Beclomethasone dipropionate attenuates airways hyperresponsiveness to neurokinin A and histamine in asthma

Gaetano Prosperini, Giuseppe Arcidiacono, Ida Ciamarra, Nunzio Crimi, Riccardo Polosa*

Dipartimento di Medicina Interna e Specialistica, Sezione di Malattie Respiratorie, Università di Catania, Via Passo Gravina 187, 95125 Catania, Italy

Received 24 September 2005; accepted 29 September 2005

Summary
Background: Inhaled corticosteroids (ICS) are the most effective anti-inflammatory agents available for the treatment of asthma but they produce only modest effects on airway inflammation and non-specific bronchial hyperresponsiveness (BHR). However, little is known about the possibility that treatment with ICS might cause additional protection on BHR to inhaled tachykinins such as neurokinin A (NKA).

Objective: Therefore, we compared the effects of beclomethasone dipropionate (BDP) on the degree of BHR to inhaled histamine and NKA in a double-blind, controlled, cross-over study of asthmatic patients.

Methods: Patients attended the laboratory before and after each 6 weeks treatment period to undertake concentration–response studies with histamine and NKA. Bronchial responsiveness to both funs was expressed as the provocative concentration producing a 20% decrease in FEV₁ from baseline (PC₂₀).

Results: BDP therapy attenuated the constrictor response to both agonists to a similar degree, their geometric mean (range) PC₂₀ values increasing from 0.47 (0.21–1.41) mg/ml to 2.43 (0.51–4.50) mg/ml (P<0.01, post-salb vs. post-BDP treatment) and from 101.7 (27.3–356.1) µg/ml to 666.7 (151.5–1000) µg/ml (P<0.01, post-salb vs. post-BDP treatment) for histamine and NKA, respectively.

Conclusion: Airway responsiveness to histamine and NKA is reduced by BDP to the same extent. As a result of these findings, provocation with NKA is unlikely to provide additional useful information in the assessment of airway inflammation in asthma.

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*Corresponding author. Tel.: +39 095 7594506; fax: +39 09330707.
E-mail address: polosa@unict.it (R. Polosa).
Introduction

Asthma is a chronic inflammatory disease of the airways characterized by bronchial hyperresponsiveness (BHR), which can be defined as an increase in the degree of airway narrowing in response to stimuli that have little or no effect in normal subjects. Although the precise mechanisms underlying BHR remain poorly understood, it is now widely accepted that distinctive inflammatory processes in the airway wall have a major role in its development. There is also evidence for a definite association between degree of BHR and severity of asthma, indicating that treatment of BHR should also aim at reducing the degree of severity of asthma.1

Asthma patients exhibit increased sensitivity (BHR), which can be defined as an increase in airways characterized by bronchial hyperresponsiveness to neurokinin A (NKA), are sensory neuropeptides exhibiting a range of features that may be relevant to the pathophysiology of asthma including contraction of airways smooth muscle, increased vascular permeability, and mucus secretion. When administered by inhalation NKA provokes dose-related bronchoconstriction in asthmatic subjects but its mode of action is not well understood. The bronchoconstrictor effect of NKA in asthma is largely inhibited by prior treatment with nedocromil sodium, suggesting that this response may be evoked indirectly rather than through direct stimulation of airway smooth muscle. This indirect bronchoconstrictor effect of NKA appears to be mediated by activation of a specific tachykinin NK2 receptor.

As for many other neuropeptides mediator of asthma, its biological function is terminated by the combined action of a variety of peptidases such as neutral endopeptidase (NEP), a membrane-bound enzyme that modulates NKA’s physiological and pathophysiological responses by degrading it to inactive metabolites. NEP has been detected in bronchial epithelial cells, sub-mucosal glands, bronchial smooth muscle and vascular endothelium, sites known to express receptors for NKA. In support of the view that enzymatic modulation of NKA responses is also important in vivo, it has been demonstrated that pre-treatment with the NEP-Inhibitor phosphoramidon enhances NKA-induced bronchoconstriction in asthmatic subjects.

NEP activity in human airways could be up regulated by corticosteroids. Borson et al. have shown that ICS increase the level of NEP expression in human airway epithelial cells. We hypothesized that, if NEP activity is also enhanced by corticosteroids in asthmatic airways in vivo, then the airway response to inhaled NKA could be further reduced upon treatment with ICS when compared with agonists, such as histamine, which are known not to be metabolized by NEP.

To test this hypothesis, we performed a 6 wks, double-blind, controlled, cross-over study comparing the effects of inhaled beclomethasone dipropionate (BDP) on airways responsiveness to NKA and histamine in a group of steroid naïve asthmatic subjects.

Methods

Subjects studied

Fifteen patients were enrolled in the study and randomized to receive BDP or salbutamol, but 4 subjects were withdrawn from the trial because of non-compliance with BDP usage and excluded from further analysis. The remaining 11 subjects (7M, 4F) were non-smokers, with a mean (±SEM) age of 31.1 (±2.7) and with mild-moderate asthma (Table 1). All were atopic, as defined by a positive skin prick test reaction (> 3 mm wheal response) to one or more of 6 common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, Parietaria pollen, mixed grass pollens, cat fur, dog hair). Inclusion criteria comprised stable asthma (having had no exacerbation or respiratory tract infection in the previous 2 months), baseline forced expiratory volume in 1 s (FEV1) of greater than 65% of their predicted values, and documented BHR to inhale NKA. Patients had never used ICS before or had stopped their use at least 2 months before entry into the study. Throughout the study, only short acting inhaled beta2-adrenoreceptor agonists were allowed for relief of symptoms but were withheld for at least 8 h prior to each visit to the laboratory. Antihistamines and/or cromones were not taken throughout the investigation. The local hospital’s ethics committee approved the study and written informed consent was obtained for each patient.
Study design

All visits to the laboratory took place at the same time of the day and outside the pollen season. As it was felt ethically unacceptable to leave such patients on placebo treatment alone for such a long period of time and considering that short acting inhaled beta2-adrenoreceptor agonists do not modify non-specific BHR,\textsuperscript{19–22} it was decided to use a short acting bronchodilators (salbutamol) as an alternative.

As our working hypothesis was to test if BHR to inhaled NKA could be further reduced (compared to histamine) due to enhanced NEP activity by ICS in asthma, we wanted to achieve the highest level of NEP induction by using very high doses of BDP (2000 mg/daily—that is BDP 250 mg two puffs q.i.d.). In order to match the BDP administration regimen, we opted to administer salbutamol 100 mg in the same fashion (two puffs q.i.d.; this gives a total of 800 mg/daily).

Patients were therefore randomised using computer-generated numbers into a double-blind, controlled, crossover study with either BDP 250 mg two puffs q.i.d. or salbutamol 100 mg two puffs q.i.d. using a standard metered-dose inhaler attached to a spacer device for 6 wks separated by a washout period of at least 3–4 wks.

Patients attended the laboratory before and after each treatment period to undertake concentration–response studies with inhaled histamine and NKA. Each patient was challenged with histamine first followed by NKA challenge at least 2 h later when FEV\textsubscript{1} had spontaneously returned to within 5% of the baseline FEV\textsubscript{1} level. The order of inhalation challenges was identical for all patients throughout the study. Treatment was commenced immediately after completion of NKA challenge and continued up for 6 wks. Treatment was discontinued at least 12 h prior to bronchial challenge.

Study inhalers were weighed throughout the study to assess compliance. Patients were considered compliant if, in their BDP arm of the study, the total number of actuations fired from the inhalers was at least 60% of the number of actuations prescribed.

Bronchoprovocation testing with inhaled histamine and NKA

BHR was evaluated by means of histamine and NKA bronchial challenge, as described previously.\textsuperscript{23} In brief, histamine (Sigma Chemical Co, St. Louis, MO, USA) and NKA (Peninsula Laboratories Ltd) were made up in 0.9% sodium chloride (1% albumin solution) to produce a range of increasing doubling concentrations of 0.03–16.00 mg/ml and 3.9–500 mg/ml, respectively. The aqueous solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron Mini-nebuliser (C.R. Bard International, Sunderland, UK) driven by compressed air at a flow rate of 8 l/min. Wearing a nose-clip, subjects inhaled the aerosolized solutions

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Baseline FEV\textsubscript{1} (%predicted)</th>
<th>Atopy*</th>
<th>PC\textsubscript{20} histamine (mg/ml)</th>
<th>PC\textsubscript{20} NKA (µg/ml)</th>
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<tr>
<td>1</td>
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<td>G-P</td>
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<tr>
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<td>F</td>
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<td>D-G</td>
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<td>D-G-P</td>
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<td>28</td>
<td>70</td>
<td>P</td>
<td>0.64</td>
<td>94.2</td>
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</tbody>
</table>

\textsuperscript{FEV\textsubscript{1} = forced expiratory volume in 1 s.}  
\textsuperscript{PC\textsubscript{20} = provocation concentration producing a 20% fall from baseline FEV\textsubscript{1}.}  
\textsuperscript{*Positive immediate skin test to one or more allergens including: G = grass pollen, P = Parietaria pollen, and D = Dermatophagoides.}
in five breaths from end-tidal volume to full inspiratory capacity via a mouthpiece. Airway calibre was recorded as the forced respiratory volume in 1 s (FEV1) using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK), the better of the two consecutive measurements being used for analysis. After 15 min rest, three consecutive baseline measurements of FEV1 were made at intervals of 3 min followed by inhalation of 0.9% sodium chloride solution and further FEV1 measurements repeated at 1 and 3 min. Provided FEV1 had not fallen by >10% from baseline, increasing doubling concentrations of agonists were administered and FEV1 measured at intervals of 1 and 3 min. The challenges were discontinued when FEV1 had fallen >20% of the post-saline baseline value or when the highest concentration of agonist had been administered. The fall in the FEV1 following each concentration of agonist was expressed as percentage of the higher of the two post-saline baseline FEV1 recordings. The percentage fall in FEV1 was plotted against the cumulative concentration of agonist on a logarithmic scale and the provocative concentration required to produce a 20% decrease in FEV1 from the post-saline baseline value (PC20) was determined by linear interpolation.

Data analyses

Results are expressed as mean (±SEM) unless otherwise stated and p values of <0.05 were accepted as the minimum level of statistical significance. Baseline values of FEV1 before and after study treatments were compared between and within study days by two-factor analysis of variance (ANOVA).

PC20 histamine and NKA values were log-transformed and compared by means of two-factor ANOVA. After treatment with BDP a 20% fall in FEV1 was not obtained in seven subjects1,3,7,11 at the highest concentration of NKA administered, and therefore a minimal estimate was obtained by calculating the cumulative PC20, on the next doubling concentration beyond the highest administered. Because of these censored data the results were also compared for significance with Wilcoxon’s signed rank test.

The protective effect of BDP against broncho-provocation with each agonist was calculated by working out the difference in PC20 before and after BDP and the control drug (salbutamol) in each individual patient and expressed in terms of mean (±SEM) doubling dilutions. Paired t tests were then used to compare post-drug variations in BHR to histamine and NKA.

Results

There was no significant difference in mean (±SEM) baseline values of FEV1 between pre-treatment study days. As expected, treatment with BDP caused a small but significant 6.4% increase in mean FEV1 from baseline (P = 0.032), whereas 6 wks administration of the control drug (salbutamol) did not produce significant changes.

Baseline PC20 values for histamine and NKA were not significantly different between pre-treatment study days (Table 2). BDP therapy for 6 wks significantly attenuated the constrictor response to both agonists, their geometric mean (range) PC20 values increasing from 0.47 (0.21–1.41) mg/ml to 2.43 (0.51–4.50) mg/ml (P<0.01, post-salb vs. post-BDP treatment) and from 101.7 (27.3–356.1) µg/ml to 666.7 (151.5–1000) µg/ml (P<0.01, post-salb vs. post-BDP treatment) for histamine and NKA, respectively (Table 2).

When changes in the protective effect of inhaled BDP on provocation responses were expressed as doubling dilutions, a mean (±SEM) protection of 2.37 (±0.29) doubling doses against histamine and of 2.71 (±0.31) doubling doses against NKA were reported (Fig. 1). These changes were not significantly different from each other.

Discussion

Six weeks’ treatment with inhaled BDP had a significant protective effect against BHR to both histamine and NKA. The magnitude of this effect was similar for histamine and NKA, their dose-response curves being displaced to the right by 2.37 and 2.71 doubling doses, respectively. In the current study, patients with mild-moderate asthma receiving BDP (2000 µg/daily) showed a small but significant improvement in FEV1, confirming the efficacy of this dose of drug.

Due to ethical restrictions, it was decided to use salbutamol as an alternative for placebo as there is ample evidence that regular use of short acting beta2 agonists does not affect BHR.19,22 Indeed, under similar experimental conditions, Tormey et al.21 were unable to demonstrate significant differences in BHR to histamine between placebo and salbutamol (800 µg/daily) treatment periods in adult asthma. Likewise, Kozlik-Feldmann et al.20 demonstrated that regular salbutamol (800 µg daily) over a period of 3 months did not deteriorate BHR to inhaled histamine in asthmatic children. In addition, when four crossover studies comparing regular short acting beta2-agonist treatment with
placebo were pooled together in a recent Cochrane review, no significant difference in change in AHR was observed, the mean change being 0.09 doubling doses (95%CI –0.18, 0.35).24 Lastly, the issue of a potential effect of salbutamol on BHR in the placebo group it is likely to be irrelevant in virtue of the cross-over design of the present study.

The magnitude of the decrease in BHR to histamine after ICS is consistent with that of previously reported studies.25–27 However, the results of the present study with BDP differ somewhat from those of the recent work by Van Schoor et al.28 who demonstrated that inhaled fluticasone propionate reduced BHR to NKA to a greater extent compared to methacholine. This discrepancy could be related to the well known superior potency of fluticasone compared to BDP as there is abundant evidence that all comparative studies in asthma have shown at least a 2:1 ratio in clinical effects (reviewed in29). In particular, the elegant study by Bootsma et al. has demonstrated that fluticasone is as effective as twice the dose of BDP on BHR assessed by provocation with both direct (histamine) and indirect (UNDW—”fog” challenge) stimuli.25 That this might be the case is also reflected by studies in which the effects of fluticasone and budesonide were tested against the airways responsiveness to bradykinin (the bronchoconstrictor effect of bradykinin is, at least in part, mediated via release of NKA—reviewed in30); in these studies fluticasone was effective at reducing BHR to inhaled bradykinin31 whereas budesonide was not.32 Thus, despite the established dogma that ICS are the most effective anti-inflammatory agents available for asthma, comparison of the effect between different ICS on NKA responses may reveal dissimilar level of protection reflecting heterogeneous response to different ICS. However, it must be noted that the use and dose of BDP and the length of BDP therapy in our study should have minimized differences in potency between the different drug preparations across studies. Taken together, the above-mentioned considerations call for rigorous trials comparing the effect of different ICS on challenges performed in the same group of patients.

### Table 2 Individual changes in airways hyperresponsiveness to histamine and NKA after 6 wks treatment with BDP and salbutamol.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt; histamine</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt; NKA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-BDP</td>
<td>Post-BDP</td>
</tr>
<tr>
<td>1</td>
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<td>4.5</td>
</tr>
<tr>
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<td>0.59</td>
<td>2.12</td>
</tr>
<tr>
<td>3</td>
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<td>3.89</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>0.35</td>
<td>0.89</td>
</tr>
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<td>9</td>
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<tr>
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</tr>
<tr>
<td>11</td>
<td>1.04</td>
<td>3.91</td>
</tr>
</tbody>
</table>

G. mean 0.52 2.43 0.40 0.47 161.4 666.7 126.6 101.7

(Range) (0.19–1.10) (0.51–4.50) (0.12–1.56) (0.21–1.41) (36.8–578.1) (151.5–1000) (32.8–389.9) (27.3–356.1)

BDP = beclomethasone dipropionate; salb = salbutamol.
Another possibility for the observed discrepancy in results may be due to dissimilar methodology and to distinctive patients’ characteristics. If Van Schoor et al.28 would have compared post-pla vs. post-fluticasone variations rather than pre vs. post-fluticasone variations, they could have seen less marked changes in BHR to NKA. In addition, it could be argued that a 2-week course of fluticasone would simply fail to elicit a maximal protective effect against methacholine in such a short period of time; for example, it is possible that a 4-week course of fluticasone could have produced a stronger protective effect against methacholine resulting in a less pronounced relative reduction in BHR to NKA. Conversely, given that 7 of 11 patients failed to show a 20% fall in FEV\textsubscript{1} at the highest dose of NKA administered after treatment with BDP, our conservative approach of assigning a PC\textsubscript{20} value to these patients means that the reduction in NKA responsiveness is likely to be underestimated.

The working hypothesis that airway responsiveness to inhaled NKA could be further reduced due to the enhanced NEP activity by ICS in asthma, it is not supported by the results of the present investigation and reflects the reported discrepancies described by a number of in vitro studies. Some authors have shown that ICS increase the level of NEP expression and of enzymatic activity in human airway epithelial cells,18,33 but others failed to confirm these observations.34,35 In addition, although NEP expression is significantly enhanced in the airway epithelium of asthmatic patients on ICS as compared with steroid naïve asthmatics,36 no differences in peptidase activities in serum or BAL fluid were observed between fluticasone propionate users and non-users in allergic asthma.37

Whatever the cause for the discrepancies observed, it is apparent that NKA challenge is not useful for monitoring changes in airway inflammation even in the hands of experienced researchers. Moreover, this should be discussed against the evidence of the superior sensitivity profile of other non-invasive markers of airway inflammation (such as AMP challenge, exhaled NO, and sputum eosinophils) for use in diagnosis, monitoring the disorder’s activity, and evaluating airways response to anti-inflammatory therapy in asthma (reviewed in7,38).

Acknowledgements

This work was supported by a research grant from the University of Catania (Grant 60%) and by a Grant-in-Aid from Chiesi Farmaceutici SpA.

References


