Effect of an inhaled glucocorticosteroid on mast cell and smooth muscle $\beta_2$ adrenergic tolerance in mild asthma

Deborah H Yates, Miin Worsdell, Peter J Barnes

Abstract

Background – Regular inhaled $\beta_2$ agonist therapy is associated with loss of bronchoprotection to indirect bronchial provocation challenges such as allergen or adenosine monophosphate (AMP), while directly acting challenge is less affected, implying preferential mast cell tolerance. Glucocorticosteroids may reverse such $\beta_2$ adrenoceptor tolerance and upregulate mast cell $\beta_2$ adrenoceptor function.

Methods – The effect of single high dose glucocorticosteroids on terbutaline induced loss of bronchoprotection was studied in a placebo controlled, double blind, crossover study. Fifteen asthmatic subjects who were not taking inhaled glucocorticosteroids underwent two 10-day treatment periods with terbutaline (800 μg four times daily via Turbohaler), each followed by a single dose of inhaled budesonide (800μg via Turbohaler) or identical placebo.

Results – Regular treatment with terbutaline resulted in significant loss of bronchoprotection to AMP (mean difference (95% CI) −1.7 (−3.0 to −0.4) doubling dilutions) but not to methacholine (mean difference −0.1 (−1.0 to 0.8) doubling dilutions). Single high dose budesonide increased the protective effect of terbutaline more to AMP than to methacholine challenge (+0.76 (0.3) doubling dilutions compared with +0.13 (0.4) doubling dilutions, respectively). The mean (SE) difference between budesonide and placebo for methacholine challenge was 0.08 (0.14) whereas that for AMP was 0.075 (0.15); p = NS. The difference in PC$_{20}$ was not statistically significant when compared with placebo for either challenge agent.

Conclusions – Inhaled glucocorticosteroids in a single dose had no significant effect in restoring terbutaline induced loss of bronchoprotection, implying that mast cell $\beta_2$ adrenoceptor sensitivity is not restored by a single dose of an inhaled glucocorticosteroid in asthma.

(Thorax 1998;53:110–113)

Keywords: $\beta_2$ agonists, tolerance, glucocorticosteroid, inhaled steroids.
AMP challenge. Investigating the effect of a single dose of glucocorticosteroid in vivo should therefore allow study of mast cell β₂ adrenoceptor rather than mast cell function in clinical asthma.

We have therefore investigated the effect of a single high dose of budesonide on terbutaline induced loss of bronchoprotection. Budesonide or identical placebo were administered after 10 days of regular therapy with terbutaline and mast cell and smooth muscle effects were measured by assessment of the response to AMP and methacholine, respectively.

Methods

PATTERNS

Seventeen non-smoking subjects with mild asthma were recruited (table 1). All consented to participate in the study which was approved by the local ethics committee. All subjects had asthma according to the criteria of the American Thoracic Society. Baseline forced expiratory volume in one second (FEV₁) for all subjects was >70% predicted. All subjects were sensitive to methacholine and AMP challenge as documented by a provocative concentration causing a 20% fall in FEV₁ (PC₂₀) of <8 ng/ml and <100 mg/ml, respectively, at screening (table 1). None had suffered an asthma exacerbation or upper respiratory tract infection within six weeks preceding the study, nor used any glucocorticosteroid within two months. All used inhaled short acting β₂ agonists for asthma control, but ipratropium bromide was substituted during the study.

STUDY DESIGN

The study was double blind, randomised, placebo controlled, and crossover. Due to potential interaction, methacholine and AMP challenges were conducted on separate days with the order of challenge randomised on entry into the study but remaining identical for each patient throughout the study, and performed at an identical time of day for each patient. The power of the study was calculated on the basis of variation of PC₂₀ values as performed in our laboratory. With an α value of 5% and power of 80% it was calculated that 15 patients would be required to detect a twofold difference in PC₂₀ which would be of clinical significance.

After a seven day run in period baseline protection by terbutaline to methacholine and AMP was assessed 15 minutes after inhalation of terbutaline 500 μg via a multidose dry powder delivery (Turbohaler, Astra Draco, Sweden). Subjects were then treated with terbutaline 500 μg four times daily via Turbohaler over 12 days. Bronchoprotection of terbutaline 500 μg was examined to both methacholine and AMP before terbutaline treatment, at days 7 and 8, and again after active or placebo treatment on days 11 and 12. On day 10 subjects also inhaled either budesonide 800 μg or identical placebo in a single dose exactly 12 hours before challenge. Terbutaline treatment was continued between the challenges (with terbutaline being taken immediately after the challenge) on the assumption that any tolerance would, if anything, be greater after a longer treatment period. There was a minimum 10 day washout period between treatment periods and the study sequence was then repeated with the alternative inhaler. Inhaled ipratropium bromide and caffeinated beverages were withheld for at least 12 hours before each challenge.

BRONCHIAL PROVOCATION CHALLENGE

Bronchial provocation challenge was performed as previously reported. Fresh solutions of methacholine and AMP (Sigma, Poole, UK) were made up in 0.9% saline in doubling dilutions (0.06–32 mg/ml and 0.39–800 mg/ml respectively). Each solution was administered from a nebuliser attached to a breath activated dosimeter (Mefar, Brescia, Italy). After resting quietly, baseline spirometric values were assessed by three forced expiratory manoeuvres using a dry wedge spirometer (Vitalograph, Buckingham, UK). Terbutaline 500 μg was administered via a Turbohaler and FEV₁ measured in an identical manner 15 minutes afterwards. Subjects then inhaled five breaths of saline followed by incremental doses of methacholine or AMP at three minute intervals. Challenges were terminated when a 20% decrease in FEV₁, from the post-saline value was reached.

STATISTICAL ANALYSIS

All values were expressed as mean (SE) apart from PC₂₀ results which were expressed as geometric means. Log dose-response curves were constructed and PC₂₀ calculated by linear interpolation. Baseline FEV₁, post-bronchodilator FEV₁, and log PC₂₀ values were compared by repeated measures two-way analysis of variance (ANOVA) with Bonferroni’s correction for multiple comparisons. A standardised computerised statistical package (NCSS) was used for the analysis. All tests of significance were two-tailed and p values of <0.05 were regarded as significant.

The effect of treatment on responses to provocation on each challenge day was calculated by comparing the difference in PC₂₀ before and after terbutaline treatment and also after the
administration of the budesonide and placebo in each subject; this was expressed as doubling doses using the formula: \( \log PC_{20} \) after budesonide − \( \log PC_{20} \) after placebo)/\( \log_{10}2 \). The response to budesonide and placebo for each challenge was calculated taking the post-terbutaline values as baseline, and differences between pre-terbutaline and post-terbutaline treatment periods were analysed for each treatment period individually.

**Results**

Two patients were withdrawn from the study due to upper respiratory tract infections. Their results were excluded from the statistical analysis.

**EFFECT OF REGULAR TERBUTALINE ON METHACHOLINE AND AMP CHALLENGE AND ON FEV\(_1\)**

Baseline geometric mean \( PC_{20} \) prior to regular treatment and without any terbutaline to methacholine was \(-0.27\) log units and to AMP was \(1.12\) log units.

After treatment with terbutaline for seven days the mean \( PC_{20} \) to methacholine changed from 0.34 log units to 0.31 (mean difference (95% CI) \(-0.1\) (−1.0 to 0.8) doubling dilutions); this small change was not statistically significant (fig 1). With AMP, however, mean \( PC_{20} \) changed from 1.56 to 1.07 log units (mean difference (95% CI) \(-1.7\) (−3.0 to −0.4) doubling dilutions; \( p<0.05 \)). The difference in loss of bronchoprotection between the two challenge agents was statistically significant (\( p<0.04 \)).

Baseline \( FEV_1 \) was \(85\) (2.6)% predicted. Terbutaline caused significant bronchodilatation which was only minimally less after the terbutaline treatment period (8.4% versus 6.6%; \( p=\text{NS} \)). No significant change in baseline \( FEV_1 \) was observed at any of the study visits and, in particular, between the pre-terbutaline and post-terbutaline treatment values (3.35 (0.11) l versus 3.38 (0.11) l; \( p=\text{NS} \)).

**EFFECT OF BUDESONIDE ON METHACHOLINE AND AMP CHALLENGE**

Changes in methacholine \( PC_{20} \) and AMP responsiveness were +0.13 (0.4) and +0.76 (0.3) doubling dilutions, respectively, on budesonide and +0.51 (0.4) and +0.6 (0.2) doubling dilutions on placebo (fig 1). Compared with placebo, budesonide had no statistically significant effect on either methacholine or AMP challenge. The mean difference between budesonide and placebo for methacholine challenge was 0.08 (0.14) whereas that for AMP was 0.075 (0.15). This difference between challenge agents was not statistically significant.

**Discussion**

Little information is available regarding the effect of glucocorticoids on bronchoprotective tolerance in asthma. We have investigated whether such tolerance could be readily restored by inhaled glucocorticosteroids and whether this is mediated by reversal of mast cell \( \beta_2 \) adrenoceptor function.

Many studies using both human and animal pulmonary and bronchial tissue have previously demonstrated reversal of \( \beta_2 \) adrenergic tolerance by glucocorticosteroids. Glucocorticosteroids also prevent desensitisation of the \( \beta_2 \) receptors and restore downregulated receptors to near normal levels. In normal Airways \( \beta_2 \) adrenergic resistance induced by regular inhaled \( \beta_2 \) agonist therapy can be restored by the use of intravenous hydrocortisone when measured between six and 48 hours and lymphocyte \( \beta_2 \) adrenoceptor function and number can be restored to normal within 16 hours by oral or intravenous high dose glucocorticosteroids.

We therefore expected that a single high dose of budesonide would restore mast cell \( \beta_2 \) adrenoceptor function. In our study bronchoprotective tolerance occurred to AMP challenge, but not to methacholine. We failed to demonstrate significant reversal of bronchoprotective tolerance with single dose inhaled budesonide, implying that mast cell \( \beta_2 \) adrenoceptor function is not readily reversed by single dose glucocorticosteroids when used in maximal recommended dosage. Whether an intravenous dose or one exceeding the recommended dosage might have been effective is uncertain. Steroid concentrations used in laboratory studies have been considerably higher than those used clinically, but we wished to study an effect which could reflect the situation in an asthmatic patient. Regular inhaled glucocorticosteroids, although more clinically applicable, could not be used in view of their effect on mast cell number and activation.

We cannot exclude the possibility of a type II error accounting for our essentially negative findings, although our power calculation would suggest that a sufficient number of subjects was studied, nor can we exclude individual variation.
in β₂ adrenoceptor susceptibility to β₂ adrenoceptor upregulation with glucocorticosteroids. Our study was, however, crossover rather than parallel in design, which should have minimised such a possibility.

Our results suggest that tolerance induced by terbutaline on the mast cell β₂ adrenoceptor may be relatively resistant to the effects of inhaled glucocorticosteroids, at least in therapeutic dosage. Airway inflammatory cell β₂ adrenoceptor tolerance may thus not be rapidly reversible in vivo, at least using inhaled glucocorticosteroids. Until further studies have been reported, our data would appear to reinforce current recommendations regarding the importance of maximising anti-inflammatory treatment and keeping regular β₂ agonist therapy to a minimum in asthma.

We thank Astra Draco, Lund, Sweden for provision of drugs and for financial support.

Effect of an inhaled glucocorticosteroid on mast cell and smooth muscle beta 2 adrenergic tolerance in mild asthma.
D H Yates, M Worsdell and P J Barnes

Thorax 1998 53: 110-113
doi: 10.1136/thx.53.2.110