Separate and combined effects of corticosteroids and bronchodilators on airflow obstruction and airway hyperresponsiveness in asthma

Johan B. Wempe, MD, Dirkje S. Postma, PhD, Nanke Breederveld, MD, Desiree Alting-Hebing, Thom W. van der Mark, PhD, and Gerard H. Koëter, PhD *Groningen, The Netherlands*

We have investigated separate and interactive effects of corticosteroids and bronchodilators on airflow obstruction and airway hyperresponsiveness. Twelve allergic subjects with asthma were treated in a double-blind, crossover, randomized study with budesonide, 1.6 mg daily for 3 weeks, prednisone, 40 mg daily, for 8 days, and placebo. After each period, dose-response curves were measured on 4 study days with doubling doses of salbutamol, ipratropium, a combination of salbutamol and ipratropium, and placebo until a plateau in FEV_1 was reached. A histamine challenge was then performed, and the provocation concentration causing a 20% fall in FEV_1 (PC_{20}) was calculated. The budesonide and prednisone regimens were equipotent. FEV_1 was 81.2% of predicted after budesonide, 81.0% predicted after prednisone, and 67.5% predicted after placebo, bronchodilatation thus being 13.7% predicted (budesonide) and 13.5% predicted (prednisone). PC_{20} improved with 2.17 doubling concentrations (DCs) after budesonide, and 1.86 DCs after prednisone, compared with that of placebo. Salbutamol caused stronger bronchodilatation than ipratropium (26.2% versus 14.7% predicted) and a better protection against histamine challenge (3.95 versus 1.12 DC). The effects of corticosteroids and bronchodilators on FEV_1 and PC_{20} were, in general, additive. This study emphasizes different modes of action on both airflow obstruction and airway hyperresponsiveness by corticosteroids and bronchodilators, and it demonstrates no enhancement of bronchodilator action by corticosteroids. (J ALLERGY CLIN IMMUNOL 1992;89:679-87.)

Key words: *Airway hyperresponsiveness, asthma, corticosteroids, bronchodilators*

Both reversibility of airflow obstruction and degree of airway responsiveness are known to be important parameters for the assessment of the severity of asthma.[1, 2] Reversibility may be tested with bronchodilating agents in different forms (e.g., metered-dose inhaler and nebulizer) and with different agents and regimens. In general, β-adrenergic agents provide more bronchodilatation than anticholinergic agents in young subjects with asthma.[3-5] The method leading to the largest bronchodilatation appears to be the administration of cumulative doses of bronchodilator until a plateau in airflow values is reached.[6] It is, however, uncertain whether the achieved bronchodilatation is really the largest possible bronchodilatation. For instance, it is not known to what extent smooth muscle relaxing agents influence the thickening of the airway wall[7] that is believed to play an important role in airflow obstruction in individuals with asthma.[8, 9] It may be necessary to add corticosteroids, which suppress inflammatory processes and may diminish

Abbreviations used

PC_{20}:	Provocative concentration causing a 20% fall in FEV_1
DC:	Doubling concentration
MANOVA:	Multivariate analysis of variance

From the Department of Pulmonology, University Hospital Groningen, Groningen, The Netherlands.

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Reprint requests: G. H. Koëter, MD, Professor of Pulmonary Medicine, University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

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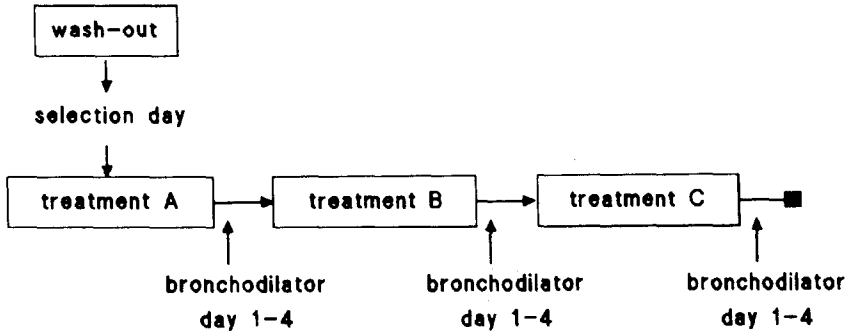

FIG. 1. Study design. Treatment consisted of budesonide, 1.6 mg/day for 3 weeks; prednisone, 40 mg/day for 8 days; and placebo. On bronchodilator-treatment days, dose-response curves were measured with ipratropium, salbutamol, and combination of salbutamol and ipratropium, which contained 50% of the concentration of each drug administered at separate days, and placebo.

airway wall thickness, to obtain maximal bronchodilatation. Moreover, in vitro data suggest even a synergistic interaction between corticosteroids and β-agonists caused by a "permissive" effect of corticosteroids on β-receptors,[10, 11] which could enhance responsiveness to β-agonists. In vivo studies have demonstrated some[12, 13] or no evidence[14-16] of such enhanced responsiveness to bronchodilators (by corticosteroids) when airflow obstruction was measured.

Corticosteroids and bronchodilating agents act differently on airway hyperresponsiveness.[17, 18] Corticosteroids have been demonstrated to reduce hyperresponsiveness, probably by suppressing continuously present inflammatory responses.[19-21] Airway diameter per se is known to influence the degree of hyperresponsiveness in subjects with asthma[22]: the greater airflow obstruction, the greater airway hyperresponsiveness. The mechanisms of action of bronchodilators on airway hyperresponsiveness, however, appear to be, at least partly, independent of bronchodilatation,[23] and β-agonists demonstrate a stronger protection against provoking stimuli than anticholinergic agents.[23] At the present time, no data have been obtained on the combined effects of corticosteroids and bronchodilators on airway hyperresponsiveness.

In the present study, separate and combined effects of corticosteroids and bronchodilators were investigated by comparing the effects of cumulative doubling doses of salbutamol and ipratropium on airflow obstruction and airway hyperresponsiveness, with and without pretreatment with large doses of inhaled budesonide and oral prednisone.

METHODS

Patients

Ten male and two female subjects, aged 20 to 41 years, were examined after giving informed consent. The study was approved by the hospital medical ethics committee. All patients had complaints of episodic wheezing, consistent with the clinical diagnosis of asthma. They were clinically stable, without any respiratory infection or exacerbation 2 months before the study. Patients were selected on positive allergy (positive skin tests for at least two common allergens, including house dust mite, and an increased level of specific IgE against house dust mite), baseline FEV_1 between 50% and 80% of the predicted value[24] after a washout period (see Study design), and the presence of airway hyperresponsiveness defined by a provocation concentration of histamine causing a 20% fall in FEV_1 (PC_{20}) of <8 mg/ml (see Methods).

Methods

FEV_1 was measured with a water-sealed spirometer (Lode BV, Lode Instruments, Groningen, The Netherlands), the best of three attempts being accepted. Bronchial challenge tests were performed by the method described by Cockcroft et al.[25] with a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa.), with an output of 0.13 ml/min. A 0.9% phosphate-buffered saline solution and doubling histamine diphosphate concentrations from 0.03 to 16 mg/ml were inhaled for 2 minutes at 5-minute intervals until a 20% fall in FEV_1 was reached, compared with the postsaline value.

Study design

Treatment. The subjects came for a selection day to the lung function laboratory after a 6-week washout period for inhaled corticosteroids and cromolyn sodium and a 2-week washout period for all bronchodilators except β-agonists. On this selection day, spirometry and histamine challenge were performed. After inclusion, subjects entered a double-blind, randomized, crossover study consisting of treatment periods of 3 weeks, each followed by 4 study days (Fig. 1). During each period, they received four puffs twice daily by metered-dose inhaler for the entire 3 weeks, and four tablets twice daily for the last 8 days. There were three treatment regimens: A, budesonide puffs (1.6 mg daily) and placebo tablets; B, placebo puffs and prednisone tablets (40 mg daily); and C, placebo puffs and placebo tablets. The trial medication was continued during the 4 study days that

TABLE I. Subjects' characteristics on selection day

No.	Age (yr)	Sex	Smoking status	FEV_1 (L)	FEV_1 (% pred)	PC_{20} histamine (mg/ml)	Eosinophil count (10^6/L)	Maintenance treatment
1	24	M	n	2.70	68.5	0.66	99	ba,ac,ic
2	22	M	n	2.95	62.4	1.12	297	ac,cs
3	20	M	n	3.15	63.5	0.65	396	ba,ac,cs,ic
4	28	F	e	1.85	60.7	0.08	429	ba,ac,cs,ic
5	41	M	n	2.25	58.7	0.35	264	ba,ac,cs,ic
6	29	M	e	3.85	77.0	0.15	473	ac,cs,ic
7	20	F	n	2.55	70.8	0.44	396	ic
8	40	M	e	2.35	61.5	0.38	231	ba,ac,cs,ic
9	31	F	n	1.95	70.7	0.56	198	ba,ac,cs,ic
10	33	M	n	3.25	80.8	0.28	198	ba
11	23	M	e	4.05	75.6	0.13	176	ba
12	31	M	c	1.65	62.5	0.24	330	ba,ic

n, Never smoked; *e*, exsmoker; *c*, current smoker; *pred*, predicted; *ba*, β_2-agonists; *ac*, anticholinergics; *ic*, inhaled corticosteroids; *cs*, cromolyn sodium.

followed each period. During the entire study, inhaled salbutamol was allowed if this were required, except during 12 hours before arrival in the clinic on the study days.

Bronchodilator days. Each treatment period was followed by 4 study days on which reversibility was tested, starting between 8 and 8:30 AM. Baseline FEV_1 was determined after 15 minutes of rest. If baseline FEV_1 on day 2 to 4 was within 10% of FEV_1 on day 1, the bronchodilator part of the study took place. Doubling doses of ipratropium, salbutamol, a combination of both drugs, and saline were administered by nebulizer on the 4 days in a double-blind, randomized, and crossover design. Solutions for nebulization were prepared immediately before use from stock solutions of ipratropium bromide, 0.25 mg/ml, without preservatives (Boehringer Ingelheim, Inc., Ridgefield, Conn.), salbutamol sulfate, 5 mg/ml (Glaxo, Inc., Research Triangle Park, N.C.), and a 0.9% sodium chloride solution. The solutions were isotonic, and pH was always 3.5. For nebulization an HICO-ultrasonic nebulizer, type 806E (Hirtz & Co., Cologne, Germany) was used, with an output of 0.6 ml/min, 1.7 MHz, emitting 3 ml of solution during 5 minutes of tidal breathing. During inhalation, patients wore a noseclip. Nebulized doses of ipratropium were 0.19, 0.38, 0.75, 1.50, and 3.00 mg. For salbutamol, the nebulized doses were 0.94, 1.88, 3.75, 7.50, and 15.00 mg. The combination (ipratropium/salbutamol) was administered in doses of 0.09/0.47, 0.19/0.94, 0.38/1.88, 0.75/3.75, and 1.50/7.50 mg, respectively. Subjects rested 1 hour after each inhalation before FEV_1 was measured again. The experiment was stopped when the increase of FEV_1 was <5% compared with the previous measurement. One hour after the final administration of bronchodilator, a histamine challenge was performed.

Data analysis

PC_{20} histamine was calculated by interpolation of the log dose-response curve, normal values considered to be >8 mg/ml. If PC_{20} was >16 mg/ml, an extrapolation was performed with the last two points of the curve. If PC_{20} was >32 mg/ml, we performed the analyses with PC_{20} = 64 mg/ml. PC_{20} values were log transformed for further analysis. The $\log_2$ was chosen because doubling concentrations of histamine were administered in the histamine challenge. One unit in $\log_2$ represents one concentration step or doubling concentration (DC). FEV_1 was expressed as a percentage of the predicted value.[24]

MANOVA with repeated measurements was used for analysis. Dependent variables were FEV_1 percent predicted and $\log_2 PC_{20}$, and explanatory factors were treatment, bronchodilator, and two-way interactions between treatment and bronchodilator. In case of significant treatment and bronchodilator effects, Student's *t* tests for paired observations were used for comparisons between treatment regimens and bronchodilators. The interactive effects of corticosteroids and bronchodilators were estimated and evaluated with MANOVA. The null hypothesis in this analysis was that the interactive effect was zero, which means that the observed value for the combination of corticosteroids and bronchodilators was equal to the sum of their separate effects; the effects are additive. An observed positive interactive effect indicates that the observed effect of the combination is larger than the sum of the separate effects ("synergistic"), whereas a negative interactive effect implies a smaller effect of the combination than was expected as the sum of separate effects ("antagonistic"). Factors for time effects and residual effects of budesonide and prednisone were included in the analysis. A p value of <0.05 was considered significant. All calculations were performed with the statistical software package (SPSS/PC+, V3.1, SPSS Inc., Chicago, Ill.).

RESULTS

The clinical characteristics of the patients after the washout period are presented in Table I.

Patients 11 and 12 had to withdraw from the trial because of an exacerbation during the placebo-treat-

TABLE II. Effects of bronchodilators and treatment with corticosteroids on FEV_1*

Treatment	Bronchodilator			
	Placebo	Ipratropium	Salbutamol	Combination
Placebo	67.5 (3.2)	82.2 (3.8)	93.7 (2.4)	93.5 (2.5)
Budesonide	81.2 (4.2)	92.5 (3.0)	93.0 (4.0)	93.0 (3.0)
Prednisone	81.0 (3.0)	93.4 (2.6)	97.7 (2.8)	97.3 (2.8)

*Mean (SEM) percent predicted.

TABLE III. Effects of bronchodilators and treatment with corticosteroids on PC_{20} histamine*

Treatment	Bronchodilator			
	Placebo	Ipratropium	Salbutamol	Combination
Placebo				
Mean (SEM)	−2.61 (0.43)	−1.49 (0.59)	1.34 (0.32)	1.41 (0.33)
Geometric mean	0.16	0.36	2.53	2.66
Budesonide				
Mean (SEM)	−0.44 (0.42)	0.21 (0.70)	2.74 (0.82)	2.96 (0.92)
Geometric mean	0.74	1.16	6.68	7.78
Prednisone				
Mean (SEM)	−0.75 (0.55)	0.38 (0.62)	3.20 (0.63)	3.04 (0.65)
Geometric mean	0.59	1.30	9.19	8.22

*Mean (SEM) $\log_2$ PC_{20} geometric mean in milligrams per milliliter.

ment period. The analysis is based on the remaining 10 patients. Patient 10 had to use a β-agonist during the placebo-treatment day of the budesonide-treatment period, and the results of this day were excluded from the analysis.

All subjects received three doses of bronchodilator by nebulization on each bronchodilator day. The third inhalation did not improve FEV_1 >5%, compared with the values obtained after the second dose. Cumulative dose-response curves of FEV_1 after placebo treatment are illustrated in Fig. 2. Mean values of FEV_1 and PC_{20} at the end of the 4 study days after each treatment are presented in Tables II and III. PC_{20} values at the end of the salbutamol or combination study day were >32 mg/ml in three subjects after budesonide treatment, and in two subjects after prednisone treatment.

Time and residual effects

During the study, FEV_1 and PC_{20} demonstrated a decrease of 0.1% predicted ($p = 0.99$), and 0.25 DC ($p = 0.84$), respectively, which indicates that a time effect was not significant. There was a residual effect of prednisone, which was, however, negative, both for FEV_1 (−5.7% predicted; $p = 0.028$) and PC_{20} (−0.58 DC; $p = 0.24$). The residual effect of budesonide was −1.6% predicted on FEV_1 ($p = 0.48$) and 0.52 DC on PC_{20} ($p = 0.21$).

Effects of corticosteroid treatment

Treatment had a significant effect on FEV_1 ($p = 0.002$) and PC_{20} ($p < 0.001$). Analysis of values measured at the placebo bronchodilator day (Table II) reveals that budesonide caused an increase in FEV_1 of 13.7% predicted and that prednisone improved FEV_1 with 13.5% predicted, compared with placebo ($p = 0.021$ and $p = 0.011$, respectively). The difference in results between budesonide and prednisone was not statistically significant ($p = 0.74$). Both corticosteroids resulted in higher values of PC_{20} histamine, compared with placebo, as presented in Table III. At the placebo bronchodilator day, PC_{20} improved with 2.17 DC after budesonide, and with 1.86 DC after prednisone treatment, compared with PC_{20} after placebo treatment ($p = 0.009$ and $p = 0.016$, respectively). As for FEV_1, the effects of budesonide and prednisone administration were not significantly different ($p = 0.91$).

Effects of bronchodilators after placebo treatment

FEV_1 and PC_{20} were significantly influenced by bronchodilators ($p < 0.001$ for both variables). Dose-response curves measured at the 4 bronchodilator days after placebo treatment are plotted in Fig. 2. The salbutamol curve appeared to reach higher

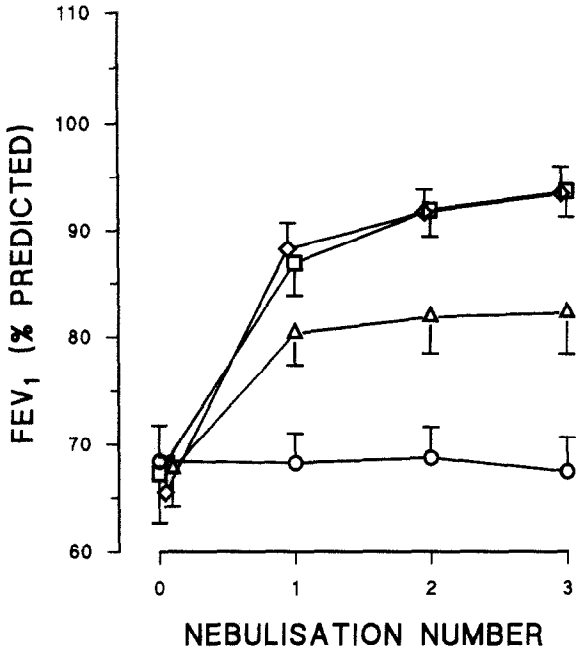

FIG. 2. Cumulative dose-response curves of FEV_1 after placebo treatment; placebo (○), ipratropium (△), salbutamol (□), and the combination of ipratropium and salbutamol (◇). Differences in final FEV_1 between bronchodilators and placebo and between ipratropium and salbutamol or the combination were statistically significant.

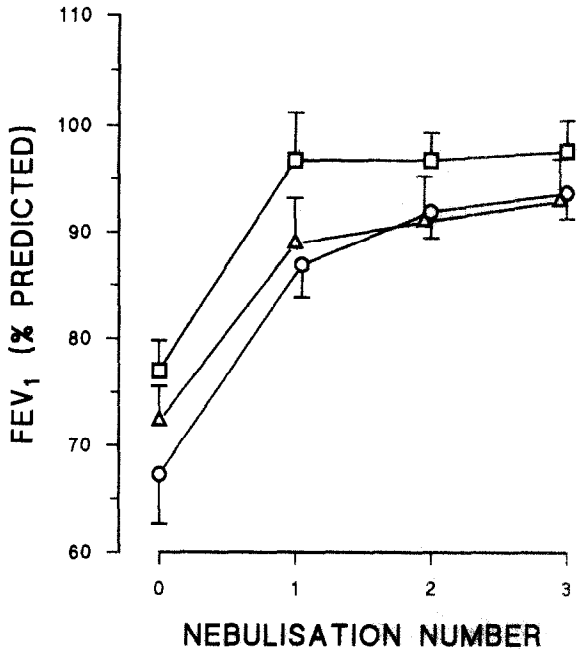

FIG. 3. Effects of treatment on the salbutamol dose-response curve; placebo (○), budesonide (△), and prednisone (□). No significant differences in postsalbutamol FEV_1 existed between the treatments.

levels than the ipratropium curve, and FEV_1 after the final nebulization was higher after salbutamol than after ipratropium treatment (Table II), 93.7% predicted versus 82.2% predicted, implying a bronchodilatation of 26.2% predicted versus 14.7% predicted ($p = 0.031$). Salbutamol resulted in an improvement of PC_{20} of 3.95 DC, compared with PC_{20} after placebo treatment ($p < 0.001$). The difference between ipratropium and placebo, 1.12 DC, was also statistically significant ($p = 0.048$). Salbutamol provided a better protection against histamine than ipratropium ($p < 0.001$). The combination of salbutamol and ipratropium mimicked the salbutamol nebulization, both for FEV_1 ($p = 0.86$) and PC_{20} ($p = 0.84$), which is illustrated in Fig. 2 and Tables II and III.

Combined effects of corticosteroids and bronchodilators

First, we compared postbronchodilator FEV_1 and PC_{20} values with and without corticosteroid treatment. Dose-response curves for FEV_1 after placebo, budesonide, and prednisone administration are plotted in Fig. 3 (salbutamol) and Fig. 4 (ipratropium). For salbutamol, final FEV_1 values (Table II) were not significantly changed after budesonide (−0.7% predicted; $p = 0.87$) or prednisone treatment (4% predicted; $p = 0.071$), compared with that after placebo treatment. Postipratropium FEV_1, however, increased with 10.3% predicted after budesonide ($p = 0.015$)

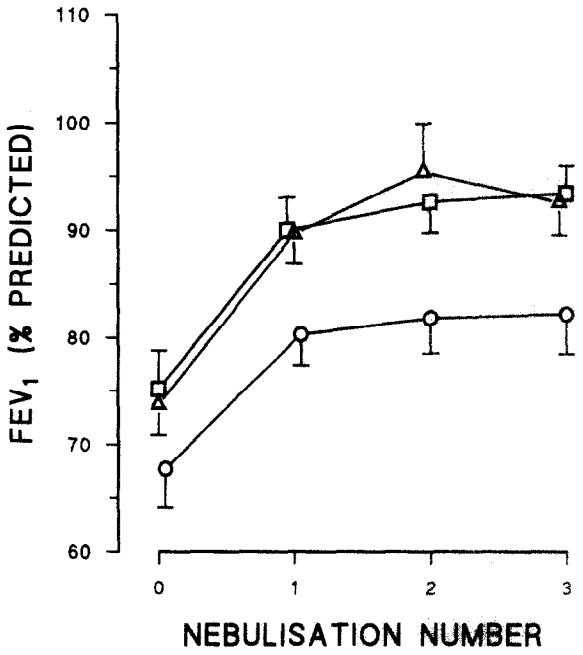

FIG. 4. Effects of treatment on the ipratropium dose-response curve; placebo (○), budesonide (△), and prednisone (□). Postipratropium FEV_1 was significantly higher after both corticosteroids than after placebo.

TABLE IV. Interactive effects between corticosteroids and bronchodilators*

	FEV_1† (% pred)	p ‡	PC_{20}† (DC)	p ‡
Budesonide plus salbutamol	−13.5	0.059	−0.78	0.76
Budesonide plus ipratropium	−3.4	0.51	−0.48	0.93
Prednisone plus salbutamol	−9.6	0.030	0.00	0.996
Prednisone plus ipratropium	−2.4	0.53	0.00	0.99

*Evaluated with MANOVA.
†Difference between actually measured value and summated value of the separate effects compared with placebo.
‡Probability; null hypothesis effects are additive; value for interactive effect is 0.

and with 11.2% predicted after prednisone treatment ($p = 0.019$), both compared with that after placebo treatment.

Postbronchodilator PC_{20} values were increased by previous corticosteroid treatment. Compared with placebo treatment, budesonide resulted in an increase in postsalbutamol PC_{20} of 1.40 DC ($p = 0.084$) and in postipratropium PC_{20} of 1.70 DC ($p = 0.032$). Prednisone was associated with an increase in postsalbutamol PC_{20} of 1.86 DC ($p = 0.007$) and in postipratropium PC_{20} of 1.87 DC ($p = 0.007$). Differences in postbronchodilator FEV_1 and PC_{20} were not statistically significant between budesonide and prednisone treatment.

Second, we analyzed interactions between corticosteroids and bronchodilators. The two-way interaction between treatment and bronchodilators was statistically significant for FEV_1 ($p = 0.008$) but not for PC_{20} ($p = 0.99$). The magnitude and p values of all possible interactive effects are presented in Table IV. The interactive effect between budesonide and salbutamol of −13.5% predicted means that the combination of the two drugs resulted in a 13.5% predicted lower value than was expected as the sum of the separate effects. All combinations demonstrated negative interactive effects on FEV_1, which were only (close to) significant for FEV_1 between prednisone and salbutamol ($p = 0.030$) and budesonide and salbutamol treatment ($p = 0.059$). The effects of corticosteroids and bronchodilators on PC_{20} were additive.

DISCUSSION

This study was designed to investigate separate and combined effects of corticosteroids and bronchodilators, and it demonstrates that cumulative doubling doses of salbutamol elicit larger bronchodilatation and a stronger protective effect against histamine than ipratropium. It demonstrates that, in the administered doses, treatments with budesonide and prednisone result in similar improvements of postbronchodilator FEV_1 and PC_{20}, and that corticosteroids and bronchodilators act, in general, in an additive way on FEV_1 and PC_{20}.

The present study was designed without an additional washout between the treatment periods, which would prolong undertreatment and a subsequent increase in dropout rate. In practice, however, there often existed such washout periods, in which only the escape medication, salbutamol, was allowed, caused by patient-related circumstances, such as holidays. On only six of 30 occasions, the next treatment period started within 2 weeks after a corticosteroid-treatment period. One placebo- and one budesonide-treatment period were preceded by a prednisone-treatment period; four prednisone-treatment periods, in which active treatment was administered only during the last 8 days, were preceded by a budesonide-treatment period. It has been suggested that after a short corticosteroid-treatment period, FEV_1 and PC_{20} return to baseline within 2 weeks.[16, 18] Therefore, the risk of residual effects of corticosteroids is probably low in our study. The surprising presence of a negative carryover effect of prednisone on FEV_1, accompanied by a not significantly, lower PC_{20} may suggest a rebound effect but could also be due to sampling error.

The effects of corticosteroids on airflow obstruction and airway hyperresponsiveness can be explained by the inhibition of virtually every stage in the inflammatory processes in the airways. A reduction of mediator release by eosinophils[26] and macrophages[27] may diminish bronchial responses to allergic and nonallergic stimuli. Furthermore, the decreased mediator release will result in increased baseline airway caliber by a decrease in smooth muscle contraction and a reduction of swelling of the airway wall. Many studies have, indeed, demonstrated that *inhaled* corticosteroids, such as beclomethasone and budesonide, diminish hyperresponsiveness.[16-19, 28, 29] The effects of inhaled corticosteroids on FEV_1 are less distinct, but in general, a small increase can be observed, as in our study. Although there is no doubt about the beneficial effect of *oral* corticosteroids in asthma, the

extent of their effects on FEV_1 and hyperresponsiveness is controversial.[30-33] Jenkins and Woolcock[33] have demonstrated that inhalation of 1.2 mg of beclomethasone daily for 3 weeks improved hyperresponsiveness, in contrast to 12.5 mg of prednisone, which had no effect at all. In our study, FEV_1 and PC_{20} improved similarly after treatment with budesonide and prednisone. Webb et al.[34] have suggested that 8 days of treatment with 40 mg of prednisolone daily results in the maximal corticosteroid response. Our results indicate that budesonide, 1.6 mg daily for 3 weeks, is equipotent to this regimen and may be used to estimate corticosteroid sensitivity in subjects with asthma.

Salbutamol and ipratropium are well established, potent bronchodilators.[35] Young subjects with asthma appear to respond better to β-agonists, whereas older patients reveal a better or at least equal response to anticholinergic drugs.[3-5, 36, 37] Bronchodilator responses are dependent on the amount of bronchodilator and the method of administration. We have used cumulative dose-response curves with doubling doses of nebulized bronchodilator to achieve maximum dilatation. This method produces a greater airway response than noncumulative and single-dose studies,[6] probably because of the fact that the bronchodilatation produced by the preceding dose of bronchodilator allows following doses to reach more peripheral bronchi.

Our study demonstrates that bronchodilatation with salbutamol resulted in higher plateau levels of FEV_1 than ipratropium. Treatment with budesonide or prednisone failed to improve the response to salbutamol, which is probably caused by a "ceiling" effect. Post-salbutamol levels of FEV_1 after placebo treatment were near normal and could not improve any further. In contrast, maximal bronchodilatation after ipratropium could only be reached after treatment with corticosteroids. These results may be explained by the different effects of these drugs on two components of airflow obstruction, that is, contraction of airway smooth muscle and airway wall thickening. The action of β-agonists on smooth muscle cells consists of stimulation of β-adrenergic receptors, and, probably less important, modification of neurotransmission in parasympathetic ganglia.[35] It has been suggested that β-agonists may also have an effect on airway wall thickness.[7] They are known as potent stabilizers of mast cells[38] and may diminish plasma extravasation and edema formation.[39, 40] If FEV_1 reflects smooth muscle contraction *and* airway wall thickening, our results suggest that β-agonists and corticosteroids do share an effect on airway wall thickness because budesonide and prednisone do not significantly increase post-salbutamol FEV_1 levels. Anticholinergic agents, such as ipratropium, influence parasympathetic tone of bronchial smooth muscle cells, which may insufficiently counteract the constrictive effects of inflammatory mediators. Moreover, anticholinergic agents probably have no effect on airway wall thickness.[7] Ipratropium may therefore have a smaller bronchodilating effect in allergic subjects with asthma in whom inflammatory mediators contribute substantially to airflow obstruction by constriction of smooth muscle cells and formation of airway-wall edema, which is in agreement with the increased postipratropium bronchodilatation by corticosteroids. Another recently suggested explanation for the relatively small effect of ipratropium on FEV_1 is the inhibition of M3 autoreceptors on vagal nerve endings, which leads to an increased acetylcholine release.[41] It is not yet clear whether this mechanism is of clinical significance.

The results of our study demonstrate that salbutamol has a stronger protective effect against histamine-induced airflow obstruction than ipratropium, even in the presence of similar FEV_1 levels, which is in agreement with other studies.[23, 42-44] The mechanisms of action of these bronchodilators on airway responsiveness are not well understood. The effect of ipratropium may be in antagonizing the contribution of cholinergic activity to histamine-induced airflow obstruction and in increasing prechallenge airway diameter. The potent functional antagonistic action of β-agonists on histamine is probably located in smooth muscle cells; however, inhibition of mast cells and prevention of microvascular leakage may also counteract histamine-induced airflow obstruction.

The combination of salbutamol and ipratropium, both administered in a concentration that is 50% of the concentration administered at the separate bronchodilator days, resulted in values for FEV_1 and PC_{20} similar to values after inhalation of salbutamol alone, suggesting that the salbutamol component is the major factor. The nebulized doses, however, were large, as deduced from bronchodilatation and side effects, such as tremor, increased heart rate, and dry mouth. For clinical practice, combined treatment may be useful, for example, with regard to prolonged bronchodilating action, reduction in side effects with lower doses, or long-term effects.

It has been demonstrated in vitro that corticosteroids are able to increase the number of β-receptors of human blood lymphocytes.[10, 11] Several in vivo studies have not produced uniform results on interactive effects of corticosteroids and β-agonists. Corticosteroids were able to restore β-agonist responsiveness in nonresponsive subjects with chronic asthma[12] or healthy volunteers.[45] Later studies could not establish this synergistic effect of corticosteroids and β-ago-

nists,[14-16] although one study has recently provided some evidence for a synergistic interaction between betamethasone and terbutaline in antagonizing methacholine-induced bronchoconstriction in rats.[46] Our study also fails to demonstrate synergistic effects. The "ceiling effect" in FEV_1 may indicate that lower doses of β-agonist are required to investigate further interactive effects. The previously demonstrated "permissive" effect is perhaps only to be observed in individuals with a markedly reduced number and function of β-receptors and may not be of clinical significance in subjects with stable disease, who still have a good response to β-agonist drugs.

In summary, this study demonstrates that the effects of large doses of corticosteroids and bronchodilators are, in general, additive on FEV_1 and PC_{20}, indicating that their mechanisms of action are largely different. The beneficial effects of corticosteroids are especially of clinical relevance. Their bronchodilating action, for instance, is as large as action caused by ipratropium. These effects of corticosteroids on long function and airway hyperresponsiveness, and especially their known ability to improve both hyperreactivity and hypersensitivity of the airways,[47] their long duration of action, and the often very satisfactory effects on symptoms, emphasize the role of (inhaled) corticosteroids in the first line of treatment in allergic subjects with asthma. Additional studies have to elucidate whether long-term treatment with corticosteroids improves morbidity and mortality of the disease, either by direct effects on underlying processes or by an induced reduction of bronchodilator comedication.

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