Eur Respir J 1997; 10: 858–865
DOI: 10.1183/09031936.97.10040858
Printed in UK - all rights reserved

Copyright ©ERS Journals Ltd 1997
European Respiratory Journal
ISSN 0903 - 1936

The effect of inhaled sodium cromoglycate on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in asthmatics

M. Hoshino, Y. Nakamura

The effect of inhaled sodium cromoglycate on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in asthmatics. M. Hoshino, Y. Nakamura. ©ERS Journals Ltd 1997.

ABSTRACT: There is no direct evidence of the anti-inflammatory effect of inhaled sodium cromoglycate (SCG). To investigate whether inhaled SCG has any effect on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in patients with asthma, biopsies of the bronchial mucosa were taken from nine patients with atopic bronchial asthma before and after treatment with inhaled SCG (8 mg·day^{-1}) from a metered-dose inhaler (MDI).

Eosinophils were stained with anti-EG2, neutrophils with anti-NP57, mast cells with anti-AA1, T-lymphocytes with anti-CD4, CD8 and CD3, and macrophages with anti-CD68. Intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and P-selectin were stained at the same time as adhesion molecules expressed in vascular endothelium. The intensity of ICAM-1 expression in the bronchial epithelium was also evaluated.

The numbers of eosinophils, mast cells, T-lymphocytes and macrophages were significantly reduced as a result of SCG administration, and the expression of ICAM-1, VCAM-1 and ELAM-1 was also significantly inhibited. A significant correlation was found between ICAM-1 expression and T-lymphocytes and between VCAM-1 expression and eosinophils.

It was concluded that sodium cromoglycate does have an effect on the infiltration of the bronchial mucosa by inflammatory cells and also on the expression of adhesion molecules.

Eur Respir J 1997; 10: 858–865.

Dept of Internal Medicine, Saiseikai Yokohama Municipal South Hospital, Japan.

Correspondence: M. Hoshino
Second Dept of Internal Medicine
Toho University School of Medicine
6-11-1 Omori-nishi
Ota-ku
Tokyo 143
Japan

Keywords: Adhesion molecules
anti-inflammation
asthma
bronchial biopsies
inflammatory cells
sodium cromoglycate

Received: July 30 1996
Accepted after revision December 10, 1996

Bronchial asthma is a chronic inflammatory disorder of the airways, in which the bronchial mucosa is infiltrated with activated inflammatory cells, which include eosinophils, mast cells and T-lymphocytes [1]. These inflammatory cells release chemical mediators of inflammation resulting in altered airways physiology, swelling of the mucosa and submucosa, stripping of and damage to the epithelium, thickening of the basement membrane, excessive production of bronchial secretions, and enlargement of the smooth muscle [2]. The migration of inflammatory cells into the bronchial mucosa is, in part, stimulated by chemotactic agents, such as platelet-activating factor (PAF) [3] and leukotriene B_4 (LTB_4) [4]. More recently, it has been shown that the migration and activation of these cells is also controlled by the increased expression of adhesion molecules to leucocytes on epithelium and on vascular endothelial cells. Adhesion molecules have also been demonstrated to have multiple receptor-ligand systems [5, 6], and are thought to play an important role in the migration of cells into selected tissues.

Anti-inflammatory drugs that modify the pathological processes and reverse the histopathological changes are regarded as the most suitable for treating asthma. Corticosteroids, sodium cromoglycate (SCG) and nedocromil sodium are recommended in the therapeutic guidelines for asthma treatment [7] as the first-line anti-inflammatory drugs to be used after the use of intermittent bronchodilators.

Whilst studies have been completed showing the positive anti-inflammatory effects of inhaled corticosteroids [8, 9], no such studies exist with the chromones, sodium cromoglycate and nedocromil sodium. In this study, we have investigated the anti-inflammatory effects of inhaled sodium cromoglycate, and have also investigated the effect of this drug on the expression of adhesion molecules.

Patients and methods

Study subjects

The subjects were nine nonsmoking adult patients with mild-to-moderate allergic asthma, who had never been treated with any anti-inflammatory drug, such as corticosteroids, sodium cromoglycate, nedocromil sodium, or any oral antiallergic drugs. No patient had any signs of upper or lower respiratory disease for at least 2 weeks before entering the study. All patients used inhaled salbutamol as required as the primary treatment

for their asthma, and four patients also used oral theophylline. The dosage of oral theophylline was maintained constant throughout the trial. No drug other than inhaled salbutamol as required was taken during the trial. All subjects demonstrated a 20% or greater increase in forced expiratory volume in one second (FEV_1) or peak expiratory flow rate (PEFR) after the inhalation of a standard dose of a β_2-agonist. All patients had allergic asthma, which was diagnosed on the basis of a positive personal or familial history of atopy, positive skin-prick tests to house dust, house dust mite or other inhaled allergen, or a positive radioallergosorbent test (RAST) (Class 2 or greater) to an inhaled allergen.

Study design

The trial was of open design. Following a 2 week baseline observation period, asthmatic patients underwent flexible bronchoscopy and bronchial biopsy before and after 12 weeks of treatment with inhaled sodium cromoglycate (Fujisawa Pharmaceuticals, Tokyo, Japan), at a dose of 2 mg (2×1 mg) *q.i.d.*. Patients kept a daily record of SCG inhalation, β_2-agonist inhalation, and theophylline usage in their patient diary.

Throughout the baseline and treatment periods, the patients recorded the severity of attacks of asthma, the presence or absence of productive cough, the amount of sputum produced, and effect of the asthma on daily activities and on sleep, using the criteria of asthma severity recommended by the Japanese Society of Allergology [10]. Patients also measured PEFR twice daily using an Assess peak flow meter (HealthScan, Cedar Grove, NJ, USA).

Scoring of symptoms

Five symptom scores were recorded daily on the diary card: 1) the severity of asthma attacks using a graded scale, severe=9, moderate=6 and mild=3; 2) cough, present=1 and absent=0; 3) sputum, large=2 and small=1; 4) the effect of asthma on daily activities, daily activities severely restricted because of asthma=18, daily activities moderately restricted because of asthma=12, daily activities only slightly restricted because of asthma=6, and no restriction of daily activity=0; 5) the effect of asthma at night, unable to sleep because of asthma=9, woke several times during the night due to asthma=6, woke 1–2 times during the night=3, and slept well all night=0.

Functional assessment

Biopsies were taken at the end of the 2 week baseline and after 12 weeks of treatment with sodium cromoglycate. Lung function tests were carried out 1 week before the biopsies were taken. The measurement of airway responsiveness followed the method of TAKISHIMA *et al*. [11]. This uses an Astograph Direct Writing Recorder (Chest Co., Tokyo, Japan), measuring dose-response curves of respiratory resistance (R_{rs}) during continuous inhalation of methacholine at stepwise incremental concentrations. Methacholine hydrochloride in isotonic saline was gradually increased to 49, 98, 195, 390, 781, 1,563, 3,125, 6,250, 12,500 and 25,000 $\mu g \cdot mL^{-1}$. Baseline values were measured after the inhalation of a solution of isotonic saline, then followed after 1 min with increasing concentrations of methacholine hydrochloride. The cumulative dose that had been administered at the point where the reciprocal of the R_{rs} decreased linearly was used as the measure of bronchial reactivity (D_{min}). D_{min} was scaled by a unit equal to 1 min of a 1.0 $mg \cdot mL^{-1}$ aerosol inhalation of methacholine.

Biopsy of bronchial mucosa

On the day of the bronchoscopy and the taking of bronchial biopsies, subjects fasted from 09:00 h. All oral and inhaled drugs were withheld from the evening of the previous day. Premedication was provided by intramuscular injection of 0.5 mg of atropine sulphate and 15 mg pentazocine. When the throat had been anaesthetized by spraying with 4% lidocaine, the subjects inhaled 200 µg of salbutamol sulphate to prevent bronchoconstriction. A bronchoscope (BF type 20; Olympus, Tokyo, Japan) was inserted through the mouth and pharynx, and the trachea and bronchi were anaesthetized with 2% lidocaine. Biopsy forceps (FB15C; Olympus) were then used to collect two biopsies, the first from the branch between the right upper lobar branch and right principal bronchus, and the second from the opening of the right central lobar branch. At the second bronchoscopy, tissue samples were taken from the same site as on the first occasion. On each occasion, the biopsy specimen showing the least damage was selected from the specimens taken from the two sites.

Tissue fixing and staining

The tissue obtained was mounted in ornithine carbamyl transferase (OCT) compound (Miles Co., Naperville, IL, USA), frozen rapidly in dry ice-acetone and kept in a deep freeze at -70°C. Haematoxylin and eosin (H&E) stained specimens were prepared from all tissues. Continuous sections of 4 µm thickness were prepared and mounted on slides, air-dried for 30 min and fixed for 15 min in cold acetone (-20°C). The tissue sections were washed five times with phosphate-buffered saline (PBS) for 5 min, and then treated for 30 min with 10% normal porcine serum.

The following monoclonal antibodies were then added and allowed to react for 60 min: for activated eosinophils, EG2 (Pharmacia, Uppsala, Sweden), which stained eosinophil cationic protein (ECP); for neutrophils, NP57 (Dako Ltd, High Wycombe, UK), which stained human neutrophil elastase (HNE); for mast cells, AA1 (Dako, Ltd), which stained human mast cell tryptase; for T-lymphocytes, CD3, CD4 and CD8 (Becton Dickinson, Cowley, UK); and for macrophages, CD68 (Dako, Ltd). In addition, monoclonal antibodies were used against the following adhesion molecules: intercellular adhesion molecule-1 (ICAM-1) (British Biotech., Abingdon, UK); vascular cell adhesion molecule-1 (VCAM-1) (Immunotech. S.A, Marseille, France); endothelial leucocyte adhesion molecule-1 (ELAM-1) (Seika-gaku, Tokyo, Japan); and P-selectin (Novocastra Ltd, Newcastle, UK).

After being washed with PBS, peroxidase-labelled anti-mouse immunoglobulin G (IgG) was allowed to react for 1 h at room temperature as the secondary antibody was then washed. NaN_3 (65 mg·dL^{-1}) was added in order to prevent nonspecific reaction due to endogenous peroxidase in the eosinophils and neutrophils. Subsequently, 3,3'-diaminobenzidine (DAB) 4HCl was allowed to react for 5 min to develop the colour. Finally, the nuclei were stained with methylene green and, after being washed in running water, dried with alcohol and treated with xylol, the specimens were examined. Staining omitting the primary antibody was used as the negative control.

Counting of positively-stained cells

Tissue specimens in a good state of preservation were selected from the biopsy specimens taken from the two sites. The specimens were all coded and examined blind using a wide-field microscope (BH2; Olympus) at a magnification of ×400. The specimens were all coded to blind the observer and then randomized. Cell counting was undertaken according to the method of DJUKANOVIĆ and co-workers [12, 13]. The observer counted the number of cells in five different fields and calculated the number of cells per field. The number of positively-stained cells was counted only in intact lamina propria in the submucosal area, and epithelial, glandular and vascular tissue was excluded. The tissues measured were traced with extraction apparatus, and a two-dimensional digital program (NEC Co., Tokyo, Japan) was used to measure the area, so that finally the number of cells per square millimetre could be recorded.

Evaluation of adhesion molecule intensity

The intensity of the expression of the adhesion molecules, ICAM-1, VCAM-1, ELAM-1 and P-selectin on vascular endothelial cells and of ICAM-1 on bronchial epithelium was evaluated "blind" using a graded scale, by two independent observers, using a semiquantitative method similar to that described by MESSADI *et al*. [14] and LEUNG *et al*. [15]. The following scale was used: 2 = strong expression, 1 = weak expression; 0 = no expression. Rare differences were reconciled by consultation between the observers.

Statistical analyses

A mean symptom score was calculated from the daily mean of the sum of the five symptoms both for the 2 weeks of the baseline period and for the last 2 weeks of the treatment period. The mean daily PEFR was calculated from the mean of the morning and evening readings, and a mean calculated for the 2 weeks of the baseline and the last 2 weeks of treatment. Diurnal variation of PEFR was calculated from the difference between the morning and evening readings, and expressed as a percentage of the daily mean. The differences between the mean values for FEV_1, vital capacity (VC), PEFR, diurnal variation of PEFR, D_{min}, and symptom score before and after treatment with sodium cromoglycate were compared using Student's 2-tailed paired t-test.

The numbers of each type of inflammatory cell per square millimetre of tissue were counted before and after treatment with inhaled sodium cromoglycate. The results were expressed as means±SEM and Wilcoxon matched-paired, signed rank tests were used to compare the differences. Mann-Whitney U-tests were used for the comparison of the intensity of the expression of each adhesion molecule. The relationship between each type of inflammatory cell and the intensity of the adhesion molecules was tested using the Pearson's correlation coefficient. A p-value of less than 0.05 was taken as representing a significant difference in the tests.

Patient consent and ethics approval

The aims of the study were explained carefully to all the subjects and their consent to participate obtained in writing. The study protocol was approved by the Ethics Committee of the Toho University School of Medicine.

Results

Patients

Nine atopic adult asthmatic patients, mean age 26 yrs (range 20–35 yrs) were recruited into the study. The individual characteristics are presented in table 1. All

Table 1. – Patient characteristics

Pt. No.	Sex	Age yrs	FEV_1 % pred	VC % pred	D_{min} units	Serum IgE IU·mL^{-1}	Treatment
1	F	20	90	110	2.56	266	$Beta_2$
2	M	35	61	132	0.34	304	$Beta_2$
3	M	25	62	107	1.25	266	$Beta_2$
4	M	25	64	88	0.32	925	$Beta_2$, Theo
5	F	31	63	108	0.52	786	$Beta_2$
6	M	20	58	102	0.21	243	$Beta_2$
7	F	30	62	111	0.15	720	$Beta_2$, Theo
8	M	26	64	124	0.33	500	$Beta_2$, Theo
9	M	29	65	106	0.64	762	$Beta_2$, Theo
Mean		26	65	110	0.7	541	
±SD		±5	±9	±12	±0.7	±250	

Pt: patient; M: male; F: female; FEV_1: forced expiratory volume in one second; VC: vital capacity; % pred: percentage of predicted value; D_{min}: dose of methacholine as a measure of bronchial reactivity; units: equal to 1 min of a 1.0 mg·mL^{-1} aerosol inhalation of methacholine; IU: immunizing unit; IgE: immunoglobulin E; $Beta_2$: inhaled salbutamol; Theo: theophylline.

patients had evidence of atopy, with raised serum immunoglobulin E (IgE) levels, mean 541 (range 243–925) immunizing units (IU)·mL^{-1}. The mean value for FEV$_1$ was 65% predicted normal, and for D_{min} 0.7 units. All patients were taking inhaled β_2-agonists as the primary treatment for their asthma, and four patients were also taking oral theophyllines. The dosage of theophyllines was kept constant in the four patients taking them, and the plasma level of theophylline was 4.2±2.0 µg·mL^{-1} in the baseline period and 3.9±2.3 µg·mL^{-1} after treatment with inhaled sodium cromoglycate. The mean daily use of inhaled β_2-agonists was 5.4 (range 2.4–8.0) puffs·day^{-1} in the baseline period, and 3.9 (range 0.4–6.4) puffs·day^{-1} at the end of the treatment period.

Clinical variables and functional examination

The results of pulmonary function tests, bronchial reactivity and symptom scores for the baseline period and the last 2 weeks of treatment, together with the results of the statistical tests, are summarized in table 2. All variables showed a significant improvement after treatment with inhaled sodium cromoglycate.

Degree of infiltration by type of inflammatory cell

After treatment with inhaled sodium cromoglycate, the cell numbers in the lamina propria of the bronchial mucosa declined as follows: eosinophils from 28.2±4.7 to 6.6±2.7 mm^{-2}; mast cells from 18.0±3.8 to 7.4±3.9 mm^{-2}; CD4+ T-cells from 55.7±5.9 to 15.4±3.3 mm^{-2}; CD8+ T-cells from 45.7±4.1 to 27.3±8.9 mm^{-2}; CD3+ T-cells from 84.9±6.8 to 39.3±4.7 mm^{-2}; and macrophages from 65.2±4.8 to 45.3±4.3 mm^{-2}. All of these changes were statistically significant. Neutrophils fell from 28.7±5.3 to 24.7±4.7 mm^{-2} (NS). The changes are presented graphically in figures 1 and 2. Figure 3a and b illustrates changes in the numbers of eosinophils from a single patient.

Staining intensity of adhesion molecules

The use of inhaled sodium cromoglycate significantly inhibited the staining intensity of ICAM-1 in the bronchial epithelium and vascular endothelium (table 3). The staining intensity of VCAM-1 and ELAM-1 was also significantly inhibited. There was no significant difference in the intensity of P-selectin (table 3). Figure 3c and d illustrates tissue specimens from a subject in whom the staining intensity for ICAM-1 went from 2+ to zero.

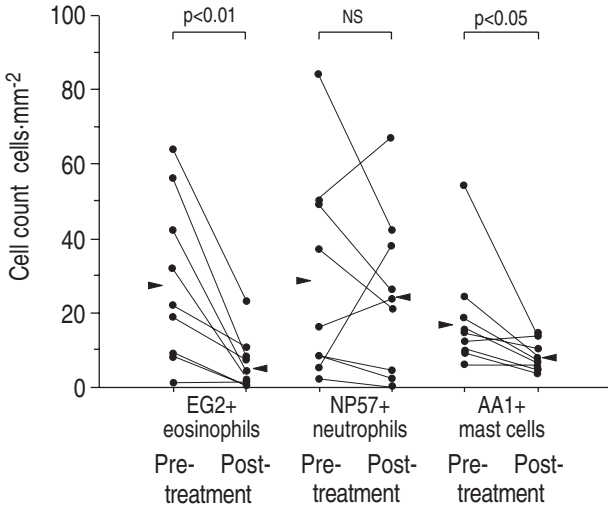

Fig. 1. – Individual cell counts of EG2+ eosinophils, NP57+ neutrophils and AA1+ mast cells expressed as numbers of cells per square millimetre of lamina propria of bronchial mucosa pre- and posttreatment with sodium cromoglycate. NS: nonsignificant. Arrowheads indicate mean values.

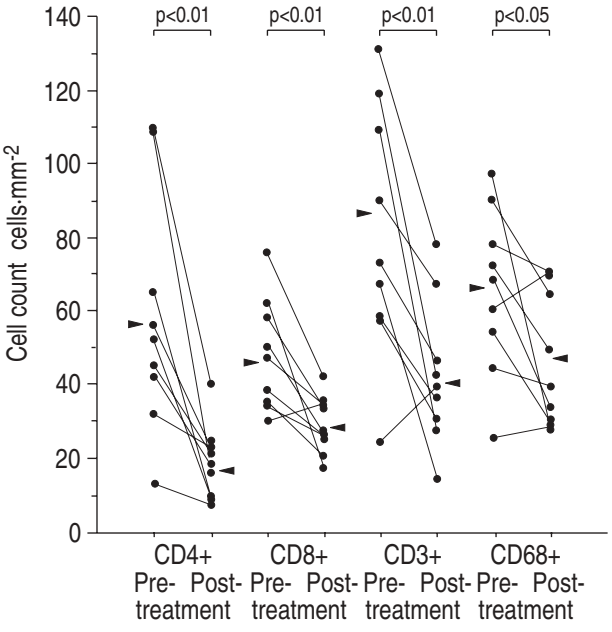

Fig. 2. – Individual T-lymphocyte (CD4+, CD8+, CD3+) and macrophage (CD68+) counts in the bronchial mucosa, expressed as numbers of positive cells per square millimetre of lamina propria pre- and post-treatment with sodium cromoglycate. Arrowheads indicate mean values.

Table 2. – Indices of asthma severity measured pre- and post-treatment

Variable	Pretreatment	Post-treatment	Mean difference	95% CL	p-value
FEV$_1$ % pred	65±9	75±8	9.3±4.6	5.8–12.8	<0.001
VC % pred	110±12	117±12	7.3±6.6	2.2–12.3	<0.05
D_{min} units	0.7±0.7	1.3±1.1	0.6±0.4	0.26–0.89	<0.001
PEFR L·min^{-1}	374±78	482±91	108±53	67.0–148.6	<0.001
Diurnal variation %	22±5	10±2	-11.6±4.5	-8.12– -15.1	<0.001
Total mean symptom score	3.8±1.5	1.2±0.7	-2.6±-1.7	-1.7– -3.42	<0.001

Data are expressed as mean±SD. Symptom score is daily mean of sum of five symptoms. PEFR: peak expiratory flow rate; 95% CL: 95% confidence limit. For further definitions see legend to table 1.

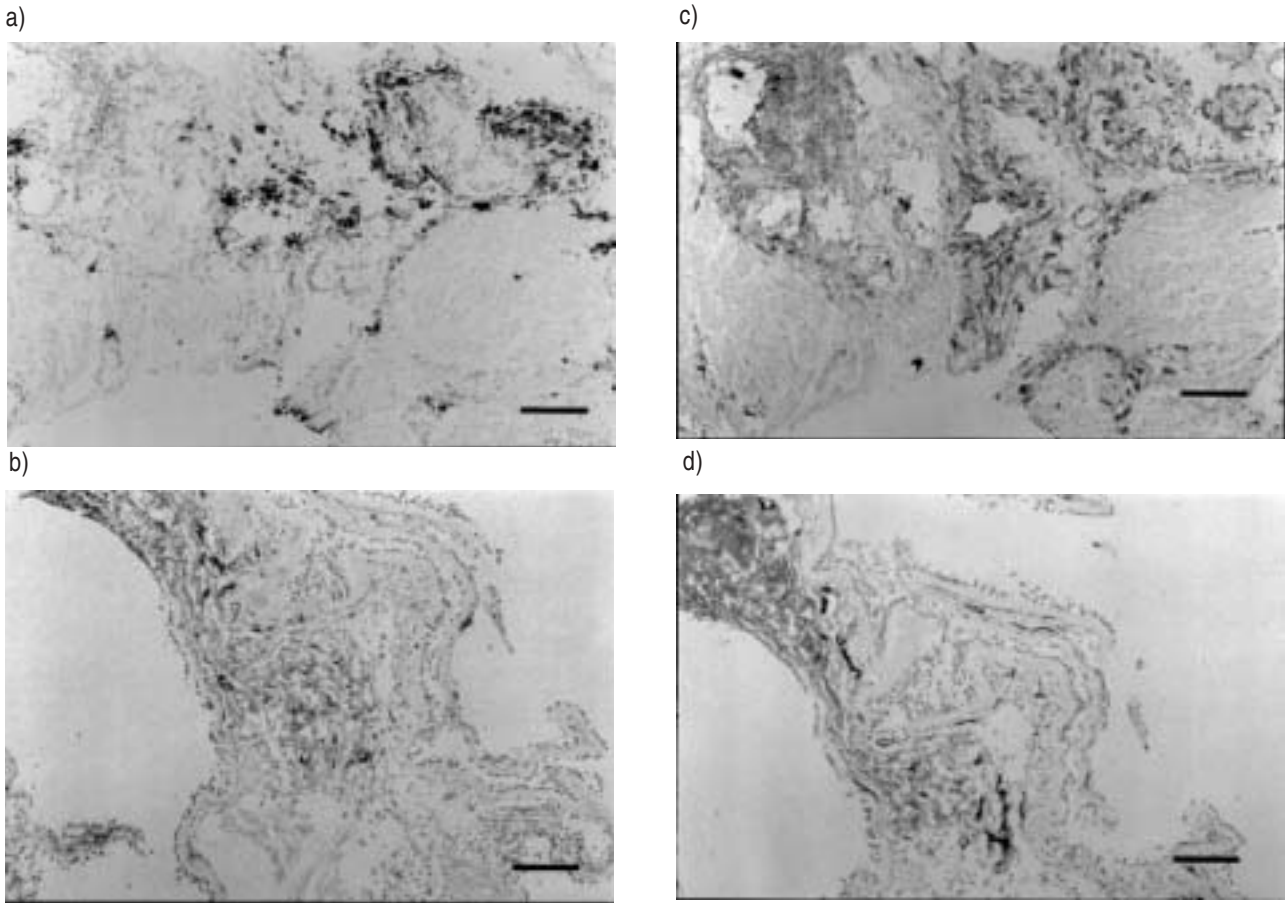

Fig. 3. – Bronchial mucosal biopsy stained for EG2+ eosinophils and ICAM-1 adhesion molecules pre- and post-treatment with sodium cromoglycate. a) EG2+ eosinophils pretreatment; b) EG2+ eosinophils post-treatment; c) intensity of ICAM-1 pretreatment; d) intensity of ICAM-1 post-treatment. ICAM-1: intercellular adhesion molecule-1. (internal scale bar=100 µm).

Table 3. – Visual analogue scores for intensity of staining of adhesion molecules ICAM-1, VCAM-1, ELAM-1 and P-selectin in bronchial biopsies from asthmatic patients pre- and post-treatment with sodium cromoglycate

Intensity of staining	ICAM-1 Epithelium		ICAM-1 Endothelium		VCAM-1		ELAM-1		P-selectin	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
0	1	5	0	1	2	6	1	3	0	2
1	5	4	2	7	2	3	2	6	2	4
2	3	0	7	1	5	0	6	0	7	3
p-value	<0.05		<0.05		<0.05		<0.05		NS	

The values presented are the absolute number of patients. ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; ELAM-1: endothelial leucocyte adhesion molecule-1; NS: nonsignificant.

Relationship between type of inflammatory cell and intensity of adhesion molecules

The coefficient of variation (CV) for repeat counting by a single observer for the immunostains were as follows: EG2 6%; NP57 4%; AA1 7%; CD4 8%; CD8 9%; CD3 11%; and CD68 9%. A significant positive correlation was found between CD4+ ($r=0.83$; $p<0.005$), CD8+ ($r=0.72$; $p<0.025$) and CD3+ ($r=0.86$; $p<0.005$) T-cells and the expression of ICAM-1 in bronchial epithelium; and between CD4+ ($r=0.76$; $p<0.005$) and CD3+ ($r=0.72$; $p<0.025$) T-cells and the expression of ICAM-1 in vascular epithelium. A significant positive correlation was also found between EG2+ eosinophils and the expression of VCAM-1 ($r= 0.87$; $p<0.005$), but none was found between the other types of inflammatory cell and adhesion molecules.

Discussion

This is the first study of the effect of inhaled sodium cromoglycate on the inflammatory cell infiltrate and

adhesion molecule expression in the bronchial mucosa of patients with mild-to-moderate allergic asthma. No group of subjects receiving placebo was included because of constraints imposed by our Ethics Committee, who considered that a placebo group was not justified. This and the lack of blinding weakens the study and means that the results have to be interpreted with some caution. Consequently, we cannot be certain that the improvement in asthma and the anti-inflammatory effects are due solely to the effects of sodium cromoglycate.

Before and during the study, all nine patients were being treated with inhaled β_2-agonists as required, and four of them with oral theophyllines at a fixed dose. Both these classes of drugs have been reported to affect inflammatory cells [8, 16, 17]. In the four patients who were taking theophyllines, the dose was maintained constant throughout and although the serum theophylline levels fell slightly this was not significant. The effects seen in these four subjects did not differ from the other five, and we therefore conclude that the theophylline is unlikely to have been responsible for the changes observed. All nine patients were using inhaled salbutamol as required for the treatment of their asthma before and during the study. After the addition of inhaled sodium cromoglycate they were able to reduce their dose of inhaled salbutamol. It is possible that this reduction accounted for some of the changes seen.

MANOLITSAS *et al*. [18] used regular inhaled albuterol in addition to "as required" inhaled albuterol in their study of the anti-inflammatory effects of inhaled nedocromil sodium and placebo. The albuterol-treated group showed an increase (nonsignificant compared to placebo) in the EG2+ eosinophils but no change in mast cells or lymphocytes. In the study by LAITINEN *et al*. [8], which was a comparison of budesonide, terbutaline and placebo, the group treated with regular terbutaline showed a mean (nonsignificant) increase in eosinophils, a (nonsignificant) reduction in mast cells, and a significant reduction in lymphocytes. In an open study, by DJUKANOVIĆ and co-workers [13], similar to the present study but with inhaled beclomethasone, 7 out of 9 patients discontinued inhaled β_2-agonists completely; the possibility that this might have contributed to the anti-inflammatory changes was considered and rejected.

Similar reductions in the numbers of eosinophils in bronchoalveolar lavage (BAL) fluid after treatment with inhaled sodium cromoglycate have been reported by DIAZ *et al*. [19]. In their study, there was also a reduction in inhaled salbutamol usage in the sodium cromoglycate-treated group and an increase in the placebo group. Changes in eosinophil numbers in the placebo group were not consistent, so that it seems unlikely that these changes were related to alteration in the doses of salbutamol. In the present study, the reduction in inhaled salbutamol dosage was only possible due to the addition of inhaled sodium cromoglycate, and it is possible that the changes observed resulted from the combined effect of the reduction of one drug and the addition of the other. On balance, we conclude that the anti-inflammatory effects seen were primarily attributable to the addition of inhaled sodium cromoglycate.

Nedocromil sodium is an alternative anti-inflammatory drug to sodium cromoglycate. It is considered to have similar pharmacological effects but to be more potent. MANOLITSAS *et al*. [18] compared inhaled nedocromil sodium with inhaled albuterol and placebo, in a study in which they took bronchial biopsies before and after 16 weeks of treatment. Compared to baseline values, treatment with nedocromil sodium resulted in a significant reduction in EG2+ eosinophils but the changes were not significantly different to placebo. There were no changes in the numbers of mast cells or in T-lymphocyte subtypes. Because of differences in technique, it is not possible to make a direct comparison of the effects seen in the study by MANOLITSAS *et al*. [18] with those in the present study. The effects of nedocromil sodium would appear to be rather less. The supposedly greater potency of nedocromil sodium is based upon *in vitro* experiments and bronchial challenge studies; these experiments may not be relevant in clinical usage or in *in vivo* biopsy changes in asthmatic patients.

In allergic inflammatory conditions, including asthma, various types of cell adhesion molecule are thought to be responsible for the selectivity of inflammatory cell movement in and through the tissues from the blood vessels [6]. The adhesion molecules investigated in the present study were ELAM-1, VCAM-1, ICAM-1 and P-selectin. Upregulation of ELAM-1 has been shown in asthmatic patients at 6 h after antigen challenge [20], and GUNDELL *et al*. [21] found a correlation between the increased expression of ELAM-1 and neutrophil infiltration in the airways of nonhuman primates showing delayed-type bronchoconstriction. However, there is very little evidence of increased neutrophils in biopsies from the bronchial mucosa of asthmatic patients [22, 23]. The present study, likewise, failed to indicate any change in the number of neutrophils before and after using sodium cromoglycate and, although the expression of ELAM-1 was significantly inhibited by sodium cromoglycate, no relationship could be found between changes in the expression of ELAM-1 and changes in the numbers of neutrophils.

The expression of ICAM-1 both on bronchial epithelium and vascular endothelium was significantly inhibited by administration of sodium cromoglycate. SAITO *et al*. [24] found a significant correlation between the distribution and staining intensity of ICAM-1 in the mucosa of the inferior nasal concha in patients with nasal allergy, and the distribution and degree of infiltration of T-lymphocytes, especially CD4+ cells. As the staining intensity of ICAM-1 on bronchial epithelium showed a positive correlation with CD4+, CD8+ and CD3+ T-cells, and the staining intensity of ICAM-1 on vascular endothelium with CD4+ and CD3+ T-cells, it is suggested that one way in which T-cell infiltration into the bronchial mucosa was inhibited following the administration of sodium cromoglycate was by a reduction in the expression of ICAM-1.

The expression of VCAM-1 was significantly inhibited by the administration of sodium cromoglycate. The analysis showed a significant positive correlation between the intensity of VCAM-1 and EG2+ eosinophils. VCAM-1 expression and its binding to the integrin ligand, very late activation antigen-4 (VLA-4) [25], has attracted attention as a mechanism to explain cellular infiltration in eosinophil- and T-lymphocyte-dominant allergic inflammation. VLA-4 does not appear in neutrophils [26]. NAKAJIMA *et al*. [27] used mice challenged

with ovalbumin (OA) sensitizing antigen, and compared a group pretreated with anti-VCAM-1 antibody or anti-VLA-4 antibody and a group pretreated with anti-ICAM-1 antibody or anti-lymphocyte function-associated antigen-1 (LFA-1) antibody. They reported that eosinophils in the airways were significantly inhibited in the former.

Our findings suggest, therefore, that the inhibition of T-lymphocyte infiltration into the airways following administration of sodium cromoglycate involved the inhibition of ICAM-1 on bronchial epithelial cells, and that the reduction in the infiltration of eosinophils involved the inhibition of VCAM-1 on vascular epithelial cells. However, as the expression of adhesion molecules was evaluated using a semiquantitative method, the interpretation of these relationships must be regarded with some degree of caution. We showed a significant reduction in mast cells but no relationship was found between the change in the number of mast cells and the expression of adhesion molecules. It is possible that this reduction is mediated by another mechanism.

The increased expression of adhesion molecules after antigen exposure is probably mediated by specific cytokines, particularly tumour necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-4 [28, 29]. The source of these cytokines is likely to be mast cells or activated macrophages in the bronchial mucosa. Mast cells from allergic individuals show strong immunostaining for a range of cytokines, specifically TNF-α, IL-4, IL-5 and IL-6 [30]. Sodium cromoglycate has been shown to inhibit TNF-α activity in antigen-stimulated mast cells [31], and in skin mast cells exposed to ultraviolet B irradiation [32]. It is, therefore, possible that the anti-inflammatory effects of the drug are related to its ability to reduce the release of inflammatory mediators and cytokines both from sensitized mast cells and possibly from other cells, such as macrophages or epithelial cells.

Detailed studies by electron microscopy [23, 33] have demonstrated that the number of macrophages increases in the bronchial epithelium and mucosa of patients with asthma. Activated macrophages produce many mediators and cytokines. The adhesion of mononucleocytes *in vitro* is dependent upon interactions with ELAM-1 and VCAM-1 [34]. The finding that sodium cromoglycate decreased the number of macrophages may be due to its inhibition of the expression of these adhesion molecules.

In conclusion, this is the first prospective study to demonstrate the anti-inflammatory effects of inhaled sodium cromoglycate in symptomatic patients with asthma by showing a significant reduction in the relevant inflammatory cells, with an associated improvement in the clinical indices of the disease. It has also provided some clues as to the likely mechanisms of the anti-inflammatory effects by correlating the reduction in the inflammatory cells with downregulation of the relevant adhesion molecules.

Acknowledgement: The authors thank A. Edwards for revising the manuscript.

References

1. Azzawi M, Bradley M, Jeffery PK, *et al*. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990; 142: 1407–1413.
2. Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989; 83: 1013–1026.
3. Arnoux B, Denjean A, Page CP, Nolibe D, Morley J, Benveniste J. Accumulation of platelets and eosinophils in baboon lung after Paf-acether challenge. *Am Rev Respir Dis* 1988; 137: 855–860.
4. O'Byrne PM, Leikauf GD, Aizawa H, *et al*. Leukotriene B_4 induces airway hyperresponsiveness in dogs. *J Appl Physiol* 1985; 59: 1941–1946.
5. Springer TA, Dustin ML, Kishimoto TK, Marlin SD. The lymphocyte function-associated, LFA-1, CD2 and LFA-3 molecules: cell adhesion receptors of the immune system. *Ann Rev Immunol* 1987; 5: 223–252.
6. Osborn L. Leukocyte adhesion to endothelium in inflammation. *Cell* 1990; 62: 3–8.
7. National Heart, Lung and Blood Institute. Guidelines for the diagnosis and management of asthma. National Asthma Education Program Expert Panel Report. *J Allergy Clin Immunol* 1991; 88: 425–534.
8. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a β_2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomised, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992; 90: 32–42.
9. Hoshino M, Nakamura Y. Anti-inflammatory effects of inhaled beclomethasone dipropionate in nonatopic asthmatics. *Eur Respir J* 1996; 9: 696–702.
10. Yamamura Y, Shida R, Mitui S, *et al*. Japanese Society of Allergology. Bronchial asthma severity. *Jpn J Allergol* 1993; 32: 1186–1199.
11. Takishima T, Hida W, Sasaki H, Suzuki S, Sasaki T. Direct-writing recorder of the dose-response curves of the airway to methacholine. *Chest* 1981; 80: 600–606.
12. Djukanović R, Lai CKW, Wilson JW, *et al*. Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. *Eur Respir J* 1992; 5: 538–544.
13. Djukanović R, Wilson JW, Britten KM, *et al*. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992; 5: 669–674.
14. Messadi DV, Pober JS, Fiers W, Gimbrone MA Jr, Murphy GF. Induction of an activation antigen on postcapillary venular endothelium in human skin organ culture. *J Immunol* 1987; 139: 1557–1562.
15. Leung DYM, Cotran RS, Kurt-Jones E, Burns JC, Newburger JW, Pober JS. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet* 1989; ii: 1298–1302.
16. Jeffery PK, Wardlaw AJ, Nelson FC, Collins J, Kay AB. Bronchial biopsies in asthma: an ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989; 140: 1745–1753.
17. Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. *Lancet* 1994; 343: 1006–1008.
18. Manolitsas ND, Wang J, Devalia JL, Trigg CJ, McAulay AE, Davies RJ. Regular albuterol, nedocromil sodium, and bronchial inflammation in asthma. *Am J Respir Crit Care Med* 1995; 151: 1925–1930.
19. Diaz P, Galleguillos FR, Cristina Gonzalez M, Pantin CFA, Kay AB. Bronchial lavage in asthma: the effect

of disodium cromoglycate (cromolyn) on leukocyte counts, immunoglobulins, and complement. *J Allergy Clin Immunol* 1984; 74: 41–48.
20. Montefort S, Gratziou C, Goulding D, *et al*. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J Clin Invest* 1994; 93: 1411–1421.
21. Gundell RH, Wegner CD, Torcellini CA, *et al*. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *J Clin Invest* 1991; 88: 1407–1411.
22. Bradley BL, Azzawi M, Jacobson M, *et al*. Eosinophils, T-lymphocytes, mast cells, neutrophils and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991; 88: 661–674.
23. Poston RN, Chanez P, Lacoste JY, Litchfield T, Lee TH, Bousquet J. Immmunohistochemical characterisation of the cellular infiltration in asthmatic bronchi. *Am Rev Respir Dis* 1992; 145: 918–921.
24. Saito H, Asakura K, Kataura A. Study on the profiles of infiltrating T lymphocytes and ICAM-1 expression in allergic nasal mucosa. *Acta Otolaryngol (Stockh)* 1994; 114: 315–323.
25. Elices MJ, Osborn L, Takada Y, *et al*. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990; 60: 577–584.
26. Bochner BS, Luscinskas FW, Gimbrone MA Jr, *et al*. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991; 173: 1553–1556.
27. Nakajima H, Sano H, Nishmura T, Yoshida S, Iwamoto I. Role of vascular cell adhesion molecule 1/very late activation antigen-4 and intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 interactions, in antigen-induced eosinophil and T cell recruitment into the tissue. *J Exp Med* 1994; 179: 1145–1154.
28. Springer TA. Adhesion receptors in the immune system. *Nature* 1990; 346: 425–434.
29. Walsh LJ, Trinchieri G, Warldorf HA, Whitaker D, Murphy GF. Human dermal mast cells contain and release tumour necrosis factor α, which induces endothelial leucocyte adhesion molecule 1. *Proc Natl Acad Sci USA* 1991; 88: 4220–4224.
30. Bradding P, Roberts JA, Britten KM, *et al*. Interleukin-4,-5,-6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10: 471–480.
31. Bissonnette EY, Enciso JA, Befus AD. Interferon and antiallergic drug regulation of histamine and tumor necrosis factor-α in rat mast cell subsets. *Int Arch Allergy Immunol* 1995; 107: 156–157.
32. Walsh LJ. Ultraviolet B irradiation of skin induces mast cell degranulation and release of tumour necrosis factor-α. *Immunol Cell Biol* 1995; 73: 226–233.
33. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993; 147: 697–704.
34. Carlos T, Kovach N, Schwartz B, *et al*. Human monocytes bind to two cytokine-induced adhesive ligands on cultured human endothelial cells: endothelial-leukocyte adhesion molecule-1 and vascular cell adhesion molecule-1. *Blood* 1991; 77: 2266–2271.