Determinants of the bronchodilation response to salbutamol on histamine-induced bronchoconstriction

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Received 22 November 2005; accepted 1 February 2006

Summary  Assessment of the bronchodilation response to short-acting β2-adrenergic receptor agonists on pharmacologically induced bronchoconstriction has often been used to investigate airway smooth muscle β2-adrenoreceptor function. However, little is known about factors affecting this response. In the present study, the bronchodilation response to 0.2 mg of salbutamol on histamine-induced bronchoconstriction was assessed in 101 steroid-naive asthmatic subjects. The associations of the response with a wide range of challenge procedure-related variables, clinical asthma severity indicators, and blood markers of airway inflammation were investigated. The response was re-assessed after 6 and 12 weeks' therapy with inhaled budesonide. Baseline FEV1, final histamine concentration, and the maximal fall in FEV1 explained 35–59% of the total variation in the response to salbutamol, depending on the index chosen to express the response. Serum concentration of myeloperoxidase, an index of neutrophilic inflammation, was associated with a poor response. The preceding week daily PEF variation, rescue bronchodilator use, severity of asthmatic symptoms, blood eosinophil count, and serum eosinophilic cationic protein and eosinophilic protein X concentrations were not associated with the response. The salbutamol response seemed to diminish during budesonide treatment but when adjusted by the challenge procedure-related variables the treatment effect vanished. In conclusion, the bronchodilation response to salbutamol on histamine-induced bronchoconstriction is largely determined by...
Introduction

The bronchodilation response to short-acting $\beta_2$-adrenoceptor agonists (SABA) can be used to assess airway $\beta_2$-adrenoceptor function in vivo. It is difficult to assess in stable asthmatic patients in whom there is usually no resting bronchoconstriction.\(^1,2\) Therefore, it has become popular to assess the bronchodilator effect of SABAs on induced bronchoconstriction, especially in studies investigating the development of bronchodilator tolerance to SABAs during regular treatment with $\beta_2$-adrenoceptor agonists.\(^3-10\) The bronchoconstriction has usually been induced pharmacologically\(^3,5-8,11\) or, less often, by exercise.\(^9,10\)

Given the popularity of the method, surprisingly little is known about the factors associated with the bronchodilation response to SABAs on pharmacologically induced bronchoconstriction. In most of the above-mentioned studies one or all of the following challenge procedure-related factors have usually been considered: baseline lung function (usually expressed as forced expiratory volume in 1 s, FEV\(_1\)), maximal fall in FEV\(_1\), and the dose/concentration of the stimulus to produce the fall. However, the number of subjects in the treatment arms has usually been small.\(^3-8\) In addition, most of these studies have been cross-over in design and monitoring changes in the bronchodilation response rather than measuring bronchodilation response as an absolute variable. Therefore, variables that are not related to the test have rarely been included in the analysis. Such variables could be the various subject characteristics, severity of the subjects’ asthmatic symptoms, as well as markers of asthmatic inflammation. Furthermore, knowledge about the effect of inhaled corticosteroids on the bronchodilation response to SABAs on pharmacologically induced bronchoconstriction is limited. This information is relevant as corticosteroids are now a routine part of the asthma management.\(^12,13\)

In the present study, the variables associated with the bronchodilation response to salbutamol on histamine-induced bronchoconstriction were determined in a large group of carefully characterised, steroid-naïve asthmatic subjects. In addition, the effect of inhaled budesonide treatment on this response was assessed. The results of the present study may help future investigators who intend to use the bronchodilation response to SABAs on pharmacologically induced bronchoconstriction in order to assess airway $\beta_2$-adrenoceptor function.

Methods

Study design

The present analysis is based on a patient population which was recruited for a randomised, parallel-group, double-blind study comparing two doses (200 or 800 $\mu$g per day) of inhaled budesonide (Pulmicort Turbuhaler\(^16\), Astra Draco, Lund, Sweden) during a study period of 12 weeks, with a run-in period of 1 week.\(^14\) Detailed information about the methods and subjects is expressed in that publication. The present analysis was divided to two parts. First, the variables associated with the bronchodilation response to salbutamol on histamine-induced bronchoconstriction were analysed using the baseline data. Second, the effect of budesonide on this response was assessed by comparing the baseline data with the data obtained during the budesonide treatment. Since the two budesonide doses were shown to attenuate the histamine responsiveness similarly,\(^14\) the two treatment arms were analysed together.

Measurements

Skin prick tests were performed against common aeroallergens and atopy was defined as at least 3 mm wheal reaction to any of the allergens.\(^15\) During the study the patients continuously recorded their peak expiratory flow rates (PEF) before they took their medication in the morning and in the evening (Mini Wright Peak Flow Meter, Clement Clark International, London, UK), their rescue SABA use, as well as adverse reactions. They also recorded their symptoms of asthma separately each day and night as a single score from 0 to 3 (0 = no symptoms, 3 = severe symptoms). Histamine responsiveness was assessed three times, during the run-in phase, and after 6 and 12 weeks’ treatment. Blood samples were collected four

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times, during the run-in phase, and after 2, 6 and 12 weeks’ treatment and the following analyses were performed: The total eosinophil count was calculated by routine differential counting with a Coulter Counter (Coulter STKS, Coulter Electronics Ltd., Luton, UK). The serum eosinophilic cationic protein (ECP), serum eosinophilic protein X (EPX) and serum myeloperoxidase (MPO) were measured with commercially available radioimmunoassay research kits (Pharmacia Diagnostics, Uppsala, Sweden).

Subjects

One hundred and five adult patients with recently diagnosed asthma were recruited (asthma diagnosed during the last 3 months preceding the study and the symptoms presenting for less than 6 months). One hundred and one patients underwent all the baseline measurements. Ninety-seven patients completed the 12-week treatment period. At baseline, all the patients had to be without regular pharmacological treatment for their asthma and they were allowed only SABAs for rescue treatment. All the patients fulfilled the criteria of asthma by the American Thoracic Society16 and showed airway hyperresponsiveness (provocative concentration of histamine to induce a 20% fall in FEV1 (PC20) less or equal to 8 mg/ml). Of the 101 patients at baseline, there were 36 men, 27 current smokers, and 68 atopic subjects. The subjects were mean (SD) 38 (12) years old and their FEV1 was 87 (10) % of predicted.17 The daily number of short-acting β2-agonist rescue inhalations was 1.4 (1.7) during the run-in phase and only ten subjects (9.9%) used equal or more than four inhalations per day. Signed informed consent was obtained from all subjects and the study was approved by the Ethics Committee of Kuopio University Hospital.

Histamine challenge and definitions of the salbutamol response indices

The subjects had refrained from taking SABAs for at least 8 h before the challenge. The airway responsiveness to histamine was assessed using a modification of the method by Cockcroft et al.,18 differing from the original in that a dosimetric nebuliser (Spira Elektro 2, Hengityshoitokeskus, Hämeenlinna, Finland) was used. The first nebulised solution was 0.9% saline and the post-saline FEV1 was taken as the baseline value. After that, histamine was inhaled in doubling concentrations from 0.03 up to 16 mg/ml. The challenge was terminated when a fall ≥20% in FEV1 was documented or when the maximal histamine concentration had been administered.

The percentage fall in FEV1 after the final histamine concentration was recorded and defined as the maximal fall. Then the subjects inhaled 0.2 mg of salbutamol from a metered-dose inhaler (Ventoline® GlaxoWellcome, London, UK). The FEV1 was measured in duplicate 5 min thereafter, and the better of the two values was used for the analysis. Two indices were used to express the bronchodilation response to salbutamol on histamine-induced bronchoconstriction (Fig. 1): The ‘bronchodilation’ was the increase in FEV1 from the maximal fall value to the post-salbutamol value, expressed as percentage of the baseline value.5 The ‘residual bronchoconstriction’ was the percentage change in FEV1 from the baseline value at 5 min after salbutamol inhalation,6 with a negative value indicating a smaller FEV1 value at 5 min post-salbutamol compared with the post-saline value.

Statistical analysis

The deviation of the distribution of the salbutamol response indices from a normal distribution was studied by the one-sample Kolmogorov–Smirnov test. To define the variables associated with the bronchodilation response to salbutamol on histamine-induced bronchoconstriction at baseline, an analysis of covariance with backwards directed stepwise procedure was used. The response variables were the bronchodilation and the residual bronchoconstriction. The explanatory variables were the baseline FEV1 (expressed as % of the predicted17), the final histamine concentration...
(expressed in doubling concentrations), the histamine-induced maximal fall in FEV₁, sex, age, smoking pack-years, presence of current smoking, presence of atopy, the mean daily number of short-acting β₂-agonist rescue inhalations during the preceding week, the mean daily symptom score during the preceding week, the mean daily PEF variation during the preceding week, blood eosinophil count, ECP, EPX, and MPO. The first three explanatory variables were defined as test-related variables since they were related to the histamine challenge procedure. To define the variables associated with the serum MPO, a similar analysis of covariance was performed. The adjusted and unadjusted changes over time in salbutamol response were examined applying mixed models.¹⁹

The test-related variables were used as time-varying covariates in the analysis of adjusted change in the bronchodilation and the residual bronchoconstriction.

The data were presented as means and 95% confidence intervals. P-values less than 0.05 were considered statistically significant. The statistical analyses were performed using SPSS for Windows 11.5.

Results

The factors associated with the bronchodilation response to salbutamol on histamine-induced bronchoconstriction at baseline

Both the bronchodilation and the residual bronchoconstriction showed a close-to-normal distribution within the study population. The following variables were statistically significantly associated with bronchodilation (P-value, percentage explained from the total variation in bronchodilation, and the direction of association): baseline FEV₁ (P = 0.018, 2.4%, the larger the baseline FEV₁, the smaller the bronchodilation), final histamine concentration (P < 0.001, 20.8%, the larger the histamine concentration, the smaller the bronchodilation), maximal fall (P < 0.001, 34.8%, the larger the fall, the larger the bronchodilation), and MPO (P = 0.017, 2.5%, the larger the MPO, the smaller the bronchodilation).

The following variables were associated with the residual bronchoconstriction: baseline FEV₁ (P = 0.016, 3.7%, the larger the baseline FEV₁, the larger the residual bronchoconstriction), final histamine concentration (P < 0.001, 31.7%, the larger the histamine concentration, the larger the residual bronchoconstriction), and MPO (P = 0.011, 4.2%, the larger the MPO, the larger the residual bronchoconstriction).

The preceding week daily PEF variation, rescue SABA use, severity of asthmatic symptoms, blood eosinophil count, and serum ECP and EPX concentrations were not associated with the salbutamol response indices.

Since serum level of MPO was the only test-unrelated variable that was associated with the salbutamol response indices, its determinants were also defined. The following variables were statistically significantly associated with MPO (P-value, percentage explained from the total variation in MPO, and the direction of association): current smoking (P = 0.005, 3.7%, MPO values were larger in current smokers than in never- or ex-smokers), blood eosinophil count and serum ECP (due to their strong interrelationship they were analysed together; P < 0.001, 54.2%, the larger the values, the larger the MPO).

The effect of a 12-week treatment with inhaled budesonide on the bronchodilation response to salbutamol on histamine-induced bronchoconstriction

When analysing unadjusted data, there were significant changes in both salbutamol response indices. After a 12-week treatment with budesonide the bronchodilation decreased from 24.7 (23.1–26.3)% to 20.9 (19.3–22.5)%, P < 0.001, and the residual bronchoconstriction changed from −1.5 (−2.8 to 0.2)% to −3.8 (−5.2 to 2.5)%, P = 0.001.

However, there were also significant changes in the test-related variables that were shown to be associated with the salbutamol response indices: The baseline FEV₁ (% of predicted) increased 2.1% (P = 0.002), the final histamine concentration increased 1.0 doubling doses (P < 0.001), and the maximal fall decreased 1.5% (though not statistically significantly, P = 0.14). The MPO values increased from 463 (419–509) μg/l before treatment to 518 (473–567) μg/l after treatment (P = 0.02). The treatment-induced change in both salbutamol response indices was statistically significantly associated with the changes in the baseline FEV₁ (P = 0.01), final histamine concentration (P < 0.001) and the maximal fall (P < 0.001). However, the treatment-induced change in the salbutamol response indices did not associate with the treatment-induced changes in MPO (P = 0.36).

When the bronchodilation and the residual bronchoconstriction were adjusted with the
test-related variables, the differences between pre-treatment, 6-week post-treatment and 12-week post-treatment values completely disappeared ($P = 0.32$ for both salbutamol response indices, Fig. 2). This result indicates that the changes in the bronchodilation and the residual bronchoconstriction were mainly explained by the changes in the test-related variables. The results did not change if the two treatment groups were analysed separately and there was no difference between the treatment groups.

**Discussion**

The present study shows that the bronchodilation response to salbutamol on histamine-induced bronchoconstriction is largely determined by variables that are directly related to the histamine challenge procedure itself, namely the baseline lung function, the final histamine concentration, and the maximal fall in FEV$_1$. These variables explained 35–59% of the total variation in the salbutamol response, depending on the index chosen. Most variables that were unrelated to the challenge procedure, including various subject characteristics, clinical asthma severity indicators, and eosinophilic inflammation variables were not associated with the response. Indeed, the only challenge-unrelated variable that showed a significant association with the salbutamol response was the serum concentration of MPO, an index of neutrophilic inflammation. The present study also shows that treatment with inhaled budesonide does not affect the salbutamol response when the treatment-induced changes in baseline FEV$_1$, final histamine concentration, and maximal fall in FEV$_1$ are taken into account.

Studies about the effect of regular treatment with $\beta_2$ adrenergic agonists on the bronchodilator effect of SABAs on pharmacologically induced bronchoconstriction have often suggested the same test-related variables as the present study. Compared with these studies, the present study includes a considerably larger study group and more detailed information about the various subject characteristics, asthma severity indicators, and markers of asthmatic inflammation. Therefore, the present authors can estimate the relative contribution of the test-related variables and asthma severity-related variables. The results clearly show that the bronchodilation response to salbutamol on histamine-induced bronchoconstriction is mainly determined by directly test-related variables. One may criticise that some of the test-related variables, like FEV$_1$ at baseline and the final histamine concentration also measure asthma severity. However, the authors believe that their association with the salbutamol response is technical by nature since the great majority of test-unrelated asthma severity indicators showed no association. Thus, the documented association of high FEV$_1$ before challenge with poor salbutamol response probably does not indicate that mild asthma would be associated with a poor response to $\beta_2$ adrenergic agonists but that there is limited "room" for FEV$_1$ improvement. Accordingly, the association of high final histamine concentration with poor salbutamol response does not indicate that mild airway hyperresponsiveness would be associated with a poor response to $\beta_2$ adrenergic agonists but that the competition between bronchoconstrictor and bronchodilator effects on airway smooth muscle is probably in favour of the former.
There was only one completely challenge procedure-unrelated variable that was associated with the salbutamol response: the serum concentration of MPO. Induced sputum neutrophil count correlates well with the sputum MPO level in asthma\textsuperscript{21,22} and MPO is widely used as an index of neutrophilic inflammation.\textsuperscript{20} In the present study, MPO was measured from serum and it is difficult to estimate how well it reflects the neutrophilic inflammation within the airways. In previous studies those asthmatic subjects with a predominantly neutrophilic airway inflammation have shown a poor increase in FEV\textsubscript{1} after a course with either inhaled\textsuperscript{22,23} or oral\textsuperscript{22} corticosteroids suggesting that neutrophilic inflammation may be involved in the pathophysiology of irreversible airflow obstruction in asthma. Assuming that serum MPO does reflect the neutrophilic inflammation within the airways, the present study is the first to suggest that neutrophil-dominated airway inflammation may also be associated with a poor bronchodilation response to salbutamol on induced bronchoconstriction.

In the present population, serum MPO level correlated closely with the indices of eosinophilic inflammation and, much less clearly, with current smoking. It has been shown previously that in severe asthma, eosinophils and neutrophils are usually found together in the airways\textsuperscript{24} and that smoking induces neutrophilic airway inflammation in mild asthma.\textsuperscript{25} Taken together, these findings suggest that neutrophilic airway inflammation is typical for an asthmatic patient with severe eosinophilic airway inflammation, and may be enhanced by smoking. Interestingly, neither indices of eosinophilic inflammation nor smoking habits were independent determinants of the salbutamol response, highlighting the role of neutrophilic inflammation in this respect.

Treatment with budesonide did not affect the bronchodilation response to salbutamol on histamine-induced bronchoconstriction though it had clear effects on the patient’s asthmatic symptoms, lung function, histamine responsiveness, and indices of eosinophilic inflammation (as previously reported by Tukiainen et al.\textsuperscript{14}). This fits to the above-mentioned concept that this response to salbutamol has little to do with the clinical severity of patient’s asthma. The lack of effect by budesonide on the salbutamol response may also be explained by the fact that inhaled corticosteroids do not suppress the airway neutrophilic inflammation whereas their effect on eosinophilic inflammation is clear.\textsuperscript{26,27} In fact, the serum MPO levels actually increased during the budesonide treatment in the present study. This could be explained by the fact that corticosteroids can inhibit neutrophil apoptosis and phagocytic removal and yet induce the same process in eosinophils.\textsuperscript{28,29}

**Limitations of the study**

One may criticise that the observation time after the salbutamol dose (5 min) was too short to fully reveal the salbutamol response. However, the baseline histamine challenge showed that the FEV\textsubscript{1} was just 1.5% lower at 5 min post-salbutamol compared with the post-saline FEV\textsubscript{1} (the ‘residual bronchoconstriction’). The authors therefore believe that a longer observation time would not have changed the results significantly. In fact, the rather short observation time after salbutamol ensured that the spontaneous recovery after histamine challenge (30–60 min in adults\textsuperscript{30}) did not interfere with the salbutamol response. The authors’ way to measure inflammatory indices from blood samples instead of sputum samples may also be considered as a weakness. However, by this way the authors could measure these indices in all subjects whereas as many as a quarter of subjects may produce inadequate samples in response to sputum induction,\textsuperscript{31} which can be a cause of a selection bias. Finally, as the present study was not placebo-controlled, the authors cannot define whether the documented increase in MPO during budesonide treatment was a true treatment effect.

**Conclusion**

In conclusion, the bronchodilation response to salbutamol on histamine-induced bronchoconstriction seems to be mainly determined by test-related technical variables. This response is unrelated to the clinical severity of asthma and is not affected by treatment with inhaled budesonide. Neutrophilic airway inflammation may be associated with a poor response.

**Acknowledgements**

The budesonide preparations were provided by Astra Finland Oy, Masala, Finland.

**References**