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ORIGINAL ARTICLES

A Double-Blind, Cross-Over Study Using Salbutamol, Beclomethasone, and a Combination of Both in Bronchial Asthma

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ABSTRACT

A double-blind, cross-over protocol was applied to 22 asthmatic patients who were previously subjected to provocation tests with methacholine. The baseline FEV_1 for mild asthma was 89.6 ± 13.6% while for moderate asthma it was 73 ± 6%. The initial provocation tests with methacholine revealed that the mild asthma group needed a greater accumulated dose of methacholine than that required by the moderate asthma group to lower the FEV_1 by 20%, stressing the enhanced bronchial hyperreactivity present in the latter group. Significant differences in the PD_{20} values were obtained in both groups of patients using the combination of salbutamol plus beclomethasone. Salbutamol alone was ineffective to change the PD_{20} values in mild asthma while beclomethasone alone was able to change significantly the PD_{20} values in these patients, stressing the importance of the inflammatory component in the pathogenesis of stable asthma. Furthermore, the combination of both drugs was also more effective in the moderate asthma group than either medication alone, confirming the pharmacological control of the obstructive and inflammatory changes that are already established in patients with moderate asthma.

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INTRODUCTION

Bronchial asthma is a disease induced by several factors and characterized by a reversible obstruction of the bronchial airways associated with an enhanced response by the trachea and the bronchi to various stimuli. The obstructive pattern may be abolished either spontaneously or by the effect of inhaled and/or systemic therapy (1). A remarkable histopathological feature recently stressed is the inflammatory component associated with the physiopathology of bronchial asthma, which may further augment the airways obstruction and the bronchial hyperreactivity (2–4).

For many years, steroids have been used parenterally, orally, and, more recently, inhaled due to their potent anti-inflammatory action with demonstrable effects in improving the symptoms of bronchial asthma (5). In addition, bronchodilators given by the same routes also provide a variable degree of clinical improvement (6,7). However, Sears et al. demonstrated that inhalation of a beta-agonist agent could be associated with deterioration of the asthma control (8).

The aim of the present investigation was to further explore the effects of inhaled salbutamol, beclomethasone, or a combination of both, following a double-blind, cross-over protocol applied to patients with bronchial asthma, who were previously subjected to provocation tests with methacholine in an ambulatory setting.

MATERIALS AND METHODS

Patients

Twenty-two patients with the diagnosis of allergic or mixed bronchial asthma from the Outpatient Department of the Institute of Immunology and from the Experimental Asthma Clinic of the José Ignacio Baldó Hospital were studied. Twelve male and 10 female patients, in the age range 7–45 years (average 21 ± 13 years) were selected in accordance with the following criteria: atopic background, serum IgE values higher than 180 IU/ml, and positive skin tests for aeroallergens, with response of ≥3+ on a scale of 0–4+. I addition, the patients were nonsmokers wit absence of infections of the lower respirator tract at least 3 months prior to the test, an with three negative stools examinations fo ova and parasites. Orally administered ste roids and disodium chromoglycate, whic some of the patients were receiving, wer discontinued at least 1 month before th patients entered the study. Any other medica tions were also discontinued 2 weeks prior t the beginning of each test. Ipatropium bro mide (Alovent, Boerhinger LA, London) wa used for intermittent symptoms or as "rescu medication." The patients were classified as mild asthmatics (n = 11) and moderate asth matics (n = 11) in accordance with the criteri of the National Heart, Lung, and Blood Insti tute, National Asthma Education Program (9)

Each individual received information abou the procedures to be performed and writte consent to participate in the study was ob tained.

Provocation Tests

All provocation tests were performed be tween 8:00 and 11:00 A.M. Initial provocatio tests were performed using serial dilutions o methacholine (Provocholine, Roche Labora tories, La Roche Inc., Nutley, NJ) from a stock of 100 mg of methacholine chloride, reconsti tuted with saline solution in a laminar-flow hood, aliquoted in amber flasks, and stored at 4°C. The final concentrations were 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 25 mg/ml. Prio to the provocation tests, a basal spirometry (Fukude Ltd, Buckingham, England) was performed to determine the forced expiratory volume in the first second (FEV_1). FEV_1 above 1 liter was a requirement to perform the tests. The protocol employed followed guidelines introduced by De Vries et al. and Altouhyan et al. (10,11). Aerosol was generated by a nebulizer (Salter Labs. One N 8900) connected to a compressor (N: 646, Vilbiss Co., Somerset), delivered directly into a mask held loosely over the mouth and clipped nose and inhaled by tidal breathing for 2 min. Nebulizer output was kept constant at 0.13 to 0.16

min. Phosphate buffered saline was inhaled first and followed at 5 min intervals by doubling concentrations of methacholine. The response was measured by FEV_1 before and 3 min after each inhalation. Inhalations were discontinued when there was a fall in FEV_1 of 20% or more below the lowest postsaline value. From the initial dilution and up to the time of stopping the test, the administered units were added reaching the PD_{20} value units in accordance with the scheme described in Table 1.

Serum Total IgE

Serum total IgE was measured by radioimmunoassay using a commercial kit (Quanticlone IgE, Kallestad, Austin) as previously reported (12).

Skin Tests

A commercially available panel of antigens was used (Hollister-Stier Laboratories, Spokane, WA) including: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria*, *Aspergillus*, *Homodendrum*, *Penicillium*, cat, dog hair and dander, Orchard, Timothy, Red Top, and Bermuda. Antigens were administered employing the scarification technique (prick test), reading the results at 10 min. A 1% solution of histamine and a 5% glycerosaline solution were included as positive and negative controls, respectively. The skin tests were interpreted on a scale of 0–4+.

Daily Record of Symptoms

Each patient was instructed to perform daily self-evaluation by means of a clinical symptoms record form, which was closely followed up in the outpatient clinic every 15 days, registering the presence of dypsnea, cough, wheezing, and expectoration.

Medications

Blinded metered-dose inhalers were supplied by Glaxo Laboratories, Caracas, Venezuela. The inhalers contained salbutamol, 100 mg per puff, beclomethasone dipropionate, 50 mg per puff, or a combination of both (salbutamol, 100 mg, and beclomethasone, 50 mg per puff). Medications were inhaled by slow, deep inspiration from functional residual capacity through the open mouth, followed by a 10 sec breath hold. The doses used were salbutamol, two puffs (200 mg); beclomethasone, two puffs (100 mg); combination, two puffs (salbutamol 200 mg, beclomethasone 100 mg) four times daily.

Ipatropium bromide was used for intermittent symptoms or as a rescue medication.

Study Design

The protocol was designed as a double-blind, cross-over, randomized study. Subjects were submitted to an initial washing period of 2 weeks without medications. On day 7 of this period, the initial provocation test was

Table 1. Scheme of Provocation Test with Methacholine

METHACHOLINE (mg/ml)	NUMBER OF RESPIRATIONS	ACCUMULATED UNITS OF METHACHOLINE (mg/ml)	TOTAL ACCUMULATED UNITS
0.05	5	0.25	0.25
0.1	5	0.5	0.75
0.2	5	1	1.74
0.5	5	2.5	4.25
1	5	5	9.25
2	5	10	19.25
5	5	25	44.25
10	5	50	94.25
25	5	75	219.25

performed. All provocation tests were done between 8:00 and 11:00 A.M. During the next 4 weeks, subjects randomly received either one of the three inhalers. Then, and after another 2 weeks of washout period, the provocation test was repeated, and a new drug was started, following the same design as the first medication, until three inhalers were administered.

Patients followed at random one of three selected sequences:

2 weeks	4 weeks
Washout	Combination
Washout	Salbutamol
Washout	Combination

2 weeks	4 weeks
Washout	Salbutamol
Washout	Combination
Washout	Beclomethasone

2 weeks	4 weeks
Washout	Beclomethasone
Washout	Beclomethasone
Washout	Salbutamol

Statistical Analysis

Results are presented as geometric means (GM) ± standard deviation (SD). Statistical analysis of the PD20 was performed on the logarithmic transformation of these data, since the raw values had a skewed distribution. Values of the transformed PD20 were then compared by the Wilcoxon's *signed*-rank test; p values < 0.50 indicated a significant difference.

RESULTS

Immunoclinical Characteristics

The total serum IgE values in both groups of patients were elevated, with values for mild asthmatics of 527 ± 188 IU/ml (mean ± SD) and for the moderate asthmatics of 628 ± 269 IU/ml. Skin test responses to aeroallergens were positive (response > 3+) for four antigens (house dust, *D. pteronyssinus*, *D. farinae*, and *Alternaria*) in all patients. In addition, 9 of the 22 patients also had positive results to animal epithelium (dog and cat), and 6 of the 22 patients responded positively to these six antigens plus the herbs (Red Top).

Initial Spirometry and Provocation Tests

The initial spirometry assessment carried out prior to the provocation tests showed that both groups exhibited a diminished FEV_1 when compared to predictive values (11,12). Thus, in the mild asthmatic group the FEV_1 reached an average ± SD of 89 ± 6% while in the moderate asthmatic group the average value was 73 ± 6%. In both groups of patients, the minimum FEV_1 value measured was always above 1 liter. The accumulated doses of methacholine required to obtain a reduction of the FEV_1 higher than 20% referred to the basal value are shown in Table 1. The mild asthmatic group needed a dose of 6.91 ± 5.6 accumulated units (PD_{20}) (lg PD_{20} = 0.8 ± 0.25) while the moderate asthmatic group required 0.65 ± 0.5 accumulated PD_{20} units (lg PD_{20} = 0.22 ± 0.28). (Table 1).

Drug Protocols Applied to Mild Asthmatics

After a salbutamol period of 4 weeks, the lg PD_{20} values of mild asthmatics were similar to the initial lg PD20 values (1.09 ± 0.44 vs. 0.80 ± 0.25), while those obtained after the administration of beclomethasone for 4 weeks were significantly different compared to the initial values (1.56 ± 0.53 vs. 0.8 ± 0.25, $p < 0.025$). (Table 2).

When the combination inhaler was used, the obtained lg PD_{20} values were similar to those of beclomethasone (1.55 ± 0.43 vs. 1.56 ± 0.53), also showing a highly significant difference when compared to the initial lg PD_{20} (1.55 ± 0.43 vs. 0.80 ± 0.25, $p < 0.0025$) (Fig. 1). Therefore, when the capacity of each drug to block the effect of methacholine was analyzed, beclomethasone alone or combined with salbutamol was significantly more effective than salbutamol alone ($p < 0.025$). (Table 2).

Table 2. Drug Protocols Applied to Mild and Moderate Asthamatics

PATIENT	INITIAL lg PD_{20}	SALBUTAMOL lg PD_{20}	BECLOMETHASONE lg PD20	COMBINATION lg PD_{20}
		Mild Asthma		
1	0.63	1.28	2.34	2.34
2	0.63	1.28	0.97	0.97
3	0.63	0.63	1.65	1.28
4	0.63	0.63	2.34	1.28
5	0.97	1.97	2.34	1.28
6	0.97	0.97	0.97	1.28
7	1.28	1.28	1.28	1.97
8	0.63	0.97	1.97	1.65
9	1.28	1.28	1.65	2.34
10	1.28	1.28	1.28	1.65
11	0.63	0.97	1.65	1.65
SM ± SD	0.80 ± 0.25	1.09 ± 0.44	1.56 ± 0.53	1.55 ± 0.43
		Moderate Asthma		
1	0.24	0.24	0.63	0.63
2	0.12	0.24	0.24	0.24
3	0.12	0.12	0.97	0.24
4	0.60	0.63	0.63	1.65
5	0.12	0.24	0.63	0.63
6	0.12	0.24	0.24	1.97
7	0.60	0.12	0.24	0.24
8	0.12	0.24	0.24	0.63
9	0.24	0.24	0.24	0.63
10	0.12	0.63	1.28	0.97
11	0.6	0.12	0.24	0.63
	0.21 ± 0.28	0.24 ± 0.25	0.42 ± 0.34	0.59 ± 0.54

PD_{20}, determined as described in Materials and Methods, in the initial and posttreatment methacholine provocation tests in patients with moderate and mild asthma. The geometric mean of the lg of the PD_{20} values observed with the use of beclomethasone and combination is statiscally different from the initial value ($p < 0.025$), $n = 11$.

Drug Protocols Applied to Moderate Asthmatics

In a similar fashion, we applied the selected drug protocols to the group of patients with moderate asthma. After a 4-week period on salbutamol, the lg PD_{20} values were significantly different when compared to the initial lg PD_{20} accumulated units of methacholine (0.24 ± 0.25 vs. 0.21 ± 0.28, $p < 0.025$). When beclomethasone was applied, the differences in lg PD_{20} (0.42 ± 0.34 vs. 0.21 ± 0.28) were even more significantly different ($p < 0.0025$). When the combination was applied, the differences in the lg PD_{20} (0.59 ± 0.54 vs. 0.21 ± 0.28) were the most significant ($p < 0.001$)

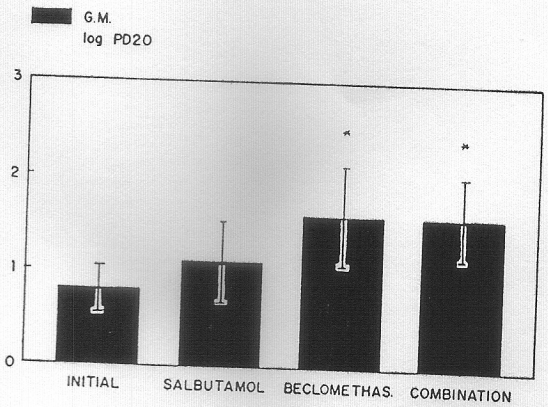

Figure 1. Results in mild asthmatics.

(Table 2) (Fig. 2). Thus, when the capacity of each drug to block the effect of methacholine in moderate asthmatics was investigated, the obtained lg PD_{20} value using either salbutamol or beclomethasone was significantly different. However, the combination of both drugs induced a significant difference in lg PD_{20} when compared with salbutamol or beclomethasone alone.

Daily Record of Symptoms

During treatment with salbutamol in the group of mild asthmatics, 5 of 11 patients (45%) had cough; 3 (27%), in addition to the cough, had dyspnea and moderate to severe wheezing. Two of 11 patients (18%), in addition to coughing, had expectoration. During treatment with beclomethasone and/or the combination, this group of patients did not show any symptoms. In the group of moderate asthmatic patients during treatment with salbutamol, 4 of 11 patients (36%) had cough; 2 (18%), in addition to coughing, had dyspnea and wheezing, while the other 2 patients, in addition to coughing, had expectoration. During the course of the treatment with beclomethasone, 3 of 11 patients (27%) had cough. Finally, with the combination, only 2 patients had cough (18%) (Table 3).

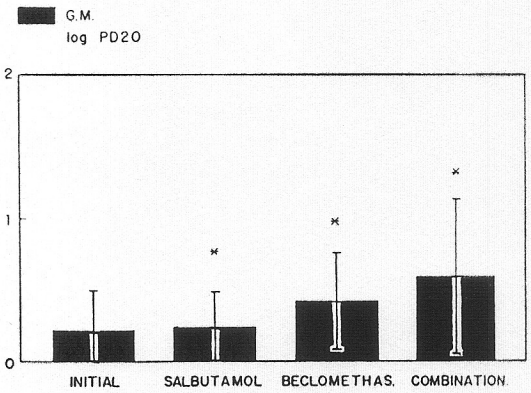

Figure 2. Results in moderate asthmatics.

DISCUSSION

Recently, measurements of the bronchial airways responsiveness have changed the concepts regarding the pathogenesis (15–19) and treatment of bronchial asthma (20–24), stressing the fact that the clinical characteristics of bronchial asthma may be secondary, at least in part, to the late-phase inflammatory component of the air passages. Studies of this late phase in the asthmatic response have implicated inflammatory cells, especially eosinophils and neutrophils (25). The inflam-

Table 3. Daily Record of Symptoms

SYMPTOM	SALBUTAMOL (PATIENTS)	BECLOMETHASONE (PATIENTS)	COMBINATION (PATIENTS)
		Mild Asthma	
Cough	5	0	0
Expectoration	2	0	0
Dyspnea	3	0	0
Wheezing	3	0	0
Total	5	0	0
		Moderate Asthma	
Cough	4	3	1
Expectoration	3	0	0
Dyspnea	2	0	0
Wheezing	2	0	0
Total	4	0	0

matory response and the release of mediators increase the bronchial hyperreactivity through mechanisms which include: an increase of the permeability of the air passages epithelium, diminution of the caliber thereof, alteration of the autonomous control, and changes in myogenic function (26). This nonspecific bronchial hyperreactivity can be quantified by means of the provocation test with methacholine (27). We investigated, in a double-blind, cross-over fashion, the blocking effect of either inhaled salbutamol, beclomethasone, or a combination of both drugs on the bronchial hyperreactivity induced by methacholine in a carefully selected group of mild and moderate atopic asthmatics. The initial provocation test with methacholine revealed that the mild asthmatic group needed a greater accumulated dose of methacholine than that required by the moderate asthmatic group to lower the FEV_1 by 20%, stressing the enhanced bronchial hyperreactivity present in the latter group. However, when applied in combined form, salbutamol plus beclomethasone were able to provoke a significant difference in the PD20 in the mild asthmatic group when compared to the baseline values or to salbutamol alone. This finding seems to be related to the addition of the beclomethasone since these patients with mild asthma did show a significant PD_{20} change with the administration of this drug alone.

These results tend to suggest that in mild asthmatic patients classified as having stable asthma with absence of or minimal bronchospasm, the inflammatory process may be partly responsible for the pathogenesis of symptoms. In fact, none of these patients showed any symptoms during treatment with beclomethasone alone or with the combination. The effectiveness of the combination was also observed in the moderate asthmatics group, whose PD_{20} significantly changed when compared either to salbutamol or to beclomethasone administered alone. The change induced by the combination of both drugs was significant although either drug was able to provoke different PD_{20} values compared to the baseline levels. Some authors have suggested that inhaled beta-agonist might increase bronchial reactivity, inducing also a rebound phenomenon when the drug is withdrawn (28). For instance, Sears et al. *recommend* that inhaled beta-agonists should be used only on demand to prevent the adverse effects that could be seen with regular inhalation (8). However, these adverse effects could occasionally be avoided by adding steroids to augment the expression of the beta-adrenergic receptors in lung membranes (29). Indeed, we found that in both groups of patients the combination of salbutamol plus beclomethasone proved more efficient by blocking the provocation methacholine test. This effect seems to depend mostly on the pharmacological control of an inflammatory compromise detected in the group with mild asthma and already established in the moderate asthmatic patients. Furthermore, in the latter group, the secondary action of the steroid on the expression of the beta-adrenergic receptors should also be considered. Therefore, the use of the combination of salbutamol plus beclomethasone seems to be beneficial in well-characterized, stable bronchial asthma.

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