Short paper

Effect of an inhaled glucocorticosteroid on mast cell and smooth muscle β_2 adrenergic tolerance in mild asthma

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Abstract

***Background* – Regular inhaled β_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 β_2 adrenoreceptor tolerance and upregulate mast cell β_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 (500µg 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 β_2 adrenoceptor sensitivity is not restored by a single dose of an inhaled glucocorticosteroid in asthma.**

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Keywords: β_2 agonists, tolerance, glucocorticosteroid, inhaled steroids.

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Beta$_2$ adrenoceptor agonists are very effective in the treatment of acute asthma symptoms. Recently, however, it has been suggested that regular inhaled β_2 agonist therapy may lead to loss of control of asthma symptoms[1] and reduced acute bronchoprotection against bronchoconstriction.[2 3] Such loss of bronchoprotection is small and its clinical significance is uncertain.[3] Other studies have suggested that regular glucocorticosteroid therapy does not prevent the development of bronchoprotective tolerance.[3 4]

Although the mechanism underlying loss of bronchoprotection in vivo is unknown, it probably relates to β_2 adrenoceptor downregulation which occurs in human airways in vitro.[5] Preferential downregulation of β_2 adrenoceptors on mast cells is suggested because loss of bronchoprotection is more easily demonstrable using indirect bronchial provocation challenges such as adenosine monophosphate (AMP), which are thought to act via mast cell activation, than methacholine which acts directly on smooth muscle.[2 3] Glucocorticosteroids increase β adrenoceptor expression in human lung cells by increasing β_2 adrenoceptor transcription.[5] High doses of glucocorticosteroid prevent and reverse β_2 receptor tolerance in desensitised animals and in human tissue in vitro.[6] Studies in both asthmatic and non-asthmatic subjects using high dose glucocorticosteroids have shown that glucocorticosteroids rapidly reverse β_2 adrenoceptor tolerance of lymphocytes, neutrophils, and normal airways.[5] Thus, high dose inhaled glucocorticosteroids would be expected to reverse loss of bronchoprotection in asthma induced by β_2 agonists.[5]

Prolonged treatment with steroids in vivo has direct inhibitory effects on mast cells, with actions not restricted to β_2 adrenoceptor transcription. In clinical studies using regular inhaled glucocorticosteroids the number and activation of mast cells are reduced,[7] along with inhibition of the early response to allergen.[8] In contrast, glucocorticosteroids appear to have little effect on non-β_2 adrenoceptor mediated mast cell function when used as a single dose, neither inhibiting IgE mediated release of histamine from cultured human lung mast cells or airway tissue,[9] nor the early asthmatic response to allergen.[5] Single high dose budesonide itself does not have a significant effect on

Table 1 Characteristics of asthmatic patients studied

Patient no.	*Age/sex*	*FEV_1 (% pred)*	*Log PC_{20} MCh**	*Log PC_{20} AMP**	*Atopy*
1	25/M	103	−0.57	0.89	+
2	21/M	73	−0.46	0.75	+
3	36/F	78	−0.91	1.51	−
4	46/M	84	−0.80	0.23	+
5	20/M	99	−0.005	0.84	+
6	23/M	75	−0.52	0.24	+
7	21/M	84	0.018	1.11	+
8	29/M	85	−1.17	0.94	+
9	41/F	89	−0.11	1.44	+
10	25/M	86	−0.48	1.48	+
11	51/M	70	0.16	2.66	+
12	21/M	72	0.38	1.51	+
13	25/F	94	−0.31	1.63	+
14	25/M	94	0.083	0.48	+
15	25/F	86	0.69	1.13	+
Mean (SE)	28.6 (2.4)	84.8 (2.6)	−0.27 (0.13)	1.12 (0.16)	

* Challenges performed at screening without prior terbutaline inhalation.

AMP challenge.[10] Investigating the effect of a single dose of glucocorticosteroid in vivo should therefore allow study of mast cell β_2 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

PATIENTS

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 (FEV_1) 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 FEV_1 (PC_{20}) of <8 mg/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 β_2 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 PC_{20} values as performed in our laboratory.[2] 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 PC_{20} 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.[4] 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 FEV_1 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 FEV_1 from the post-saline value was reached.

STATISTICAL ANALYSIS

All values were expressed as mean (SE) apart from PC_{20} results which were expressed as geometric means. Log dose-response curves were constructed and PC_{20} calculated by linear interpolation. Baseline FEV_1, post-bronchodilator FEV_1, and log PC_{20} 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 PC_{20} 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)/$\log_{10}2$).[4] 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 log 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 log 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=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=NS).

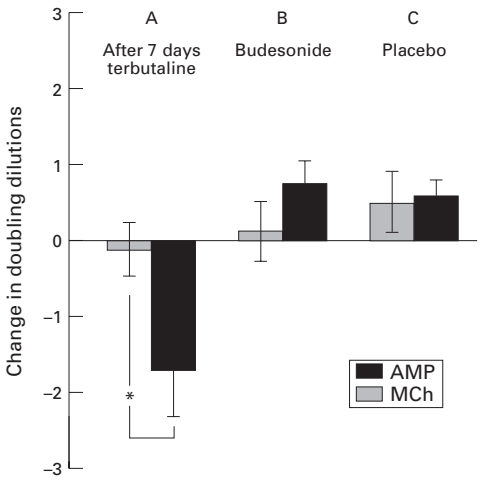

*Figure 1 Effect of regular inhaled terbutaline (500μg q.i.d. via Turbohaler over seven days) on (A) methacholine and adenosine monophosphate (AMP) challenge (*p<0.05 for comparison between challenges), and of adding (B) budesonide (800μg via Turbohaler) or (C) identical placebo to methacholine and AMP challenge; p=NS. Changes in doubling dilutions are shown.*

EFFECT OF BUDESONIDE ON METHACHOLINE AND AMP CHALLENGE

Changes in methacholine PC_{20} and AMP reactivity 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 glucocorticosteroids 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 β_2 adrenoceptor function.

Many studies using both human and animal pulmonary and bronchial tissue have previously demonstrated reversal of β_2 adrenergic tolerance by glucocorticosteroids.[5 9] Glucocorticosteroids also prevent desensitisation of the β receptor and restore downregulated receptors to near normal levels.[5 6] In normal airways β_2 adrenergic resistance induced by regular inhaled β_2 agonist therapy can be restored by the use of intravenous hydrocortisone when measured between six and 48 hours and lymphocyte β_2 adrenoceptor function and number can be restored to normal within 16 hours by oral or intravenous high dose glucocorticosteroids.[5]

We therefore expected that a single high dose of budesonide would restore mast cell β_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 β_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 β_2 adrenoceptor susceptibility to β_2 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 β_2 adrenoceptor may be relatively resistant to the effects of inhaled glucocorticosteroids, at least in therapeutic dosage. Airway inflammatory cell β_2 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 β_2 agonist therapy to a minimum in asthma.

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