

Original article

Induced sputum in children with newly diagnosed mild asthma: the effect of 6 months of treatment with budesonide or disodium cromoglycate

Background: There are few controlled studies on the effects of anti-inflammatory treatment on airway inflammation in newly diagnosed childhood asthma.

Methods: Sixty children with newly diagnosed mild persistent asthma, 5–10 years of age, and 17 healthy control subjects were studied. Asthmatic children were randomized into an open study with two treatment groups: (1) budesonide 400 µg twice daily for 1 month, 200 µg twice daily for 5 months and (2) disodium cromoglycate (DSCG) 10 mg three-times daily for 6 months. All exacerbations were treated with budesonide 400 µg twice daily for 2 weeks. Symptoms and lung function were recorded throughout the study.

Results: Sputum induction was safe and the overall success rate was 71%. This improved with age and decreased after treatment. At baseline, the asthmatic children had more eosinophils in blood (0.26 vs $0.18 \times 10^9/l$, $P = 0.03$) and sputum (1.1 vs 0.0 %, $P = 0.0001$) than the control subjects. The numbers of sputum eosinophils correlated with bronchial responsiveness ($R = -0.58$, $P = 0.0002$). Eosinophils were higher in children with atopic asthma than with nonatopic asthma ($P < 0.0001$), and in children with a history wheezing than in children without wheezing ($P = 0.02$). Six months of budesonide treatment, but not of DSCG, improved lung function ($P = 0.007$), decreased symptoms ($P = 0.007$) and sputum eosinophils ($P = 0.003$). The effects of budesonide were pronounced in children with intense sputum eosinophilia ($> 3\%$).

Conclusion: Sputum eosinophilia is present in children with newly diagnosed mild persistent asthma. Treatment with inhaled budesonide, but not with DSCG, decreases sputum eosinophils along with clinical and functional improvement.

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The role and characteristics of airway inflammation in paediatric asthma are less well established than in adults (1). Induced sputum analysis has been recently introduced as a direct and relatively noninvasive way of assessing airway inflammation. Methods for sputum induction have developed and are reasonably reproducible (2). Previous studies using induced sputum in childhood asthma have been cross-sectional, in stable asthma during treatment or during acute exacerbations (3–7). Induced sputum has not been used to characterize airway inflammation in newly detected childhood asthma, or to monitor treatment effects.

Inhaled corticosteroids (ICS) are considered as first line anti-inflammatory therapy in childhood asthma as in adults (8, 9). Studies of adults and children with asthma have shown that some functional reversibility may be lost if anti-inflammatory treatment is postponed (10, 11). The ICS have been shown to suppress eosinophilic airway inflammation along with lung function improvement in adult asthma (12, 13). However, high-dose ICS may have

systemic effects in paediatric patients (14). There are no controlled longitudinal studies on the effects of ICS or disodium cromoglycate (DSCG) on airway inflammation in childhood asthma.

We assessed airway inflammation by induced sputum in children with newly diagnosed mild persistent asthma and compared them with healthy children. The relationship between airway inflammation and clinical characteristics was examined. We also investigated, in an open study, the effect of 6 months of treatment with inhaled budesonide or DSCG on airway inflammation.

Materials and methods

Subjects

The study population consisted of 60 children between 5 and 10 years with newly diagnosed asthma and 17 healthy control

Table 1. Baseline characteristics of subjects

Characteristics	Budesonide (<i>n</i> = 39)	DSCG (<i>n</i> = 21)	Healthy (<i>n</i> = 17)
Age (years)*	7.3 (5–10)	7.5 (5–10)	9.4 (5–13)
Male/female	23/16	12/9	7/10
Atopic	27 (69%)	10 (48%)	7 (41%)
History of rhinitis	13 (33%)	5 (24%)	0 (%)
Duration of symptoms, months	9.7 (1–40)	11.4 (3–72)	–
FEV ₁ (% pred) [†]	90.9 (71–111)	91.9 (74–113)	95.4 (79–118)
Δ PEF (%) [‡]	10.1 (3.2–24)	10.9 (4.7–25)	0.3 (0–1)
PD ₂₀ PEF (mg) [§]	0.97 (0.04–1.6)	1.00 (0.02–1.6)	1.50 (0.41–1.6)

* *P* = 0.01.

[†] Reference values by Koillinen (16), *P* = 0.04.

[‡] Peak expiratory flow variability between morning and evening during the baseline period, *P* < 0.0001.

[§] *P* < 0.0001.

Data are expressed as mean and range or number and percentage of patients. *P*-values are calculated for differences between all asthmatics and healthy subjects using the Mann–Whitney *U*-test or χ^2 -test, as appropriate. Only statistically significant *P*-values are shown.

FEV₁, forced expiratory volume in 1 s.

subjects (Table 1). The asthmatic children presented asthma symptoms (wheezing and/or prolonged cough) for at least 1 month prior to entry into the study, and significant bronchial liability: (1) diurnal variation of $\geq 20\%$ in peak expiratory flow (PEF) on 3 days during a 2-week follow-up period, or (2) bronchial reversibility of $\geq 15\%$ as measured by PEF or forced expiratory volume in 1 s (FEV₁) in response to β_2 -agonist, or (3) a fall of $\geq 15\%$ in FEV₁ during an exercise test (15). Children with an FEV₁ < 50% of the predicted (16) and with treatment during the preceding 2 months with corticosteroids, cromones, or long-acting β_2 -agonists were excluded. Children with seasonal asthma symptoms only or hairy pets at home were also excluded. The healthy control children exhibited no respiratory symptoms and had normal lung function. None of the children had presented symptoms of a respiratory tract infection within 4 weeks preceding the study.

Signed informed consent was obtained from the parents and the children over 7 years of age. The local ethics committee approved the study.

Study design

Cross-sectional study (baseline). Patients and healthy controls were evaluated in relation to various baseline parameters during a 2-week run-in period. During these visits, flow-volume spirometry, skin prick tests, a histamine challenge test, blood sampling and sputum induction were performed.

Longitudinal study (treatment). After the baseline evaluation, children with asthma were randomized into an open study with two treatment groups: (1) Budesonide group (*n* = 49), where patients received inhaled budesonide 400 μ g twice daily for the first month, then 200 μ g twice daily for 5 months (Pulmicort Turbuhaler®; AstraZeneca, London, UK), (2) DSCG group (*n* = 21), where patients received DSCG 10 mg three-times daily for 6 months (Lomudal® with Fisonair®; Aventis Pharma Ltd, Holmes Chapel, UK). Randomization was performed using a computerized list based on a block size of 10.

All patients were given rescue medication of terbutaline 0.25 mg/dose (Bricanyl Turbuhaler®, AstraZeneca). For all groups, during asthma exacerbation, the study medication was replaced by

budesonide, 400 μ g twice daily, for 2 weeks. Parents suspected an exacerbation of asthma if asthma symptoms were not controlled by terbutaline. In such cases, the patient was examined at the clinic by the paediatrician and lung-function measurements were performed. The paediatrician made a decision about whether a patient needed treatment for an exacerbation.

Parents were asked to record lung function, symptom scores (Visual Analogue Scales, score 0–10), and use of rescue medication twice daily monitored using a Vitalograph Data Storage Spirometer (Vitalograph Ltd, Buckingham, UK), as previously described (17).

Skin prick tests

At baseline, skin prick tests were made using 10 common inhalant allergens (Soluprick SQ, 10 HEP; ALK, Hørsholm, Denmark), and positive (histamine dihydrochloride, 10 mg/ml) and negative (solvent) control solutions. A subject was classified as atopic if any allergen caused a weal of 3 mm or more in diameter (18).

Lung-function measurements

Standard flow-volume spirometry (19) was performed at the clinic with a pneumotachograph (Spirotrac III®; Vitalograph Ltd). Airway responsiveness to histamine, expressed as the histamine dose (mg) resulting in a 20% decrease in PEF (PD₂₀PEF), was determined at baseline using an automated, inhalation synchronized, dosimetric jet nebulizer (Spira Electro 2; Respiratory Care Center, Hämeenlinna, Finland), as previously described (20, 21). Bronchial responsiveness was increased if PD₂₀PEF was < 1.6 mg of histamine.

Sputum induction

Sputum was induced by inhalation of nebulized 5% hypertonic saline via an ultrasonic nebulizer (Spira Ultra; Respiratory Care Center) (22). This dosimetric nebulizer produces particles with a mass median diameter of 2 μ m and has an output of 0.6 ml/min. To ensure the safety of the procedure, all subjects were pre-treated with 0.5 mg terbutaline (Bricanyl Turbuhaler®) and PEF was measured before the pretreatment and after induction. The sputum induction was carried out for 15 min but stopped earlier if bothersome symptoms appeared.

Sputum processing

We used the sputum processing method modified from the one described by Fahy et al. (23) and validated by in't Veen et al. (24). Briefly, an entire sputum sample was weighed and mixed with an equal volume of dithiothreitol for 15 min (Sputolysin 10%; Calbiochem Corp., San Diego, CA) and with phosphate-buffered saline. The resulting suspension was centrifuged, the cell pellet was resuspended, and the viabilities and absolute numbers of cells/mg of sputum processed were calculated by trypan-blue exclusion. Coded cytopins were prepared and stained using the May-Grünwald-Giemsa (MGG) method and toluidine blue to obtain cell differential counts. A minimum of 400 nonsquamous cells was counted on MGG-stained slides, and 1500 cells on toluidine-blue-stained slides. The sputum sample was considered adequate if it had < 80% squamous epithelial cells (24). All analyses were carried out blind to the clinical characteristics or treatment. The results are expressed as a percentage of individual cells in relation to total nonsquamous cell count. Blood samples were drawn by venipuncture to obtain blood eosinophil counts.

Statistical analysis

Data are expressed as mean and standard error of the mean (SEM) or medians and ranges. In the case of bronchial responsiveness following histamine challenge, geometric mean values were calculated. Significances of differences between more than two groups were assessed using Kruskal–Wallis one-way analysis by ranks. Comparisons of two groups were assessed using Mann–Whitney *U*-test or χ^2 -test, as appropriate. Correlation was assessed using Spearman's test. Wilcoxon's test for paired data was used to analyse the effects of different therapies. The control group was used to establish upper limits of the normal ranges (90th percentiles) for blood and sputum eosinophils. Power calculations based on our previous experience with induced sputum showed that we had 90% power to detect significant differences within groups (25).

Results

Baseline

Clinical characteristics of subjects. There were no significant differences in the two treatment groups of asthmatic children at baseline (Table 1). The mean duration of symptoms before diagnosis was 10 months. Increased responsiveness to histamine was found in 40 (62%) of the asthmatic children and two (12%) of the healthy children ($P < 0.0001$). The atopic asthmatic children were more responsive to histamine than the nonatopic asthmatic children ($P = 0.009$). According to symptoms and lung function tests, these asthmatic children could be categorized as having mild persistent asthma (9).

Sputum induction. All children co-operated in the sputum induction procedure. One girl reported nausea during induction, but otherwise there were no notable side effects. At baseline, the mean change in PEF during induction was +3.8% (range from –22 to +38%). Six children had a fall in PEF of more than 10% but all recovered quickly.

At baseline, an adequate sputum sample was obtained from 63 of 77 (82%) children. The success rate was 67% for 5-year-old children, 75% for 6-year-old children, 86% for children from 7 to 10 years, and 100% for children above 10 years. After 6 months of treatment the success rate was 68%.

Inflammatory cells. Blood eosinophils and sputum total and differential cell counts at baseline are shown in Table 2. Cell viability, as assessed by trypan blue exclusion, was always above 70%. Compared with healthy subjects, asthmatic children had more blood and sputum eosinophils and sputum metachromatic cells (Table 2). Atopic asthmatics had more blood ($P = 0.02$) and sputum eosinophils ($P < 0.0001$) than nonatopic asthmatics. No differences were seen between atopic and nonatopic healthy children or between the two treatment groups of asthmatic children at baseline.

Table 2. Blood and sputum measurements at baseline

	All asthmatic children ($n = 60$)	Healthy ($n = 17$)
Blood eosinophils ($\times 10^9/l$) *	0.26 (0.13–1.38)	0.18 (0.03–0.36)
Sputum		
Total cell count ($\times 10^3/mg$)	1.0 (0.04–41)	0.9 (0.06–16)
Eosinophils (%)**	1.1 (0–39)	0.0 (0–0.4)
Neutrophils (%)	19.2 (0–87)	26.0 (12–88)
Lymphocytes (%)	0.3 (0–2.5)	0.0 (0–1.3)
Macrophages (%)	73.6 (11–98)	72.3 (12–86)
Epithelial cells (%)	0.0 (0–4.0)	0.3 (0–3.3)
Metachromatic cells (%)***	0.0 (0–0.6)	0.0 (0–0.0)
Squamous cells (%)	22.4 (0–75)	40.0 (1–76)

* $P = 0.03$, ** $P = 0.0001$, *** $P = 0.04$.

Data are expressed as median and range. *P*-values are calculated for differences between all asthmatics and healthy children using the Mann–Whitney *U*-test. Only statistically significant *P*-values are shown.

There was a significant correlation between blood and sputum eosinophils in children with asthma ($r = 0.56$, $P < 0.0001$). The correlation was stronger for atopic asthmatics ($r = 0.65$, $P = 0.0003$), and it disappeared when only nonatopic asthmatics were included ($r = 0.22$, $P = 0.39$). Blood and sputum eosinophils correlated with airway hyperresponsiveness ($r = -0.42$, $P = 0.002$ for blood, $r = -0.58$, $P = 0.0002$ for sputum).

At baseline, 37 asthmatic children (62%) had a history of wheezing. Children who wheezed were significantly more responsive to histamine ($P = 0.009$) and had more sputum eosinophils ($P = 0.02$) than those without wheezing.

The upper reference limit (see Methods) in healthy subjects for blood eosinophils was $0.35 \times 10^9/l$, for the percentage of sputum eosinophils the limit was 0.31%. Seventy per cent of asthmatic children and none of the healthy controls had increased sputum eosinophils at baseline. The 18 asthmatic children (30%) with high sputum eosinophils ($> 3\%$) at baseline also had more blood eosinophils ($P = 0.0001$) and lower $PD_{20}PEF$ ($P = 0.008$) than the asthmatic children with low sputum eosinophils.

Treatment data

After 6 months of treatment, the budesonide group had significantly fewer exacerbations than the DSCG-group (Table 3). The FEV_1 improved in both groups but only in the budesonide group was the change from baseline in terms of percentage of predicted FEV_1 (with adjustment for child's height) significant. Symptoms and use of rescue medication decreased significantly from baseline only in the budesonide group. The total number of sputum cells and the numbers of sputum eosinophils decreased significantly in the budesonide group, but not in the cromoglycate group (Fig. 1). Numbers of blood eosinophils and other sputum cells did not change significantly. Two children in the DSCG group were hospitalized during the

Table 3. Changes in clinical status, lung function and induced sputum cells after 6 months of treatment with inhaled budesonide or disodium cromoglycate (DSCG)

Characteristics	Budesonide (n = 39)	P-value	DSCG (n = 21)	P-value
FEV ₁ , (l)	0.11 (−0.7–0.5)	0.007	0.05 (−0.9–0.3)	0.01
FEV ₁ , % pred	3.5 (−21–28)	0.01	−0.4 (−11–12)	
PEF, (l/min)	5.6 (−89–62)		−3.4 (−76–57)	
Symptom score	−2.1 (−9.3–3.6)	0.007	−1.6 (−8.0–5.6)	
Daily use of rescue medication	−0.6 (−3.6–2.0)	0.007	−0.5 (−4.4–3.7)	
Blood eosinophils (×10 ⁹ /l)	−0.16 (−1.9–0.6)		−0.07 (−0.80–0.43)	
Sputum				
Total cell count (×10 ³ /mg)	−3.1 (−29–8.3)	0.006	−3.5 (−29–35)	
Eosinophils (%)	−4.02 (−29–0.9)	0.003	−3.58 (−22–2.2)	
Neutrophils (%)	2.5 (−71–58)		−6.5 (−52–24)	
Metacromatic cells (%)	−0.03 (−0.6–0.07)		−0.06 (−0.4–0)	
Exacerbations*	0.6 (0–2)		1.4 (0–4)	

* $P < 0.01$ comparison between the two treatment groups.

Data are expressed as mean and range. P -values are calculated for changes within groups. Only significant P -values are shown.

FEV₁, forced expiratory volume in 1 s.

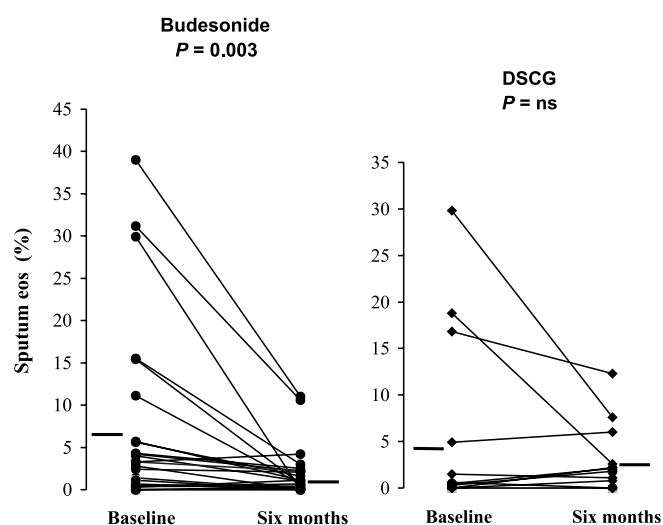


Figure 1. Changes in the percentage of sputum eosinophils with both treatments. Horizontal lines represent mean values. P -values indicate changes within groups. The difference in changes between the groups was not significant. The percentage of sputum eosinophils decreased in 15 of 23 (65%) of patients in the budesonide group, and in three of 11 (27%) patients in the disodium cromoglycate (DSCG) group. The mean number of exacerbations (use of high dose budesonide for 2 weeks) for these subjects was 0.5 in the budesonide group, and 3.7 in the DSCG group, respectively.

study because of an exacerbation of asthma, none in the budesonide group.

High vs low sputum eosinophils. In the budesonide group, the asthmatic children with high sputum eosinophils at baseline ($> 3\%$) improved more in their lung function than the children with low sputum eosinophils at baseline

($P = 0.04$ for change in FEV₁ % predicted and $P = 0.02$ for change in PEF between the high and low eosinophil groups). The numbers of blood ($P = 0.03$) and sputum eosinophils ($P = 0.005$) decreased more in children with high baseline eosinophils than in children with low baseline eosinophils. Sputum neutrophils or other sputum cells were not different between these two groups.

Discussion

Only little information is available of using induced sputum to assess airway inflammation in a group of children with newly diagnosed asthma, and to study the effects of anti-inflammatory treatments. Increased sputum eosinophils and metacromatic cells were found in the majority of asthmatic children at the time of diagnosis. Eosinophils were present in 70% of asthmatic children but in no healthy children. The percentage of sputum eosinophils correlated with blood eosinophils and increased bronchial responsiveness. Atopy and history of wheezing predicted high numbers of eosinophils in sputum.

Six months of inhaled budesonide treatment decreased numbers of eosinophils in sputum with contemporaneous improvement in clinical status. Children with high sputum eosinophils at baseline responded best to treatment with inhaled budesonide. Blood eosinophils did not change during the treatments. Patients treated with DSCG were not improved and experienced more exacerbations. In some patients in the DSCG group, there were marked decreases in numbers of eosinophils in sputum. However, there had been supportive treatment with two-week inhaled budesonide courses during exacerbations (up to 8 weeks during 6 months). Fifteen of the 21 children (71%) who were treated with DSCG needed at least one course of budesonide during the 6 months of the study whereas only 15 of the 29 children (38%) treated with budesonide needed exacerbation treatment. The number of patients in the DSCG group was only half of patients in the budesonide group. However, it was sufficient to give 90% power to detect significant changes within groups.

Sputum has previously been induced mainly in children of 6 or more years of age. We included 15 five-year-old children. Ten of them (67%) produced adequate sputum samples. Success rate improved with age and decreased with therapy. It was 82% for the entire group at baseline and 68% after 6 months of treatment. This is slightly better than reported by others in children on asthma medication (4). Sputum induction was found to be safe when asthmatic children were pretreated with a β_2 -agonist. This has previously been demonstrated in adolescents with severe stable asthma and even in acute childhood asthma (6, 7).

In asthma, airway remodelling occurs during chronic inflammation even in children (26). One of the main

goals in asthma treatment is to identify those children with an asthmatic phenotype and to initiate early treatment in order to avoid irreversible airway remodelling. Induced sputum and exhaled nitric oxide (FENO) are the most common noninvasive methods for measuring airway inflammation. In children with asthma, FENO has been found to correlate with sputum eosinophilia (3). Our recent results emphasise the importance of measuring airway inflammation in children in order to diagnose asthma in the early stage of the disease (27). The FENO was found to be superior to baseline respiratory function in identifying preschool children with probable asthma (27). The measurement of FENO is especially attractive in young children because it is relatively easy to perform.

As compared with FENO, induced sputum analysis allows a more accurate immunological characterization of asthma phenotype in children over 5 years. This may have important clinical implications, especially in cases where the diagnosis or the effects of treatments are uncertain. Eosinophilia in sputum was associated with atopy and wheezing. The treatment effect on eosinophils was seen better in sputum than in blood. According to earlier studies, eosinophilia seemed to be a marker for asthma severity and the effect of anti-inflammatory treatment (6, 28). A high sputum eosinophil count may

indicate the need for effective anti-inflammatory treatment but also predict good treatment response (29).

In conclusion, we have shown that the sputum induction method can be safely and successfully applied for studying airway inflammation in children with newly diagnosed mild persistent asthma from 5 years of age. Induced sputum showed eosinophilic inflammation, especially in atopic asthmatics, and this correlated with increased bronchial responsiveness. Six months of treatment with inhaled budesonide, but not with DSCG, improved lung function, controlled asthma well, and decreased numbers of eosinophils in sputum. These effects were especially found in children with intense sputum eosinophilia at baseline.

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