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# Dose-dependent Inhibitory Effect of Inhaled Beclomethasone on Late Asthmatic Reactions and Increased Responsiveness to Methacholine Induced by Toluene Diisocyanate in Sensitised Subjects

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*SUMMARY.* **To determine whether inhaled beclomethasone, both at low and at high doses, inhibits late asthmatic reactions and the associated increase in airway responsiveness induced by toluene diisocyanate (TDI), we studied 9 sensitised subjects. Low dose beclomethasone (200 μg bid), high dose beclomethasone aerosol (1000 μg bid), and placebo were administered for 7 days before TDI inhalation challenge to each subject, according to a double-blind, crossover study design. The washout period between the treatments was at least 1 week. When the subjects were treated with placebo, forced expiratory volume in 1 sec ($FEV_1$) markedly decreased after exposure to TDI. By contrast, high dose beclomethasone prevented the late asthmatic reaction and the low dose partially inhibited the reaction. With placebo the mean (±SE) value of $FEV_1$ 4 h after exposure to TDI was 2.6±0.17 L, which went to 3.3±0.12 after low dose beclomethasone, and to 3.5±0.15 L after high dose of beclomethasone (significant difference in the decrease of $FEV_1$ in the 8 h after exposure to TDI, between treatments: $F=9.87$, ($P<0.001$), After treatment with placebo or with low dose beclomethasone, airway responsiveness to methacholine increased 8 h after exposure to TDI. With placebo, the $PD_{20}$ decreased from 0.66 mg (Geometric Standard Error of the Mean [GSEM], 1.38) to 0.18 mg (GSEM, 1.46); with low dose inhaled beclomethasone, the $PD_{20}$ decreased from 0.93 mg (GSEM, 1.42) to 0.36 mg (GSEM, 1.63). By contrast, airway responsiveness to methacholine did not change 8 h after exposure to TDI in subjects treated with high dose inhaled beclomethasone: the $PD_{20}$ was 0.78 mg (GSEM, 1.51) before and 0.71 mg (GSEM, 1.58) after exposure to TDI. The protective effect of beclomethasone was obtained without side effects and without changes in serum cortisol levels (08.00) in any of the nine examined subjects. These results suggest that the inhibitory effect of inhaled beclomethasone on TDI-induced late asthmatic reactions and increased responsiveness is dose-dependent.**

## INTRODUCTION

Corticosteroids were first used for asthma in the 1950s. Current evidence suggests that these drugs, either inhaled or taken orally, are powerful effective agents for the treatment of moderate to severe attacks of asthma.

Airway inflammation plays an important role in bronchial asthma and, therefore, anti-inflammatory glucocorticoids are considered the drugs of choice for the treatment of exacerbations of asthma and for the prophylaxis of asthma attacks.[1]

Occupational asthma is a significant health problem in industrialised countries, and asthma induced by isocyanates is particularly frequent.[2,3] Even if recommended to interrupt exposure, subjects sensitised to substances present in the workplace often continue to be directly or indirectly exposed to the sensitised agent and, similarly to subjects sensitised to environmental allergens, need pharmacologic treatment or prophylaxis of further asthma attacks.

To investigate the mechanism of asthmatic reactions induced by toluene diisocyanate (TDI) and the prophylactic effect of antiasthma drugs on them, we recently completed some studies in sensitised workers. We observed that oral prednisone (50 mg once a day for three days) inhibits late asthmatic reactions and the increase in airway responsiveness to methacholine induced by exposure to isocyanates[4]; and that, at the same time, prednisone prevents the associated migration of neutrophils and eosinophils, and the increase of albumin concentration in bronchoalveolar fluid.[5] Then, we observed that a high dose (1 mg bid for 7 days) inhaled beclomethasone, but not cromolyn, verapamil or theophylline, completely prevents late asthmatic reactions and the associated increase in airway responsiveness induced by exposure to TDI.[6]

As oral prednisone and high dose inhaled beclomethasone, although very effective, may cause significant side-effects,[1,7] we decided to determine and to compare the protective effect of a low dose (400 μg) and a high dose (2000 μg) inhaled beclomethasone on

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dual or late asthmatic reactions and on the associated increase in airway responsiveness induced by toluene diisocyanate in sensitised subjects.

## SUBJECTS AND METHODS

### Study design

The study was conducted according to a placebo-controlled, randomised double-blind, crossover design in 9 subjects. Each subject was given 400 μg, or 2000 μg beclomethasone (corresponding to 4 puffs bid of the two commercially available canisters), or the appropriate placebo for 7 days. Placebo was made with the excipient of the active drug: a mixture of sorbitan trioleate, trichlorofluoromethane and dichlorodifluoromethane. The washout period between the treatments was at least 1 week. Canisters were weighed before and after each treatment to assess proper compliance.

The regimen of treatment was 4 puffs (ie 200 μg, or 1000 μg, or placebo) at 08:00 and at 20:00. In each series of experiments, all subjects were treated with the active drug or placebo for 7 days. The day before the beginning of the treatment, and on the sixth day of treatment, airway responsiveness to methacholine was measured in each subject at 17:00. On the seventh day, blood samples were obtained at 08:00 for measurement of cortisol levels, and inhalation challenge with TDI was performed at 09:00, ie 1 h after each subject was given the last four puffs. $FEV_1$ was measured hourly for 8 h after exposure to TDI, and an inhalation challenge with methacholine was repeated at 17:00. The same procedure was repeated with the alternative treatment, starting at least 1 week later. All patients gave informed written consent.

### Subjects

All subjects had been free of clinical, symptomatic respiratory infections for at least 8 weeks, or exposure to TDI for at least 2 weeks before the first study day. No subject took sympathomimetics, antihistamines, or cromoglycate within 24 h of any study. Normal lung volumes, good cooperation and reproducibility of pulmonary function tests, and history of sensitisation to isocyanates with the demonstration of a dual or a late asthmatic response after exposure to TDI were requested for admittance to the study. All subjects underwent detailed medical and occupational history, lung function measurement and intradermal skin tests with common allergens on a preliminary visit. A subject was considered to be atopic if there was a positive skin reaction to two or more common allergen extracts; a skin reaction was considered to be positive when, at the site of injection of any of the allergens, a wheal developed that was greater than that of the diluent control and equal or greater to the histamine control solution. The characteristics of each subject are reported in Table 1.

### Inhalation challenge with methacholine

Airway responsiveness to methacholine aerosol was measured according to the slightly modified protocol of Chai and colleagues,[9] and has been described in detail previously.[4] Briefly, aerosols were generated by a DeVilbiss 646 nebuliser (DeVilbiss Co., Somerset, PA) connected to a Rosenthal-French dosimeter (John Hopkins University, Baltimore, MA) driven by compressed air. Five inhalations were taken for phosphate-buffered saline and for each increasing doubling dose of methacholine (0.5 to 64 mg/ml), administered at 5 min intervals, until at 20% fall in $FEV_1$ was observed. $FEV_1$ was measured 3 min after the beginning of each set of inhalations of aerosolised methacholine. Ventilatory function was monitored by measurements of $FEV_1$ using a dry bellows spirometer (Ohio 840, Airco, Houston, TX).

### Inhalation challenge with TDI

Subjects were exposed to TDI ($0.014 \pm 0.001$ ppm) in a 9-cubic meter exposure chamber. TDI was generated into the atmosphere by blowing air over the surface of TDI (20 ml) in a 200 ml glass washing bottle. The flow rate of air was controlled by a rotameter calibrated at a flow rate of 2 L/min. A fan in the chamber ensured adequate mixing and circulation. The temperature in the chamber was maintained at about 24°C. The concentration generated was measured with a MDA Model 7005 isocyanate detection equipment (MDA Scientific Inc., Glenview, IL). The subject was seated close to the sampling of the monitor. Subjects were observed through the windows of the glass chamber.

$FEV_1$ was measured immediately before and after exposure to TDI, then hourly for 8 h. The duration of exposure was 30 min in subjects who developed only a late asthmatic reaction or until the appearance of symptoms of asthma in subjects who developed a dual asthmatic reaction. Each subject was exposed at the same time of the day, and the duration of exposure was the same for each set of experiments.

### Serum cortisol levels

Blood samples were obtained after 1 week of treatment with placebo, low dose and high dose of beclomethasone. Serum was separated by centrifugation. Serum sample were then frozen at −20°C for later analysis. Serum cortisol levels were measured by a radioimmunoassay.[10] All the assays were performed according to the method of the manufacturer (Coat-Count, D.P.C., Los Angeles, CA), using a gamma-counter (Mod. Cristall Packard). Using this method, normal values of cortisol at 08:00 are 138–662 nM/L. All samples were analysed in duplicate and results were averaged.

### Data analysis

An immediate or dual asthmatic reaction of TDI was considered to occur when $FEV_1$ decreased by at least 20% from baseline within 30 min (early component of a dual reaction or early alone reaction) and one or more hours (late component of a dual reaction or late alone reaction) after exposure to TDI.

To assess airway responsiveness, dose-response to methacholine were constructed by plotting the baseline value for $FEV_1$ and the peak value after each methacholine dose against the cumulative dose (nebuliser output × solution concentration) of methacholine delivered, on a log scale. The cumulative dose of methacholine producing a 20% fall in $FEV_1$ ($PD_{20}FEV_1$) was calculated by interpolation from the dose-response curve and was used as a measure of airway responsiveness. In our laboratory, the day-to-day variability of $PD_{20}FEV_1$ was never greater than a doubling inhalation dose.[11]

To test for changes in airway responsiveness during the treatments and after TDI inhalation, the log values for methacholine provocative doses were analysed using analysis of variance and covariance with repeated measures and using two-way analysis of variance (Friedman's test).[12] Mean values for provocative doses are reported as geometric means (GMean) and GSEM. To test for changes of $FEV_1$ before and after 1 week of treatment, before and after TDI inhalation, the values of $FEV_1$ were compared using analysis of variance and covariance with repeated measures and using two-way analysis of variance (Friedman's test).

### Drugs and chemicals

The following drugs were used: Becotide and Becloforte, delivering respectively 0.05 mg and 0.25 mg beclomethasone dipropionate per puff (Glaxo, Verona, Italy). The following chemicals were used: toluene diisocyanate (a mixture of 80% 2,4 isomer and 20% 2,6 isomer) (Montedison, Porto Marghera, Italy); acetyl-beta-methacholine chloride (Sigma, St. Louis, MO).

## RESULTS

The characteristics of the subjects examined are shown in Table 1. Eight subjects were males and one subject was female. None was atopic. Lung volumes were normal in all the subjects. Airway responsiveness was in the asthmatic range in five subjects and in the normal range in four subjects.

After exposure to TDI, subjects treated with placebo developed late or dual asthmatic reactions, by contrast, subjects treated with high dose inhaled beclomethasone developed no late asthmatic reaction,

**Table 1.** Anthropometric, clinical, and spirometric data at the preliminary visit of the examined subjects

| Subject No. | Sex | Age (yr) | Weight (kg) | Height (cm) | Atopy | $FEV_1$ (%pred)* | $PD_{20}FEV_1$ (mg) |
|---|---|---|---|---|---|---|---|
| 1 | F | 20 | 69 | 173 | — | 97 | 0.41 |
| 2 | M | 23 | 84 | 184 | — | 95 | 0.35 |
| 3 | M | 34 | 75 | 170 | — | 91 | 0.07 |
| 4 | M | 48 | 87 | 178 | — | 89 | 1.34 |
| 5 | M | 38 | 70 | 166 | — | 115 | 2.88 |
| 6 | M | 39 | 84 | 167 | — | 102 | 2.63 |
| 7 | M | 48 | 77 | 169 | — | 100 | 2.67 |
| 8 | M | 38 | 64 | 172 | — | 78 | 0.23 |
| 9 | M | 24 | 87 | 178 | — | 103 | 0.02 |

*Predicted values from reference 8

and subjects treated with low dose developed a less severe asthmatic reaction (Fig. 1); as shown by a significant difference in the decrease of $FEV_1$ between the three treatments ($F = 9.87$; $p < 0.001$). By using Friedman's test, $FEV_1$ decreased significantly after exposure to TDI in subjects treated with placebo ($\chi^2 = 56.2$, $p < 0.001$); and in subjects treated with low dose beclomethasone ($\chi^2 = 34.6$, $p < 0.001$) but not after treatment with high dose beclomethasone ($\chi^2 = 15.4$, n.s.). The treatments had no effect on airway responsiveness ($F = 2.61$, n.s.); but there was a significant difference in $PD_{20}$ $FEV_1$ between days (day 0 = before the beginning of any treatment, day 6 = 6 days after the beginning of treatment, and day 7 = 7 days after the beginning of treatment and 8 h after exposure to TDI) ($F = 5.29$, $p. < 0.01$). Airway responsiveness to methacholine increased 8 h after TDI inhalation when the subjects were treated with placebo ($F = 6.2$, $p < 0.01$) or with low dose beclomethasone ($F = 4.7$,

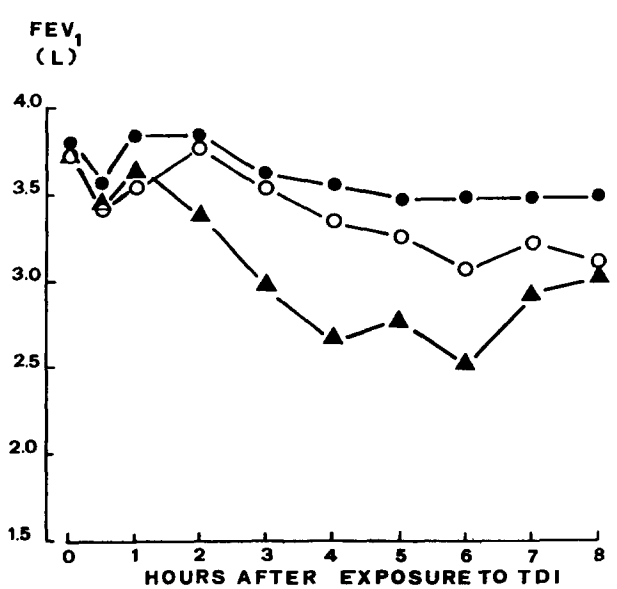


**Fig. 1**—Mean values of $FEV_1$ before and after exposure to toluene diisocyanate (TDI), after 1 week treatment with placebo (closed triangles), low dose of beclomethasone (open circles) and high dose of beclomethasone (closed circles). The horizontal axes indicate time in hours and the vertical axes indicate $FEV_1$ in litres.

**Table 2.** Individual values of $PD_{20}$ $FEV_1$ methacholine (mg) measured before and after 1 week treatment with placebo, low and high dose of inhaled beclomethasone

| | Placebo | | | Beclomethasone (low dose) | | | Beclomethasone (high dose) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day | | | Day | | | Day | | |
| | 0 | 6 | 7 | 0 | 6 | 7 | 0 | 6 | 7 |
| Subject | | | | | | | | | |
| 1 | 0.19 | 0.20 | 0.04 | 0.40 | 0.21 | 0.05 | 0.40 | 0.39 | 0.13 |
| 2 | 0.59 | 0.22 | 0.04 | 0.35 | 1.01 | 0.09 | 0.44 | 1.64 | 0.14 |
| 3 | 0.06 | 0.56 | 0.07 | 0.19 | 0.31 | 0.14 | 0.03 | 0.41 | 0.97 |
| 4 | 1.33 | 2.88 | 0.15 | 2.68 | 2.88 | 2.88 | 2.87 | 0.85 | 1.19 |
| 5 | 2.88 | 1.11 | 0.31 | 0.22 | 2.88 | 0.36 | 2.87 | 2.87 | 2.87 |
| 6 | 2.88 | 1.62 | 1.36 | 1.43 | 2.87 | 2.87 | 2.62 | 2.87 | 2.87 |
| 7 | 2.88 | 1.44 | 0.61 | 1.19 | 1.65 | 1.16 | 2.66 | 2.87 | 2.87 |
| 8 | 0.28 | 0.23 | 0.14 | 0.33 | 0.33 | 0.18 | 0.19 | 0.11 | 0.11 |
| 9 | 0.21 | 0.48 | 0.21 | 0.77 | 0.52 | 0.26 | 0.19 | 0.16 | 0.83 |
| GMEAN | 0.63 | 0.66 | 0.18* | 0.58 | 0.93 | 0.36″ | 0.62 | 0.78 | 0.71 |
| GSEM | 1.60 | 1.38 | 1.46 | 1.35 | 1.42 | 1.63 | 1.69 | 1.51 | 1.58 |

0 = before the beginning of any treatment; 6 = after 6 days of treatment; 7 = after 7 days of treatment, 8 hours after exposure to TDI.
* = $p < 0.01$; ″ = $p < 0.02$.

**Table 3.** Individual values of $FEV_1$ (L) measured before and after 1 week treatment with placebo, low and high dose of inhaled beclomethasone

| | Placebo | | | Beclomethasone (low dose) | | | Beclomethasone (high dose) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day | | | Day | | | Day | | |
| | 0 | 6 | 7 | 0 | 6 | 7 | 0 | 6 | 7 |
| Subject | | | | | | | | | |
| 1 | 3.50 | 3.18 | 3.35 | 3.70 | 3.57 | 3.45 | 3.85 | 3.60 | 3.55 |
| 2 | 4.85 | 4.67 | 2.53 | 4.90 | 4.90 | 2.82 | 4.65 | 4.80 | 3.95 |
| 3 | 3.53 | 3.45 | 2.65 | 3.51 | 3.55 | 3.35 | 3.20 | 3.57 | 3.55 |
| 4 | 3.70 | 3.35 | 2.35 | 3.57 | 3.35 | 3.65 | 3.57 | 3.75 | 3.20 |
| 5 | 4.15 | 4.20 | 2.80 | 4.10 | 4.07 | 2.20 | 3.90 | 3.80 | 3.15 |
| 6 | 3.75 | 3.90 | 3.40 | 3.80 | 3.60 | 2.70 | 3.51 | 3.55 | 3.20 |
| 7 | 3.60 | 3.55 | 3.25 | 3.60 | 3.62 | 3.35 | 3.55 | 3.55 | 3.20 |
| 8 | 3.07 | 3.10 | 2.80 | 3.15 | 3.10 | 2.90 | 3.08 | 3.13 | 3.15 |
| 9 | 4.50 | 4.81 | 4.00 | 4.30 | 4.20 | 3.90 | 4.80 | 4.65 | 4.20 |
| Mean | 3.85 | 3.80 | 3.01 | 3.84 | 3.77 | 3.14 | 3.79 | 3.82 | 3.46 |
| SEM | 0.18 | 0.21 | 0.17 | 0.17 | 0.18 | 0.18 | 0.20 | 0.18 | 0.13 |

0 = before the beginning of any treatment; 6 = after 6 days of treatment; 7 = after 7 days of treatment, 8 hours after exposure to TDI.

$p < 0.02$) (Table 2, Fig. 2). By contrast, with the high dose beclomethasone, airway responsiveness did not change 8 h after exposure to TDI. Baseline $FEV_1$ before measurement of airway responsiveness to methacholine was not different on day 0 ($\chi^2 = 2.0$, n.s.), day 6 $\chi^2 = 0.5$, n.s.) or day 7 $\chi^2 = 4.6$, n.s.) of any treatment (Table 3).

Cortisol levels did not change after 1 week treatment of placebo, low dose or high dose of beclomethasone (Table 4). The weight of the inhalers before and after 1 week treatment was respectively: before and after placebo: $38.3 \pm 0.15$ g and $33.9 \pm 0.38$ g (mean ± Standard Error of the Mean [SEM]); before and after low dose: $38.2 \pm 0.17$ g and $34.6 \pm 0.43$ g; before and after high dose beclomethasone: $38.5 \pm 0.08$ g and $34.6 \pm 0.33$ g. All subjects tolerated treatments without side effects.

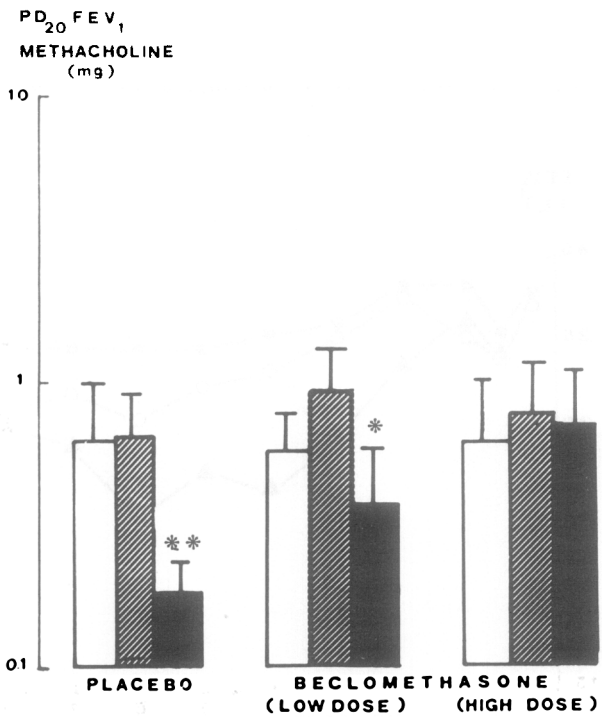


**Fig. 2**—$PD_{20}$ $FEV_1$ methacholine before treatment with placebo or with the active drug (open bars), 6 days after treatment (the day before inhalation challenge with TDI (stippled bars), and 7 days after treatment (8 h after exposure to TDI) (solid bars). Each bar represents geometric mean and GSEM. ** = $p < 0.01$; * = $p < 0.02$ (analysis of variance and covariance with repeated measures).

## DISCUSSION

The results of the present study show that treatment with inhaled beclomethasone (2000 μg) blocks the late asthmatic reaction and the associated increase in airway responsiveness to methacholine induced by TDI in sensitised subjects. We have previously shown that other drugs used in the treatment of asthma such as theophylline, cromolyn and verapamil were ineffective against both the phenomena.[6] Appropriate manage-

**Table 4.** Individual and mean values of serum cortisol levels (nM/L) measured 7 days after treatment with placebo, low dose and high dose beclomethasone

| Subject No. | Placebo | Beclomethasone (low dose) | Beclomethasone (high dose) |
|---|---|---|---|
| 1 | 501 | 474 | 494 |
| 2 | 269 | 451 | 262 |
| 3 | 740 | 627 | 553 |
| 4 | 602 | 727 | 350 |
| 5 | 401 | 464 | 465 |
| 6 | 338 | 300 | 474 |
| 7 | 614 | 529 | 674 |
| 8 | 482 | 415 | 501 |
| 9 | 924 | 667 | 750 |
| Mean | 541 | 517 | 502 |
| SEM | 68 | 45 | 49 |

ment of asthma patients may prevent chronic irreversible airflow limitation which occurs in patients with asthma.[13] Because TDI-induced asthma is a frequent occupational disease, we believe that a good management of the TDI-patient and a good treatment of TDI-induced asthma are important goals. Moreover, TDI-induced asthma rarely disappears even after cessation of exposure.[14-16] It is possible that an adequate pharmacologic prophylaxis and/or treatment of TDI-induced asthma may also affect the outcome of the disease. The observation that even a low dose of inhaled beclomethasone (400 μg daily) partially inhibits the late asthmatic reaction induced by TDI, suggests that this dosage may be employed to prevent attacks of asthma at work. However, this dose does not completely prevent the increase in airway responsiveness associated with the late asthmatic reaction. The late asthmatic reaction is likely to be due to a combination of effects of bronchoconstrictor and inflammatory mediators,[17] while the increase in airway responsiveness is probably due mainly to the effects of inflammatory mediators.[18] It has been suggested that perennial asthma may be caused by a vicious circle connecting late asthmatic reactions, increased airway responsiveness, and airway inflammation.[19] If this hypothesis is true, prophylaxis of asthma should be directed to interrupt completely this circle. The high dose inhaled beclomethasone used in the present study prevented both the phenomena without side-effects or changes in circulating cortisol, suggesting that this drug may be employed effectively and safely. However, whether a long term treatment with this high dose may cause side effects, or alter cortisol secretion, or whether a dosage in between the two used in the present study or a long treatment with the low dose would be effective without side-effects, remains to be determined.

In the strategy of treatment and control of bronchial asthma, the inhalation therapy is important for control of symptoms, low systemic bioavailability and absence of drug side effects. In the present study, the first finding is that the aerosol route was effective in our TDI patients. Secondly, the subjects examined, were able to use their metered-dose inhalers adequately, as assessed by weighing the inhalers before and after 1 week of treatment. Because doses of 1200 μg, and probably larger of beclomethasone dipropionate daily are reported not to produce significant side effects (e.g. adrenal suppression, Cushing's syndrome);[20,21] the result that a dose of 2 mg daily for 1 week completely prevents the effects of exposure to TDI in sensitised subjects suggests that this drug, presumably without significant local and general side effects, is very useful in the management of TDI-induced asthma. The elimination of the occupational exposure is, of course, important, and it is the best prophylaxis in occupational asthma. Among TDI patients, however, many subjects are self-employers and it is sometimes difficult for them to change job. Treatment with inhaled steroids may also improve the prognosis of the disease, that, at the present time, is very poor.[14-16]

The precise mechanism of action of corticosteroids still remains unclear. It has been suggested that chronic asthma results from the infiltration of activated leukocytes that have been recruited from peripheral blood into the bronchial tissues.[22-24] Among the wide range of actions, recently both the inhibition of neutrophil locomotion and eosinophilia have been demonstrated with prednisolone, suggesting that, presumably steroids reverse the inflammatory process observed in bronchial asthma.[25] In previous studies, we have shown that, like allergen-induced late asthmatic reactions,[26,27] TDI-induced late asthmatic reactions may be caused by the combination of bronchoconstriction and airway inflammation.[4,5,11]

Allergen-induced asthmatic reactions are associated with an increase in polymorphonuclear leukocytes in bronchoalveolar lavage[26,27] and are inhibited by steroids.[28] Similarly to allergen, TDI causes an early increase of neutrophils followed by eosinophils in bronchoalveolar lavage during a late reaction.[29] Orally prednisone blocks this inflammatory reaction, suggesting an inhibition of neutrophil and eosinophil chemotaxis.[5]

Late asthmatic reactions after TDI exposure are a feature of the disease and are associated with an increase in airway responsiveness.[30,31] We previously reported that, in subjects sensitised to TDI, there is a transient, steroid-sensitive component, and a long lasting, steroid-unsensitive component of airway hyperresponsiveness, and we suggested that the former might be due to airway inflammation, and the latter to unknown mechanisms.[4] This study shows that high dose beclomethasone blocks the transient component of airway hyperresponsiveness, and prompts us to examine in long term studies whether inhibition of the transient component may also modify the long lasting component of airway hyperresponsiveness. Previous studies on the effect of steroids on airway hyperresponsiveness have been performed,[32-36] and all report that treatment with inhaled steroids of asthmatic patients up to 6 months, reverse only partially airway hyperresponsiveness. In asthmatic children treated for 6 months with inhaled corticosteroids, a decrease in airway responsiveness has been reported.[37] None of these studies, however, were performed in subjects with occupational asthma.

In conclusion, inhaled beclomethasone is effective against TDI-induced late asthmatic reactions and the associated increase in airway responsiveness to methacholine and the effect is dose-related. Future studies including treatment with inhaled steroids of patients with TDI-asthma may help to understand the role of airway inflammation for the long lasting increase of airway hyperresponsiveness induced by TDI.

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## References

1. Reed C E. New therapeutic approaches in asthma. J Allergy Clin Immunol 1985; 77: 537–42.
2. Fabbri L M and Salvaggio J E. Occupational asthma. Folia All Immunol Clin 1985; 32: 1–66.
3. Chan-Yeung M, Lam S. Occupational asthma. Am Rev Respir Dis 1986; 133: 686–703.
4. Fabbri L M, Chiesura-Corona P, Dal Vecchio L, et al. Prednisone inhibits late asthmatic reactions and the associated increase in airway responsiveness induced by toluene diisocyanate in sensitized subjects. Am Rev Respir Dis 1985; 132: 1010–4.
5. Boschetto P, Fabbri L M, Zocca E, et al. Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. J Allergy Clin Immunol 1987; 80: 261–7.
6. Mapp C, Boschetto P, Dal Vecchio L, et al. Protective effect of antiasthma drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. Am Rev Respir Dis 1987; 136: 1403–7.
7. Enden P, Davies B H. High-dose corticosteroid inhalers for asthma. Lancet 1984; ii: 576.
8. Commissions des Communautes Europeenes (CECA). Tables de reference pour les examens spirometriques. Luxembourg: Office des publications officielles des Communautes Europeennes, 1971.
9. Chai H, Farr R S, Froehlich L A, et al. Standardization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 1975; 56: 323–7.
10. Tilden R L. New advantageous approach to the direct assay to cortisol. Clin Chem 1977; 23: 211–5.
11. Mapp C E, Di Giacomo G R, Broseghini C, et al. Late, but not early, asthmatic reactions induced by toluene diisocyanate are associated with increased airway responsiveness to methacholine. Eur J Respir Dis 1986; 69: 276–84.
12. BMDP Statistical Software, University of California, Press 1985.
13. Brown P J, Greville H W, Finucane K E. Asthma and irreversible airflow obstruction. Thorax 1984; 39: 131–6.
14. Moller D R, Brooks S M, McKay R T, et al. Chronic asthma due to toluene diisocyanate. Chest 1986; 90: 494–9.
15. Paggiaro P L, Loi A M, Rossi O, et al. Follow-up study of patients with respiratory disease due to toluene diisocyanate (TDI). Clin Allergy 1984; 14: 463–9.
16. Mapp C E, Chiesura Corona P, De Marzo N, Fabbri L M. Persistent asthma due to isocyanates. A follow-up study of subjects with occupational asthma due to toluene diisocyanate (TDI). Am Rev Respir Dis 1988; in press.
17. Cockcroft D W, Ruffin R E, Dolovich J, Hargreave F E. Allergen-induced increase in non-allergic bronchial reactivity. Clin Allergy 1977; 7: 503–13.
18. Kirby J G, Hargreave F E, O'Byrne P M. Indomethacin inhibits allergen-induced airway hyperresponsiveness but not allergen-induced asthmatic response. Am Rev Respir Dis 1987; 1354: 312 (abstract).
19. Cockcroft D W. Mechanism of perennial allergic asthma. Lancet 1983; 2: 253–6.
20. Toogood J H, Jennings B, Baskerville J, Lefcoe N M. Personal observations on the use of inhaled corticosteroid drugs for chronic asthma. Eur J Respir Dis 1984; 65: 321–38.
21. Clark T J H, ed. Steroids in asthma: a reappraisal in the light of inhalation therapy. Auckland, New Zealand: ADIS Press, 1983.
22. Kay A B. Mediators of hypersentivity and inflammatory cells in the pathogenesis of bronchial asthma. Eur J Respir Dis (Suppl) 1983; 129: 1–44.
23. Carroll M, Durham S R, Walsh G M, Kay A B. Activation of neutrophils and monocytes after allergen- and histamine-induced bronchoconstriction. J Allergy Clin Immunol 1985; 75: 290–6.
24. Durham S R, Carroll M, Walsh G M, Kay A B. Leucocyte activation in allergen-induced late-phase asthmatic reactions. N Engl J Med 1984; 311: 1398–1402.
25. Gin W, Shaw R J, Kay A B. Airways reversibility after prednisolone therapy in chronic asthma is associated with alterations in leukocyte function. Am Rev Respir Dis 1985; 132: 1199–1203.
26. De Monchy J G R, Kauffman H F, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reaction. Am Rev Respir Dis 1985; 131: 373–6.
27. Metzger W J, Richerson H B, Worden K, Monick M, Hunninghake G W. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. Chest 1986; 89: 477–83.
28. Booj-Noord H, Orie NGM, de Vries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48: 344–54.
29. Fabbri L M, Boschetto P, Zocca E, et al. Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. Am Rev Respir Dis 1987; 136: 36–42.
30. Mapp C E, Maestrelli P, Fabbri L M. Late asthmatic reactions in occupational asthma. Folia Allergol Immunol Clin 1985; 32: 53–9.
31. Mapp C E, Polato R, Maestrelli P, et al. Time course of the increase in airway responsiveness associated with late asthmatic reactions to toluene diisocyanate in sensitized subjects. J Allergy Clin Immunol 1985; 75: 568–72.
32. Juniper E F, Frith P A, Hargreave F E. Long-term stability of bronchial responsiveness to histamine. Thorax 1982; 37: 288–91.
33. Easton J G. Effect of an inhaled corticosteroid on methacholine airway reactivity. J Allergy Clin Immunol 1981; 67: 388–90.
34. Ryan G, Latimer K M, Juniper E F, et al. Effect of beclomethasone dipropionate on bronchial responsiveness to histamine in controlled nonsteroid-dependent asthma. J Allergy Clin Immunol 1985; 75: 25–30.
35. Svendesen U G, Frolund L, Madsen F, et al. A comparison of the effects of sodium cromoglycate and beclomethasone dipropionate on pulmonary function and bronchial hyperreactivity in subjects with asthma. J Allergy Clin Immunol 1987; 80: 68–74.
36. Boschetto P, Broseghini C, Di Blasi P, et al. One month treatment with high vs low dose inhaled beclomethasone combined with salbutamol: significant improvement in lung function without side-effects in asthmatics. Proceedings of the 6th Congress of the European Society of Pneumology (SEP), Amsterdam, 31 August–5 September, 1987, p. 121.
37. Kerrebijn K F, Van Essen-Zandvliet E E M, Neijens H J. Effect of long-treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. J Allergy Clin Immunol 1987; 79: 653–659.

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