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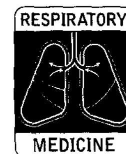

Original Articles

Regular albuterol or nedocromil sodium — effects on airway subepithelial tenascin in asthma

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Both albuterol and nedocromil sodium have been recognized to possess certain anti-inflammatory properties. However, there are no data on the impact of these drugs on the pathophysiology of the bronchial extracellular matrix in asthma characterized by enhanced tenascin (Tn) expression, known to occur proportional to the severity of asthma. This paper reports data from a morphometric study on the effects of regular treatment with inhaled albuterol or nedocromil sodium on the extent of bronchial subepithelial deposition of Tn, collagen types III, IV, and VII and mucosal infiltration with macrophages.

Thirty-two patients (14 women) with chronic asthma, aged 38·7 years (median) with a median forced expiratory volume in 1 sec (FEV_1) of 74·4% predicted, were selected to undergo fibre-optic bronchoscopy with bronchial biopsies before and after 12 weeks of treatment with either inhaled albuterol 0·2 mg or nedocromil sodium 4 mg four times daily according to a double-blind protocol. Cryostat sections of the biopsy specimens were studied by indirect immunostaining techniques using monoclonal antibodies and computer-assisted quantitative image analysis.

Albuterol treatment significantly reduced the median thickness of subepithelial Tn expression from 9·7 to 6·3 μm (P=0·023) and macrophage numbers in the epithelium (P=0·034), lamina propria (P=0·039) and entire mucosa (P=0·033), whereas nedocromil sodium had no effect. Expression of the collagen types was not affected by either treatment. There was no identifiable statistical difference between the two treatments for any of the outcome variables measured. Nevertheless, the results demonstrate that even a short-acting β_2-agonist may exert anti-inflammatory potential sufficient to interfere with the basic mechanisms of asthma as shown by reduction of subepithelial Tn content and mucosal macrophage count.

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Introduction

The recognition of chronic inflammation as the underlying pathological basis of asthma has advocated the regular use of anti-inflammatory therapy (1). Both β_2-adrenoreceptor agonists and nedocromil sodium are known to exert certain anti-inflammatory effects. However, based on available clinical experience and published information, various guidelines for asthma management (1,2) support the use of short-acting β_2-agonists for symptomatic relief on an as-needed basis only because the regular administration of these drugs has resulted in increased non-specific bronchial responsiveness (3), enhanced late response to inhaled allergen (4), loss of asthma control (5) and an increased risk of asthma death (5). Nevertheless, numerous studies have demonstrated that the β_2-agonists, in addition to directly inducing relaxation of bronchial smooth muscle and inhibiting of vascular permeability, may also benefit patients with asthma by inhibition of release or synthesis of pro-inflammatory, profibrogenic, and spasmogenic mediators from the lung (6). This is achieved by their action at β_2-adrenoreceptors on particular human cell types, resulting in inhibited release of histamine, prostaglandins, and leukotrienes from mast cells (6), inhibited generation of tumour necrosis factor-α (TNF-α) (7) and interferon-γ (IFN-γ) (8,9) by monocytes and B-lymphocytes, and depressed expression of platelet-derived growth factor (PDGF) mRNA from monocytes and alveolar macrophages (10).

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On the other hand, nedocromil sodium, an anti-inflammatory agent without direct bronchodilating properties, appears to be effective in ameliorating the clinical measures of asthmatic airway inflammation without significant side-effects (11) and is therefore recommended for daily treatment of mild persistent asthma (1). The anti-inflammatory activities of nedocromil sodium, a disodium salt of a pyranoquinoline dicarboxylic acid, have been suggested to relay on blocking cell membrane chloride channels (12). This leads to inhibition of mediator release from a range of cell types involved in the pathogenesis of asthma: eosinophils (13), T lymphocytes (14), B lymphocytes (15), mast cells (16), monocytes (17), macrophages (17), platelets (17) and bronchial epithelial cells (18).

A morphological approach, based on bronchial biopsies, provides the most reliable assessment of local pathophysiological processes at the site of asthmatic inflammation (19) as well as demonstrating the effects of drug therapy (20). Previously, we have shown a deposition of heightened amounts of tenascin (Tn) in the subepithelial reticular basement membrane (BM) of asthma patients (21,22). The extent of Tn expression was proportional to the severity of asthma and could be reduced with inhaled corticosteroid treatment (22). Only a limited number of biopsy studies using albuterol or nedocromil sodium have been performed on asthma patients. In a 16-week study by Manolitsas *et al.* (23), treatment with nedocromil sodium was associated with a decrease in airway infiltration with eosinophils, whereas an increase occurred among the albuterol-treated patients so that the change in eosinophil count was significantly different between the active treatments. Our recent 12-week trial also revealed a trend toward a decrease in the number of EG2-positive eosinophils with nedocromil sodium and an increase with albuterol, but without a statistical difference between the treatments (24). In contrast to the report of Manolitsas *et al.* (23), we demonstrated a significant reduction of T lymphocytes in the nedocromil sodium group, although without a concomitant change in the albuterol group (24). We were not able to detect any changes in mast cell numbers and, as in the study by Manolitsas *et al.* (23), no between-treatment differences were detected in lymphocyte and mast-cell numbers.

As for macrophages, the impact of β_2-agonists or nedocromil sodium on the inflammatory reaction of the airway extracellular matrix (ECM) with enhanced Tn content and collagen deposition remains unknown. In addition, a close association between macrophage-derived profibrogenic cytokines and the fibroproliferative response (25) which leads to subepithelial fibrosis and remodelling of the bronchial ECM makes the knowledge of the potential effects of these drugs on airway macrophages a valuable adjunct in validation of their position in the management of asthma. Here, we report separately unpublished results obtained from our recently implemented protocol (24) in order to emphasize the effect of treatment with albuterol or nedocromil sodium on the subepithelial expression of Tn and collagen types and on mucosal macrophage numbers in patients with chronic asthma.

Methods

STUDY DESIGN

The study was a part of our previously reported, randomized, double-blind, group comparative study conducted as a co-operative investigation between the Departments of Medicine, University Central Hospital, Helsinki, Finland and University of Tartu, Estonia (24). Briefly, the protocol included patients of either gender, aged 18 years or older, with an established diagnosis of asthma for at least 6 months. Entry criteria required the presence of asthma symptoms of a certain severity, defined as a total symptom score of at least 10 for any 10 days during a 2-week baseline period. The total symptom score was defined as the sum of four symptoms, including daytime symptoms, night-time symptoms, morning chest tightness and cough, each rated on a five-point scale (from 0=absence of symptoms to 4=very severe). All patients had to have a provocative dose causing a reduction of 15% in forced expiratory volume in 1 sec (FEV_1) ($PD_{15}FEV_1$) of less than 1·6 mg histamine to verify their bronchial hyper-responsiveness (26). In addition, each patient had to demonstrate reversibility of airways obstruction of at least 15%, measured by FEV_1 in response to an inhaled bronchodilator. Alternatively, patients were required to have documented evidence of at least 20% variability in peak expiratory flow rate (PEFR) taken daily over a 7-day period. Systemic or inhaled corticosteroids, nedocromil sodium, ketotifen and oral bronchodilators were withdrawn 6 weeks before entry into the trial.

After a 2-week baseline period, during which the patients were continued on their allowed therapy, eligible patients were randomized using computer-generated sequentially coded study numbers to receive either 4 mg nedocromil sodium (2 mg dose^{-1}, two inhalations) four times daily (q.i.d.), or 0·2 mg albuterol (0·1 mg dose^{-1}, two puffs; Ventoline®, Glaxo Operations, London, U.K.) q.i.d. for the next 12 weeks. Both drugs were provided by Fisons Plc. (Loughborough, Leicestershire, U.K.) and delivered from coded metered dose inhalers.

Patients visited the clinic at the start of the baseline period, at randomization and 4, 8 and 12 weeks after treatment. Bronchial biopsies were taken at the beginning and end of treatment (at visits 2 and 5). On each visit, the investigator examined the patient and checked his or her inhalation technique to ensure adequate delivery of the study drug. Patient compliance was assessed by having patients record their use of the test medication on the diary card and by weighing the returned aerosol canisters.

During the treatment phase, all patients had access to rescue therapy with inhaled albuterol (0·1 mg dose^{-1}) on an as-needed basis. Other permitted medications included oral prednisolone, oral or intravenous bronchodilators, antihistamines and antibiotics. If oral prednisolone was needed, according to protocol, it was given at a daily starting dose of 30 mg, followed by a decrease in dose of 5 mg each day. Antihistamines, nasal disodium cromoglycate (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 concomitant medications were recorded by the patients on the diary card.

The research protocol was approved by institutional ethical review committees at both centres, and all patients provided written informed consent.

BRONCHOSCOPY AND BRONCHIAL BIOPSIES

Bronchoscopic examination and collection of biopsy samples conformed to the international guidelines for investigative use of fibre-optic bronchoscopy in asthma and other airway diseases (27). All patients were premedicated with intravenous atropine sulphate 0·5–1 mg and diazepam 5–10 mg. After local anaesthesia of the oropharynx and vocal cords with lidocaine spray (Xylocain®, Astra Draco AB, Södertälje, Sweden) and lidocaine 2% solution (Orion OY, Espoo, Finland), an Olympus BF-20 fibre-oscope (Olympus Optical Co., Tokyo, Japan) was introduced into the right bronchial tree, where the bronchi were anaesthetized with lidocaine 2% solution instilled through the bronchoscope channel. Three bronchial biopsies were taken by the same bronchoscopist at each bronchoscopy: one from the right middle and two from the right upper lobe bronchi. The regions very close to carinae and those previously touched by the bronchoscope were avoided. At the post-treatment bronchoscopy, the particular biopsy sites utilized at the pre-treatment bronchoscopy were also avoided. All specimens were obtained with Olympus FB-19C forceps.

LABORATORY METHODS

Sample processing and immunohistochemistry

Biopsy specimens were immediately snap-frozen in liquid nitrogen (Estonian Academy of Science, Tartu, Estonia) and stored at -70°C until sectioned. After embedding in Tissue Tek ornithyl-carbamyltransferase (O.C.T.) medium, 5-μm serial sections were cut on a Leitz 1720 Digital Cryostat (Ernst Leitz GmbH, Wetzlar, Germany). Immunostaining with a mouse monoclonal antibody (mAb) 100EB2 reacting with the fourth and fifth fibronectin-like domains in Tn-C molecule (28) was used to detect Tn. An IgG2a mAb against human type III collagen (1:50, HEYL Vertriebs GmbH, Iserlohn, Germany), an IgG2b mAb which recognizes human type IV collagen (1:50, Boehringer Mannheim GmbH, Mannheim, Germany), and an IgG1 mAb against the carboxy-terminus and the major helical domain of human type VII collagen (1:1000 Gibco BRL, Gaithersburg, MD, U.S.A.) were applied to label the respective collagen types. The sections were fixed in pre-cooled acetone at -20°C for 10 min, exposed first to the primary mAb at room temperature for 30 min, washed in phosphate-buffered saline (PBS) and exposed to 1:50 diluted fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA, U.S.A.). Finally, the sections were mounted in sodium-veronal-buffered glycerol (1:1, pH 8·4) and examined under a Leitz Aristoplan fluorescence microscope equipped with an appropriate filter system for FITC fluorescence. In order to label macrophages, the mAb Ber-MAC3 (1:50, Dako A/S, Glostrup, Denmark) was used. This mAb recognizes CD163, a 130-kDa macrophage-associated integral membrane protein of the scavenger receptor superfamily, and enables distinguishing tissue macrophages from non-activated monocytes, starry sky macrophages, epithelioid macrophages, and multinucleated macrophages (29,30). Before our core experiments, the reactivity of this antibody was tested on cryosections of peripheral lung where it was supposed to stain alveolar macrophages. After fixation in acetone at room temperature for 10 min, the samples were exposed to the primary antibodies for 30 min and then processed by the alkaline phosphatase anti-alkaline phosphatase technique (Dako). The specific immunoreactivity was visualized by using a substrate solution containing naphthol AS-BI phosphate, levamisole, and new fuchsin (all from Sigma Chemical Co., St. Louis, MO, U.S.A.) reacted with sodium nitrite (E. Merck, Darmstadt, Germany). Finally, the slides were mounted in Dako Glycergel and examined under a Leitz Dialux 22 EB light microscope.

Quantification of the results

As previously described (21,22) for quantitating ECM proteins, the cross-sections of the BM area containing positive immunoreactivity were photographed with Kodak T-MAX 400/800 black-and-white film (Eastman Kodak Co., Rochester, NY, U.S.A.) at a preliminary magnification of $\times$ 80 with care to avoid sections where the BM zone was not cut perpendicularly. Paper photocopies were reproduced to reach the final magnification of $\times$ 643 for Tn and type III collagen and $\times$ 1310 for type IV and VII collagen. The ECM proteins were quantified by semiautomatic drawing of the margins of specifically stained areas of the BM from photomicrographs with a properly calibrated Kurta IS/THREE digitizing tablet (Kurta Corp., Phoenix, AZ, U.S.A.) and a pointing device linked to a computer. With use of the AutoCad program, version 10.0n, (Autodesk Inc., Sausalito, CA, U.S.A.), minimal distances were calculated between each point on the superficial limit and the closest point on the deeper border of the specific immunoreactivity in the BM. The mean value was considered as the thickness of expression of the ECM protein.

Mucosal macrophages were counted using the method we recently reported for quantitating eosinophils, mast cells and T lymphocytes (24). Briefly, the cells were counted from the whole biopsy sections photographed on Kodak EPN 100 colour slide film and projected without image distortion onto a Kurta digitizing tablet to reach a magnification of $\times$ 695. The photomicrograph images containing the bronchial epithelium and lamina propria (LP) with the specifically stained macrophages were charted using the pointing device. Areas where tissue was stretched, squeezed or double-folded were excluded. Macrophage densities were first calculated separately in the epithelium and LP with the AutoCad program and then pooled to express the results for the entire mucosa as well (as cell counts per square millimeter).

TABLE 1. Patient demographic characteristics

Characteristic	Nedocromil sodium group (n=16)	Salbutamol group (n=16)
Age (years)*	45·6 (23·6–57·2)	35·7 (19·0–58·3)
Sex (men/women)	10/6	8/8
Duration of asthma (years)*	5·0 (2–44)	7·5 (1–30)
Asthma severity		
Aas 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
Forced vital capacity (% predicted)*	100·7 (62–124)	95·0 (77–165)
FEV_1 (% predicted)*	75·0 (32–104)	74·0 (41–122)
PD_{15} (FEV_1) (mg histamine)*	0·03 (0·03–0·52)	0·04 (0·03–0·5)

*Values are medians, and range in parentheses.
†From (31).
‡Determined by skin-prick testing with 12 common allergen extracts.

For proper calibration of the tablet, a microscopic scale was photographed and reproduced at the same magnifications as applied to the sections. All measurements were performed at the Department of Anatomy, University of Helsinki, Finland, by the same analyser, who was blinded to the origin of the specimens or treatment status.

STATISTICAL ANALYSIS

Before comparisons, the distributions of all variables were tested for normality with the Shapiro–Wilk test. Due to the relatively low number of patients in the groups and the skewed distribution of the data, resulting in a sufficiently high proportion of variables in which the hypothesis of normal distribution was to be rejected, non-parametric statistics were used. The Mann–Whitney U-test was applied to differences between the treatment groups. Changes within groups (post-treatment *vs.* pre-treatment) were tested using Wilcoxon's signed-ranks test for matched pairs. For each patient, the use of the rescue medication was expressed as the mean number of daily doses of the 14 baseline days and the 14 days prior to each visit up to the end of the study. All statistical hypothesis tests were two-tailed and a value of $P<0·05$ was taken as significant. All analyses were performed using the SPSS package, version 4.0 (SPSS Inc., Chicago, IL, U.S.A.).

Results

PATIENTS

Thirty-two adult patients, aged between 19 and 58 years and with an established diagnosis of asthma with a duration from 1–44 years (median 5·5 years), were included in the study. None of the patients had received inhaled steroids or regular systemic steroids during the previous year. Instead, the patients were maintained on as-needed β_2-agonists alone or in combination with long-acting theophyllines (Table 1). The two treatment groups were considered similar for former use of asthma medication, as well as all demographic, baseline clinical and histological variables. Sixteen patients were atopic, as determined by skin-prick testing with 12 common allergen extracts (ALK, Allergologisk Laboratorium A/S, Hørsholm, Denmark). Most patients had mild or moderately severe asthma (11 and 20 patients, respectively), as assessed by a specialist in pulmonary medicine on the Aas five-point scale (31). The patients demonstrated 15–104% (median 24·3%) reversibility of FEV_1 in response to inhalation of 0·2 mg albuterol.

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 resulting in use of disallowed medication. 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 from the study did not differ demographically from those who completed the study. Of those who completed, none received allowed corticosteroids in any form or nasal disodium cromoglycate during the entire treatment period. Rescue medication with albuterol was used equally in both groups throughout the study and no significant changes occurred in rescue albuterol consumption within any group (Table 2).

TABLE 2. The use of rescue medication during the study in albuterol (n=16) and nedocromil sodium (n=16) groups (doses day^{-1})*

	Baseline	Week 4	Week 8	Week 12	Change	P-value‡
Albuterol	3·58	2·46	2·00	0·58	0·97	0·13
Nedocromil sodium	3·75	2·82	3·29	4·02	2·95	0·31
P-value†	0·55	0·51	0·38	0·09	0·15	

*Presented as medians of variables. For each patient, the use of rescue medication was expressed as the mean number of daily doses of the 14 baseline days and the 14 days prior to each following visit.
†Analysed between groups by the two-tailed Mann–Whitney U-test.
‡Analysed within groups between trial end and baseline using Wilcoxon's signed-ranks test.

MORPHOLOGY AND MORPHOMETRY OF BRONCHIAL BIOPSIES

All bronchial biopsy specimens were of sufficient quality for assessment of Tn expression. In all specimens stained for Tn, an intense immunoreactivity associated with the BM zone could be seen, which continued deeper and joined with staining of connective tissue in the LP. Positive staining was also present in the bronchial epithelium, blood vessels, and smooth muscle. With regard to the distribution of Tn, no visual differences were observed either between the specimens obtained over the course of treatment within each group or between those from the nedocromil sodium- and albuterol-treated patients at the trial end. However, quantitative analysis revealed that the thickness of subepithelial Tn expression decreased significantly (P=0·023) with albuterol treatment (Fig. 1; Table 3). The two treatments, however, did not show significantly different changes in Tn expression.

Diffuse immunoreactivity for type III collagen was localized in the entire BM and continued to the LP, where the staining pattern was more reticular and clearly distinguishable from that visible in the BM. The thickness of the collagen type III layer in the BM did not change significantly during treatment within either group when compared with pre-treatment values. Immunostaining for collagen types IV and VII was seen at the superficial margin of the subepithelial BM. In addition, immunoreactivity for type IV collagen was detectable in vessels, smooth muscle cells and the BM of bronchial glands. In almost all specimens, dot-like bodies positive for collagen type IV, suggestive of the anchoring plaques, were present close to the bottom of the linear subepithelial deposition. The thickness of both collagen types IV and VII was not affected by treatment (Table 3).

The macrophage count decreased significantly in the epithelium (P=0·034), LP (P=0·039) and entire mucosa (P=0·033) when patients were treated with albuterol (Fig. 2). No significant changes occurred in the nedocromil sodium group. The two treatments did not differ significantly with regard to changes in macrophage number in either the epithelium, LP or entire mucosa.

Discussion

The results of this immunohistochemical study have shown for the first time that treatment with the short-acting β_2-agonist albuterol significantly diminishes the thickness of the subepithelially located Tn layer and reduces the number of macrophages infiltrating the airways of patients with chronic asthma. Tn, a large ECM glycoprotein, is known to exert an enhanced expression in tissues during inflammation and tissue repair (32,33). Increased subepithelial Tn deposition has been demonstrated in the bronchi of patients with different types of asthma, in proportion to the severity of the disease (21,22). In addition, the thickness of the

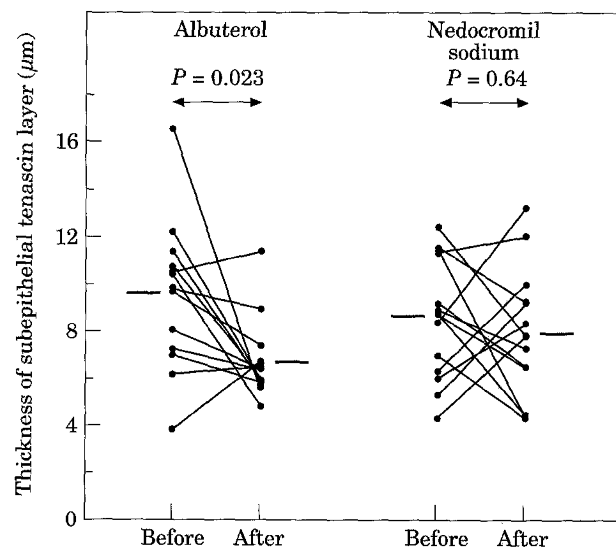

FIG. 1. Effect of treatment with albuterol or nedocromil sodium on the thickness of the subepithelial layer of tenascin in bronchial biopsies taken before and after 12 weeks of treatment. The difference between the two treatments did not reach statistical significance. Wilcoxon's signed-ranks test was applied to evaluate the significance of changes within groups and the Mann–Whitney U-test to estimate the between-treatment differences. Values shown are for individual patients. Bars, median values.

TABLE 3. Changes in thickness of subepithelial immunoreactivity for tenascin and collagen types III, IV, and VII (μm) in albuterol (n=13) and nedocromil sodium (n=14) groups*

	Baseline	Week 12	Change	*P*-value‡
Tenascin				
Albuterol	9·74	6·34	− 1·58	0·02
Nedocromil sodium	8·76	7·77	− 1·92	0·64
Median difference	0·93	− 1·31	− 2·24	
95% CI	− 1·46, 3·34	− 2·78, 0·88	− 4·78, 1·43	
P-value†	0·52	0·16	0·30	
Type III collagen				
Albuterol	8·98	9·58	0·23	0·70
Nedocromil sodium	8·78	9·80	0·85	0·68
Median difference	− 0·01	− 0·36	− 0·71	
95% CI	− 1·52, 1·56	− 1·83, 1·64	− 3·15, 2·49	
P-value†	0·98	0·58	0·72	
Type IV collagen				
Albuterol	2·28	1·80	− 0·23	0·09
Nedocromil sodium	2·29	2·19	0·03	0·51
Median difference	0·02	− 0·39	− 0·36	
95% CI	− 0·52, 0·33	− 0·70, − 0·06	− 0·78, 0·07	
P-value†	0·91	0·01	0·07	
Type VII collagen				
Albuterol	2·67	3·01	0·37	0·08
Nedocromil sodium	3·48	3·36	0·38	0·43
Median difference	− 0·76	− 0·40	0·25	
95% CI	− 1·44, − 0·06	− 1·27, 0·51	− 0·64, 1·27	
P-value†	0·11	0·33	0·65	

*Values are medians; CI, confidence interval.
†Analysed between groups by the two-tailed Mann–Whitney *U*-test.
‡Analysed within groups using Wilcoxon's signed-ranks test.

subepithelial Tn layer has been shown to diminish significantly in response to treatment with inhaled corticosteroids (22). To date, neither a direct nor an indirect action of a β_2-adrenoreceptor agonist on Tn synthesis or degradation has been described, in contrast to the effect demonstrated experimentally with corticosteroids (34). Nevertheless, albuterol inhibits various cell types which secrete pro-inflammatory cytokines. It has been shown to suppress both the release of IFN-γ (8,9) and TNF-α (7) from human blood mononuclear cells and the enhanced expression of PDGF mRNA by alveolar macrophages and adherent monocytes (10). These cytokines are widely expressed in the airways of asthma patients and function as a part of the complex cytokine network, which maintains chronic asthmatic inflammation. The fact that these cytokines also operate as Tn production stimulators (35–37) provides one explanation as to how treatment with albuterol may produce a decrease in the level of Tn expression in asthmatic airways. Analogously, the decrease in macrophage numbers found in all tissue compartments when patients were treated with albuterol may be explained by suppression by the drug via the release of cytokines, particularly IFN-γ, to the site of inflammation. On the other hand, the fact that the macrophages are themselves a major source of cytokines responsible for Tn upregulation (25) raises the possibility that the decrease in Tn thickness and decline in macrophage number are related events, where Tn downregulation is secondary to macrophage decrease and inactivation by albuterol. The importance of the decreased macrophage numbers may also be related to the role of these cells as a potential source of profibrogenic cytokines, which cause the enhanced elaboration of interstitial collagens and subepithelial fibrosis characteristic of asthma (38). Although significant effect was found on the expression of the collagen types with either drug, the present results for Tn and macrophage count support the possibility that treatment with albuterol suppresses, to some extent, the fibroproliferative response and postpones airway ECM remodelling in asthma.

In this study, the reduction of subepithelial Tn and macrophage numbers by albuterol treatment conflicts with the results obtained for EG2-positive eosinophils, mast cells and T lymphocytes (24), which showed a significant decrease in T lymphocyte number in the nedocromil sodium-treated group. This evidence suggests that albuterol and nedocromil may either downregulate different cytokine profiles or suppress certain cytokines to a different extent, resulting in their exertion of inhibitory effects on different inflammatory cells or markers in asthma. Also, in context with the lack of changes in subepithelial Tn in the

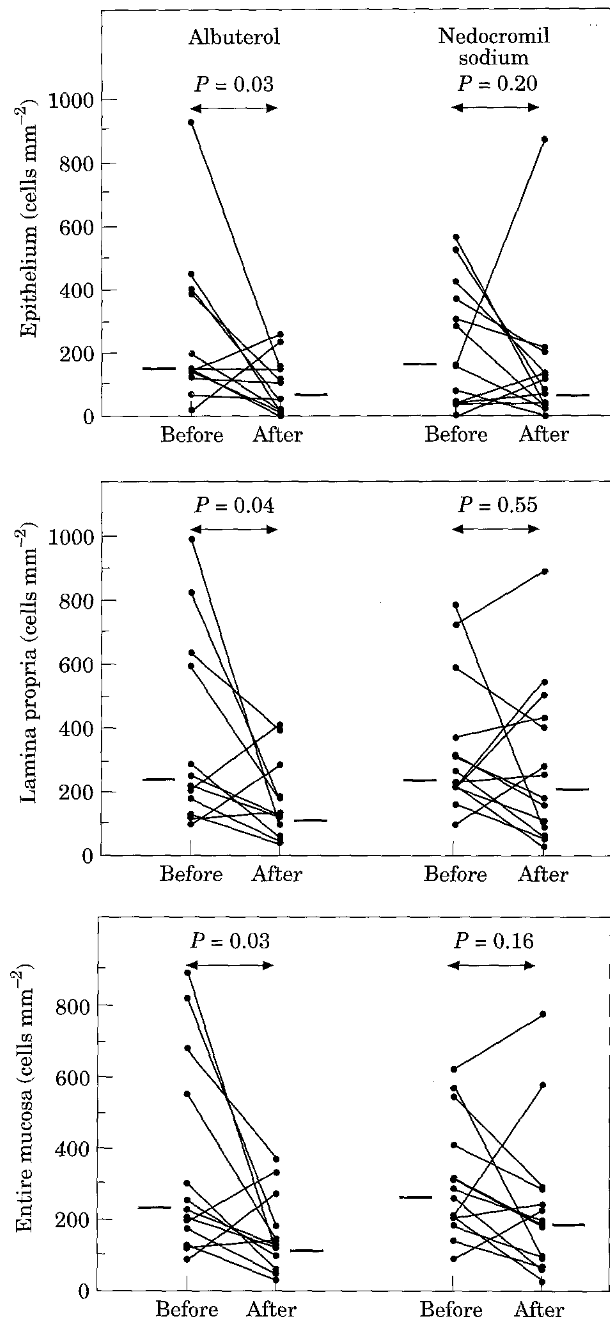

FIG. 2. Effect of treatment with albuterol or nedocromil sodium on macrophage counts in the bronchial epithelium, lamina propria and entire mucosa in bronchial biopsies taken before and after 12 weeks of treatment. Differences between the two treatments did not reach statistical significance. The Mann–Whitney U-test was used to estimate the between-group differences, and Wilcoxon's signed-ranks test was applied to the changes within groups. Values shown are for individual patients. Bars, median values.

nedocromil sodium group analogous to those which occurred with albuterol treatment, or the inability of either drug to affect the collagens, one could speculate that this may be due to an insufficient inhibitory capacity of the drug to limit the release of profibrogenic or pro-inflammatory mediators by different cells. Another possible explanation is the treatment phase duration which was insufficient for significant change to become apparent.

Given that both nedocromil sodium and β_2-adrenoreceptor agonists have certain effects associated with exertion of anti-inflammatory potential in asthmatics, the study was designed to compare only the two treatment regimens for their ability to reduce Tn thickness or macrophage count without involving a placebo group for reference. In a previous biopsy report (23) comparing the effects of albuterol, nedocromil sodium and placebo, the authors, despite finding nedocromil sodium superior to albuterol in the reduction of eosinophilia, did not detect any difference between the active treatments and placebo for eosinophil, mast cell or T-lymphocyte numbers. This lends support to the position that omitting the placebo group does not interfere substantially with the interpretation of the major factors behind our results in the present study. Although not revealing any significant differences between the albuterol and nedocromil sodium groups, this is the first study of the effects of these two treatments on Tn in asthma. It was therefore hard to perform calculations to determine the optimal duration of treatment, sufficient number of participating patients or their asthma severity as an inclusion criterion. In this study, the relatively small number of patients in both groups and the skewed distribution of data, necessitating the use of non-parametric statistics, may have been associated with insufficient statistical power to detect changes caused by both treatments. The present results are unlikely to have been biased by concomitant treatment with rescue albuterol or corticosteroids as the use of rescue medication was relatively low and similar in both groups and as steroids had not been used regularly for 1 year prior to the start of trial and were not used by the patients in any form during the study. The use of steroids was similar in both groups. Another concern is that, because the majority of patients had had asthma for more than 5 years, it is probable that the effects of the short-acting β_2-agonist albuterol and nedocromil sodium on the ECM may, in part, have remained masked by characteristic airway structural remodelling. This being the case, the lack of effects of nedocromil sodium on histological variables noted in the current study needs to be confirmed by more extensive investigations. Principally, the relatively high macrophage numbers currently found in both the epithelium and LP, compared to our former results using electron microscopy in patients with newly detected asthma (19,20), could provide excellent scope for reduction by any of the study drugs. These high pretreatment macrophage counts seem to be true because the epithelial/submucosal macrophage ratio was roughly similar to that found in previous reports (19,20) and the numbers expressed for the entire mucosa are in keeping with the results of other immunohistological studies (22,39).

There were no statistically significant differences between the effects of either regularly administered albuterol or nedocromil sodium on any of the outcome variables studied here. In the light of the reasons discussed above, it remains possible that differences between the active treatments or

those versus placebo would become apparent in studies with greater power based on a longer treatment period and larger and more homogeneous groups of patients. Nevertheless, the primary evidence from this study does not support the concept of nedocromil sodium as an effective anti-inflammatory drug and urges some caution in the recommendation of its use as basic anti-inflammatory therapy for asthma, although the recent guidelines laid out in the Global Initiative for Asthma (1) recommend inhaled nedocromil sodium for the daily treatment of mild persistent asthma.

In conclusion, the results of this immunohistochemical study complement the current knowledge of the spectrum of action of the short-acting β_2-agonist albuterol. The significant reduction of bronchial Tn expression and suppression of mucosal infiltration with macrophages found during albuterol treatment indicate that the drug is not only a directly acting bronchodilator, but may also interfere with the basic inflammatory mechanisms in asthma. This study also suggests a need for further investigation of the effects of nedocromil sodium on the airway ECM and inflammatory cells.

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