Short paper

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

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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 ($500\mu g$ four times daily via Turbohaler), each followed by a single dose of inhaled budesonide ($800\mu g$ 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 a 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.

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Keywords: $\beta_2$ agonists, tolerance, glucocorticosteroid, inhaled steroids.

Beta2 adrenoceptor agonists are very effective in the treatment of acute asthma symptoms. Recently, however, it has been suggested that regular inhaled $\beta_2$ agonist therapy may lead to loss of control of asthma symptoms1 and reduced acute bronchoprotection against bronchoconstriction.2,3 Such loss of bronchoprotection is small and its clinical significance is uncertain.4 Other studies have suggested that regular glucocorticosteroid therapy does not prevent the development of bronchoprotective tolerance.5

Although the mechanism underlying loss of bronchoprotection in vivo is unknown, it probably relates to $\beta_2$ adrenoceptor downregulation which occurs in human airways in vitro.3,6 Preferential downregulation of $\beta_2$ 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.2,23 Glucocorticosteroids increase $\beta_2$ adrenoceptor expression in human lung cells by increasing $\beta_2$ adrenoceptor transcription.1,3,7,8 High doses of glucocorticosteroid prevent and reverse $\beta_2$ receptor tolerance in desensitised animals and in human tissue in vitro.4 Studies in both asthmatic and non-asthmatic subjects using high dose glucocorticosteroids have shown that glucocorticosteroids rapidly reverse $\beta_2$ adrenoceptor tolerance of lymphocytes, neutrophils, and normal airways.3 Thus, high dose inhaled glucocorticosteroids would be expected to reverse loss of bronchoprotection in asthma induced by $\beta_2$ agonists.

Prolonged treatment with steroids in vivo has direct inhibitory effects on mast cells, with actions not restricted to $\beta_2$ adrenoceptor transcription. In clinical studies using regular inhaled glucocorticosteroids the number and activation of mast cells are reduced,5 along with inhibition of the early response to allergen.5 In contrast, glucocorticosteroids appear to have little effect on non-$\beta_2$ 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,5 nor the early asthmatic response to allergen.5 Single high dose budesonide itself does not have a significant effect on...
AMP challenge. 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 <8mg/ml and <100mg/ml, respectively, at screening (table 1). None had suffered an asthma exacerbation or upper respiratory tract infection over 12 days. Bronchoprotection of terbutaline over 12 days. Bronchoprotection of terbutaline was assessed by three forced expiratory manoeuvres on each challenge day and log PC_{20} values were computed as previously reported. 4 Fresh solutions of methacholine and AMP (Sigma, Poole, UK) were made up in 0.9% saline in doubling dilutions (0.06–32mg/ml and 0.39–800mg/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 500μg was administered via a Turbodisk inhaler, and methacholine or AMP challenge followed 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.

**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. 5 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 500μg via a multidose dry powder delivery (Turbodisk, Astra Draco, Sweden). Subjects were then treated with terbutaline 500μg four times daily via Turbodisk over 12 days. Bronchoprotection of terbutaline 500μg 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 800μg 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 caffeine beverages were withheld for at least 12 hours before each challenge.

**BRONCHIAL PROVOCATION CHALLENGE**

Bronchial provocation challenge was performed as previously reported. 1 Fresh solutions of methacholine and AMP (Sigma, Poole, UK) were made up in 0.9% saline in doubling dilutions (0.06–32mg/ml and 0.39–800mg/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 500μg was administered via a Turbodisk inhaler 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.

### Table 1 Characteristics of asthmatic patients studied

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

* Challenges performed at screening without prior terbutaline inhalation.
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 FEV₁**

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 FEV₁ 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 FEV₁ 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).

**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 broncho-protective 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 β₂ adrenoceptor function.

Many studies using both human and animal pulmonary and bronchial tissue have previously demonstrated reversal of β₂ adrenergic tolerance by glucocorticosteroids. Glucocorticosteroids also prevent desensitisation of the β₂ receptors and restore downregulated receptors to near normal levels. In normal airways β₂ adrenergic resistance induced by regular inhaled β₂ agonist therapy can be restored by the use of intravenous hydrocortisone when measured between six and 48 hours and lymphocyte β₂ 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 β₂ adrenoceptor function. In our study broncho-protective tolerance occurred to AMP challenge, but not to methacholine. We failed to demonstrate significant reversal of broncho-protective tolerance with single dose inhaled budesonide, implying that mast cell β₂ 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.

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

in $\beta_2$ adrenoceptor susceptibility to $\beta_2$ 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 $\beta_2$ adrenoceptor may be relatively resistant to the effects of inhaled glucocorticosteroids, at least in therapeutic dosage. Airway inflammatory cell $\beta_2$ 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 $\beta_2$ agonist therapy to a minimum in asthma.

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