l) was clinically a relevant improvement, but statistically proved to be insignificant ($p = 0.15$). The FEV$_1$/FVC ratio remained quite steady or in fact slightly reduced in spite of the corticosteroid therapy.

Serum ECP levels tended towards reduction during ICS therapy but the change was insignificant. Table 2 demonstrates the association between changes in lung function and changes in serum ECP levels. The change between reduced ECP levels and improved FVC values proved to be statistically significant ($p = 0.001$).
Regional pulmonary deposition patterns of the radiolabelled liposome particles are demonstrated in Fig. 1. Immediately after inhalation, the ratio between central and peripheral deposition (C/P ratio) in mild asthmatics prior to the anti-inflammatory therapy was 0.77 ± 0.10; after the 4-month medication period, 0.78 ± 0.13. Thereafter, pulmonary distribution patterns remain similar during the 24-h follow-up period being 0.69 ± 0.06 and 0.67 ± 0.06 at 24 h, respectively. No statistically significant changes in pulmonary deposition patterns were observed in the follow-up period. The AUC (0–4h) values for C/P ratios were 2.96 ± 0.38, 2.96 ± 0.29 and 2.92 ± 0.37 at baseline, 2 and 4 months (time effect \( P = 0.93 \), ANOVA for repeated measurements).

### Table 1. Lung functions of the asthmatic patients before inhaled steroids, and after 2 and 4 months of treatment

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline</th>
<th>2 months</th>
<th>4 months</th>
<th>Mean</th>
<th>95%CI</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>4.75 (1.10)</td>
<td>4.88 (1.25)</td>
<td>5.11 (1.09)</td>
<td>0.2</td>
<td>0.02 to 0.38</td>
<td>0.04</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>93.8 (1.4)</td>
<td>96.4 (1.31)</td>
<td>100.3 (1.24)</td>
<td>4.78</td>
<td>1.04 to 8.52</td>
<td>0.02</td>
</tr>
<tr>
<td>FEV1</td>
<td>3.53 (0.70)</td>
<td>3.77 (0.84)</td>
<td>3.89 (0.73)</td>
<td>0.24</td>
<td>-0.11 to 0.59</td>
<td>0.15</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>83.4 (1.15)</td>
<td>88.5 (0.98)</td>
<td>91.4 (0.95)</td>
<td>6</td>
<td>-0.68 to 12.68</td>
<td>0.07</td>
</tr>
<tr>
<td>FEV1/FVC%</td>
<td>75.4 (91)</td>
<td>778 (5.6)</td>
<td>770 (7.7)</td>
<td>1.13</td>
<td>-0.90 to 2.30</td>
<td>0.4</td>
</tr>
<tr>
<td>FEF 50% predicted</td>
<td>64.5 (25.4)</td>
<td>74.2 (23.5)</td>
<td>73.3 (26.1)</td>
<td>6.11</td>
<td>-90.4 to 21.26</td>
<td>0.38</td>
</tr>
<tr>
<td>ECP</td>
<td>16.76 (11.34)</td>
<td>16.17 (11.55)</td>
<td>11.93 (6.33)</td>
<td>-5.37</td>
<td>-12.86 to 2.12</td>
<td>0.14</td>
</tr>
</tbody>
</table>

### Table 2. Association between the changes between S- ECP and lung functions during 4 months therapy on inhaled steroids

<table>
<thead>
<tr>
<th>Change in S-ECP vs. Change in</th>
<th>Spearman’s rho</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>-0.934</td>
<td>0.001</td>
</tr>
<tr>
<td>FVC % of the predicted</td>
<td>-0.587</td>
<td>0.126</td>
</tr>
<tr>
<td>FEV1</td>
<td>-0.619</td>
<td>0.012</td>
</tr>
<tr>
<td>FEV1 % of the predicted</td>
<td>-0.491</td>
<td>0.217</td>
</tr>
<tr>
<td>FVC/ FEV1%</td>
<td>0.048</td>
<td>0.911</td>
</tr>
<tr>
<td>FEF 50%</td>
<td>-0.108</td>
<td>0.799</td>
</tr>
</tbody>
</table>

*Group of healthy subjects (n=11) from the earlier published data by author (19)

**Fig. 1.** C/P ratio as a function of time in novel asthmatics during 4 months inhaled steroid therapy and in healthy volunteers.
DISCUSSION

Asthma is a chronic inflammatory disease characterized by airway obstruction of varying degrees and bronchial hyperresponsiveness. Asthmatic inflammation is now known to be present in the small airways in addition to the large and intermediate size airways (25). The efficacy of inhaled corticosteroid treatment of asthma is well documented (26), with national and international asthma guidelines recommending their use as a first choice therapy. Corticosteroids are usually administrated by inhalation via a metered dose inhaler (MDI) aerosol with a large-volume spacer or in dry powder (DPI) form. Evidence from both bronchial biopsy tissue and BAL samples shows that certain inflammatory markers are increased in both large and small airways in asthma, and that anti-inflammatory treatment should be directed at both large and small airways to achieve suppression in inflammation throughout the entire respiratory tract (1). However, not all MDI or DPI inhalers are efficient enough to deposit medication in the peripheral airways of the lung (11,27,28). Ventilation scans show that in asthma a greater aerosol deposition occurs in central than in peripheral airways (29,30) and this central accumulation increases with the severity of asthma (10).

Present methods for assessing inflammation and obstruction in peripheral airways are limited. No reliable direct or surrogate markers for airway inflammation are so far available. Standard lung function tests focus more on large airway events; improvements in these parameters may not be reflected by small airway pathology (31). Pulmonary gamma scintigraphy provides a functional method of evaluating aerosol deposition in small airways provided that the inhalation mode and aerosol particle size are beneficial for peripheral lung penetration (32). In our study, the jet nebulizer (Aerotech II) was chosen for aerosol delivery because it produces aerosol particles likely to produce an alveolar deposition of the inhaled liposomes (MMAD approximately 2 μm). In addition, a slow inspiratory flow rate was used to minimize impaction in the upper parts of the respiratory tract.

In earlier studies we demonstrated the efficacy of inhaled radiolabelled-DLPC liposomes in assessing regional lung distribution by means of the C/P ratio at different stages of asthma and in healthy subjects. Lung deposition with DLPC-liposome aerosols produced by the Aerotech II nebulizer immediately after inhalation proved to be uniform throughout the lung tissue, yielding a central vs. peripheral deposition ratio of 0.66 (19) in healthy subjects. Central deposition is increased in asthma and enhanced by the severity of the disease. The C/P ratio differs between severe and mild asthmatic patients at 1.07 and 0.76, respectively (10). In our recent study (33), we assessed the lung deposition of Bec–DLPC liposomes in chronic stable asthma. Patients had normal or slightly reduced lung function with no asthma symptoms. A 1-week treatment of inhaled formoterol was added to their regular corticosteroid therapy and lung scintigraphy was repeated before and after treatment. Formoterol enhanced peripheral lung deposition and thus lowered the C/P ratio, which was 0.87 at the baseline. After long-acting beta2-therapy, the C/P ratio decreased to 0.77 — the same fractional lung distribution as in our novel asthmatic group of the present study. As a summary, the data concerning the regional distribution of Tc-labelled DLPC liposomes is given in Table 3.

In the current study, steroid-naïve asthmatic patients with normal or slightly reduced lung functions were treated for 4 months with inhaled corticosteroids. Clinical asthma symptoms expired in all patients. There was a significant improvement in FVC in association with a reduction in concentration of serum ECP. Other lung function values also improved, except a slightly reduced FEV1/FVC ratio present before the corticosteroid therapy, which may indicate a minor peripheral obstruction resistant to the administered anti-inflammatory therapy. In pulmonary scintigraphy no evidence of change in peripheral aerosol deposition was demonstrated. Whether this is due to a lack of inflammatory control resulting from insufficient peripheral deposition of inhaled corticosteroids is still an open question.

Recent studies suggest that the particle size of inhaled steroids is critical at the delivery site in airways (34). Seventy percent of the particles emitted from a standard chlorofluorocarbon (CFC)-MDI are larger than 5 μm (35). Even with an optimal inhalation technique, only about 10% of the corticosteroid dose generated by

| Table 3. | C/P ratio in healthy subjects and in mild and severe asthmatics measured using technetium-labelled DLPC liposomes |
|-----------------|----------------------|-------------------|------------------|-----------------|-------------------|
| Subjects       | C/P ratio           | Before ICS        | After ICS        | Before therapy  | After 1-week formoterol therapy |
| Healthy        | 0.66                |                   |                  |                 |                                 |
| Mild asthmatics| 0.77                |                   |                  |                 |                                 |
| Mild asthmatics on ICS | 0.87        |                   |                  |                 |                                 |
| Severe asthma on ICS | 1.07        |                   |                  |                 |                                 |
CFC-based inhalers reaches the lower respiratory tract, with most medication deposited in the oropharynx (32). Esmaipour et al. (36) investigated the deposition pattern of fluticasone dipropionate administered via a large volume spacer as a single dose. Their study revealed fluticasone concentrations three to four times lower in peripheral airways than in central airways. Although we are aware of the intersubject and intrasubject variability of the pulmonary deposition studies (32,37) our results correspond well with previously mentioned data. Small airways may be undertreated even in mild asymptomatic asthmatics. Recent findings with extra-fine hydrofluoroketamine (HFA)-propelled corticosteroid aerosols suggest that these newer small particle formulations have enhanced peripheral lung deposition and are a reduction in the number of asthma exacerbations — even at the lower doses of inhaled corticosteroids than CFC-propelled steroids (27,38). The downside of enhanced peripheral drug targeting may be an increase in side effects due to the increased absorption of inhaled drugs by lung tissue and a resultant increased systemic bioavailability of the drug.

In conclusion, the knowledge concerning drug delivery to the small airways is still relatively limited. Our results correspond to recently published data that the relatively large particle size of inhaled steroids using conventional chlorofluorocarbon (CFC)-MDI might leave the small airways undertreated and thereby obstructed due to inefficient drug targeting in peripheral lung regions.

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