

Sodium cromoglycate attenuates pulmonary inflammation without influencing bronchial responsiveness in healthy subjects exposed to organic dust

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Summary

Background Inhalation of organic dust from a pig house induces airway inflammation and increases bronchial responsiveness to methacholine in healthy subjects.

Objective To study whether sodium cromoglycate influences the airway inflammatory reaction and the increase in airway responsiveness induced by inhalation of organic dust.

Methods Bronchoalveolar and nasal lavages, and bronchial methacholine challenges were performed and blood samples were drawn in 32 healthy subjects before and after exposure to dust in a pig farm. Sodium cromoglycate was inhaled (20 mg, twice a day) and administered intranasally (5.2 mg, twice a day) by 16 and a corresponding placebo was given to the other 16 healthy controls for two weeks prior to exposure.

Results Exposure induced a significant increase in inflammatory cells and soluble components (pro-inflammatory cytokines, inflammatory mediators) in bronchoalveolar and nasal lavage fluid in both groups. The increase in neutrophils, interleukin (IL)-6 and tumour necrosis factor (TNF)- α as well as myeloperoxidase and soluble intracellular adhesion molecule (ICAM)-1 in bronchoalveolar lavage (BAL) fluid was significantly reduced by treatment with sodium cromoglycate. Although sodium cromoglycate inhalation largely influenced a variety of inflammatory indices in bronchoalveolar lavage fluid it had no effect on the increase in bronchial responsiveness to methacholine.

Conclusion Sodium cromoglycate alters the airway inflammatory response to inhaled organic dust without influencing the dust-induced increase in bronchial responsiveness to methacholine.

Keywords: organic dust, inflammation, hyper-responsiveness, sodium cromoglycate, pig.

Clinical and Experimental Allergy, Vol. 31, pp. 1356–1368. Submitted 21 July 2000; revised 6 October 2000; accepted 15 February 2001.

Introduction

Inhalation of dust in a swine confinement building may lead to symptoms such as mental fatigue, headache and chills. The exposure also induces increased body temperature and an intense inflammatory reaction in the lower and

upper airways in healthy subjects [1,2]. The cellular reaction is dominated by neutrophils but there is also an increase in alveolar macrophages and activated T cells in bronchoalveolar lavage (BAL) fluid [1,3]. Pro-inflammatory cytokines (tumour necrosis factor (TNF)- α , interleukin (IL)-1 α , IL-1 β , IL-6) and IL-8 are elevated in bronchoalveolar and nasal lavage fluid following a few hours exposure to the dust [2,4] and TNF- α and IL-6 also increase in peripheral blood [5]. Furthermore, exposure to

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swine house dust induces a multifold increase in bronchial responsiveness to methacholine without major influence on lung function in healthy subjects [6].

It is not known what agents in the swine house dust are responsible for the airway reaction. Possible agents are feed components (grain dust, glucans) and microorganisms or microbial products in the dust. The dust contains bacteria and bacterial products such as lipopolysaccharide (LPS) mainly from Gram-negative bacteria and peptidoglycans mainly from Gram-positive bacteria [7]. Grain dust has been shown to increase chemotaxis for neutrophils *in vitro* [8] and, following inhalation, it causes an acute inflammatory airway response and influences lung function in non-asthmatic, non-atopic grain handlers [9]. LPS from bacteria, mainly Gram-negative species, causes an acute influx of neutrophils into the airways [10] but does not increase bronchial responsiveness [11,12] as does inhalation of dust from swine confinement houses. Glucans and grain dust are only weak stimulators of IL-8 release from airway epithelial cells and alveolar macrophages [13]. There are weak, but significant, correlations between the exposure to peptidoglycans, a marker for Gram-positive bacteria, and the increase in body temperature as well as IL-6 and granulocyte levels in peripheral blood [14]. Furthermore, a significant relationship has been reported between the number of neutrophils in BAL fluid and the levels of muramic acid which is a marker for peptidoglycan [4].

Sodium cromoglycate (SCG) has been used in the treatment of asthma and rhinitis for almost 30 years. It has been repeatedly shown that SCG reduces bronchial responsiveness especially to indirect, but also to direct, bronchoconstrictor stimuli in asthmatic subjects [15]. It has also been demonstrated that SCG interferes with the inflammatory process in many aspects. Thus, it inhibits mediator release from mast cells [16] and reduces cytotoxicity of eosinophils and neutrophils [17]. In addition, SCG inhibits activation [18] and chemotaxis [19] of eosinophils and neutrophils, inhibits the release of a neutrophil chemotactic factor from alveolar macrophages [20] and reduces the number of T lymphocytes and alveolar macrophages in the airway wall in asthmatic subjects [21]. Sodium cromoglycate also decreases the expression of adhesion molecules on airway epithelial and endothelial cells in patients with asthma [21].

Sodium cromoglycate is thus capable of influencing bronchial responsiveness and has also the capability of influencing different indices of the inflammatory process in asthma. The aim of the present study was to evaluate whether SCG also influences the airway inflammatory response to inhaled organic dust and if such possible effects influence the increase in bronchial responsiveness to methacholine following exposure.

Materials and methods

Subjects

Thirty-two healthy, non-smoking subjects (17 men), 32 years (range 22–50) of age, gave informed consent to participate in the study which was approved by the Ethics Committee of the Karolinska Institute. None had a history of asthma or allergy and none had a respiratory tract infection during the last four weeks prior to the study.

Study design

The study design is shown in Fig. 1. Lung function measurements, a bronchial methacholine provocation test and nasal lavage were performed on the first day and bronchoalveolar lavage were performed and blood samples were drawn 3–7 days later. The subjects were then randomised to treatment with SCG inhalation (20 mg twice a day from a metered dose inhaler) and SCG intranasally (5.2 mg twice a day as an aqueous aerosol) or corresponding inhaled and intranasal placebo in a single blind manner. The age and gender distribution was similar in the two groups. After two weeks of treatment the subjects were exposed to swine dust while weighing pigs for three hours in a swine confinement building with approximately 900 pigs. The participants assisted the farmer and guided the pigs through a weighing box. The exposure started approximately 2 h after the last dosing. Two subjects, one from each group, were exposed at each occasion. Personal samplers were used for exposure measurements. Four and a half hours after the start of the exposure a blood sample was drawn and 2.5 h later lung function measurements, a bronchial methacholine provocation test and a nasal lavage were performed. Twenty-four hours after the start of exposure BAL was performed. Headache, chills, mental fatigue, muscle pain and nausea were asked for by using a questionnaire. The symptoms were graded from 1 (no symptoms) to 5 (severe symptoms). Only rates of 4 or 5 were classified as significant. Oral temperature was measured before and repeatedly, 4–9 h after the start of the exposure and the subjects were asked to measure the temperature at home, if they suspected an increased body temperature.

Exposure measurements

Dust was sampled at an air flow of 2.0 L/min with personal samplers using 25 mm filter cassettes (IOM) and air suction pumps (SKC Ltd, Dorset, UK). The cassettes were carried in the breathing zone and were equipped with polycarbonate filters (Nuclepore®, Costar Corp. Headq., Pleasanton, CA, USA). Total dust was measured by

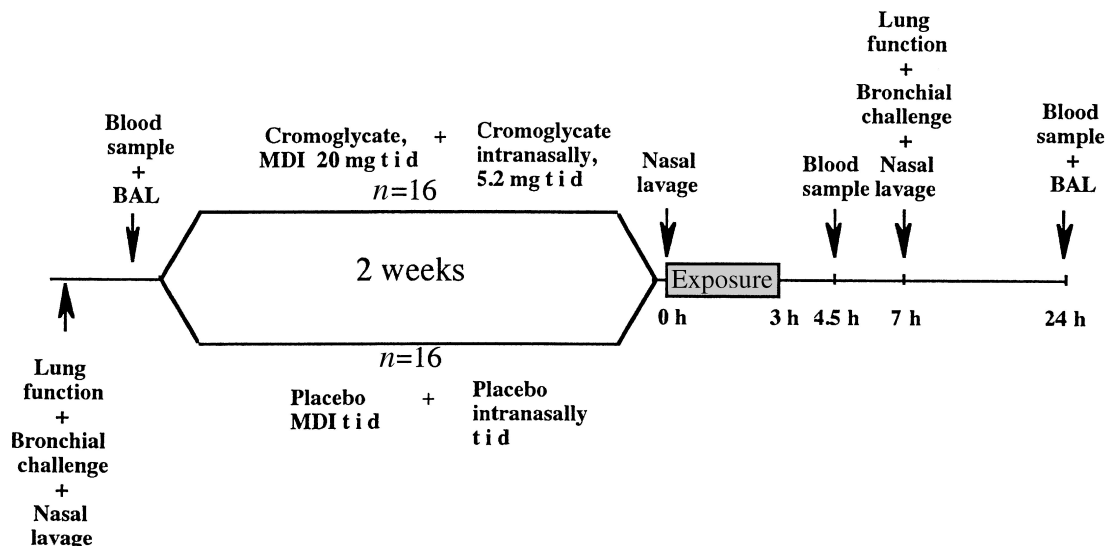


Fig. 1. Study design. Thirty-two healthy subjects treated with sodium cromoglycate ($n = 16$) or placebo ($n = 16$) for two weeks prior to swine dust exposure. BAL = bronchoalveolar lavage. The time schedule of the study has been designed according to experiences from a number of previous studies [1,2,4–6,33].

weighing (Mettler® ME 22 balance, Mettler, Greisensee, Switzerland) after 24 h of conditioning and compared with reference filters.

Endotoxin was extracted from filters by shaking in 10 mL of pyrogen-free water for 60 min. The extracts were centrifuged for 10 min at 1000 *g* and the supernatants were frozen at -70°C until analysis. Endotoxin was measured after dilution with a chromogen version of *Limulus amoebocyte* lysate assay (QCL-1000, Endotoxin, BioWhittaker, Walkersville, MD, USA with *E. coli* 0111:B4 as standard).

Lung function and bronchial responsiveness

Lung function (FEV_1 and VC) was measured according to the American Thoracic Society (ATS) criteria [22] with a low-resistance rolling-seal spirometer (OHIO model 840, Airco, Madison, WI, USA). Residual volume (RV) was measured at a breathing frequency of 25 breaths/minute in a volume constant body plethysmograph (Erich Jaeger, GmbH, Würzburg, Germany) connected on line to a computer. The reference values by Hedenström *et al.* were used [23,24].

Bronchial responsiveness was assessed by a methacholine provocation test. Inhalation of the diluent was followed by inhalation of doubling concentrations of methacholine starting at 0.5 mg/mL. The challenge was stopped at a FEV_1 decrease of 20% compared to the value obtained after inhalation of the diluent or after inhalation of the highest methacholine concentration (64 mg/mL). The cumulative

dose causing a 20% decrease in FEV_1 ($\text{PD}_{20}\text{FEV}_1$) was calculated. The method is standardized with regard to inhalation flow (0.4 L/s) and volume (0.8 L/breath, 15 breaths during 60 s). The procedure has been described in detail elsewhere [25].

Bronchoalveolar lavage

Bronchoscopy was performed through the mouth or the nose with a flexible fiberoptic bronchoscope (Olympus Type 4B2) under local anaesthesia with lignocaine (Xylocain®, Astra, Södertälje, Sweden) after premedication with morphine-scopolamine. The bronchoscope was wedged in a middle lobe subsegmental bronchus and 250 mL of sterile saline at $+37^{\circ}\text{C}$ was instilled in five aliquots of 50 mL. After each instillation the fluid was gently aspirated and collected in a siliconized plastic bottle kept on ice.

After filtering through a single layer of gauze, the BAL fluid was centrifuged at 400 *g* for 5 min at $+4^{\circ}\text{C}$. The supernatant was kept frozen at -70°C until analysis. The pellet was resuspended in Tris-Hanks balanced salt solution at pH 7.4 and the cells were counted in a Bürker chamber. Smears for differential cell counts were prepared by cytocentrifugation. After staining with May-Grünwald Giemsa, 400 cells were counted.

Nasal lavage

A nasal lavage procedure described by Bascom and

Pipkorn [26,27] was used with minor modifications. The subject flexed the neck 45 degrees backwards and closed the soft palate while 5 mL 0.9% NaCl was instilled into one nostril, using a needleless syringe. After 10 s, the neck was flexed forwards and the liquid was expelled into a plastic basin which was placed on ice during processing. The procedure was repeated on the other side. The volume of the combined lavage portions were measured and centrifuged for 10 min at 200 *g* at +4 °C and the supernatant was frozen at -70 °C until analysis. The pellet was resuspended in 0.9% NaCl with 0.1% human serum albumin and the cells were counted in a Bürker chamber and the number of cells/mL of recovered lavage fluid were calculated. Cytocentrifuge-prepared slides were stained with May-Grünwald Giemsa stain and 300 cells were counted for cell differentials. Less than 100 cells were considered too few cells to make an accurate differential count.

Analyses in lavage fluids

TNF- α , IL-6, IL-1 β and IL-8 were measured in duplicate by enzyme-linked immunosorbent assay in lavage fluids. A Quantikine™ two-site (sandwich) ELISA kit (R & D Systems Europe, Abingdon, UK) was used. For IL-1 β in lavage fluid a Quantikine high sensitivity, two-site (sandwich) ELISA kit (R & D Systems) was used. The lower detection limit of the assay for TNF α , IL-6, IL-1 β and IL-8 was 0.25, 0.156, 0.125 and 31.3 ng/L, respectively.

Myeloperoxidase (MPO) was analysed using an MPO radioimmunoassay (Pharmacia AB, Uppsala, Sweden). The detection limit was 8 mg/L. Soluble intracellular adhesion molecule (sICAM)-1 were measured using an ELISA kit (R & D Systems, Inc., Minneapolis, MN, USA). The sensitivity of the assay was 0.35 μ g/L. Methyl-histamine was analysed using Methyl-Histamine RIA (Pharmacia AB). The detection limit was 0.1 mg/L. Eosinophilic cation protein (ECP) was analysed with the ECP RIAC (Pharmacia AB). The detection limit was 0.50 μ g/L. Fibronectin (FN) was analysed with a modified double-sandwich ELISA technique (Pharmacia AB). In short, microtitre plates (NUNC, Roskilde, Denmark) were coated with rabbit-anti-human fibronectin antibodies (Dakopatts, Glostrup, Denmark) diluted at 1 : 2000 in carbonate buffer, pH 9.6. After incubation at room temperature (21–23°C) for 24 h, the titre plates were carefully washed. Phosphate buffer saline (PBS) containing 0.005% albumin was added for one hour and then washed away. BAL fluid samples were added in diluted series together with horseradish peroxidase-labelled anti-human fibronectin 1 : 2000 (Dakopatts) as the secondary antibody and the plates were incubated for 90 min. The amount of bound peroxidase, which is proportional to the amount of fibronectin in the

sample, was determined by analysing the enzymatic activity on ortho-phenyldiamine. Plasma fibronectin of nephelometric quality was provided by Sigma Chemicals (Poole, UK) and was used as standard. The detection limit was 10 mg/L. Albumin was quantified using an in-house inhibition ELISA method which has been described previously [2].

Analyses in blood

Blood samples were kept at room temperature for 30–60 min and then centrifuged. Aliquoted serum samples were stored in closed glass tubes at -70 °C until analyses. Commercial ELISA kits with monoclonal antihuman antibody were used in assaying IL-6 in serum (R & D Systems). The standard curves were constructed with dilution of recombinant human IL-6 supplied with the kit. All assays were done in duplicate, using four-parameter logistic curve fit. The minimum detectable concentration of IL-6 is 0.156 ng/L.

Drugs

Sodium cromoglycate and placebo for inhalation and intranasal administration were a kind gift from Fisons, Sweden. All participants were asked to bring their drugs back after treatment, i. e. the day of exposure and drug consumption was estimated by weighing the canisters.

Statistics

Results of the bronchial methacholine provocation and concentrations of cells and soluble products in lavage fluid and blood are given as median values (25th – 75th percentiles). Lung function data, oral temperature, exposure measurements and consumed medication are given as mean values (95% confidence intervals). Comparisons were performed by means of analysis of variance, Students *t*-test for paired observations, Mann–Whitney *U*-test for unpaired observations or Wilcoxon's signed rank test for paired observations depending on the distribution of data which was assessed by Shapiro–Wilk *W*-test. When a skew distribution was found analysis of variance was performed on logarithmically transformed data. Blood data were compared by means of Friedman's test followed by Wilcoxon's signed rank test for paired observations when appropriate. Concentration values below detection limit (cytokines before exposure) were assigned the value of the lowest detection limit. If 20% decrease in FEV₁ was not obtained at the highest methacholine concentration (64 mg/mL), a value for PD₂₀FEV₁ of 25.5 mg (corresponding to the cumulated dose after inhalation of 64 mg/mL at a nebulizer output of 0.4 L/s) was assigned for non-parametric statistical

calculations. A P -value < 0.05 (or < 0.01 when multiple comparisons were performed) was considered significant.

Results

Symptoms

Prior to the exposure, oral temperature was 36.1 (35.8–36.4) °C in the SCG-group and 36.9 (36.6–37.2) °C in the placebo group and increased significantly ($P < 0.001$) by 0.76 (0.49–1.03) °C in the SCG group and 1.05 (0.74–1.36) °C in the placebo group ($F = 2.36$; $P = 0.14$ between the groups). There were no differences in post-exposure symptoms between the groups.

Exposure and medication

Airborne total dust measured with personal samplers in the subjects was 23.5 (19.2–27.9) mg/m³ in the placebo group and 26.4 (19.3–33.5) mg/m³ in the SCG group. Corresponding values for endotoxin were 1.17 (0.97–1.38) µg/m³ and 1.17 (0.92–1.42) µg/m³.

The 'drug consumption' (calculated as the pre- and post-treatment weight difference of the drug + device) for nasal use was 13.1 (11.0–15.2) g in the placebo group and 13.3 (10.8–15.8) g in the SCG group. Corresponding values for inhaled drugs were 21.7 (19.8–23.5) g in the placebo group and 21.8 (19.4–24.1) g in the SCG group. No significant

differences were found between the groups regarding exposure or drug consumption ($P > 0.7$).

Lung function and bronchial reponsiveness

Lung function (FEV₁ and VC) was normal in both groups (Table 1). Exposure to swine dust induced a small, but significant, decrease of FEV₁ in both groups and a significant decrease of VC in the placebo group. Residual volume did not change significantly in either group. There was no significant difference in lung function changes between the two groups.

Prior to exposure methacholine inhalation did not induce 20% decrease in FEV₁ in 4 subjects in the placebo group and one in the SCG group (Fig. 2). Before exposure PD₂₀FEV₁ was 5.6 (1.2–21.7) mg in the placebo group and 2.1 (1.3–6.5) in the SCG group (Fig. 2). Inhalation of swine house dust increased bronchial responsiveness to methacholine in every subjects, PD₂₀FEV₁ was 0.62 (0.22–1.24) in the placebo group and 0.21 (0.02–0.30) mg in the SCG group (Fig. 2). This represents more than 3.2 and 3.3 halvings of the methacholine dose, respectively. The dose-response slope, i.e. the percentual FEV₁-decrease as a function of the cumulated dose of methacholine, increased from 8.4 (2.8 to 14.7)%/mg to 84.0 (72.7–294.6)%/mg in the SCG group and from 3.4 (0.7 to 14.7) to 32.2 (13.9–85.1)%/mg in the placebo group ($F = 0.77$; $P = 0.39$, ANOVA, log-transformed data, interaction treatment/occasion).

	Placebo <i>n</i> = 16	SCG <i>n</i> = 16
FEV ₁		
Pre-exposure	103	101
% of predicted	96–110	96–105
Change	– 5.2 ($P < 0.001$)	– 7.0 ($P < 0.001$)
% of pre-exposure	– 7.9 to – 2.6	– 9.8 to – 4.3
VC		
Pre-exposure	101	98
% of predicted	97–105	92–104
Change	– 2.5 ($P = 0.014$)	– 1.0 ($P = 0.33$)
% of pre-exposure	– 4.5 to – 0.4	– 3.6 to + 1.6
RV		
Pre-exposure	115.5	118.9
% of predicted	94–137	95–143
Change	– 2.8	+ 3.9
% of pre-exposure	– 10.5 to + 11.5 ($P = 0.80$)	– 10.4 to + 18.2 ($P = 0.24$)

Table 1. Baseline, pre-exposure, lung function (in percent of predicted value) and percent change compared with pre-exposure value in 32 healthy subjects of whom 16 regularly inhaled SCG and 16 placebo two weeks prior to exposure

P -values indicate differences between pre- and post-exposure values (Students t -test for paired observations). There was no significant difference between the two groups with regard to the change of FEV₁ ($F = 0.79$; $P = 0.38$), VC ($F = 0.89$; $P = 0.35$) or RV ($F = 0.94$; $P = 0.34$). Mean and 95% confidence intervals.

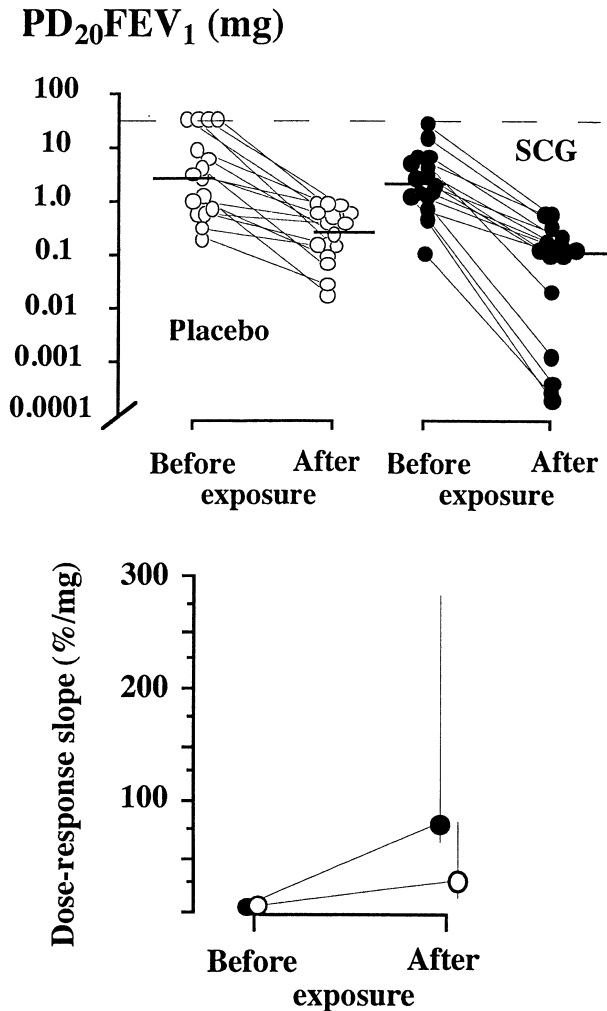


Fig. 2. Upper panel: PD₂₀FEV₁ before and after exposure to swine dust in 16 healthy subjects treated with SCG and 16 healthy subjects treated with corresponding placebo. The exposure induced a decrease in PD₂₀FEV₁ in all 32 subjects. Horizontal lines indicate median values. Horizontal dashed line represents the highest inhaled dose of methacholine. Four subjects in the placebo group and one in the SCG group did not react with 20% decrease in FEV₁ on the highest methacholine dose prior to dust exposure. Lower panel: Dose–response slope, i. e. percentual decrease of FEV₁ as a function of the cumulative dose of methacholine before and after exposure to swine dust in 32 healthy subjects, 16 treated with SCG (filled circles) and 16 with corresponding placebo (open circles). Median and 25th – 75th percentiles. No significant difference (ANOVA, log-transformed data, interaction treatment/occasion) between the groups ($F = 0.77$; $P = 0.39$).

BAL fluid analyses

Cellular reaction There were no significant differences in pre-exposure cellular concentration in BAL fluid between the two groups (Table 2). Cellular findings in BAL fluid

Table 2. Basal, pre-exposure, pre-treatment concentrations of cells and soluble products in BAL fluid in 32 healthy subjects who inhaled placebo or SCG during two weeks prior to swine dust exposure

	Placebo (<i>n</i> = 16)	SCG (<i>n</i> = 16)
Alveolar macrophages ($\times 10^6$ cells)	84.5 68.2–94.7	93.0 75.9–112.8
Neutrophils ($\times 10^6$ cells)	1.6 0.7–2.5	1.6 1.0–3.6
Lymphocytes ($\times 10^6$ cells)	6.2 2.8–9.0	5.3 3.2–6.2
Eosinophils ($\times 10^6$ cells)	0.092 0–0.375	0.065 0–0.328
TNF- α (ng/L)	< 0.25 < 0.25–0.30	< 0.25 < 0.25
IL-6 (ng/L)	0.63 0.50–1.61	1.61 0.23–0.87
IL-8 (ng/L)	< 31.3 < 31.3	< 31.3 < 31.3
sICAM-1 (μ g/L)	35.8 28.8–42.4	38.2 31.6–56.3
MPO (μ g/L)	2.1 1.9–3.7	3.7 2.5–5.4
ECP (μ g/L)	0.82 0.78–0.90	0.83 0.79–0.97
Fibronectin (mg/L)	1.24 0.98–1.96	1.47 1.06–2.30
Methyl-histamine (ng/L)	33 30–35	40 29–44

There were no significant difference between the two groups with regard to any of the measured parameters. Median (25th – 75th percentiles).

are shown in Fig. 3. Alveolar macrophages and lymphocytes in BAL fluid increased 1.4–2.3 fold following exposure in both groups. Neutrophils in BAL fluid increased 32-fold in the SCG group and 71-fold in the placebo group ($P = 0.02$ between the groups). The increase in alveolar macrophages and lymphocytes did not differ significantly between the two groups.

Cytokines Pre-exposure TNF α , IL-6 and IL-8 concentrations did not differ significantly between the groups (Table 2). Cytokine findings in BAL fluid are shown in Fig. 4. All three cytokines increased significantly in both groups and there was a significant difference between the groups with regard to TNF- α ($P = 0.0003$, Mann–Whitney *U*-test on post-exposure values due to a majority of values below detection limit before exposure) and IL-6 ($F = 17.3$; $P < 0.001$). Post-exposure TNF- α was 0.60 (< 0.25–1.51)

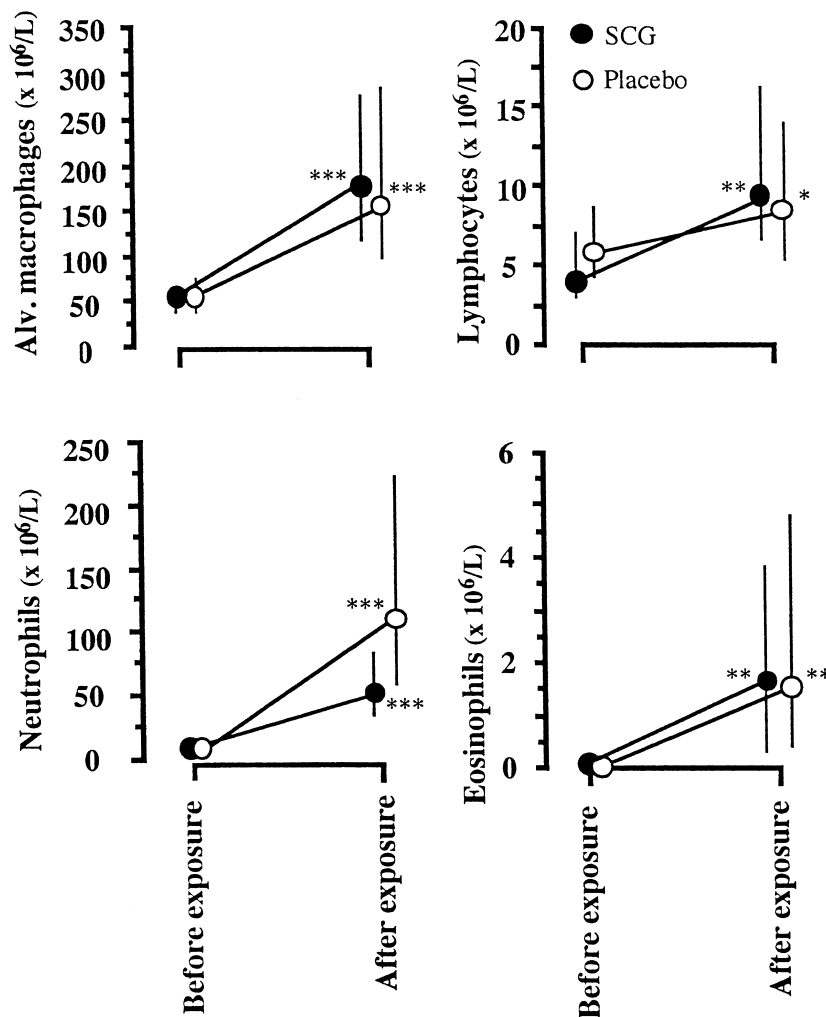


Fig. 3. Cellular findings in BAL fluid before and after exposure to swine dust in 32 healthy subjects of whom 16 were treated with inhaled SCG inhalation and 16 with inhaled placebo for 2 weeks prior to the exposure. Median values and 25th–75th percentiles. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with pre-exposure values. There is a significant difference between the groups with regard to the increase in BAL fluid neutrophils ($P = 0.02$), but not the other cell types.

ng/L in the SCG group in which 8 out of 16 subjects had TNF- α levels below detection limit. In the placebo group post-exposure TNF- α was 4.00 (2.27–8.41) ng/L, all subjects having a post-exposure concentration above the detection limit. The post-exposure IL-6 concentration in BAL fluid was 6.55 (3.90–10.67) ng/L in the SCG group and 12.71 (8.14–21.47) ng/L in the placebo group. The increase in BAL fluid TNF- α and IL-6 correlated significantly with the increase in neutrophils ($r = 0.72$ and $r = 0.64$, respectively, $P < 0.0001$ for both, $n = 32$, data not shown). Significant correlations remained also in each group. No correlation was found between the increase in BAL fluid neutrophils and IL-8 ($r = 0.11$, $P = 0.55$).

Other soluble products Pre-exposure concentration of MPO, ECP, methyl-histamine, soluble ICAM-1, and fibronectin in BAL fluid did not differ significantly between the groups (Table 2, Fig. 4). Fibronectin, MPO and ECP levels in BAL fluid increased significantly

following dust exposure in both groups ($P < 0.01$) whereas a significant increase in sICAM-1 only was found in the placebo group ($P < 0.01$). Swine dust exposure did not change methyl-histamine concentration in BAL fluid in either group. The increase in BAL fluid levels was significantly greater in the placebo group than in the SCG group for sICAM-1 ($F = 11.94$; $P = 0.002$) and MPO ($F = 7.07$; $P = 0.012$) while no significant differences between the groups were found with regard to the change in fibronectin ($F = 4.23$; $P = 0.05$), methyl-histamine ($F = 0.09$; $P = 0.77$) and ECP ($F = 1.74$; $P = 0.20$).

Nasal lavage fluid analyses

Pre-treatment cellular concentration in nasal lavage fluid did not differ significantly between the groups (5.2 (2.0 – 20.7) $\times 10^6$ cells/L in the SCG group and 7.7 (4.0 – 16.0) $\times 10^6$ cells/L in the placebo group). Treatment *per se* did not change the nasal lavage cellular content but

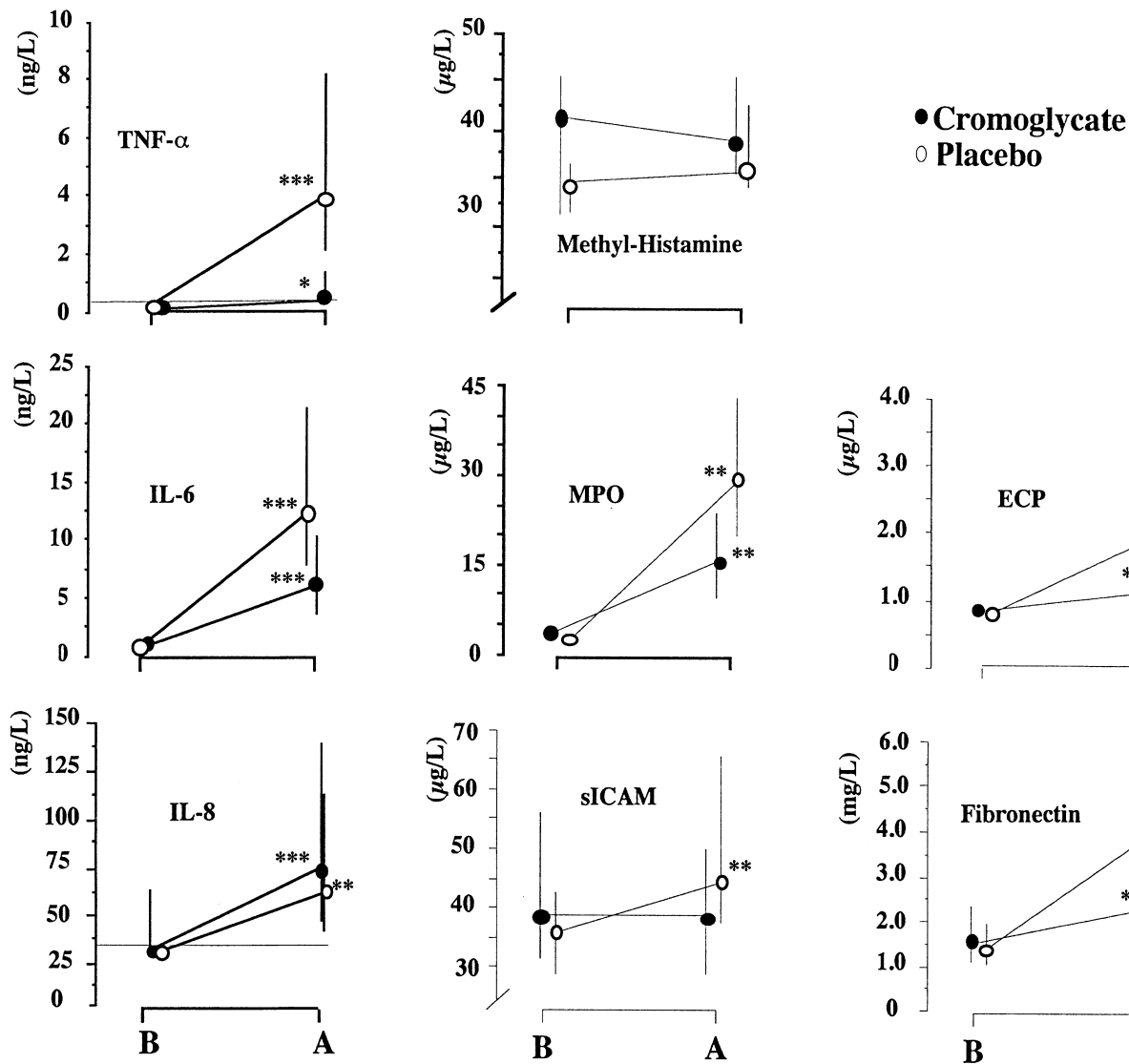


Fig. 4. Soluble components in BAL fluid before (B) and after (A) exposure to swine dust in 32 healthy subjects, 16 pre-treated with SCG and 16 with placebo on a regular base for two weeks. Median values and 25th–75th percentiles. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with pre-exposure values. Significant difference between the groups were found for TNF- α ($P = 0.0003$, Mann–Whitney U -test calculated on post-exposure values) and IL-6 ($F = 17.3$; $P < 0.001$) but not for IL-8 increase in BAL fluid. Significant differences were also found between the groups for MPO ($F = 7.07$; $P = 0.012$) and sICAM-1 ($F = 11.94$; $P = 0.002$). The exposure-induced change in methyl-histamine, fibronectin, and eosinophilic cationic protein (ECP) levels did not differ significantly between the groups.

exposure induced a significant increase in nasal lavage cell content in both groups ($P < 0.01$). Because of low cell numbers differential count was not possible to perform in approximately half of the subjects before treatment and before exposure. After exposure the cells were counted in all but one subject and neutrophil numbers were $102.2 (35.5–193.0) \times 10^6$ cells/L in the SCG group and $75.8 (33.2–219.6) \times 10^6$ cells/L in the placebo group ($P = 0.96$ between the groups).

Albumin and cytokines (IL-1 β , TNF- α , IL-6 and IL-8) in

nasal lavage fluid were not influenced by treatment but increased in both groups following exposure ($P < 0.01$ for all cytokines) with no differences between the groups (Table 3).

Blood analyses

The two weeks of treatment with SCG or placebo before exposure did not change any parameter measured in peripheral blood (data not shown). Following exposure

Table 3. Albumin and cytokine concentration in nasal lavage fluid after two weeks intranasal treatment (SCG or placebo) before and after exposure to swine dust in 32 healthy subjects

	Placebo (<i>n</i> = 16) Before exposure	After exposure	SCG (<i>n</i> = 16) Before exposure	After exposure
Albumin (mg/L)	15.0 11.1–26.5	35.8** 24.9–76.1	13.0 9.1–21.2	35.8*** 20.6–71.8
IL-8 (ng/L)	155 103–227	1064*** 874–1390	142 111–290	1134*** 718–1618
IL-1 β (ng/L)	2.6 0.7–4.0	8.5*** 6.4–28.1	2.4 1.7–3.9	11.0*** 3.9–18.1
IL-6 (ng/L)	1.43 0.56–3.30	26.4*** 16.2–49.4	0.85 0.34–1.87	18.0*** 11.7–35.8
TNF- α (ng/L)	< 0.25 < 0.25	1.50** 0.71–2.97	< 0.25 < 0.25	1.15** 0.46–2.42

Pre-exposure levels did not differ significantly. ** $P < 0.01$, *** $P < 0.001$ compared with pre-exposure values. No significant difference between the groups for any parameter. SCG = sodium cromoglycate. Median (25th – 75th percentiles).

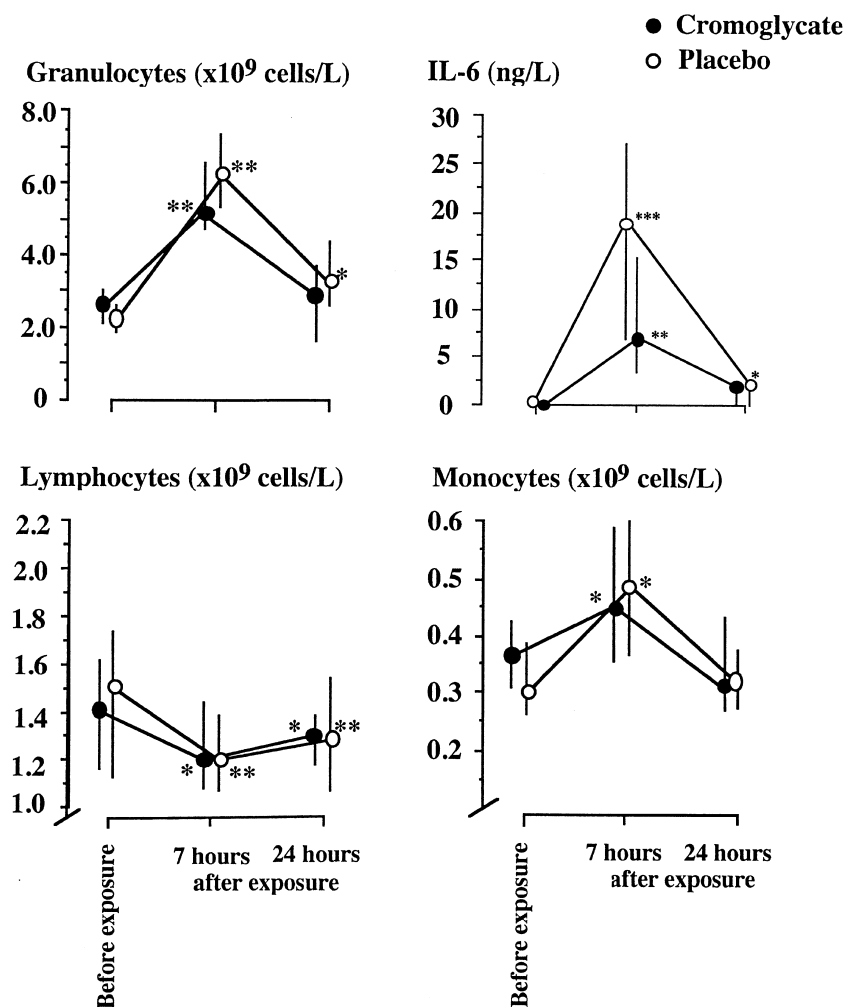


Fig. 5. Cell and IL-6 concentration in peripheral blood during treatment (before exposure) with SCG (*n* = 16) or placebo (*n* = 16) and 7 and 24 h after exposure to swine dust in healthy subjects. There were significant differences over time for all three cell types (granulocytes $P < 0.001$, lymphocytes $P < 0.001$, monocytes $P < 0.01$) and IL-6 ($P < 0.001$) as assessed by Friedmans test. Post-hoc tests were performed with Wilcoxon's signed rank sum test. *denotes $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with values before exposure. There were no differences between the groups (ANOVA, log-transformed data, interaction treatment/occasion) with regard to the changes in cellular concentration (granulocytes $F = 1.41$; $P = 0.24$, lymphocytes $F = 0.54$; $P = 0.63$, monocytes $F = 1.34$; $P = 0.27$) or in post-exposure IL-6 blood concentration ($P = 0.13$, Mann-Whitney).

there was a significant increase in the number of granulocytes and monocytes and a significant decrease in lymphocytes in peripheral blood in both groups (Fig. 5). The number of monocytes in both groups and granulocytes in the SCG group had returned to pre-exposure levels 24 h after exposure.

Pre-exposure blood levels of IL-6 were below detection limit in both groups and increased to 7.1 (3.6–16.3) ng/L in the SCG group ($P < 0.01$) and 19.2 (5.8–26.9) ng/L in the placebo group ($P < 0.001$) following exposure. The IL-6 increase was twice as high in the placebo group as in the SCG group but the difference between the groups was not statistically significant ($P = 0.13$, Mann–Whitney).

Discussion

In the present study it has been shown that SCG inhibits the influx of neutrophils and the release of pro-inflammatory cytokines (TNF- α and IL-6) in the airways without influencing the increase in bronchial responsiveness to methacholine following exposure to organic dust. Furthermore, it has been demonstrated that exposure to dust in a swine confinement building induces increased levels of MPO, ECP, sICAM-1 and fibronectin and that SCG attenuated the response with regard to neutrophil activation (MPO) and sICAM-1.

Sodium cromoglycate almost completely inhibited the release of TNF- α and inhibited the IL-6-release into BAL fluid substantially. Alveolar macrophages are a major source for TNF- α in human airways and swine dust induces the release of TNF- α from alveolar macrophages stimulated *in vitro* [28]. Recently mast cells have also been shown to be an important source for TNF- α in human airways [29], which have been demonstrated both in patients with asthma and in healthy subjects [30]. Sodium cromoglycate inhibits TNF- α release from mast cells *in vitro* [31] and has the capability to inhibit TNF- α release in the airways *in vivo* in newborn children at risk from bronchopulmonary dysplasia [32]. The target cell for this *in vivo* effect of SCG in the lung is not known. We have previously shown that mast cells are probably involved in the response to inhaled swine dust in healthy subjects. Thus, we found that the urinary concentration of 9 α ,11 β -prostaglandin F₂ [33], a metabolite of prostaglandin D₂ which is almost exclusively produced by mast cells in humans [34], increased following inhalation of organic dust from swine houses in healthy subjects. The SCG-inhibition of TNF- α release in BAL fluid in the present study could consequently be expected, at least partly, to be due to inhibition of release from mast cells. The lack of elevated BAL fluid levels on methyl-histamine, the stable and deactivated metabolite of histamine by N-methyltransferase does not, however, lend support for mast cell degranulation

in the airways. As the swine dust exposure leads to systemic reactions our previous findings of 9 α ,11 β -prostaglandin F₂ in urine could be explained by mast cell degranulation at locations not resulting in a spillover into the airspaces. If, however, mast cells are not involved in the reaction to swine dust SCG must inhibit TNF- α release from another cell type, presumably alveolar macrophages. There are data indicating that SCG may influence the alveolar macrophage traffic in the lungs in asthmatic subjects [21] but to our knowledge, there are no data on the effect of chromones on the release of TNF- α in alveolar macrophages obtained from healthy subjects. This question has to be addressed in *in vitro* trials. Epithelial cells are another suggested source for TNF- α which is released upon stimulation with an effect that is inhibited by nedocromil sodium [35]. We have not been able to show that swine dust induces TNF- α release from human epithelial *in vitro*. The possibility that TNF- α emanates from epithelial cells *in vivo* and that SCG acts on these cells can, however, not be excluded.

The effect of SCG on IL-6 levels in BAL fluid and peripheral blood may be a direct effect on IL-6 production or a consequence of the inhibition of TNF- α release since this cytokine induces increased expression of IL-6 [36,37]. Nedocromil sodium acts by the same mechanism as SCG does and has been shown to inhibit IL-6 release from alveolar macrophages obtained from asthmatic subjects *in vitro* [38]. To our knowledge there are no data on the effect of SCG on cytokine release from alveolar macrophages in healthy subjects.

The influx of neutrophils into the airways in the SCG group was almost half the increase observed in the placebo group. The exposure was similar in both groups and the difference is thus not explained by different exposure in the two groups. Sodium cromoglycate inhibits the chemotaxis and activation of neutrophils *in vitro* [19]. This is consistent with the present *in vivo* study in which neutrophil (but not lymphocyte and macrophage) influx into the airways was inhibited by SCG. Swine dust exposure also induced activation of neutrophils (increased levels of MPO in BAL fluid) which was partly counteracted by SCG. The mechanism for this effect on neutrophils is unclear. It could be assumed that IL-8, which is increased in BAL fluid following exposure, is responsible for the neutrophil influx into the airways. The pro-inflammatory cytokine TNF- α is of importance for the release of IL-8 as shown in human bronchial epithelial cells *in vitro* [39]. In the present study, however, inhibition of TNF- α by SCG did not influence BAL fluid levels of IL-8 which was unaffected by treatment with SCG. In agreement with the present findings we were, in a previous study, not able to demonstrate a correlation between the increase of neutrophils and IL-8 in BAL fluid following exposure to swine dust. This suggests

that IL-8 may have a minor role for the swine dust-induced lung neutrophilia [4]. However, the time course for the turnover of different cytokines could differ which makes conclusions of cytokine interaction, based on sampling in one timepoint, difficult.

The significant relationship between changes in TNF- α /IL-6 and the numbers of neutrophils in BAL fluid is in agreement with previous findings. A causal relation between these cytokines and the influx of neutrophils into the airways has been suggested in bronchopulmonary dysplasia [32]. The correlation between SCG-mediated effects on neutrophils and TNF- α levels in BAL fluid indicates that this cytokine may be of importance for the influx of neutrophils caused by inhalation of swine dust. Since neutrophils are also capable of producing TNF- α an inhibition of neutrophil influx would probably lead to lower levels of this cytokine in BAL fluid. However, the small effect on neutrophil influx compared with the dramatic effect on BAL fluid TNF- α concentration does not support this assumption. Swine dust exposure induced an increase of sICAM-1 in BAL fluid. Soluble ICAM is known to be shedded from the endothelium into the blood and also into the air spaces during migration of inflammatory cells, especially neutrophils. Sodium cromoglycate was demonstrated to significantly attenuate the release of a sICAM-1 in BAL fluid, which is in agreement with the recently shown inhibition of sICAM-1 release of endothelial cells *in vitro* [40]. This observation is also paralleled by the reduction of neutrophil recruitment into the air spaces and BAL fluid.

Fibronectin was, for the first time, evaluated, and found to be slightly elevated in BAL fluid following swine dust exposure in healthy subjects. Also, the increase of this general inflammation-associated factor was reduced by pretreatment with the SCG. This may suggest either a SCG action on a large number of different cell types capable of producing fibronectin, or a reduction of cytokine signals responsible for full activation of those cells.

Bronchial responsiveness to direct stimuli such as methacholine and histamine is related to airway inflammation although there is no evidence of a causal relationship. In the present study, dust exposure induced a large, similar, increase in bronchial responsiveness in both groups. Thus, the attenuation of several inflammatory indices in the SCG group was not reflected by a corresponding effect on bronchial responsiveness to methacholine. In animal experiments, TNF- α can cause increased bronchial methacholine responsiveness [41] and it has been discussed whether this is by a direct action on the bronchial smooth muscle or by indirect action via the ability of TNF- α to stimulate the release of other mediators [42]. We found an almost total inhibition of BAL fluid TNF- α without any alteration of increase in bronchial responsiveness in the SCG group. We suggest that this finding may speak against

a major importance concerning the involvement of TNF- α in the development of increased bronchial responsiveness following exposure to swine dust.

The increase in bronchial responsiveness to methacholine was not related to the number of neutrophils, SCG reduced neutrophil influx without effect on the increased bronchial responsiveness. The findings of the present study confirm that swine dust inhalation induces only minor increase in BAL eosinophils [1]. It can not be excluded that the eosinophils could be of importance for the development of increased bronchial responsiveness following dust inhalation. It has been claimed that eosinophilic granulocytes are important for bronchial hyper-responsiveness in asthma [43] but it is not clear whether increased bronchial responsiveness to methacholine following swine dust exposure is mediated by the same mechanisms as bronchial hyper-responsiveness to methacholine in asthma.

We found no differences in cellular or cytokine response in nasal lavage fluid between the two groups. The lack of a SCG effect in the nose may be a fast clearance of the drug. There is also a possibility that the mechanism for cellular recruitment and cytokine release differ in the upper and lower airways. We have previously demonstrated a correlation between the number of neutrophils and the IL-8 levels in nasal lavage fluid while such a correlation was not found in BAL fluid [4]. Since SCG did not influence the IL-8 release in the upper or the lower airways but reduced the neutrophil influx into the lower airways the mechanism for neutrophil migration may be different in the lungs and the nose.

The blood level of IL-6 is increased following dust exposure and may play a role for the increase in body temperature. The airways are the probable source for increased blood levels of IL-6. The dust-induced increase in BAL fluid IL-6 level was lower in the SCG group than in the placebo group whereas IL-6 blood levels and the increase in body temperature only showed a non-significant tendency towards greater increase in the placebo group. It could be assumed that the pulmonary effects of SCG may have minor influence on the fever reaction but that the material in the present study is too small to detect this.

In conclusion, we have found that SCG alters the airway reaction to inhaled organic dust by attenuating the inflammatory cellular and cytokine response without affecting the increase in bronchial responsiveness and without affecting symptoms induced by the exposure. We also found that SCG did not have this anti-inflammatory effect in the nose after intranasal application.

Acknowledgements

This study was supported by the Swedish Heart Lung

Foundation, The Swedish Council for Work Life Research and Fisons Ltd, Sweden. The expert technical assistance of Siw Siljerud is gratefully acknowledged.

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