

Effect of regular nedocromil sodium or albuterol on bronchial inflammation in chronic asthma

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Nedocromil sodium is recommended for daily treatment of mild persistent asthma but its effect on cellular changes in asthmatic airways is poorly understood. We compared the antiinflammatory effects of nedocromil sodium and albuterol in 32 patients with asthma who received either nedocromil sodium 4 mg or albuterol 0.2 mg four times daily for 12 weeks according to a double-blind protocol. Patients underwent fiberoptic bronchoscopy, and lung function and bronchial responsiveness to histamine were measured. Numbers of inflammatory cells were studied by immunohistochemistry and image analysis. We were unable to identify any statistical differences between treatment groups for any of the variables measured. We conclude that our immunohistochemical study does not support the concept of nedocromil sodium as a potent/antiinflammatory drug. (J Allergy Clin Immunol 1996;98:S58-66.)

Key words: Nedocromil sodium, albuterol, antiinflammatory therapy, bronchial biopsy, asthma

Because asthma is a chronic inflammatory disorder of the airways,¹ there is a need for a safe topical agent to prevent or reverse this process. Nedocromil sodium has long been recognized as an antiinflammatory agent without direct bronchodilating properties, but in contrast to the wealth of in vitro data,² there is limited knowledge about its effect on the cellular changes in the airways of patients with asthma. In one study on allergic asthma, nedocromil sodium reduced the numbers of eosinophils in bronchoalveolar lavage fluid after segmental allergen challenge.³ Recently, the effects of 16 weeks of treatment with nedocromil sodium, albuterol, or placebo were studied in patients with asthma by means of bronchial biopsies.⁴ There was a reduction in the numbers of total and activated eosinophils in the bronchial mucosa in the nedocromil sodium-treated group and an increase in the albuterol-

Abbreviations used

BHR: Bronchial hyperresponsiveness
mAb: Monoclonal antibody
PEF: Peak expiratory flow

treated group, demonstrating in significant differences between the two treatments.

Morphologic study of bronchial biopsy specimens provides a reliable assessment of local conditions at the site of asthmatic inflammation⁵ as well as a means of studying the effects of drug therapy.⁶ In this investigation, we used bronchial biopsy specimens to study the effect of 12 weeks of treatment with nedocromil sodium or the bronchodilator albuterol in patients with asthma.

MATERIALS AND METHODS

Study design

This randomized, double-blind, group-comparative study was conducted as a cooperative investigation between University Central Hospital, Helsinki, and the University of Tartu. Entrance criteria included asthma symptoms of a certain severity, defined as a total symptom score of ≥ 10 for any 10 days during a 2-week baseline period. The total symptom score was the sum of four symptoms (daytime symptoms, nighttime symp-

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toms, morning chest tightness, and cough), each rated on a five-point scale, from 0 = no symptoms to 4 = very severe. The presence of atopy was determined by skin prick testing with 12 common allergen extracts (ALK, Allergologisk Laboratorium A/S, Hørsholm, Denmark). Seasonal variability of symptoms was documented from the patients' case histories. Eligible patients were then randomly assigned, by computer-generated sequentially coded study numbers, to receive either nedocromil sodium or albuterol for the next 12 weeks. The study was approved by institutional ethical review committees at both centers, and all patients provided written informed consent.

Patients visited the clinic at the start and the end of the baseline period and after 4, 8, and 12 weeks of treatment. Bronchial hyperresponsiveness (BHR) and lung function were measured on visits 1, 2, 4, and 5, and bronchial biopsies were performed at the beginning and end of treatment (visits 2 and 5). On each visit, the investigator examined the patient and checked his or her inhalation technique.

Oral or inhaled corticosteroids, nedocromil sodium, ketotifen, and oral bronchodilators were discontinued 6 weeks before entry into the trial. The study medications were nedocromil sodium 4 mg (2 puffs) or albuterol 0.2 mg (2 puffs; Ventoline, Glaxo Operations, London, United Kingdom), both administered four times daily from coded metered-dose inhalers. Both drugs were provided by Fisons plc (Loughborough, United Kingdom). Patient compliance was assessed by having patients record their use of the test medication on a diary card and by weighing the returned aerosol canisters.

During the treatment phase, all patients had access to rescue therapy with inhaled albuterol on an as-needed basis. Other permitted medications included oral corticosteroids, oral or intravenous bronchodilators, antihistamines, and antibiotic agents. If oral prednisolone was needed, it was given at a daily starting dose of 30 mg, decreasing in dose by 5 mg each day. Antihistamines, nasal cromolyn sodium (Lomudal Nasal, Fisons), and nasal corticosteroids were allowed to control allergic rhinitis. The use of all concomitant medications other than as-needed inhaled albuterol was not permitted for longer than 12 days on two occasions during the trial. All patients were instructed to record the use of additional medications on the diary card.

Clinical methods

Measurements of forced vital capacity, FEV₁, and peak expiratory flow (PEF) were performed with a Jaeger Flowscreen 2.1 gG spirometer (Erich Jaeger Laboratories, Würzburg, Germany). Bronchodilators were withheld for 4 hours before lung function testing.

Histamine challenge was performed with a Spira Elektro automatic inhalation-synchronized dosimetric jet nebulizer (Respiratory Care Centre, Hämeenlinna, Finland) according to the method described previously.⁷ All challenge tests were performed at the same time of day (± 2 hours) for each patient. The patients were not

allowed to use an inhaled bronchodilator for 12 hours before the test.

Bronchoscopic examination and collection of biopsy samples conformed to the international guidelines for investigative use of fiberoptic bronchoscopy in asthma and other airway diseases.⁸ All patients were premedicated with intravenous atropine sulfate 0.5 to 1 mg and diazepam 5 to 10 mg. The oropharynx was anesthetized immediately before the procedure with a topical lidocaine spray (Xylocain, Astra Draco AB, Södertälje, Sweden), and lidocaine 2% solution (Orion OY, Espoo, Finland) was applied through the bronchoscope channel to the vocal cords and upper airways. The Olympus BF-20 bronchoscope (Olympus, Tokyo, Japan) was used for the examination. Bronchial biopsy specimens were taken from the right upper- and middle-lobe bronchi. The regions very close to carinae and those previously touched by the bronchoscope were avoided. All specimens were obtained with Olympus FB-19C forceps.

Laboratory methods

In all samples, activated eosinophils, mast cells, and T lymphocytes were counted separately in bronchial epithelium and lamina propria, and results were expressed for the entire mucosa as total count per square millimeter. Cryostat sections (5 μ m thick) of bronchial biopsy samples were immunostained with a panel of mouse monoclonal antibodies (mAbs) against human cell-specific markers. The alkaline phosphatase anti-alkaline phosphatase technique (Dako A/S, Glostrup, Denmark) was used to visualize inflammatory cells. Activated eosinophils were stained with mAb EG2 (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden), which reacts with the activated form of eosinophil cationic protein. Mast cells were stained with mAb AA1 (Dako A/S) raised against mast-cell tryptase. T lymphocytes were recognized with an anti-CD3 mAb (Dako A/S). The dilutions applied for these mAbs were 1:50, 1:500, and 1:1000, respectively.

Inflammatory cells were quantified by semiautomatic charting of images from photomicrographs with a fully calibrated Kurta IS/THREE digitizing tablet (Kurta Corp., Phoenix, Ariz.) and a pointing device linked to a computer. With use of the AutoCad program, version 10.0n (Autodesk, Inc., Sausalito, Calif.), inflammatory cells were counted from the entire sections, and densities were calculated separately for epithelium and lamina propria. All measurements were carried out at the Department of Anatomy, University of Helsinki, by the same person, who had no knowledge of the origin of the specimens.

Statistical analysis

Changes in inflammatory cell count (posttreatment compared with pretreatment) were analyzed by non-parametric methods within and between groups; the between-group comparisons were emphasized. BHR PD₁₅ values were analyzed by *t* tests after the data were transformed (log_e transformation). Pulmonary function tests were analyzed by *t* tests. All statistical hypothesis tests were two-tailed, and a value of *p* <

TABLE I. Patient demographic characteristics

Characteristic	Nedocromil sodium group (n = 16)	Albuterol group (n = 16)
Age (yr)*	45.6 (23.6-57.2)	35.7 (19.0-58.3)
Sex (men/women)	10/6	8/8
Duration of asthma (yr)*	5.0 (2-44)	7.5 (1-30)
Asthma severity		
Aas ⁹ 5-point scale score	2.0 (1-4)	2.0 (1-2)
Mild/moderate/severe/very severe	5/10/0/1	6/10/0/0
Presence of atopy†	6	10
Presence of seasonal variation (from case histories)	6	6
Asthma medication in past year		
Inhaled β_2 -agonists	16	14
Theophylline	6	7
FVC (% of predicted)*	100.7 (62-124)	95.0 (77-165)
FEV ₁ (% of predicted)*	75.0 (32-104)	74.0 (41-122)
PD ₁₅ (mg histamine)*	0.03 (0.03-0.52)	0.04 (0.03-0.5)

*Values are medians and range in parentheses.

†Determined by skin prick testing with 12 common allergen extracts.

TABLE II. Results of pulmonary function tests in nedocromil sodium (n = 16) and albuterol (n = 16) groups

	Baseline	Week 8	Week 12	Week 8 vs baseline	Week 12 vs baseline
FEV ₁ (L)					
Nedocromil sodium	2.60	2.77	2.76	0.17	0.16
Albuterol	2.97	3.11	3.26	0.15	0.29
Mean difference (SE)	-0.37 (0.296)	-0.34 (0.349)	-0.50 (0.330)	0.02 (0.203)	-0.13 (0.197)
95% CI	-0.97, 0.24	-1.05, 0.37	-1.17, 0.17	-0.39, 0.44	-0.54, 0.27
p Value	0.22	0.33	0.14	0.91	0.23
Forced vital capacity (L)					
Nedocromil sodium	3.78	4.01	4.03	0.23	0.25
Albuterol	4.16	4.21	4.42	0.04	0.26
Mean difference (SE)	-0.38 (0.350)	-0.10 (0.364)	-0.39 (0.399)	0.19 (0.154)	-0.01 (0.182)
95% CI	-1.10, 0.33	-0.94, 0.55	-1.21, 0.42	-0.13, 0.50	-0.38, 0.36
p Value	0.28	0.60	0.33	0.23	0.28
PEF (L/min)					
Nedocromil sodium	406	410	409	3	2
Albuterol	447	454	477	6	29
Mean difference (SE)	-41 (34.3)	-44 (36.6)	-68 (37.3)	-3 (13.9)	-27 (21.0)
95% CI	-111, 30	-119, 31	-144, 31	-31, 25	-70, 15
p Value	0.25	0.24	0.08	0.82	0.20

CI, Confidence interval; SE, standard error.

0.05 was used to determine the presence of a treatment effect.

RESULTS

Patients

Thirty-two adult patients were included in this study. The two treatment groups were consid-

ered similar for all demographic, baseline clinical (Table I), and histologic variables. Most patients had mild or moderately severe asthma as assessed by a specialist in pulmonary medicine on the Aas⁹ five-point scale. The patients demonstrated 15% to 104% (median 24%) reversibility of FEV₁ in response to inhalation of

TABLE III. Bronchial hyperresponsiveness (PD₁₅ [milligrams histamine]) in nedocromil sodium (*n* = 16) and albuterol (*n* = 16) groups

	Baseline*	Week 8*	Week 12*	Week 8/ Baseline	Week 12/ baseline
Nedocromil sodium	0.034	0.072	0.055	2.09	1.61
Albuterol	0.068	0.099	0.225	1.45	3.31
Mean ratio (nedocromil/albuterol)				1.44	0.49
95% CI				0.42, 4.87	0.14, 1.73
<i>p</i> Value				0.55	0.26

CI, Confidence interval.

*Geometric means. Analysis completed on log_e-transformed data.

TABLE IV. Mucosal inflammatory cell counts (median number of cells per square millimeter) in nedocromil sodium (*n* = 14) and albuterol (*n* = 13) groups

	Baseline	Week 12	Week 12 vs baseline
Activated eosinophils			
Nedocromil sodium	76.0	42.9	5.1
Albuterol	86.2	116.3	15.0
Median difference (SE)	-10.2 (34.6)	-73.4 (41.6)	-9.9 (48.1)
95% CI	-81.4, 61.2	-159.0, 12.2	-109.0, 89.2
<i>p</i> Value	0.77	0.09	0.84
Mast cells			
Nedocromil sodium	142.2	111.4	-6.6
Albuterol	103.7	95.6	-17.4
Median difference (SE)	38.5 (38.6)	15.8 (51.0)	10.8 (73.6)
95% CI	-40.9 117.9	-89.3, 120.9	-140.7, 162.4
<i>p</i> Value	0.33	0.76	0.88
T lymphocytes			
Nedocromil sodium	1023.1	651.0	-270.4
Albuterol	899.6	556.5	-147.3
Median difference (SE)	123.6 (229.1)	94.5 (207.1)	-123.1 (137.9)
95% CI	-348.2, 595.3	-331.9, 520.9	-407.2, 160.1
<i>p</i> Value	0.59	0.65	0.38

CI, Confidence interval; SE, standard error.

Untransformed data. Analyzed by nonparametric (Wilcoxon) test.

albuterol 0.2 mg. All patients were hyperresponsive, as verified by histamine challenge.⁷

Two patients in the nedocromil sodium group and three in the albuterol group withdrew after randomization. Reasons for withdrawal in the nedocromil sodium group were failure to comply with the protocol and worsening of asthma with use of disallowed medications. In the albuterol group, patients withdrew because of worsening of asthma, laboratory values significantly outside the normal range, and lack of compliance with the protocol. The patients who withdrew did not differ demographically from those who completed the study.

BHR and lung function

Neither nedocromil sodium nor albuterol caused any statistically significant change in FEV₁, forced vital capacity or PEF, or BHR (Tables II and III), and there were no significant differences between treatments for these variables.

Inflammatory cell counts

All bronchial biopsy specimens were of good quality except for those from one patient in each treatment group that lacked sufficient epithelium for assessment. The T-lymphocyte count decreased significantly (*p* = 0.026) with nedocromil sodium treatment (Table IV and Fig. 1). A similar trend in

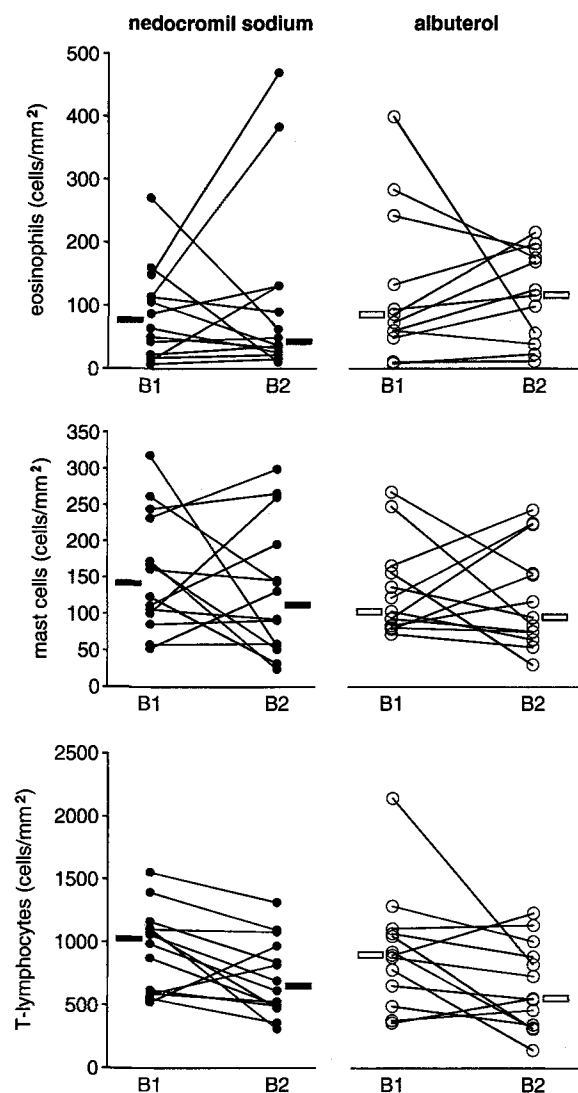


FIG. 1. Effect of treatment with nedocromil sodium or albuterol on inflammatory cell numbers in bronchial biopsy specimens taken before (B1) and after (B2) 12 weeks of treatment. Data shown for individual patients. Bars, Median values.

the albuterol group did not reach significance. However, the numbers of patients showing a decrease were similar in the two groups, and the between-group difference was not significant. The number of activated eosinophils and mast cells did not change significantly during treatment within either group when compared with pretreatment values. The two treatments did not differ significantly for changes in any cell type in either the epithelium, lamina propria, or entire mucosa.

DISCUSSION

The results from this study for inflammatory cell counts and for BHR show some variability both within and between patients. This variability is demonstrated by the big size of the 95% confidence intervals for the between-treatment comparisons.

The current study showed a statistically significant reduction in the number of T lymphocytes in the bronchial mucosa when patients were treated with nedocromil sodium. A direct action of nedocromil sodium on T cells has not been shown previously. Nedocromil sodium inhibits the release of IL-8¹⁰⁻¹³ and tumor necrosis factor- α from bronchial epithelial cells^{10,13} and decreases tumor necrosis factor- α secretion by mast cells.¹⁴ It could be speculated that these changes may lead to down-regulation of tissue T lymphocytes because these cytokines appear to enhance T-cell chemotaxis¹⁵ and proliferation.¹⁶ Studies based on experimental models, however, have shown that nedocromil sodium fails to inhibit the proliferation of human cultured T lymphocytes¹⁷ or to protect guinea-pig lungs from T-cell infiltration.¹⁸ A recent immunohistochemical study in patients with asthma was also unable to demonstrate any significant differences between nedocromil sodium or albuterol in their effect on T lymphocytes (CD3⁺, CD4⁺, CD8⁺ or CD25⁺ cells), either in the epithelium or in the submucosa.⁴ However, in the current study there was also a nonsignificant reduction in T-cell number in the albuterol group, and there was no difference between the two treatments in the effect on T cells.

The eosinophil counts were not significantly reduced in either group, and there was no difference between the groups despite a trend toward a decrease with nedocromil sodium and an increase with albuterol. Previous results⁴ have shown a significant reduction of eosinophil number in the bronchial mucosa after 16 weeks of treatment with nedocromil sodium when compared with albuterol, although both that study and the current one showed no difference between treatment groups in mast cell count. As mentioned earlier, particularly for BHR the ability to detect treatment differences in the current study was limited by the large variability in results.

This study showed no statistically significant differences between treatments on BHR or lung function. Although some studies have also failed to show significant differences between the effects of treatment with nedocromil sodium and a β_2 agonist on these variables,^{4,19,20} other studies have

found treatment with nedocromil sodium to be significantly more effective than treatment with albuterol.^{21, 22} It is possible that the discrepancy in these results may be due to differences in patients' baseline characteristics. However, when patient data from recent studies^{21, 22} are compared with ours, the patients' age, duration of asthma, severity of asthma, and presence of atopy do not seem to be factors in determining the clinical response to nedocromil sodium.

The guidelines laid out in the Global Initiative for Asthma¹ recommend inhaled nedocromil sodium in the daily treatment of mild persistent asthma, the clinical features of which include FEV₁ or PEF $\geq 80\%$ of the predicted value with a variability of 20% to 30% and with the occurrence of asthma symptoms more than once per week but less than every day. Indeed, many patients in our study did not fulfil any of these criteria because they had more severe asthma at baseline. In particular, 20 (63%) patients had a baseline FEV₁ $< 80\%$ of the predicted value and 27 (84%) had a diurnal variability in PEF of $< 20\%$. Therefore, we separately analyzed data from a subgroup of patients who at the time of random group assignment had an FEV₁ $> 60\%$ of the predicted value. These patients did not differ from the whole study population for age, asthma duration, symptom scores, PEF and PEF variability, BHR, or the presence of atopy. This supplementary analysis, although reduced in power by the lower patient number, suggests that the efficacy of nedocromil sodium is not necessarily determined by asthma severity.

In conclusion, our immunohistochemical study does not appear to support the concept of nedocromil sodium as a potent antiinflammatory drug because we were unable to show any significant superiority of treatment with nedocromil sodium compared with treatment with the β_2 agonist albuterol. It remains possible that differences would be detected in different conditions, for example in a more homogeneous group of patients or with a longer treatment period or in a study with greater power and lower variability. To endorse the recommendation of nedocromil sodium as basic antiinflammatory therapy of asthma, however, we require more exact knowledge about relevant indications, which in turn would require more intensive investigation of the effect of nedocromil sodium on asthmatic airway inflammation.

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DISCUSSION

R. Djukanović. I very much liked the study design used by Manolitsas et al. (*Am J Respir Crit Care Med* 1995;151:1925-30), and one of the very important features that should not be underestimated with nedocromil sodium and other drugs is their ability to attenuate the rises in inflammatory markers. They may not be as potent as corticosteroids, but the latter, while extremely potent, are not without possible side effects. Studies showing that cells do come in and become activated either after allergen challenge or after withdrawal from treatment are well worthwhile. I would like to ask Robert Davies whether any of the patients in the Manolitsas et al. study were asked to stop taking inhaled corticosteroids 2 or 3 months before the study.

R. J. Davies. They had never taken any antiinflammatory treatment.

R. Rodriguez-Roisin. The patients in both studies had a wide range of FEV₁ values. Did the data show a different trend if you differentiated between those with normal and those with low FEV₁ values?

A. Laitinen. It made no difference if we analyzed only those patients with FEV₁ values above 60% or 70%. However, we did have one exceptional patient whose eosinophils increased dramatically with treatment, and

he had an FEV₁ of only 32% of predicted, and at the trial end, his PD₁₅ was 0.025 mg of histamine.

L. M. Fabbri. Laitinen et al. (*J Allergy Clin Immunol* 1992;90:32-42) have shown previously that terbutaline may reduce the number of lymphocytes in the airway mucosa of asthmatic persons. Thus, I wonder whether there might be a molecular difference between terbutaline and albuterol because the Manolitsas et al. study has shown that albuterol may increase the number of eosinophils.

J. C. Virchow. With respect to the similarity or not of terbutaline and albuterol, Newhouse did a study recently in rabbits. He showed that oleic acid, which is an adjunct to albuterol, can cause airway damage (*Am J Respir Crit Care Med* 1994;150:790-4).

R. Aalbers. Is it possible that by treating our patients we could induce asthma? If treatment with albuterol alone could be increasing the number of eosinophils, and if this is an important aspect of inflammation in asthma, then it could be that by treating our patients with mild or early asthma only with albuterol, we induce asthma.

R. J. Davies. I am rather inclined to agree with you.

L. M. Fabbri. I have a problem with Davies' suggestion that nedocromil sodium may be very effective in the epithelium and Annika Laitinen's suggestion that we should consider the whole tissue. The rationale for us to look separately at the epithelium, the lamina propria, and the submucosa was to see whether there was heterogeneity, which in fact there was (Saetta et al. *Am Rev Respir Dis* 1992;145:160-8). For example, mast cells may be increased in the epithelium but not in the submucosa, if nedocromil sodium is highly active in the epithelium. I would be interested to see the results of analyses by Davies et al. in the epithelium.

A. Laitinen. We analyze the whole biopsy sample by counting the epithelium and lamina propria area separately. Our computer expresses the results in the epithelium and the lamina propria separately and also counts the results put together. The reliability of epithelial results depends on the quality of the biopsy and the amount of epithelium present in the biopsy sample. If we have epithelium that is at least half the length of a biopsy sample then it can be analyzed.

R. J. Davies. We count the cells in a very similar way. We measure the whole area of the biopsy; we split it into the epithelium and the lamina propria and we count by eye the number of cells throughout the whole biopsy sample. I would like to stress again, from the in vitro studies that I showed you, that we might not expect a particularly dramatic effect from nedocromil sodium unless we looked over a period of time longer than 4 months. We would like to look at studies of more than a year or two, although it would be impossible to have people receive no treatment for 2 years. I do not know how to answer the question about albuterol, except to say that our results are consistent throughout. It is possible that there is some irritant effect on the epithelium.

S. T. Holgate. This issue of an increase in inflammation with β -agonists has been going on for a number of years now. I have been very impressed with the work from Barnes's laboratory on activator protein-1 and more recently some of the work from Kay on cAMP response element binding protein. It would appear that β -agonist exposure over prolonged periods can reduce the ability of a cell to respond to a corticosteroid because of competition at the level of transcription factors, probably in the cytoplasm. Fan Chung might like to comment on that because his group has been involved.

K. F. Chung. Yes, you can certainly demonstrate the effect in vitro on various cell types, including epithelium. High concentrations of β -agonists appear to inhibit the effect of steroids, for example loss of inhibition of the stimulated release of IL-8. It may be that in vivo this relates to high doses of inhaled β -agonists that impinge on the epithelium as the aerosol deposits. That leads me to the question I would like to ask Robert Davies. His in vitro data show that nedocromil sodium inhibits the stimulated release of various cytokines from the epithelium. Does that occur at the level of transcription? In other words, do you find that the messenger RNA is inhibited? Just as β -agonists can interfere with steroid effects, maybe β -agonists could also interfere with the effects of nedocromil sodium, perhaps through transcription factors?

R. J. Davies. We have not measured messenger RNA so I do not have an answer to that, although it may well affect messenger RNA. We shall hear more about this, but it is through the chloride channels that there is activation of the cells, which in the end will lead to changes in transcription.

K. F. Chung. Did nedocromil sodium have any effect on the other variables that were measured in the study?

R. J. Davies. No, and again this is similar to the preliminary results that Annika Laitinen has shown. We did not show any significant effect on mast cell numbers, lymphocyte numbers, or basement membrane thickness or a significant change in airway hyperresponsiveness. We also looked at peak flows, and evening peak flows were higher in the group receiving regular albuterol group.

S. E. Wenzel. You started out with a baseline FEV₁ very close to normal, around 90%. Perhaps part of the problem is that you are starting from a very high baseline, and you are just not able to see improvement.

L. M. Fabbri. The study presented by Manolitsas et al. (Am J Respir Crit Care Med 1995;151:1925-30) showed no effect of nedocromil sodium—no reduction of symptoms and bronchial responsiveness and no improvement in lung function. I would suggest that a better strategy for clinical trials with nedocromil sodium might be to take the patients to their best possible clinical condition with an aggressive treatment, for example with steroids, and then to measure the effect of nedocromil sodium on keeping (rather than putting) asthma under control. The preclinical studies suggest that nedocromil sodium may

prevent rather than reverse inflammation. Prevention of exacerbations is one of the major aims of asthma treatment (National Institutes of Health: Global initiative for asthma. January 1995: publication 95-3659).

R. Pauwels. When we look at the results of studies we tend far too much to look at the means of different variables rather than looking at what percentage of patients in which the disease is actually controlled, as we define it, and is not exacerbated. For the patient with asthma what is actually very important is that the disease is controlled and does not fluctuate too much.

J. P. Seale. During the albuterol arm of the study the BHR did not change at all and the peak flow rates increased. Should we still be worried about the patients whom we never see in our clinics, who still use albuterol? Our current guidelines indicate that it is probably all right for patients with very mild disease to be taking β -agonists. In your study, despite the increase in eosinophils in the 4-month study period, there did not seem to be any alarming symptomatic or functional changes.

R. J. Davies. I would suggest again that, if we are going to do more in the way of these studies, we ought to be looking at a much longer period of time. We need to know whether there are more fundamental changes over 1- to 2-year periods. What this study has done for me is to alter the way that I look at people with asthma who are taking regular β -agonists. My policy now is not to allow people to take regular β -agonists, and this is the group that really should be starting to take nedocromil sodium.

S. T. Holgate. I wonder whether the study by Calhoun et al. (J Allergy Clin Immunol 1996;98:S46-50) missed an opportunity to detect an effect on the late response by studying the airways at 48 hours? We have compared lavage and biopsy findings at 24 hours after segmental antigen challenge, and we found that by that time, apart from an increase in IL-2 receptor expression, the lung tissue on biopsy was almost devoid of the influx of eosinophils, neutrophils, and so on, whereas in lavage fluid the eosinophilia was very prominent. It is as if by 24 hours the airway had cleaned itself out. What we saw at 24 hours, and you may be looking at 48 hours, is the residuum of something that happened some time before. If you had studied this at, say, 6 hours, when the late reaction was evolving rather than when it had completed, you might have had rather stronger findings.

W. J. Calhoun. I agree with you about the efflux of eosinophils from blood vessels through the interstitium and into the airway, but the kinetics of eosinophil recruitment after segmental antigen challenge are such that, at least in the bronchoalveolar lavage fluid, it has not peaked by 48 hours. We selected 48 hours because we were looking for a maximal signal in the bronchoalveolar lavage fluid, and we knew that at 24 hours the recruitment of eosinophils was significantly less than it would be at 48 hours. We felt that if we were looking for an effect of reduction of eosinophils then should choose the time period with the biggest signal. In our studies in

which we have compared biopsy samples of the bronchus with bronchoalveolar lavage findings, we have not found a very impressive relation. There may actually be something quite different about the basement membrane and epithelium in asthma compared with the basement membrane and epithelium in atopy without asthma, in terms of the ability of the epithelium to trap the eosinophils in a site where they can do damage.

A. A. Norris. The release of granule mediators is a later event that could well take place at a time when there is very little drug present, so you might expect to see not an effect on granule mediators but an effect on eosinophils, which correlates quite nicely with what we know about the effect of the drug on mast cell activation. The effect on the eosinophilia might be explained by the ability of the drug to prevent IL-5 release, which we have seen in preliminary studies on human lung mast cells. It might be interesting to give the drug a second time, perhaps at 24 or 36 hours, and see if it has an effect on granule release.

E. W. F. W. Alton. Could you speculate on why you

recovered an increased volume of fluid with nedocromil sodium?

W. J. Calhoun. It has to do with the degree to which histamine release was blocked. Histamine causes mucosal edema, which can reduce volume returns dramatically.

R. Djukanović. Perhaps we now need to redefine the terms "prophylactic" and "antiinflammatory," which we use rather loosely. We have been using these terms to mean basically the same, which may not necessarily be the case. There are data forthcoming to show that drugs like nedocromil sodium and theophylline, which, while they may not be antiinflammatory in the sense that they decrease the eosinophils in ongoing asthma, may prevent the surges in cellular inflammation. One study recently conducted by Doull in Southampton looked at the effect of inhaled corticosteroids on asthma exacerbations in children. To our great surprise they did not have any effect. This is not conclusive, but it is stimulating enough for us to rethink the way in which we use terms "prophylactic" and "antiinflammatory," and we need to design trials with that in mind.