LONG-TERM INTERVENTION IN CHILDHOOD ASTHMA

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LONG-TERM INTERVENTION IN CHILDHOOD ASTHMA

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Ter nagedachtenis aan mijn vader

Aan mijn moeder
Aan Hans

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General introduction

Asthma is a chronic inflammatory disorder of the airways in which many cell types play a role, including mast cells and eosinophils. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. It also causes an associated increase in airway responsiveness to a variety of stimuli.[1] The disease is usually diagnosed by a history of episodes with symptoms of cough, wheeze, breathlessness and sputum production, caused by chronic or recurrent airway obstruction. Symptoms can be provoked by viral respiratory infections, exposure to allergens, cigarette smoke, SO_2 and other irritants. Exercise-induced asthma[2] and nocturnal symptoms[3] are frequently seen.
This thesis deals with the effect of long-term pharmaceutical intervention on childhood asthma.

1.1. Genetics

Asthma seems to be inherited in a multifactorial way.[4-6] Allergy to one or more allergens is a common feature in asthmatic patients, and recently Cookson et al.[7] identified a gene locus on chromosome 11 which codes for IgE hyperresponsiveness, represented by an elevated level of IgE . They showed that 85 percent of subjects with this gene had symptoms of allergy, 60 percent had a wheeze and in 20 percent the diagnosis asthma had been established. Asthma and IgE hyperresponsiveness can be transmitted from donor to recipient of an allogenic bone marrow transplant. This suggests that specific cells which proliferate in bone marrow induce asthma and IgE hyperresponsiveness in the recipient.[8] These findings suggest that asthma is not merely a disease of the lungs, but might be considered as a dysfunction of bone marrow cells, probably of a lymphocyte subset.[9]

1.2. Environmental factors

It is unknown what exactly causes the variation in expression of asthma. There is a positive relation between the number of allergens to which one is sensitized and the severity of asthma,[10] which suggests that external factors like allergen exposure contribute to the expression of asthma. This has recently been proven for house

dust mite[11]. There is much discussion about the role that viral infections play in the expression of asthma. Respiratory syncytial (RS) virus is associated with recurrent wheeze. In an uncontrolled study Rooney et al.[12] showed that this occurred in 35 of 62 children but they could not exclude confounding by genetic factors as both patients and their families had an increased prevalence of atopy. In another uncontrolled study Webb[13] et al. did not find any difference in personal or family history of atopy between children with and without respiratory symptoms three and a half year after RS-bronchiolitis. Several controlled studies with a follow-up of 7-10 years have shown an increase in respiratory symptoms amongst children after a proven infection with RS virus independent of atopy in these children or their relatives[14-16]. It was not clear whether there was a causal relation between the viral infection and persisting symptoms or whether certain children had a preexisting vulnerability of airways. The results of a study of Martinez et al.[17] showed a negative relation between lung function prior to any lower respiratory tract illness and subsequent wheezing during the first year of life. In a longer follow-up study they also showed that the number of viral infections and the time at which the first viral infection manifests itself are associated with the subsequent degree of abnormality of lung function.[18] Another external factor contributing to the expression of the disease is the exposure to cigarette smoke.[19-21] Children of smoking mothers are more likely to develop asthma than children of non-smoking mothers,[22,23] the relative risk being 2.1 to 2.5. Murray et al. showed recently that children with atopic dermatitis were more prone to develop asthma when their mother smoked.[22] Martinez et al.[23] found that this risk was increased independent of self-reported respiratory symptoms by the parents. Cross-sectional studies have shown that active cigarette smoking in children and adolescents is associated with chronic respiratory symptoms.[24,25] Other pollutants that may influence the expression of asthma symptoms are SO_2, which has been shown to increase airway hyperresponsiveness after accidental exposure to a high concentration of this gas,[26] and NO_2.[27] Both pollutants potentiate the effect of exercise in asthmatic subjects with known exercise-induced asthma.[27,28] In children exercise is an important provoking factor for asthma symptoms. Exercise-induced asthma occurs in 70-80 percent of children with asthma.[29,30] Cold air inhalation may provoke asthma symptoms, and is considered as an important trigger for exercise-induced asthma.[31,32]

It can be concluded that the expression of asthma can be influenced by many environmental factors.

1.3. Prevalence of asthma

The prevalence of asthma varies considerably in the various studies,[33-40] dependent on the definition used. One study[41] suggests that by the age of 10 at least 20 percent of children will have had symptoms due to asthma. The estimated prevalence of doctor's diagnosed asthma decreases with age, being 5-10 percent for 0-4 year old children, 5-6 percent for 5-14 year old children and 3-5 percent for adolescents in the age of 15-20 years. In childhood more boys than girls are affected, but such a difference is not seen during adolescence. Longitudinal studies which could explain why this difference in prevalence between sexes disapperars with age are lacking. Some studies have looked at the change in prevalence of asthma over time.[42-44] Burr et al.[42] showed an increase among 12 year old children in South Wales from 6 percent in 1973 to 12 percent in 1988. Robertson et al.[43] found a huge increase in the history of asthma in 7 year old children in Melbourne: from 19.1 percent in 1964 to 46 percent in 1990, an increase of 141 percent. Among 8-13 year old Aberdeen children the asthma prevalences were 4.1 percent in 1964 and 10.2 percent in 1989.[44] It is difficult to estimate to what extent the increase is attributable to a better recognition of asthma, but all authors show an increase in asthma prevalence.

Schwartz et al.[45] showed that in a large subgroup of the Second National Health and Nutritional Examination Survey the prevalence of asthma was 3 percent among white and 7.2 percent among black children. After adjusting for age, sex, younger maternal age, residence in the central city and family income black children still had a 1.7 relative odds of having asthma, which suggests that asthma prevalence may partly depend on race.

1.4. Characteristics of asthma

1.4.1. *Morphology*

Asthma is an inflammatory disease of the airways. From studies of bronchoalveolar lavage fluid and bronchial biopsies it has become clear that inflammation is a feature even in mild asthmatics.[46-48] The inflammation in asthma is characterized by activated eosinophils and mast cells in the bronchial mucosa, increased vascular permeability, mucosal edema and epithelial damage.[46] Activated T-lymphocytes are found in the submucosa and are now considered to play an important regulatory

role in the inflammatory process. The cytokines IL3, IL5 and GM-CSF control eosinophil production and survival time in bone marrow and play a role in mast cell differentiation after antigen contact. Other lymphokines have chemotactic effects on neutrophils and eosinophils. Activation of mast cells and macrophages leads to the release of inflammatory mediators, which seem to be crucial in the initiation of the early response after allergen provocation. These mediators cause constriction of airway smooth muscle, mucus hypersecretion, stimulation of afferent nerve endings, they recruit inflammatory cells and increase the permeability of airway epithelium and endothelium of small blood vessels; this results in edema of the airway wall and a change in airway wall mechanics.[49] During the late reaction after allergen exposure both the number and the activation of eosinophils are increased;[50] these cells are still present in broncho-alveolar lavage fluid after more than 24 hours, whereas neutrophils disappear within 4 hours. This suggests that eosinophils are more important than neutrophils in the sustained inflammation of the late response, which is associated with an increase in airway responsiveness.[51] The role of neutrophils in asthma is controversial. They are present in bronchoalveolar lavage fluid (BAL)[52] and in tissue sections of asthmatic airways.[47] In stable asthma the percentage of neutrophils is not increased,[47] whereas the percentage of neutrophils in BAL during the late phase reaction after allergen[53] and toluene diisocyanate[54] is increased but not after plicatic acid in red cedar asthma.[55]

The surface of airway epithelium is partly damaged in asthma.[47,56] A biopsy study comparing epithelial tight junctions in asthmatics, bronchitics and normal subjects showed that these tight junctions might be deranged, which could be an explanation for epithelial fragility.[57] A recent study in mild to moderately severe asthmatics shows that with increasing density of eosinophils in bronchial mucosa, both the incidence of opening of tight junctions of epithelial ciliary cells and the degree of widening of intercellular spaces in epithelium increased significantly.[58] Thickening and hyalinization of the reticular layer (i.e., lamina reticularis) beneath the basal membrane is a constant finding in asthma. This lesion is characteristic for asthma, being present early on even when symptoms are mild.[58-60] The thickening is due to subepithelial fibrosis as a result of myofibroblast activity.[60] The percentage of bronchial wall occupied by bronchial smooth muscle shows a striking increase during severe asthma attacks.[61-63] The increase in muscle mass is largely attributable to hyperplasia of individual muscle fibers and less to hypertrophy.[63]

Although the pathophysiology of asthma becomes more and more established, the intricate play of inflammatory cells, nervous system and extracellular matrix has not yet fully been disclosed.

1.4.2. *Pathophysiology*

Airway caliber in patients with asthma is permanently or intermittently decreased. This is considered to be mainly the consequence of airway inflammation.[64] Because in asthma the amount of airway smooth muscle is increased, its contractile responses to histamine, methacholine and exercise may be exaggerated.[65,66] In the assessment of airway obstruction, forced expiratory volume in 1 second (FEV_1) and baseline peak expiratory flow (PEF) are the most commonly used indices of airway caliber; other indices, such as airway resistance and specific airway conductance, are more sensitive but less reproducible. Another way to look at airway obstruction is the bronchodilator response, i.e. the improvement in airway caliber after a bronchodilating agent. The bronchodilator response can be expressed in different ways: as an absolute difference in FEV_1 before and after bronchodilation (delta FEV_1 in liters), as a percentage of predicted FEV_1 (delta FEV_1 percent predicted) and as a percentage of baseline FEV_1 (delta FEV_1 percent baseline). In children the best way seems to be delta FEV_1 percent predicted,[67] as this is not dependent on height, age and sex and is the least influenced by baseline value of FEV_1 of all ways of expressing the bronchodilator response.

Another characteristic of asthma is airway hyperresponsiveness (AHR). This can be distinguished in an increased sensitivity of airways to contractile stimuli like viral infections, cold air, chemical irritants such as SO_2 and cigarette smoke, and an increased maximal response plateau. In the laboratory the sensitivity can be assessed by inhalation of increasing concentrations or dosages of histamine or methacholine. This is usually expressed as the provocative concentration or dose of histamine or methacholine which causes a decrease in FEV_1 of 20 percent from baseline (airway sensitivity)($PC_{20}FEV_1$ or $PD_{20}FEV_1$). There is a weakly negative relation between airway caliber and AHR to histamine or methacholine: this is more important in patients with chronic airflow obstruction with an initial FEV_1 below 70 percent of predicted[68] than in patients with asthma.[69] The maximal response plateau indicates the degree of maximally achievable airway narrowing. It is unmeasurable in moderate to severe asthmatics, intermediate in mild asthmatics and low in non-asthmatics.[70] Apart from histamine or methacholine sensitivity airway sensitivity can be assessed by inhalation of cold air[71], hyper- or hypotonic saline,[72] and by exercise.[73] Mellis et al.[74] compared the responses to exercise and histamine in 50 asthmatic children. They found a higher incidence of a positive response with histamine (90 percent) than with exercise (74 percent). Others had similar findings.[75] Galdès-Sebaldt et al.[75] compared the responses to methacholine and cold air

hyperventilation in 21 children with asthma; 95 percent of the patients were hyperresponsive to methacholine and 57 percent to cold air. From these and other studies[29,76-78] it can be concluded that histamine and methacholine challenges are the most sensitive tests: the percentage of patients with current symptomatic asthma who are hyperresponsive to histamine or methacholine[76] is almost 100, while only 70-80 percent have exercise-induced asthma[29,77,78] and 57-100 percent are hyperresponsive to cold air.[32,75] Diurnal or between days variation of PEF is another indicator of AHR.[103]

The mechanisms which determine airway responsiveness and the reaction to triggers are complex. In their review article Sterk et al.[70] give a schematic explanation by distinguishing pre- and postjunctional mechanisms, which determine a leftward shift of the dose-response curve (increased sensitivity) and an increase in maximal response plateau, respectively. Prejunctional mechanisms include epithelial damage or malfunction, neural control, number and activation grade of inflammatory cells, cell-cell interaction, and metabolism or absorption of mediators, whereas postjunctional mechanisms include smooth muscle contraction, swelling of the airway wall, viscous and elastic load on the airway and intraluminal exsudate and secretions. Histamine is likely to have its primary site of action on neural control and smooth muscle,[79] methacholine on smooth muscle,[79] and exercise at the mucosal level.[2] PEF variation might well be the expression of the spontaneous changes in smooth muscle contraction and the swelling of airway mucosa as the result of various interacting mechanisms.

1.5. Airway hyperresponsiveness and inflammation

The relation between AHR (sensitivity) and inflammation is well established.[80,81] Mast cells and eosinophils are considered as the primary effector cells for allergic asthma. T-cells play an orchestrating role causing inflammation in asthma.[82] (see also 1.4.1.) This concept led to several studies which looked at the relation between cellular events (numbers of inflammatory cells, cell activation) and AHR. A highly significant positive relation was found between methacholine responsiveness and the number of metachromatic cells and eosinophils in BAL fluid.[83] In this study the concentration of major basic protein (MBP) in BAL fluid as an indicator of eosinophilic activation, was not different in asthmatics and non-asthmatics. In a study by Wardlaw et al.[84] mean eosinophil count in BAL fluid was higher in mild symptomatic hyperresponsive asthmatics than in non-symptomatic asthmatics, hay

fever patients and healthy subjects. This difference was due to 2 symptomatic asthma patients, most symptomatic asthmatics having similar eosinophil counts compared to the subjects in the other groups. More important is their finding that MBP was significantly increased in the symptomatic group and differed significantly between normal and hyperresponsive patients. This suggests that AHR is mediated by products derived from eosinophils. This study also suggests that the activation state and not just the presence of inflammatory cells is important in causing AHR. Bradley et al.[85] found a positive correlation between the ratio of activated leukocytes/eosinophils in bronchial biopsies and methacholine PC_{20}. An acute inflammatory reaction (such as after influenza vaccination[86,87]), the late reaction after exposure to allergen[50,88-91] and the late reaction after inhalation of toluene-diisocyanate[92] cause both an inflammatory reaction and a temporary increase in AHR in patients with asthma. A recent study[93] showed a reduction in the number of epithelial and mucosal mast cells, eosinophils and submucosal T lymphocytes together with a decrease in airway responsiveness to methacholine after 8 weeks of treatment with inhaled corticosteroid, but no change in the extent of mast cell and eosinophil degranulation, which means that the inflammatory process was only partly switched off. Lundgren et al.[94] performed a biopsy study in 6 adult patients with severe intrinsic asthma before and after 10 years treatment with 200-1600 μg inhaled corticosteroid per day, and compared the findings with biopsies taken from healthy controls. They found that the number of inflammatory cells (which was increased in the asthmatic patients before the treatment) had normalised and epithelial damage had decreased although PC_{20} to methacholine was still highly abnormal. In this study cell activation was not measured. These observations suggest that airway inflammation, expressed as the presence of an increased number of inflammatory cells in the airways, and AHR are not completely interchangeable.

1.6. Indicators for the activity of asthma

Assessment of asthma severity usually includes history, physical examination, drug requirement and lung function tests. Physical examination in asthma is often misleading. Assessment of airway caliber by lung function tests is the most objective measurement but shows a considerable variation over time. Drug requirement is influenced by the ability of the patient to perceive airway obstruction[95] and to tolerate symptoms, and by personal lifestyle. Not all patients

with symptoms of asthma exhibit AHR when challenged with histamine, methacholine or exercise. Conversely, not all patients with AHR have symptoms. A review of the literature[96] on both cross-sectional and longitudinal studies shows a positive overall relation between the severity of asthma symptoms and the degree of airway responsiveness, but within subjects the relationship is weaker.[97,98] This might be explained by a discrepancy in the degree in which a certain stimulus influences airway caliber and the complicated mechanism that determine AHR,[70] and also by differences in sensitivity of measurement methods and in patient perception of airway narrowing.[95] This implies that histamine/methacholine hypersensitivity is not synonymous with asthma.[99-102] PEF variation is another indicator which is associated with the severity of asthma.[103] But as for AHR, PEF variation in normals and asthmatics shows a large overlap.[104] Both severe AHR to histamine/methacholine and a large diurnal PEF variation are associated with the frequency of asthma exacerbations and asthma deaths.[105,106]

1.7. Relation between asthma in children and asthma in adults

1.7.1. *The natural history of asthma from childhood to adulthood*

Several studies addressed the natural history of asthma. Because of intervention which has changed with time, "natural history" must be read as the history of asthma patients under changing conditions. Data were partly collected retrospectively by means of questionnaires, which has the potential risk of preferential recall or underreporting of symptoms. Several retrospective studies have shown that after a follow-up period of 5-27 years 30-55 percent of subjects who had asthma in childhood were symptom free.[107-112] Results of prospective studies are in agreement with these figures. Blair performed a prospective study in 244 children under 12 years of age from a London general practice.[113] He followed them up for 20 years and found that 52 percent had become symptom free for at least the last two years, but that another 27 percent had a relapse of asthma after a symptom free interval of at least 3 years. Bronnimann et al.[114] did a prospective study in 3454 subjects of the general population in Tucson, of whom 2300 were followed up for a mean period of 9.4 years. Of this last group 136 had active asthma in the first survey, and at the final survey 22 percent had a remission, defined as no asthmatic attacks, no "frequent" attacks of shortness of breath with wheezing and no need for medication during the preceding year. Age ranged from

below 10 years to 79 years. The remission rate was highest in those between 10 and 19 years of age (65 percent) and lowest in those between 40 and 49 years of age (6 percent). The longest prospective study, the ongoing study in Melbourne[115,116], showed that in a sample (401 subjects) of a birth cohort 54 percent with wheeze in childhood was symptom free at the age of 21 and 32 percent at the age of 28. Gerritsen et al.[117] completed a follow-up study of 119 asthmatic children between 6 and 14 years of age. Of these patients 101 were reinvestigated at an age between 22 and 31 when 43 percent still had asthma symptoms. Peat et al.[118] conducted a prospective study in a population-based sample of 380 schoolchildren in whom they investigated the prevalence of respiratory symptoms and AHR to histamine (sensitivity) on 3 occasions at 2-year intervals. They found a high correlation between the initial degree of AHR and ongoing respiratory symptoms: of those children who had severe or moderate AHR on the first occasion 87 percent had current respiratory symptoms 4 years later. An observational study[119] that was published recently suggests that long-standing asthma may lead to chronic persistent airway obstruction and thereby mimic chronic bronchitis and emphysema.

1.7.2. *Predictive factors for the course of asthma*

From the prospective study in Australia it has become clear that airway caliber (FEV_1 percent predicted) at the age of 14 has predictive value for the presence of asthma symptoms at the age of 21.[115,120] Gerritsen et al.[117] have shown that both airway caliber and AHR (sensitivity) to histamine at ages of 6 to 14 years are predictors for the presence of asthma symptoms between 22 and 31 years of age. Their data do not clarify whether these factors predict the course of asthma independently. Although these predictors may be valid for groups of patients their value for individuals has not been studied. A further follow-up of the Australian study cohort at age 28 years[116] reveals that wheeze at the age of 14 has a predictive value for the severity of asthma in later life: of those with few symptoms at age 14, 73 percent continued to have little or no asthma at age 28, and 68 percent of those with frequent wheezing at age 14 still had recurrent asthma at age 28.

Eczema in early childhood is another predictive factor for persisting asthma in later life.[111,113,121]

1.8. Treatment of asthma

Current treatment of asthma aims to reduce the inflammatory process.[1,122,123] Because of the importance of allergy in maintaining inflammation and symptoms, allergen avoidance would be the first measure to be taken, according to advice of international consensus reports.[1,122,123] A number of studies show that avoidance of allergen diminishes asthma symptoms and the need for drug therapy.[124-126] However, in most asthmatics this is difficult to achieve and therefore drugs cannot be avoided. The following drugs are currently used:

Bronchodilators

$Beta_2$-agonists are the most frequently used drugs for the treatment of asthma in children and adults. They are the most potent bronchodilators available. Administration by inhalation is preferable to other routes because of the better dose effect ratio and the quicker effect.

Pharmacology

$Beta_2$-agonists act directly on $beta_2$-adrenoceptors which activate the adenylate cyclase system and then cause an increase in the intracellular concentration of cyclic AMP. This leads to activation of protein kinase, which inhibits the phosphorylation of myosin and decreases intracellular ionic calcium concentrations. These processes result in relaxation of all airways from trachea to terminal bronchioles.[127] $Beta_2$-agonists relax airway smooth muscle via $beta_2$-adrenergic receptors,[128] increase mucociliary transport,[129] inhibit cholinergic transmission at the ganglionic level[130] and may modulate mediator release from mast cells and basophils[131,132] but not from eosinophils[133] or macrophages.[134] Of these actions, the smooth-muscle-relaxing effect is considered to be the predominant mechanism. The smooth muscle relaxing effect is present after contraction by various mediators involved in asthma, such as acetylcholine, histamine, 5-hydroxytryptamine, prostaglandine F_2alpha, LTC4, LTD4 and substance P, as has been shown in isolated human airway preparations.[135] $Beta_2$-agonists have been shown to inhibit plasma leakage in several microvascular beds in a number of animal models,[136] but data on humans are lacking. Long-acting $beta_2$-agonists seem to have no effect on inflammatory cells in BAL fluid and in bronchial biopsies.[137,138]

Clinical effects.

The potent immediate bronchodilating effect of short-acting $beta_2$-agonists causes quick relief of symptoms of asthma, which explains the favourite role these drugs

play in asthma treatment. The maximal effect is seen after 20 minutes, and the duration of bronchodilation is 4-8 hours. The magnitude of the bronchodilating effect is dependent on the severity of the airway obstruction and the dose of agonist. In periods of increased disease activity high doses of an inhaled $beta_2$-agonist may be needed for a limited degree of improvement because of diminished $beta_2$-receptor responsiveness[139] or other mechanisms (possibly mucosal swelling). Short-acting $beta_2$-agonists have an acute protective effect but no long-term effect on AHR (sensitivity). Some studies have reported a slight increase in AHR or a transient rebound effect (Chapter II). The additional effect of a $beta_2$-agonist to inhaled corticosteroid on AHR has not yet been investigated. Short-acting $beta_2$-agonists prevent the early, but not the late, asthmatic response after antigen challenge.[140,141] They also prevent the early asthmatic reaction after exercise.[142,143] They decrease daily PEF variation slightly.[144] Long-acting $beta_2$-agonists, like short-acting $beta_2$-agonists, relieve symptoms of asthma by their bronchodilating action, with a maximal effect after about 1h and a duration of action of at least 12 hours.[145] They have a protective effect on AHR which lasts for 12-24h.[146] No data are available on the long-term effect of these drugs on AHR. They inhibit the allergen-induced early and late response[147] and decrease diurnal PEF variation.[148-150]

Side effects

Side effects are minor after inhalation of the recommended doses. Tremor is sometimes mentioned; other rare side effects are palpitations, nervousness and hypokalemia (the last especially after systemic administration). In recent discussions some authors drew attention to the potentially harmful effects of $beta_2$-agonists on symptoms, decline of airway caliber and fatal course of asthma in adults.[151-153] Similar observations in children have not been reported. Arguments have been raised to prefer an on-demand prescription of $beta_2$-agonists to daily treatment.[151,152] Prospective long-term studies that can answer this question are lacking.

Ipratropium bromide is an anticholinergic drug which is inhaled; it has bronchodilating and mucus secretion inhibiting properties. Its bronchodilating effect in asthma is inferior to that of $beta_2$-agonists and therefore this is not the first choice as an asthma drug. No antiinflammatory effects have been demonstrated.

Theophylline has been widely used as a bronchodilator. The precise mode of action is not known. Side effects include gastro-intestinal symptoms, disorders of sleeping, behaviour and learning. Therefore it is no longer recommended as a therapy of choice in asthma management.[1,122,123]

Antiinflammatory agents

Sodium cromoglycate and nedocromil sodium

Disodium cromoglycate (DSCG) is recommended as a front-line antiinflammatory drug for children with moderate asthma when maintenance treatment is indicated.[1,122,123] Nedocromil sodium (NSO) is a pyranoquinoline dicarboxylic acid which is recommended as first line antiinflammatory drug for adults with moderate asthma.[1] The effect of this drug in children has not yet been definitely established.

Pharmacology

DSCG and NSO partly inhibit the IgE mediated release from human mast cells in a dose-dependent way.[154] They also stabilize mast cells and inhibit activation of eosinophils, neutrophils, macrophages and monocytes.[155-158] It has been suggested that both drugs modulate inflammatory cell function by inhibition of protein kinase C.[156,157] Treatment of asthma patients with DSCG during 4 weeks resulted in a significant decrease in the number of eosinophils in BAL fluid.[52] Additional effects on nerve reflexes have been suggested. This theory derives from experiments in dogs in which reflex-induced bronchoconstriction following stimulation of C-fiber sensory nerve endings with capsaicin was blocked by prophylactic treatment with DSCG.[159] The cough reflex which is provoked by inhalation of citric acid in the dog is blocked by NSO and codeine phosphate but not by DSCG,[160] which suggests that NSO suppresses cough-related nerve reflex activity in the bronchial tree. Chatterjee et al.[161] showed an antitussive effect of NSO in humans.

Clinical effects

DSCG and NSO have an immediate protective effect against bronchoconstriction caused by various stimuli. NSO is 2-10 times more potent than sodium cromoglycate in preventing some forms of acute bronchoconstriction although comparative studies are scarce.[162-164] The duration of the protective effect is 6-8h. During maintenance treatment with either drug asthma symptoms improve.[165,166] They have no effect on airway caliber. DSCG does not have an acute protective or long-term effect on AHR (sensitivity)(see Chapter II). Data on the effect of NSO on AHR are limited, and the results are conflicting (Chapter II). In non-allergic adult patients long-term treatment with NSO reduces AHR.[167] Both DSCG and NSO diminish PEF variation.[165,168] Prophylactic treatment with DSCG and NSO results in inhibition of the early and late response after allergen provocation,[169-171] and the acute bronchoconstriction after exercise.[172] The blockade of the late response after allergen exposure is an important effect as the late response may cause a sustained increase in AHR.[173] DSCG and NSO may also prevent an increase in AHR during birch pollen season in patients who are sensitized for this allergen.[174,175] In

patients with exercise-induced airway narrowing DSCG and NSO have similar effects on reducing the decrease in FEV_1.[176,177] Not all patients benefit equally from DSCG, and there are no predictors that can help identify patients who will benefit from this drug. Therefore, a trial period of 6-8 weeks is recommended.[1,122]

Side effects

No serious side effects have been reported. The only occasional complaint is cough after inhalation of sodium cromoglycate as dry powder.

Corticosteroids

Corticosteroids are currently the most effective anti-inflammatory drugs. Inhaled corticosteroids are now recommended as first-line drug in all patients with severe asthma and in those with moderate asthma who do not respond adequately to a maintenance treatment with DSCG (children) or NSO (adults).[1,122,123]

Pharmacology.

Corticosteroids exert their antiinflammatory effect through a variety of actions, including interaction with arachidonic acid metabolism. They inhibit the production of T-cell growth factor (interleukine 2) by lymphocytes,[178] which induces proliferation of responsive T-lymphocytes and reduces migration of monocytes to sites of inflammation.[179] Corticosteroids suppress monocyte and neutrophil activation, manifested by a reduction in complement receptor expression.[180] In vitro effects that have been shown are the suppression of release of mediators from alveolar macrophages[181] and human basophils[182] but not from mast cells.[183] Other effects are the reduction of the number of eosinophils in blood and tissues, and in a rat model they inhibit eosinophil degranulation.[184] Corticosteroids reduce microvascular leakage caused by inflammatory mediators,[185] stimulate bronchial ciliogenesis,[186] repair epithelial damage[187] and potentiate the relaxing effect of $beta_2$-receptors on airway smooth muscle.[188]

Clinical effects.

A single dose of beclomethasone dipropionate causes a small improvement of airway caliber and AHR (sensitivity)[189] and does not influence exercise-induced airway narrowing.[190] Long-term treatment with inhaled corticosteroids improves airway caliber and reduces AHR (sensitivity).[191-194] The effect on AHR depends on dose and duration.[195] Data on the effect of long-term use of inhaled corticosteroid on exercise-induced airway obstruction are conflicting. Some investigators did find an effect,[196,197] while others did not.[198] Long-term treatment with inhaled corticosteroids abolishes the early and late response after allergen exposure[199], improves the tolerance to an inhaled allergen (house dust mite)[200] and decreases

the diurnal PEF variation.[144,200]

Side effects

In children the maximal daily dose that is considered to be safe during long-term treatment is 600-800 μg.[201] At these dosages a slight dose-dependent suppression of baseline adrenal corticosteroid production with preservation of the stress response has been detected, without any sign that this has clinical implications.[202-205] Growth has always been a point of concern in children treated with corticosteroid. An overview of the literature shows that long-term administration of inhaled corticosteroid with daily doses up to 800 μg at a maximum usually does not affect height growth.[201,206] A delay of puberty that occurs frequently in children with asthma[207] is likely to be the explanation for the disturbed growth pattern that was seen by Littlewood et al..[208] Some studies have been published on the short-term effect of inhaled corticosteroid on growth of the lower leg measured by knemometry.[209,210] In a double-blind cross-over study shows a mean decrease of growth of 0.36 mm per week on 800 μg and of 0.11 mm per week on 200 μg budesonide was shown, compared to a normal growth of 0.63 mm per week during the run-in period and 0.64 mm per week in the wash-out period.[209] Every period (both run-in, treatment and wash-out) lasted 18 days. A more recent 8 week study[210] showed no influence of 400 μg, but a reduction of 0.26 mm per week due to 800 μg budesonide compared to a growth of 0.39 mm per week on placebo. These publications led to a number of letters: one[211] mentioned a similar decrease of growth under 400 μg beclomethasone dipropionate (BDP) during 4 weeks but no reduction after 200 μg BDP, others[212,213] emphasized the importance of adequate treatment of asthma symptoms, rather than treatment that does not produce any detectable effect on height increment. Another point that was made was that if the findings of knemometry were extrapolated to a longer period, this would imply serious growth disturbance in large groups of asthma patients, which is simply not seen.[201,214] A recent study from our own group has shown that 400-600 μg budesonide does not impair height growth in asthmatic adolescents.[214] Inhaled corticosteroids reduce total body calcium, osteocalcin and bone density in a dose-dependent way.[215-219] Most studies were performed in healthy volunteers over a short period. It is unclear what the clinical implications of these findings are. No data exist in children because of methodological difficulties in studying this phenomenon in growing subjects. Other side effects are dysphonia and oropharyngeal candidiasis, both with a low incidence in children. Rare side effects are cataract[220] and skin atrophy,[221] but they have never been reported in children.

Other anti-asthma drugs

Antihistamines have a weak effect on asthma symptoms after several weeks of therapy. Most antihistamines have side effects on the central nervous system, especially sleepiness. They are mainly indicated for patients who, besides asthma, have additional symptoms of allergy, like eczema and rhinitis, which cannot be treated adequately with local therapy.

1.9. Purpose and design of the study

In this study the hypothesis was tested that in children with asthma, long-term intervention directed at suppression of inflammation and increase of airway caliber is superior to long-term intervention directed at increase of airway caliber only.
The study was part of a national multicenter study on long-term intervention in chronic non-specific lung disease in which children with asthma and adults with asthma and chronic obstructive pulmonary disease participated. Furthermore growth of lungs and airways in children with asthma was investigated. All studies were sponsored by the Dutch government, as part of the so-called "Stimulation Program", a program which supports clinical research in selected areas with a high burden of costs. For the intervention study there were two coordinators: one for the children's part, one for the adult's part. The data and coordinating center for children was in Rotterdam, and for adults in Groningen. The children's study was carried out in three centers for pediatric respiratory medicine. Clinical endpoints were symptoms (scale from 0 = no symptoms to 3 = many symptoms), use of additional $beta_2$-agonist, temporary need for oral steroid (prednisolone), absence from school and hospital admission because of asthma. Physiological endpoints were airway caliber (FEV_1, PEF), bronchodilator response (postbronchodilator FEV_1 and PEF) and AHR (PD_{20} histamine, PEF variation). AHR was only measured as histamine sensitivity. When we planned the study the technique of measurement of maximal response plateau (with methacholine instead of histamine) had not yet been used in children so that we decided not to use this method in a large multicenter study. Power calculations for group size were based on a 5 percent difference in mean FEV_1 percent predicted after 3 years of treatment. Intervention consisted of either a $beta_2$-agonist (salbutamol 0.2 mg) plus an inhaled corticosteroid (budesonide 0.2 mg) 3 times daily, or a $beta_2$-agonist (salbutamol 0.2 mg) plus placebo 3 times daily for a predefined study period of 3 years. Drugs were administered by dosisaerosol, and all canisters were identical. Additional to

the study medication patients received a $beta_2$-agonist (fenoterol 0.2 mg) in a different outfit (dry powder), to be taken on demand with a maximum of 4 times daily. In case of a severe exacerbation patients were treated with a course of prednisolone according to a standardized protocol (starting at 30 mg on the first day and reducing to zero mg in one week according to a scheme that depended on body weight). The study was double-blind and randomized with stratification for center, sex and previous use of cromoglycate and/or inhaled corticosteroid. Before entering the baseline period, patients discontinued their usual maintenance treatment for at least 1 month (antihistamines), 2 weeks (sodium cromoglycate, inhaled corticosteroid) or 2 days (theophylline). During the baseline period which lasted 2-4 weeks the only medication allowed consisted of inhaled $beta_2$-agonist on demand. Protocols were designed for tapering off cromoglycate and inhaled corticosteroid, for intervention of exacerbations, and for withdrawal from the intervention study. All measurements were performed during clinically stable periods (i.e. not within 4 weeks after an exacerbation and/or discontinuation of prednisolone). Before the start of the study considerable effort was put into the standardization of methods. Spirometry was performed using water sealed or dry spirometers according to standard guidelines.[222] Written guidelines for all lung function measurements in the hospital were handed out to the respiratory function technicians, who were trained at one training session. Furthermore, site visits were made by a group of lung function experts before the study and yearly thereafter to check the methods used. In the various participating centers hospital pharmacists were asked to prepare histamine solutions using an identical method. Output of all histamine nebulizers was measured before the start of the study and checked once a year in the pediatric lung function laboratory in Rotterdam. Nebulizers with an output variation of $\geq$ 10 percent were replaced. All parts of each nebulizer were marked with waterproof paint to prevent interchanging. Patients were instructed and checked in the use of dosisaerosols, in performing PEF measurement and in the registration of symptoms, use of additional $beta_2$-agonist and prednisolone, absence from school, hospital admission because of asthma and PEF. They were instructed both verbally and written, by handing out an instruction and registration booklet.

Randomization with stratification was performed by calling an independent telephone center, where a computer had been installed for this purpose. Before the start of the study, a feasibility study was undertaken to test patient selection procedures and to investigate the number of patients that each center could include into the study, to test lung function methods, data entry forms,

randomization procedures, and to look for general shortcomings of the protocol. This led to several adaptations in the final protocol.
All data were collected and checked by the pedatric coordinating center in Rotterdam to ensure completeness and to prevent bias due to local procedures. Interim analyses of the study data were made by an independent statistical center every six months and reviewed by an independent data-monitoring committee. Investigators were kept blinded of the results of the interim analyses.

In this thesis we first describe what is known from the literature on the effect of asthma drugs on airway responsiveness to histamine and methacholine (Chapter II).
Chapter III describes the results of an acute effect study of an inhaled corticosteroid on AHR (sensitivity) to methacholine.
In Chapter IV a study in children in which the effect of an inhaled corticosteroid was compared with the effect of a $beta_2$-agonist on airway caliber, airway responsiveness to methacholine and symptoms during a period of 6 months is described.
Chapter V describes the results of the first part of the multicenter study, in which the effect of long-term treatment with an inhaled $beta_2$-agonist plus an inhaled corticosteroid was compared with the effect of an inhaled $beta_2$-agonist-only on clinical endpoints, airway caliber, bronchodilator response and airway responsiveness to histamine. This part had to be discontinued after a median treatment period of 22 months because of the high withdrawal rate in the $beta_2$-agonist-only group.
The second part of the study was concerned with the continuation of the patients on $beta_2$-agonist plus inhaled corticosteroid up to 28 to 36 months after randomization, in which the further course of endpoints was studied and the remission rate was evaluated. The results are described in Chapter VI.
The third part deals with the effect of cessation of inhaled corticosteroid in those children on inhaled corticosteroid who had a follow-up of at least 28 months on July 1, 1991 (Chapter VII).

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Chapter II

The effect of anti-asthma drugs on bronchial hyperresponsiveness

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2.1. Summary

The effects of the currently used anti-asthma drugs on BHR to histamine or methacholine are disappointing. Short-acting $beta_2$-agonists give acute protection which lasts only for a limited period of time. Long-term administration does not improve BHR. Long-acting $beta_2$-agonists have an acute protective effect which lasts for at least 24 hours. No data are available on their effect on BHR after long-term administration. Other bronchodilators such as anticholinergic drugs exert either no or a small acute short-lasting protection, but no change in BHR when administered for weeks or months. The same is true for H_1 antihistamines.
Treatment with inhaled corticosteroids, which have an anti-inflammatory action, often results in a modest decrease in BHR. The duration of the improvement after discontinuation of treatment seems to be short-lasting, but only three studies are available in which this has been looked at systematically. Further studies are needed to establish the influence of dose and duration of treatment in relation to the severity of asthma.
Results of treatment with disodium cromoglycate, which also has anti-inflammatory properties, are disappointing. DSCG protects against the transient increase in BHR after contact with allergen but offers no acute protection against histamine or methacholine challenge. The limited data on nedocromil do not yet allow a conclusion about the value of this drug in the modulation of BHR. Studies on the long-term effect of doses of DSCG and nedocromil which are higher than those currently used are warranted.

2.2. Introduction

One of the characteristics of asthma is non-specific bronchial hyperresponsiveness (BHR). BHR is defined as an increased tendency of airways to constrict after contact with certain stimuli such as viral infections, cold air, cigarette smoke, and chemical irritants such as SO2. The degree of BHR is generally measured by inhalation of histamine or methacholine in increasing dosages or concentrations. Methods used are the dosimeter method and the tidal breathing technique, both of which are equally valid with a within-subject reproducibility of one dose-step.[12,14,75] Values are often expressed as $PD_{20}FEV_1$ or $PC_{20}FEV_1$, indicating the dose or concentration, respectively, of histamine or methacholine at which a decrease of 20 % in baseline FEV_1 is reached, or as change in specific airway conductance

(sGaw). The change in PD_{20}, PC_{20} or sGaw after drug administration can be expressed as doubling dose (DD).
The cause of BHR is unknown. Inflammation seems to be an important factor. Studies of biopsy specimens indicate that in patients with stable asthma and BHR a low grade chronic inflammation of the airway wall may exist.[36,51,55] Exacerbations are probably often accompanied by an acute inflammatory response during which BHR increases transiently.
In adults with chronic obstructive lung disease, BHR is associated with progressive loss of pulmonary function.[70] BHR is also considered to be a risk factor for the outcome of childhood asthma.[30] Treatment which aims at suppression of BHR seems therefore appropriate. This review summarizes the data published on the effect of anti-asthma drugs on BHR to histamine or methacholine in subjects with asthma.

Studies on the effect of drugs on BHR are of two types:

a) Acute protection studies, in which the shift of the dose-response curve is determined immediately after drug administration.
b) Long-term protection studies in which patients have been treated for weeks or months and BHR measurements are performed after a drug-free interval of several hours.

2.3. Beta$_2$-agonists.

2.3.1. *Short-term effect.*

A shift of the dose-response curve of histamine or methacholine to the right after a single dose of a short-acting beta$_2$-agonist has been demonstrated in a number of studies[1,2,6,7,9,13,14,17,20,21,37,42,67,74,77,78,79] (Table 1). The increase in PD_{20} or PC_{20} varies from 0.3 to 4.3 DD. The effect is more pronounced when the drug is inhaled than after oral administration. Two studies[6,77] indicate that it depends on the dose delivered. Few data are available on the duration of the protection. Salome et al.[78] found this to be shorter than 3 hours in all subjects, whereas the improvement in FEV, was well maintained in most subjects 4 hours after drug administration. Ahrens et al.[1]

and Joad et al.[42] obtained similar results. Britton et al.[9] showed a logarithmic relation between the dose of beta$_2$-agonist and the shift of the dose-response curve. The observation by Salome et al.[78] that BHR returns more rapidly towards baseline than FEV, indicates that the protection is not or only partially dependent on smooth muscle relaxation. The few studies with long-acting beta$_2$-agonists show an acute effect on BHR that lasts 9.5 to at least 24 hours.[7,21,74,85,92] (Table 1). The magnitude of the effect, which is maximal after 1 hour, is 2.6-4.0 DD.

2.3.2. *Long-term effect*

A number of studies have been published on the effect of long-term administration of short-acting beta$_2$-agonists on BHR to histamine or methacholine[34,45,46,65,72,86,89,93,96] (Table 2).

Peel et al.[65] found no significant difference in PC_{20} after 4 weeks of treatment with 200 μg salbutamol 4 times daily, and two weeks after discontinuation of the drug. Wiebicke et al.[96] had similar results with 200 μg salbutamol 4 times daily during 3 weeks. Kraan et al.[46] looked at BHR in adults who received 500 μg terbutaline 4 times daily for 4 weeks. Measurements were performed 12-16 hours after discontinuation of the drug. A small decrease in PC_{20} after 2 and 4 weeks (-0.8 DD and -0.5 DD), which was however within the intra-subject reproducibility of the method, was observed. Van Schayck et al.[89] conducted a study of 1 year, during which period patients were treated with 400 μg salbutamol 4 times daily. Their histamine responsiveness increased slightly. The longest study until now was performed by Haahtela et al.[34] who investigated the effect of 22 months treatment with 375 μg terbutaline twice daily in adults. They found a small decrease in airway responsiveness in those patients who remained in the study, which could have been influenced by the high drop-out rate (10/39) because of symptoms. Kerrebijn et al.,[45] investigating the effect of 6 months treatment with 500 μg terbutaline 3 times daily in children, also found a small decrease in PD_{20}. Tattersfield[93] suggested that the findings of Kraan et al.[46] and Kerrebijn et al.[45] might be a result of patient selection. Patients enrolled for long-term studies have to be in a stable condition, so that the risk of deterioration is greater than the chance of improvement. Neither study had a control group which might have shown a similar decrease in PD_{20}. Evidence supporting this conjecture comes from Raes et al.[72] who looked at the effect of 200 μg inhaled fenoterol 3 times daily for 4 months and found a slight increase in PD_{20} after 3 and 4 months. This was however within the intra-subject

Table 1. **Acute protection of beta$_2$-agonists against BHR to histamine or methacholine.**

Reference	Drug	Route	Dose (µg)	Interval (minutes)	Test	Mean change in BHR (DD)
Casterline et al.(9)	salbutamol	IN	170	0	histamine	+3.6 (PD)
Cockcroft et al.(17)	salbutamol	PO	4000	60	histamine	+1.1 (PC)
		IN	200	15	histamine	+3.6 (PC)
DeCotiis et al. (20)	terbutaline	SC	5/kg	NS*	methacholine	+2.4 (PD)
Bandouvakis et al.(2)	fenoterol	IN	800	45	histamine	+3 (PC)
					methacholine	+4 (PC)
Salome et al.(77)	fenoterol	IN	400	15	histamine	+2.4 (PD)
		PO	5000	90	histamine	+0.5 (PD)
		IN	400	15	methacholine	+3.8 (PD)
		PO	5000	90	methacholine	+1.5 (PD)
Chung et al.(13)	salbutamol	IN	200	30	histamine	+2.5 (PD_{35}) ♦
Salome et al.(78)	fenoterol	IN	100	15	histamine	+1.8 (PD)
			200	15	histamine	+1.9 (PD)
			400	15	histamine	+3.1 (PD)
Chung et al.(14)	salbutamol	IN	200	30	methacholine	+3 (PD_{35})
Ahrens et al.(1)	salbutamol	IN	90	30	histamine	+3 (PD)
				120	histamine	+1.1 (PD)
				240	histamine	0 (PD)
			180	30	histamine	+4.3 (PD)
				120	histamine	+2.7 (PD)
				240	histamine	+0.7 (PD)
	metaproterenol	IN	1300	30	histamine	+2.6 (PD)
				120	histamine	+1.3 (PD)
				240	histamine	0 (PD)
			2600	30	histamine	+3.6 (PD)
				120	histamine	+2 (PD)
				240	histamine	0 (PD)
Joad et al.(42)	salbutamol	IN	200	30	histamine	+2.8 (PD)
				240	histamine	0 (PD)
Britton et al.(6)	salbutamol	IN	5	15	histamine	+0.3 (PD)
			30	15	histamine	+1.1 (PD)
			200	15	histamine	+1.5 (PD)
			1000	15	histamine	+3.0 (PD)

Salome et al.(79)	fenoterol	IN	200	5	histamine	+3.6	(PD)
				180	histamine	+1.5	(PD)
				360	histamine	+0.6	(PD)
	salbutamol	IN	200	5	histamine	+3.8	(PD)
				180	histamine	+1.9	(PD)
				360	histamine	+0.2	(PD)
Phillips et al.(67)	salbutamol	IN	2500	40	histamine	+3.2	(PC)
				40	methacholine	+2.9	(PC)
Higgins et al.(37)	salbutamol	IN	1855¶	60	histamine	+2.3	(PD)
Twentyman et al.(85)	salmeterol	IN	50	90	histamine	+4.0	(PC)
				570	histamine	+3.0	(PC)
Ramsdale et al.(74)	salbutamol	IN	200	30	methacholine	+3.1	(PC)
				12h	methacholine	0	(PC)
	formoterol	IN	12	30	methacholine	+3.8	(PC)
				12h	methacholine	+2.6	(PC)
	formoterol	IN	24	30	methacholine	+4.3	(PC)
				12h	methacholine	+3.0	(PC)
Campos Gongora et al.(7)	salbutamol	IN	200	60	histamine	+1.8	(PD)
				12h	histamine	−0.2	(PD)
	salmeterol	IN	50§	60	histamine	+2.4	(PD)
				12h	histamine	+1.8	(PD)
	salmeterol	IN	50#	60	histamine	+1.8	(PD)
				12h	histamine	+1.5	(PD)
Derom et al.(21)	salbutamol	IN	200	60	methacholine	+1.9	(PC)
				12h	methacholine	+0.5	(PC)
	salmeterol	IN	50	60	methacholine	+2.4	(PC)
				12h	methacholine	+1.6	(PC)
	salmeterol	IN	100	60	methacholine	+3.2	(PC)
				12h	methacholine	+2.4	(PC)
Verberne et al.(92)	salmeterol	IN	50	60	methacholine	+3.8	(PD)
				12h	methacholine	+2.0	(PD)
				24h	methacholine	+1.2	(PD)

* NS = not stated
♦ PD_{35} = PD_{35}sGaw; sGaw = specific airway conductance
¶ cumulative dose
§ metered dose inhaler
\# dry powder

variability of the method. A study performed by Waalkens et al.[83] showed no effect of 8 weeks treatment with 500 μg terbutaline 4 times daily on histamine responsiveness in children. Van Essen-Zandvliet et al.[86] performed a long-term double blind parallel study in children. 58 patients were treated with salbutamol 200 μg plus placebo 3 times daily and 58 patients with a combination of 200 μg salbutamol plus 200 μg budesonide for a median period of 22 months. In the group treated with salbutamol-only no change in PD_{20} histamine was seen. Baseline airway caliber remained unchanged in the majority of patients in all long-term studies.
Vathenen et al.[91] looked at the effect of stopping $beta_2$-agonist after a maintenance treatment of 2 weeks. In a double-blind cross-over study 8 patients were treated with terbutaline 750 μg or placebo 2 times daily. They concluded that treatment for 2 weeks impaired the ability of terbutaline to protect against histamine-induced bronchoconstriction and was followed by a rebound increase in bronchial responsiveness 23 hours after cessation of treatment. This increase, which was maximal 23 hours after stopping terbutaline (1.5 DD difference between the two treatments) might well be attributable to day-to-day variation, as was discussed by Postma et al.[71]. Wahedna et al.[95] did a similar cross-over placebo-controlled study with salbutamol and broxaterol, a new $beta_2$-agonist during 3 weeks. They found a progressive increase in PD_{20} histamine after cessation of salbutamol with a significant maximum difference of 1.65 DD compared to placebo, whereas this difference after cessation of broxterol was smaller (0.79 DD) and not significant. The clinical relevance of these findings is not clear.
No studies are available on the effect on BHR of long-term treatment with long-acting $beta_2$-agonists.

2.3.3. *Conclusion*

$Beta_2$-agonists exert an acute protection against histamine- or methacholine induced bronchoconstriction which lasts up to 3 hours. One study showed a logarithmic relation between the dose of $beta_2$-agonist and the degree of protection. Long-term administration has no effect on BHR if this is measured more than 6 hours after drug discontinuation. Only short-term studies have been performed with the recently developed long-acting inhaled $beta_2$-agonists.

Table 2. **Long-term effect of inhaled beta$_2$-agonists on BHR to histamine or methacholine.**

Reference	Drug and frequency	Daily dose (μg)	Duration	Test	Interval	Mean change in BHR (DD)
Adults:						
Peel et al.(65) n = 8	salbutamol q.i.d.	800	1 mo	histamine	>6 h 2 wk	-0.3 (PC) +0.5 (PC)
Kraan et al.(48) n = 17	terbutaline q.i.d.	2000	2 wk	methacholine	>12 h >12 h 2 wk 4 wk	-0.8 (PC) -0.5 (PC) +0.1 (PC) -0.3 (PC)
Van Schayck et al.(89) n = 15	salbutamol q.i.d.	1600	12 mo	histamine	>8 h	-0.3 (PC)
Wiebicke et al. (96) n = 12	salbutamol q.i.d.	800	3 wk	histamine methacholine	>6 h >6 h	+0.6 (PC_{100})* 0 (PC_{100})*
Haahtela et al. (34) n = 43	terbutaline b.i.d.	750	22 mo	histamine	6 h	+0.3 (PC_{15})¶
Children:						
Kerrebijn et al.(45) n = 7	terbutaline t.i.d.	1500	1 mo 3 mo 6 mo	methacholine	12 h 12 h 12 h	-0.9 (PD) -0.8 (PD) -0.8 (PD)
Raes et al.(72) n = 8	fenoterol t.i.d.	600	1 mo 2 mo 3 mo 4 mo	histamine	12 h 12 h 12 h 12 h	+0.1 (PD) +0.2 (PD) +1.1 (PD) +1.9 (PD)
Waalkens et al.(93) n = 12	terbutaline q.i.d.	2000	4 wk 8 wk	histamine	>12 h >12 h	-0.5 (PC) -0.1 (PC)
Van Essen-Zandvliet et al. (86) n = 58	salbutamol	600	22 mo	histamine	8 h	0 (PD)

* PC_{100} = provocative concentration which causes an increase in sRaw by 100%; sRaw = specific airway resistance
¶ PC_{15} = provocative concentration which causes a decrease in FEV_1 by 15%

2.4. Anticholinergic drugs

2.4.1. *Short-term effect*

Several investigators have looked at the acute effect of anticholinergic drugs on BHR to histamine and methacholine[2,6,9,13,15,17,26,37,38,68,98]. Table 3 summarizes the results of the studies with histamine, which show a maximal increase in PD_{20}/PC_{20} of 1.9 DD. Britton et al.[6] looked at 4 different doses of ipratropium bromide and found that increasing dosages resulted in further improvement of airway caliber, but not in PD_{20}. These figures support the observation of Salome et al.[78] that protection and

bronchodilatation are not closely associated. Ihre et al.[38] also looked at the effect of different doses of ipratropium bromide, but they did not find a relation between dose and airway caliber. In contrast to what Britton et al.[6] found they showed a higher protective effect on histamine sensitivity, without differences between the doses. Bandouvakis et al.[2] found a late bronchoconstrictive reaction 5-12 hours after methacholine inhalation in 4 patients with a PC_{20} to methacholine of 75-320 μg. They termed this a late asthmatic response comparable to the late response after allergen inhalation. In two other patients with PC_{20}s of 420 and 480 μg no late reaction was seen. It was suggested that this could be an effect of stimulation of cholinergic receptors on mast cells and subsequent degranulation and mediator release. No data are available on the duration of the protective effect.

2.4.2. *Long-term effect.*

Hardly any data exist on the effect of long-term treatment with anticholinergic drugs on BHR. (Table 4). Newcomb et al.[62] treated 9 asthmatic adults with 60 μg ipratropium bromide 4 times daily for 3 weeks and found a slight decrease of PC_{20} to methacholine 24 hours after discontinuation of medication (-0.9 DD) but no decrease after 48 hours. They explained this as a transient supersensitivity because of cholinergic receptor upregulation. 12 hours after withdrawal of medication sensitivity to methacholine was still somewhat reduced. This indicates that the effect of ipratropium on muscarinic receptors may last longer than its bronchodilator effect. BHR to histamine was not measured. Sly et al.[60] treated 31 asthmatic children with 40 μg ipratropium bromide 3 times daily for 4 weeks. Airway caliber did not increase but there was a slight increase in PD_{20} to histamine of +0.7 DD; measurements were performed after a drug-free interval of 8 hours. Raes et al.[72] looked at the effect of 40 μg ipratropium bromide 3 times daily for 4 months on PD_{20} to histamine in 9 asthmatic children. No significant effect (+ 0.5 DD) was seen and no muscarinic receptor upregulation was observed.

2.4.3. *Conclusion.*

Anticholinergic drugs have only a weak acute protective effect on BHR to histamine in spite of bronchodilatation. There is no relation between the degree of bronchodilatation and protection. Data on a possible transient increase in BHR to

methacholine after discontinuation of ipratropium bromide are incomplete. Long-term treatment with anticholinergic drugs does not seem to have a significant effect on BHR to histamine.

Table 3. **Acute protection of anticholinergics against BHR to histamine.**

Reference	Drug	Route	Dose (μg)	Interval (minutes)	Mean change in BHR (DD)
Casterline et al.(9)	atropine sulfate	IN	5000	10	+1.5 (PD)
Cockcroft et al.(17)	ipratropium bromide	IN	40	60	+1.5 (PC)
	atropine sulfate	IN	290	60	+1.2 (PC)
Woenne et al.(97)	ipratropium bromide	IN	80	30	–0.2 (PD)
Bandouvakis et al.(2)	ipratropium bromide	IN	80	45	+0.7 (PD)
Chung et al.(13)	atropine methonitrate	PO	4000	45	+1.5 (PD)
Clarke et al.(15)	ipratropium bromide	IN	40	10	+1.0 (CBU)
Eiser et al.(26)	atropine methonitrate	IN	1500	30	*
Britton et al. (6)	ipratropium bromide	IN	5	40	+0.2 (PD)
			30	40	–0.5 (PD)
			200	40	+0.3 (PD)
			1000	40	+0.2 (PD)
Salome et al.(79)	ipratropium bromide	IN	80	5	+1.8 (PD)
				180	+1.4 (PD)
				360	–0.3 (PD)
Higgins et al. (37)	ipratropium bromide	IN	1855¶	60	+0.8 (PD)
Ihre et al. (38)	ipratropium bromide	IN	40	45	+1.6 (PC)
			200	45	+1.7 (PC)
			800	45	+1.9 (PC)
Polosa et al. (68)	ipratropium bromide	IN	500	180	+1.4 (PC)

* Prevention of mean histamine response but with considerable intersubject variation (no data given)
¶ Cumulative dose

Table 4. **Long-term effect of ipratropium bromide on BHR to histamine.**

Reference	Frequency	Daily dose (μg)	Duration (weeks)	Interval (hours)	Mean change in BHR (DD)
Newcomb et al. (62)	q.i.d.	240	3	24	–0.9 (PC)
n = 9 adults				48	0 (PC)
Sly et al.(80)	t.i.d.	120	4	8	+0.7 (PD)
n = 31 children					
Raes et al.(72)	t.i.d.	120	17	>12	+0.5 (PD)
n = 9 children					

2.5. Inhaled corticosteroids

2.5.1. *Short-term effect*

Three studies have been published on the acute effect of inhaled corticosteroids on BHR. Casterline et al.[10] compared 20 mg diphenhydramine with 100 μg beclomethasone dipropionate (BDP) 15 minutes before a histamine challenge. In 5 subjects PD_{20} to histamine increased after BDP, but the mean difference before and after protection did not reach statistical significance (+ 0.4 DD). Vathenen et al.[90] investigated whether a single dose of 800 μg budesonide had an acute effect on histamine responsiveness in adults. They found an increase of 1.0 DD in PD_{20} to histamine after 6 hours. Van Essen-Zandvliet et al.[67] did a similar study in children. A dose of 800 μg budesonide increased PD_{20} to methacholine with 0.6 DD after 5 hours, which difference was not statistically significant.

2.5.2. *Long-term effect*

Various studies have been published on the effect of long-term inhaled corticosteroids on BHR in patients with mild, moderate and severe asthma. In all studies BHR decreased, although in most studies the decrease was limited [3,4,22,24,25,26,34,41,43,44,45,46,48,49,50,60,76,81,82,86,88,90,93,99] (Table 5). The increase in PD_{20} or PC_{20} to histamine or methacholine varied between 0.3 and 3.2 DD. Kraan et al.[49] found an increase in PC_{20} to histamine of + 0.4 DD after 2 weeks treatment with 100 μg budesonide twice daily, and a further increase to + 0.8 DD after 8 weeks. With 400 μg twice daily the improvement was significantly greater. In the study of Kerrebijn et al.,[45] PD_{20} to methacholine increased gradually with time during treatment with 200 μg budesonide 3 times daily. A plateau was reached after 2 months. In all studies there was considerable inter-subject variability. The conflicting results of two studies in which patients were treated for several months[25,45] may be explained by differences in the severity of asthma. The patients treated by Easton et al.[25] had moderately severe asthma whereas those in the study of Kerrebijn et al.[45] were only mildly affected. Furthermore, Easton et al.[25] performed an open study, which may also have influenced their results. In another study in children with moderate to severe asthma, Kerrebijn et al.[49] found little improvement after 2 months of treatment with 150 or 600 μg budesonide twice daily. The results of Kraan et al.[49] suggest that the decrease in BHR is not only associated with the duration of treatment but also

with the dose of inhaled corticosteroid. They compared the effect of 100 μg and 400 μg budesonide twice daily during a treatment period of 8 weeks on PC_{20} histamine and found a consistent difference in favor of the highest dosage. These results differed from those of Kerrebijn et al.[48] who found no difference between the effects of a high and a low dose. More recently two longer-term studies have been published.[34,43] Juniper et al.[43] treated adult patients for one year with 200 μg budesonide twice daily. Methacholine responsiveness decreased gradually over time, PD_{20} showed an increase by 2 DD after 1 year. Haahtela et al.[34] studied the effect of 600 μg budesonide twice daily during 22 months. They showed that histamine responsiveness diminished by 1.6 DD. In the study by Juniper et al.[43] PD_{20} did not reach a plateau after one year treatment with inhaled corticosteroids, while the Haahtela study[34] suggests plateau formation after 72 weeks. This may be explained by the longer duration of treatment. A recent long-term study (median follow-up 22 months) in 116 children by Van Essen-Zandvliet et al.[86] compared the effect of budesonide 200 μg plus salbutamol 200 μg 3 times daily with that of salbutamol 200 μg 3 times daily plus placebo. In the group with budesonide plus salbutamol mean PD_{20} to histamine increased steadily over time to a maximum of 2 DD after 20 months (without reaching a plateau), which was still in the abnormal range. This effect was completely attributable to the inhaled corticosteroid, as histamine responsiveness in the other group remained unchanged. In an extended follow-up of this group up to 28-36 months they showed that histamine responsiveness stabilised at that level (+2 DD) beyond 20 months.[88] In only a few patients in the studies summarized in Table 5 did BHR reach a value within the normal range. Vathenen et al.[90] looked at the effect of stopping inhaled corticosteroids on histamine sensitivity: they treated 32 adults with 800 μg budesonide twice daily during 6 weeks and first showed an increase of 3.2 DD in PD_{20} histamine after this period. After stopping budesonide, PD_{20} showed a fast decrease to baseline within 2 weeks. This is in contrast with the findings of the follow-up study by Juniper et al.[44] after their 1-year study with 200 μg budesonide twice daily.[43] After one year budesonide was stopped in 8 patients and histamine responsiveness measured 3 months thereafter. At that moment PC_{20} to methacholine was not significantly different from the value at the time budesonide was stopped. A 6 months study by Waalkens et al.[94] in 21 children who had received 200 ug budesonide 3 times daily for 28-36 months showed a decrease in PD_{20} to histamine of 1.6 DD compared to the moment that the dose of inhaled corticosteroid was gradually diminished and stopped. These findings suggest that the effect of inhaled corticosteroid on BHR disappears within months after stopping.

Table 5. **Long-term effect of inhaled corticosteroids on BHR to histamine, methacholine or carbachol**

Reference	Drug* and frequency (μg)	Daily dose	Duration	Test	Interval (h)	Mean change in BHR (DD)
adults:						
Easton et al.(25) n=7	BDP q.i.d.	400	4 mo	methacholine	NS♦	+ 0.4 (CBU)
Kraan et al.(48) n=17	budesonide q.i.d.	400	1 mo	histamine	>12	+ 1.3 (PC)
Ryan et al.(76) n=10	BDP b.i.d.	400	1 mo	histamine	NS	+ 0.3 (PC)
Dutoit et al.(24) n=26	BDP b.i.d.	800	10 wk	histamine	NS	+ 3.0 (PD)
Svendsen et al.(81) n=38	BDP b.i.d.	400	2 mo	histamine	6	+ 0.5 (PC)
Jenkins et al.(41) n=18	BDP q.i.d.	1200	3 wk	histamine	8	+ 1.4 (PD)
Kraan et al.(49) n=15	budesonide b.i.d.	200	2 wk	methacholine	NS	+ 0.4 (PC)
			2 mo	methacholine	NS	+ 0.8 (PC)
n=15	budesonide b.i.d.	800	2 wk	methacholine	NS	+ 1.0 (PC)
			2 mo	methacholine	NS	+ 1.6 (PC)
Molema et al.(60) n=22	budesonide q.i.d.	400	6 wk	histamine	8	+ 1.5 (PC)
Bel et al.(3) n=8	BDP q.i.d.	400	4 mo	methacholine	NS	+ 1.2 (PC)
Svendsen et al.(82) n=20	BDP b.i.d.	400	6 wk	histamine	6	+ 1.4 (PC)
ZuWallack et al.(99) n=18	triamcinolone q.i.d.	800	6 wk	methacholine	NS	+ 1.5 (PC)
Juniper et al.(43) n=16	budesonide b.i.d.	400	3 mo	methacholine	NS	+ 1.2 (PC)
			6 mo	methacholine	NS	+ 1.5 (PC)
			9 mo	methacholine	NS	+ 1.6 (PC)
			12 mo	methacholine	NS	+ 2.0 (PC)
Haahtela et al.(34) n=49	budesonide b.i.d.	1200	22 mo	histamine	6	+ 1.6 (PC_{15})¶
Vathenen et al.(90) n=32	budesonide b.i.d.	1600	3 wk	histamine	24	+ 1.4 (PD)
			6 wk	histamine	1	+ 2.5 (PD)
				histamine	6	+ 2.9 (PD)
				histamine	12	+ 3.2 (PD)
				histamine	24	+ 2.8 (PD)
				histamine	36	+ 1.7 (PD)
				histamine	1 wk	− 0.3 (PD)
				histamine	2 wk	+ 0.3 (PD)

Juniper et al.(44) n=8	budesonide b.i.d.	400	12 mo	methacholine	3 mo	+ 2.0 (PC)
Fuller et al.(28) n=10	budesonide b.i.d.	1200	3 wk	histamine	NS	+ 2.0 (PD_{35})§
Djukanovic et al.(22) n=10	BDP	2000	2 wk	methacholine	NS	+ 2.9 (PC)
children:						
Kerrebijn et al.(45) n=12	budesonide t.i.d.	600	1 mo	methacholine	12	+ 0.6 (PD)
			3 mo	methacholine	12	+ 1.5 (PD)
			6 mo	methacholine	12	+ 1.6 (PD)
Kraemer et al.(50) n=7	BDP t.i.d.	300-600	2 mo	carbachol	NS	+ 2.6 (PD_{100})#
Kerrebijn et al.(46) n=13	budesonide b.i.d.	300	1 mo	methacholine	12	+ 0.6 (PD)
			2 mo	methacholine	12	+ 1.0 (PD)
	budesonide b.i.d.	1200	1 mo	methacholine	12	+ 0.9 (PD)
			2 mo	methacholine	12	+ 0.4 (PD)
Bennati et al.(4) n=10	BDP t.i.d.	300	1 mo	methacholine	12	+ 1.5 (PC)
Waalkens et al.(93) n=12	budesonide b.i.d.	1000	4 wk	histamine	12	+ 1.3 (PC)
			8 wk	histamine	12	+ 2.1 (PC)
Van Essen-Zandvliet et al.(86) n=58	budesonide t.i.d.	600	22 mo	histamine	8	+ 2.0 (PD)
Van Essen-Zandvliet et al.(88) n=55	budesonide t.i.d.	600	28-36 mo	histamine	8	+ 2.0 (PD)

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* BDP = beclomethasone dipropionate
♦ NS = not stated
¶ PC_{15} = provocative concentration of histamine which causes a 15% decrease in FEV_1
§ PD_{35} = PD_{35}sGaw; sGaw = specific airway conductance
# PD_{100} = PD_{100}Raw; sRaw = specific airway resistance

Djukanovic et al.[22] compared the effect of 2000 μg BDP on methacholine responsiveness to that on indicators of inflammation in bronchial biopsies. They found an increase of 2.9 DD in PC_{20} to methacholine and a decrease in number of epithelial and mucosal mast cells and eosinophils and submucosal T-lymphocytes, whereas the activation grade of mast cells and eosinophils did not change. These findings have to be reconfirmed by placebo-controlled studies, as this study was not controlled.

2.5.3. *Conclusion*

Inhaled corticosteroids seem to have a slight acute protective effect on BHR to histamine. After long-term administration most studies report a modest decrease in BHR. The severity of asthma, the duration of treatment and the dose administered seem to influence the degree of improvement, but far more data are needed to establish the dose-effect relationship with time in relation to the severity of the disease. BHR has not yet been shown to be restored to the normal range in most patients. After discontinuation of treatment the effect seems to wear off but insufficient data exist on the relation of duration and dose of treatment and the time course of wearing off. Therefore more studies are needed.

2.6. **Disodiumcromoglycate (DSCG) and nedocromil sodium**

2.6.1. *Short-term effect*

A number of studies has looked at the acute effect of DSCG on BHR to histamine or methacholine[18,19,33,52,98] (Table 6). Woenne et al.[98] concluded from a study in children that acute protection to methacholine occurred in 10 out of 17 patients, and to histamine in 8 out of 13. This is in contrast to the findings of Cockroft et al.,[18] Griffin et al.[33] and Lemire et al.[52] who found no acute protection in any of the subjects studied. Nedocromil sodium (10 mg) did not inhibit methacholine responsiveness in 6 asthmatics according to a study by Crimi et al..[19]

2.6.2. *Long-term effect*

A number of studies on long-term treatment with DSCG also showed no effect on BHR[3,11,29,33,40,53,60,81,82] (Table 7). Duration of treatment varied from 4 to 16 weeks. No studies were done with doses higher than 80 mg daily. Cockcroft et al.[18] showed that 10 mg DSCG protects against the increase in BHR to histamine after allergen inhalation when given before the challenge. The mean decrease in PC_{20} was 0.16 DD compared to 0.59 DD after placebo at 7 hours, and 0.07 DD compared to 0.34 DD at 30 hours. Mattoli et al.[58] investigated the effect of two different doses of DSCG on the late allergic reaction and the subsequent increase in BHR to methacholine. Five asthmatic children received 20 or 40 mg DSCG one hour before the expected late allergic reaction, which was delayed by 3-4 hours after 40 mg DSCG mg but not after 20 mg. The mean decrease in PC_{20} to methacholine was 1.4 DD and 0.6 DD, after 20 and 40 mg DSCG respectively, compared to 3.3 DD after placebo. The degree of bronchoconstriction with the late allergic reaction was the same after both doses of DSCG and was similar to that after placebo. This indicates that the degree of BHR was not determined by the degree of bronchoconstriction. Löwhagen et al.[54] treated 22 asthmatic adults who had grass pollen atopy with 10 mg DSCG or placebo for 6 weeks during pollen season. BHR to histamine remained unchanged in the patients on DSCG but increased in those on placebo, showing that DSCG protects against pollen-induced increase in BHR.
Three studies were done with nedocromil sodium. Bel et al.[3] found that 4 mg nedocromil four times daily increased PC_{20} to methacholine by 1.4 DD in adults with non-steroid dependent non-atopic asthma after 2 months. Their study also suggests that responsiveness is attennuated by distinct mechanisms. Svendsen et al.[82] concluded that 4 mg nedocromil twice daily during 6 weeks caused an increase in PC_{20} to histamine by 0.5 DD in 19 adult asthmatics. Dorward et al.[23] compared 4 mg nedocromil sodium twice daily to placebo in 12 asthmatics with grass pollen atopy for two periods of two weeks during the grass pollen season. Mean PC_{10} FEV_1 to histamine decreased with placebo (- 0.2 DD) whereas it increased (+ 0.7 DD) with nedocromil sodium. The dose used was low and studies with higher doses are needed before definite conclusions about the value of nedocromil sodium on the modulation of BHR following exposure to allergen can be reached.

Table 6. **Acute protection of disodiumcromoglycate and nedocromil sodium against BHR to histamine or methacholine.**

Reference	Drug*	Dose	Interval (minutes)	Test	Mean change in BHR (DD)
Cockcroft et al. (18)	DSCG	20 mg	15	histamine	+ 0.1 (PC)
Woenne et al. (98)	DSCG	40 mg	10	methacholine	+ 1.3 (PD)
				histamine	+ 0.8 (PD)
Griffin et al. (33)	DSCG	20 mg	15	methacholine	0 (PD)
Lemire et al. (52)	DSCG	40 mg	10	histamine	− 0.1 (PC)
Crimi et al. (19)	NSO	4 mg	10	methacholine	0 (PD_{15})♦

Table 7. **Long-term effect of disodiumcromoglycate and nedocromil sodium on BHR to histamine or methacholine in adults.**

Reference	Drug* and frequency	Daily dose (mg)	Duration (months)	Test	Interval (hours)	Mean change in BHR
Griffin et al. (33) n = 11	DSCG q.i.d.	80	1	methacholine	NS¶	0 (PD)
Furukawa et al(29) n = 22	DSCG b.i.d-q.i.d.	40-80	3	methacholine	4	+0.8 (PC)
Löwhagen et al(53) n = 10	DSCG q.i.d.	80	1	histamine	NS	0 (PC)
Jenkins et al(40) n = 44	DSCG q.i.d.	80	4	histamine	6	+ 0.5 (PC)
Svendsen et al(81) n = 38	DSCG q.i.d.	8	2	histamine	NS	− 0.4 (PC)
Molema et al. (60) n = 22	DSCG q.i.d.	8	1½	histamine	8	+ 0.3 (PC)
Chabra et al. (11) n = 11	DSCG q.i.d.	80	3	histamine	NS	+ 1.2 (PD_{35})§
Bel et al.(3) n = 9	NSO q.i.d.	16	4	methacholine	NS	+ 1.4 (PC)
Svendsen et al. (82) n = 19	NSO b.i.d.	8	1½	histamine	6	+ 0.5 (PC)

* DSCG = disodiumcromoglycate, NSO = nedocromil sodium
♦ PD_{15} = $PD_{15}FEV_1$
¶ NS = not stated
§ PD_{35} = PD_{35}sGaw; sGaw = specific airway conductance

2.6.3. *Conclusion*

Results of investigations into the acute protection of DSCG to histamine or methacholine are conflicting. The only study published with nedocromil sodium showed no acute effect on BHR to methacholine. Long-term treatment with DSCG with doses up to 80 mg per day does not decrease BHR to histamine or methacholine. DSCG does, however, protect against the increase in BHR to histamine or methacholine after allergen challenge and during the pollen season. Nedocromil sodium also protects against pollen-induced increase in BHR, and one study reported a significant decrease in BHR after treatment for 2 months. Data are needed on the dose-effect relationship of DSCG and nedocromil sodium on BHR, as it cannot be excluded that doses which are higher than those currently used would be more effective.

2.7. **Xanthines**

2.7.1. *Short-term effect*

Few studies have been performed on the acute protective effect of xanthines on BHR to methacholine or histamine[6,17,20,47,56,59] (Table 8). An acute effect on BHR that varies between - 0.1 and + 2.7 DD when the interval between the drug administration and test is 60 minutes or more has been demonstrated. Cockcroft et al.,[17] looking at the effects of two doses of short-acting theophylline that gave different serum concentrations (<10 and >10 mg/l), found only a small difference (+ 0.4 and + 1.1 DD, respectively). McWilliams et al.[59] compared the effect of 2 different doses of hydroalcoholic theophylline solution. At the time of challenge with methacholine the serum concentrations varied between 7.0 and 18.7 µg/ml, and with histamine between 4.0 and 22.1 mg/l. Both BHR to methacholine (+ 1.6 DD) and to histamine (+ 0.9 DD) decreased. The change in BHR was not related to the serum theophylline concentration. No correlation existed between the change in BHR and the degree of bronchodilatation. Magnussen et al.[56] administered different doses of theophylline intravenously. The mean increase in PD_{100}Raw to histamine was + 1.1 DD at a serum concentration of 6.14 and + 2.7 DD at a concentration of 12.9 $mg.l^{-1}$. PD_{100}Raw to methacholine increased + 0.8 DD and + 2.0 DD at serum concentrations of 5.83 and 12.0 mg/l respectively. Because airway resistance before

Table 8. **Acute protection of xanthines against BHR to histamine or methacholine.**

Reference	Drug	Route	Serum concentration (μg/ml)	Interval (minutes)	Test	Mean change in BHR (DD)
Cockcroft et al. (17)	choline theophyllinate	PO	> 10	180	histamine	+1.1 (PC)
			< 10	180	histamine	+0.4 (PC)
	anhydrous theophylline	PO	13.4	NS*	methacholine	−0.1 (PD)
DeCotlis et al. (20)	hydroalcoholic theophylline	PO	13	60	methacholine	+1.6 (PD)
McWilliams et al. (59)					histamine	+0.9 (PD)
	theophylline ethylenediamine	IV	6.1	60	histamine	+1.1 (PD_{100})♦
Magnussen et al. (56)			12.9	60	histamine	+2.7 (PD_{100})
			5.8	60	methacholine	+0.8 (PD_{100})
			12.0	60	methacholine	+2.0 (PD_{100})
	slow-release theophylline	PO	12.9	180-240	histamine	+0.4 (PC)
Cartier et al. (8)	theophylline	IV	5.3	0	methacholine	+0.4 (PC)
Koëter et al. (47)			10.2	0	methacholine	+1.5 (PC)
			15.0	0	methacholine	+1.7 (PC)
	enprofylline	IV	1.2	0	methacholine	+0.6 (PC)
			2.4	0	methacholine	+1.5 (PC)
			3.7	0	methacholine	+1.5 (PC)

* NS = not stated
♦ PD_{100} = PD_{100}Raw,CBU; Raw = airway resistance, CBU = cumulative breath units

Table 9. **Long-term effect of xanthines on BHR to histamine and methacholine.**

Reference	Drug and frequency	Daily dose (mg)	Serum concentration	Duration (weeks)	Interval (hours)	Test	Mean change in BHR (DD)
(adults)							
Furukawa et al. (29) n = 18	slow-release theophylline b.i.d.	200-600	10-15 mg/l	12	4	methacholine	+1.6 (PC)
Dutoit et al. (24)	slow-release	400-1200	55-110	4	5	histamine	+0.4 (PD)
n = 26	theophylline b.i.d.		μmol/l	10	5	histamine	+0.2 (PD)

medication was within the normal range no relationship could be established between the protective and bronchodilator effects. Cartier et al.[6] treated 16 patients with 200-450 mg long-acting theophylline twice daily or with placebo for 3 days in a cross-over double-blind design. The serum concentration of theophylline was kept between 10 and 20 μg/ml. They found a small but significant protection against BHR to histamine (+ 0.4 DD) 3-4 hours after theophylline intake. The effect was independent of the degree of bronchodilatation. Koëter et al.[47] investigated the effect of different serum concentrations of theophylline and enprofylline after intravenous administration on BHR to methacholine in eight adult asthmatic patients. They showed that PC_{20} increased with both drugs. The improvement was related to the serum concentrations achieved and to the degree of bronchoconstriction. The maximum protective effect was 1.7 DD for theophylline at a serum concentration of 15.0 μg/ml, and 1.5 DD for enprofylline at a serum concentration of 3.75 μg/ml in comparison with values obtained after placebo. Cockcroft et al.[16] treated six atopic asthmatics with 300 mg theophylline twice daily during 4 days. The last dose was taken 1 hour before allergen inhalation. Mean serum concentrations obtained were 10.3 μg/ml 8 hours after the last tablet. They concluded that at these serum concentrations the allergen-induced increase in airway responsiveness to methacholine was not inhibited. No data are available on the duration of the protective effect of theophylline on BHR. From the study of Cockcroft et al.[17] it seems likely that it is shorter than 3 hours.

2.7.2. *Long-term effect*

Table 9 shows the results of two long-term intervention studies. Furukawa et al.[29] treated 18 children who had moderately severe asthma with 200-600 mg long-acting theophylline for 12 weeks. They did not find a statistically significant change in BHR to methacholine with time. Dutoit et al.[24] looked at the effect of long-term treatment with theophylline on BHR to histamine. Twenty-six patients suffering from severe asthma were treated with long-acting theophylline for 10 weeks. In each patient the dose after which a therapeutic serum concentration was reached was determined before the study. After 10 weeks BHR to histamine had not changed, nor were there significant changes in FEV_1.

2.7.3. *Conclusion*

Xanthines have a small acute protective effect on BHR to histamine and methacholine. This is not associated with the bronchodilatory effect. Data on the relationship between the protective effect and serum theophylline concentrations are conflicting. Long-term treatment seems to have no effect on BHR.

2.8. Histamine, receptor blocking antihistamines

2.8.1. *Short-term effect*

Several studies have been done on the acute effect of antihistamines on BHR to histamine and methacholine[5,9,31,35,57,61,63,64,66,68,69,73,84,97,98] (Table 10). BHR to histamine is (almost) totally blocked 10-45 minutes after an inhaled antihistamine and 2-4 hours after oral administration. However, BHR to methacholine is not influenced.

2.8.2. *Long-term effect*

Esau et al.[27] treated 8 asthmatics for two 3 week-periods with 1 mg clemastine twice daily and 7 other patients for the same period of time with 1 mg ketotifen twice daily. Histamine challenges were performed before and after each treatment period. PC_{20} increased + 2.7 DD in the patients on clemastine and + 2.3 DD in the patients on ketotifen. The interval between the last drug dose and histamine challenge was not mentioned, so the observed effect could be due to acute protection. Graff-Lonnevig et al.[32] looked at the effect of 1 mg ketotifen twice daily for 12 weeks on BHR to methacholine. Methacholine challenges were performed 4-weekly and showed no change with time, the interval between the last drug intake and test being 3 hours. Iwata et al.[36] treated 21 asthmatics with 2 mg azelastine twice daily over 8 weeks. Methacholine sensitivity expressed as cumulative dose after which respiratory resistance begins to increase did not change. Ghosh et al.[31] studied the effect of 15 mg terfenadine twice daily for one week on BHR to histamine. PC_{20} 14 hours after the last medication increased by 5 DD (Table 11).

Table 10. **Acute protection of histamine$_1$ receptor blocking antihistamines against BHR to histamine or methacholine.**

Reference	Drug	Dosage (mg)	Route	Interval (minutes)	Test	Mean change in BHR (DD)
Casterline et al.(9)	diphenhydramine	20	IN	15	histamine	+1.8 (PD)
Woenne et al(97)	chlorpheniramine	5	IN	30	histamine	fully blocked in 7/8 subjects
Nogrady et al(63)	clemastine	1	IN	30	methacholine	-0.4 (PD)
					histamine	+3.3 (PC_{20})♦
Nathan et al(61)	chlorpheniramine	8	PO	120	methacholine	+0.1 (PC_{20})
					histamine	+1.7 (PC)
Thomson et al(84)	clemastine	0.5 g/l 5 min.	IN	30	histamine	+2.3 (PD)
Popa(69)	chlorpheniramine	10-15	IV	NS*	histamine	+2 (PC_{10})¶
Hartmann et al(35)	clemastine	0.6	IN	10	histamine	see text
		1	IV	10	histamine	
		2	PO	240	histamine	
Phillips et al(66)	clemastine	0.5	IN	45	histamine	+2.1 (PD)
		0.5	IN	45	histamine	+2.8 (PD)
Patel(64)	ketotifen	60	PO	240	methacholine	+0.1 (PD)
	terfenadine	120	PO	240	methacholine	+0.1 (PD)
		180	PO	240	methacholine	+0.9 (PD)
Rafferty et al(73)	terfenadine	60	PO	180	histamine	+2.7 (PC)
					methacholine	-0.3 (PC)
		120	PO	180	histamine	+3.8 (PC)
					methacholine	+0.6 (PC)
		180	PO	180	histamine	+3.9 (PC)
					methacholine	+0.6 (PC)
Brik et al.(5)	cetirizine	5	PO	120	histamine	blocked up to
		10	PO	120	histamine	10 mg/ml
		20	PO	120	histamine	(dose-related)
Magnussen et al.(57)	azelastine	8.8	PO	300	histamine	+4.4 (PD_{100})§
	terfenadine	180	PO	180	histamine	+4.3 (PC)
Polosa et al.(68) Ghosh et al.(31)	cetirizine	15	PO	120	histamine	+6.2 (PC)

* NS=not stated ♦ PC_{20}=PC_{20}sGaw; sGaw=specific airway conductance ¶ PC_{10}=$PC_{10}FEV_1$ § PD_{100}=PD_{100}sRaw; sRaw=specific airway resistance

Table 11. **Long-term protection of histamine$_1$ receptor blocking antihistamines against BHR to histamine and methacholine in adults.**

Reference	Drug and frequency	Daily dose (mg)	Duration (weeks)	Test	Interval	Mean change in BHR (DD)
Esau et al.(27)	ketotifen b.i.d.	2	6	histamine	NS*	+ 2.7 (PC)
n = 8	clemastine b.i.d.	2	6	histamine	NS	+ 2.3 (PC)
n = 7						
Graff-Lonnevig	ketotifen b.i.d.	2	12	methacholine	3 hours	– 0.6 (PC)
(32) n = 18						
Iwata et al.(39)	azelastine b.i.d.	4	8	methacholine	NS	+ 1.3 (U)♦
n = 21						
Ghosh et al.(31)	terfenadine b.i.d.	30	1	histamine	14 hours	+ 5.0 (PC)
n = 10						

* NS = not stated

♦ U = cumulative dose after which respiratory resistance begins to increase.

2.8.3. *Conclusion*

H_1 receptor blocking antihistamines protect against BHR to histamine but not to methacholine. Long-term treatment with H_1 receptor blocking antihistamines does not influence BHR to methacholine.

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Chapter III

Minor acute effect of an inhaled corticosteroid (budesonide) on bronchial hyperresponsiveness to methacholine in children with asthma

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3.1. Summary

Several studies have shown that long-term administration of inhaled corticosteroid reduces airway hyperresponsiveness. This study was performed in order to exclude an acute effect of inhaled corticosteroid.
In a double-blind, randomized, cross-over study, children with asthma who had never used inhaled or oral corticosteroid, received a single dose of 0.8 mg budesonide or placebo on two separate days, with an interval of at least 48 h. On each test day, baseline FEV$_1$ and methacholine responsiveness (expressed as PD_{20} to methacholine in doubling dose) were measured. Both measurements were repeated 2 and 5 h after administration of the drug.
Twenty children were included in the study. FEV$_1$ showed a mean increase of 1% at 5 h on the budesonide day, and a decrease of 2% on the placebo day ($p=0.01$). PD_{20} increased by 0.1 doubling dose on the budesonide day, and decreased by 0.4 doubling dose on the placebo day. These changes are within the measurement variation ($p=0.06$).
We conclude that a single dose of 0.8 mg budesonide has a minor effect on methacholine responsiveness 5 h after administration in children with asthma. It is unlikely that such an effect interferes with the interpretation of data collected in long-term studies.

3.2. Introduction

Asthma is characterized by airway hyperresponsiveness to methacholine or histamine (AHR). Airway inflammation plays a central role in asthma, and is associated with AHR[1]. Several studies have shown that inhaled corticosteroid (ICS) gradually reduces AHR, after weeks or months[2], possibly by its antiinflammatory action[3]. In these studies, it was assumed that ICS has no acute effect on AHR. However, in children this was never investigated. If an acute effect exists, this might hamper the interpretation of the effect of long-term drug administration on AHR. This acute effect, if any, is not likely to be pronounced because protein synthesis, which will be activated by corticosteroid, is a slow process[4]. We investigated the acute effect of a single dose inhaled corticosteroid on airway responsiveness, in order to test this assumption.

3.3. Methods

3.3.1. *Patients*

Twenty children with a history of asthma were selected from the out-patient clinic of the subdivision of paediatric respiratory medicine of the Sophia Children's Hospital. They were atopic for one or more allergens and had been in a stable condition for at least 4 weeks. Their forced expiratory volume in 1 second (FEV_1) was >70% of predicted, and AHR expressed as the provocative dose of methacholine which caused a fall in FEV_1 of 20% (PD_{20} methacholine) was $\leq$150 µg, i.e. more than two standard deviations below the mean value in healthy children[5]. The patients had never used inhaled corticosteroid before, and were treated with cromoglycate and/or inhaled $beta_2$-agonist. All participants and their parents gave their informed consent. The study was approved by the medical ethics committee of the hospital. Patient characteristics are shown in table 1.

3.3.2. *Design*

All medication was stopped 8 h before each test day. FEV_1 was measured using water sealed spirometer, according to the ECCS recommendations[6]. The largest value resulting from 3-5 attempts was recorded. Reference values were those of Zapletal et al[7]. PD_{20} methacholine was measured by inhalation of methacholine in doubling doses (DD), according to a standard protocol[8]. Methacholine solutions were stored at 4°C and nebulized at room temperature. Methacholine was nebulized with a deVilbiss 646 nebulizer, with 5 ml solution per vial. The nebuliser was attached to a Rosenthal-French dosimeter, driven by air at 138 kpa (20 psi). The aerosol was delivered directly to the mouth, via a mouth tube. The child inspired as slowly as possible from functional residual capacity to total lung capacity, and held the breath for 5-10 s before expiration. During inspiration, the dosimeter was triggered for 0.6 s. A total of 20 µl of aerosolized solution was delivered to the mouth, in four consecutive breaths. After baseline measurement of FEV_1 subjects started with saline, followed by increasing doses of methacholine from 0.25-64 µg. FEV_1 was measured 3 min after saline and after each dose of methacholine. The test was stopped when FEV_1 had fallen by 20% from the prechallenge value. PD_{20} methacholine was calculated by a computer programme, which used linear interpolation.

The study was double-blind, randomized, placebo-controlled, and cross-over.

Randomization was performed by allocating each patient to the next number on a randomization list, which dictated the sequence in which drugs were delivered. Patients were tested on two different days within one week, with an interval of at least 48 hours. Baseline measurements of FEV_1 and PD_{20} were performed on both days. At least 60 min after the last dose of methacholine (when FEV_1 had returned to within 10% of baseline) patients received 0.8 mg budesonide or placebo, in random order. In an earlier study, we showed that with this design the reproducibility of the test is within 1 DD[8]. Budesonide and placebo were administered using a metered dose inhaler with a spacer (Nebuhaler®). One puff budesonide contained 0.2 mg. During a slow inspiration from functional residual capacity to total lung capacity, budesonide or placebo was inhaled immediately after actuation. Subsequently, the breath was held for about 10 s before expiration. This was repeated three times. Two and five hours after inhalation of budesonide or placebo methacholine challenges were repeated. The complete test procedure took 7-8 h. Power calculations led to a study size of 20 patients. At alpha = 0.05 (two-sided t-test), the power for a difference of means of 0.75 SD equals 90%.

3.3.3. *Statistical methods*

PD_{20} measurements were evaluated after logarithmic transformation. Comparisons of baseline FEV_1 and the logarithm of PD_{20} between the two study days were made using paired t-tests. Results after treatment with budesonide and placebo were compared using t-tests, as appropriate for cross-over studies[9]. Repeated measurements analysis of variance (ANOVA) was used to evaluate changes over time. The limit of statistical significance was set at $p=0.05$ (two-sided).

3.4. Results

There was no statistically significant difference in baseline values of FEV_1 and PD_{20} between the two test days (table 2).

Table 1. **Patient characteristics**

Number (nxmg)	Sex	Age (years)	FEV_1% predicted	Daily medication
1	m	7	90	DSCG* 2x5, S 2x0.1
2	m	10	86	T 0.25 i.n.♦
3	m	8	111	DSCG 3x5, T 0.25 i.n.
4	m	13	74	DSCG 3x5, T 3x0.25
5	m	8	78	DSCG 2x10, S 0.1 i.n.
6	m	10	94	DSCG 3x10, T 0.25 i.n.
7	m	7	81	S 0.1 i.n.
8	f	7	107	S 0.1 i.n.
9	m	11	87	DSCG i.n.
10	m	7	96	DSCG 3x10, S 0.1 i.n.
11	f	9	84	DSCG 3x5
12	m	9	90	T 0.5 i.n.
13	m	10	106	T 0.5 i.n.
14	f	10	105	DSCG 2x5, S 0.1 i.n.
15	f	10	103	DSCG 3x20, T 0.5 i.n.
16	m	10	83	DSCG 3x5, S 0.1 i.n.
17	f	9	109	T 0.5 i.n.
18	m	11	91	T 0.5 i.n.
19	m	7	71	DSCG 3x10, S 4x0.2
20	m	8	98	DSCG 2x5, T 2x0.5

* DSCG = disodium cromoglycate. S = salbutamol. T = terbutaline.
♦ i.n. = if needed.

Table 2. **Baseline data**

	Budesonide	Placebo
FEV_1, L; mean (SD)	1.82 (0.34)	1.80 (0.33)
FEV_1, % of predicted; mean (SD)	92 (11)	92 (13)
PD_{20}, µg; geometric mean (range)	27 (5-81)	27 (8-270)

3.4.1. *Baseline FEV_1*

The change of FEV_1 in % from baseline is reflected in figure 1, and shows a small but significant difference (p=0.01) at 5 h between the budesonide day (when a mean increase of 1% compared to baseline was seen) and the placebo day (when there was a decrease of 2%).

3.4.2. *PD_{20} methacholine*

Changes in PD_{20} from baseline, expressed as DD, are shown in figure 2. After 2 h, no significant change had occurred with either treatment. Five hours after budesonide, a mean $\pm$SEM increase in PD_{20} of 0.1 ± 0.2 DD was found, while after placebo a mean decrease of 0.4 ± 0.2 DD occurred. Both changes are within the individual measurement variation[10]. The difference of 0.5 DD between treatments was only marginally significant (p=0.06). Neither baseline FEV_1 nor baseline PD_{20} was significantly correlated with the change in PD_{20}. This applies to both test days. Individual data show an increase of PD_{20} in 10, a decrease in 4 and no change in 6 patients, after budesonide. These numbers are 3, 10 and 7, respectively, after placebo. Analysis of the data according to the sequence in which both treatments were given revealed no differences. This excludes a carry-over effect.

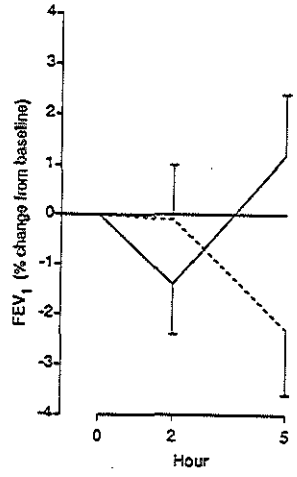

Figure 1. Mean change from baseline of FEV_1 % predicted (SEM) during treatment with budesonide (solid line) or placebo (dashed line).

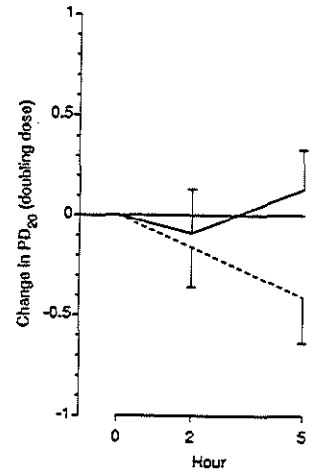

Figure 2. Mean change from baseline of $PD_{20}FEV_1$ methacholine (SEM) during treatment with budesonide (solid line) or placebo (dashed line) in doubling dose.

3.5. Discussion

This study shows a minor effect of a single dose of inhaled corticosteroid on AHR to methacholine 5 h after administration. A small increase in FEV, was also seen. These changes were within the individual measurement variation.

In asthma, inflammation of the airways seems to be a persistent feature, even in patients with mild symptoms[11] [12]. AHR to a number of stimuli, such as histamine and methacholine, is a characteristic of asthma, and there is an association between AHR and airway inflammation[1]. Nowadays, inhaled corticosteroid is the most effective drug, probably due to its antiinflammatory properties. Long-term administration of inhaled corticosteroid suppresses the inflammatory reaction[3] and causes a decrease in AHR to histamine and methacholine[2]. The effect of inhaled corticosteroid on the degree of AHR is time- and dose related[13]. The long-term effect of inhaled corticosteroid on AHR might be misinterpreted if these drugs also had an acute effect on AHR. This has never been investigated in children with asthma.

Recently, Vathenen et al.[14] studied the acute effect of a single dose of inhaled corticosteroid on AHR to histamine. In a double-blind, placebo-controlled study they compared the effect of 800 μg budesonide to placebo, in 20 adult patients with asthma. They measured PD_{20} to histamine before and 1, 6, 12 and 24 h after administration of the drug. They found a small but significant increase in median FEV, (+0.2 l, 95% confidence interval 0.05-0.40) and in median PD_{20} histamine (+1 DD, 95% confidence interval 0.2-1.6) after budesonide, which was maximal at 6 h. These differences were statistically significant at the 1% level.

We selected children with mild asthma, who had never used inhaled or oral corticosteroid. Their treatment consisted of $beta_2$-agonist on demand and/or regular cromoglycate. They were all hyperresponsive to methacholine. The interval between the two test days was at least 48 h, in order to prevent a carry-over effect of inhaled corticosteroid in the patients who received placebo on the second day. No such carry-over effect was found. The decrease in PD_{20} during the placebo day is fully attributable to the diurnal variation in airway responsiveness. Van Aalderen et al.[14] showed a spontaneous decrease in PC_{20} of 0.5 DD between 8.00 a.m. and 4.00 p.m., in two groups of children with asthma. The slight increase of PD_{20} on the budesonide day suggests that even a single dose of inhaled corticosteroid diminishes the diurnal variation in airway responsiveness. The change in PD_{20} in this study is less than that found by Vathenen et al.[14], who used the same dose of inhaled corticosteroid. We measured AHR 2 and 5 h after drug administration, so

that the possibility remains that the maximal effect was missed because we do not have data over a longer time interval. We would have preferred to measure AHR for a longer period than 2-5 h after drug administration, or to determine AHR in the morning after drug administration the night before. This was, however, not feasible, because children of this age would not cooperate if the study took longer than 7-8 h or if they had to come twice on two consecutive days. Indeed most children needed encouragement for the last AHR measurement on a test day. Ellul-Micallef[18] showed that a single dose of budesonide of 1.6 mg or 0.1 mg has an acute effect on peak expiratory flow, which is maximal after 5-8 h and lasts about 12 h. In another study the immediate effect of 1.6 mg budesonide on FEV_1 was maximal after 5 h and showed a plateau until at least 9 h after administration[15]. This effect probably also disappeared after 12 h. The results of these studies indicate that the maximal effect of inhaled corticosteroid occurs by 5 h after administration. The reason that we studied the effect of 0.8 mg budesonide, which is higher than the dose usually prescribed, is that we wanted to avoid the risk of missing an acute effect due to underdosing. We conclude that it is unlikely that the results of long-term studies on the effect of inhaled corticosteroid on AHR are biased by an acute drug effect.

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Chapter IV

Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma

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4.1. Summary

Airway inflammation is assumed to be an important determinant in increased bronchial responsiveness (BR). We tested the hypothesis that treatment with an inhaled anti-inflammatory drug (i.e., budesonide) but not with an inhaled $beta_2$-agonist (i.e., terbutaline) would reduce BR in children with asthma and with minimal or no bronchoconstriction. Twelve patients were treated with budesonide and seven with terbutaline for 6 months. BR decreased in 11 patients receiving budesonide and this decrease was significant in seven patients. BR decreased in none of the patients receiving terbutaline. FEV, demonstrated a small increase with budesonide but remained unchanged with terbutaline. Except in one patient who received terbutaline, the clinical effect was good. We conclude that inhaled corticosteroids but not inhaled $beta_2$-agonists will decrease persistent BR in most children with asthma.

4.2. Introduction

It is generally accepted that asthma has a hereditary basis, presenting itself in immune responses that cause atopic reactions,[1] as well as in bronchial hyperresponsiveness (BHR)[2]. Hyperresponsive patients are more sensitive to the effect of allergens, viral infections, and other environmental exposures than patients with asthma in whom bronchial responsiveness (BR) is normal[2].

Airway inflammation is assumed to be an important determinant in the increase in BR[3] and, therefore, it could be prevented if contact with causal environmental components could be avoided. Since this is hardly possible, additional drug treatment is needed to suppress the inflammatory reaction and, hence, decrease BR. This is also evidenced by the fact that corticosteroids, which have antiinflammatory properties, inhibit the late inflammatory reaction after allergen inhalation[4], but $beta_2$-agonists, which have no anti-inflammatory effect[5], do not.

Data on the effect of long-term treatment with these drugs on BR are limited. In this study, we tested the hypothesis that long-term treatment with an inhaled corticosteroid but not with a $beta_2$-agonist will reduce BR.

4.3. Methods

4.3.1. *Patients*

The study was performed in 19 patients with allergic asthma, aged 7 to 16 years, selected from the outpatient department of respiratory disease, Sophia Childrens Hospital, University of Rotterdam. The patients had to be capable of performing pulmonary function tests in a reproducible way (i.e. coefficient of variation of FEV_1 in three consecutive flow-volume curves <5%). Since BR is not only determined by inflammation but also by the degree of bronchial smooth muscle contraction[6], we selected children with allergic asthma who had a consistent increase in BR but little or no bronchoconstriction, i.e., (1) a dose of inhaled methacholine after which FEV_1 decreased 20% from baseline (PD_{20} FEV_1 methacholine) <150 μg (2) FVC and FEV_1 80% or more of predicted, (3) FEV_1/FVC 70% or more of predicted, and (4) a 20% or less increase in FEV_1 after 0.5 mg of inhaled terbutaline. All criteria were fulfilled on one occasion in the 3-week period before the start of the study and on at least two earlier occasions. Although none of the patients needed continuous medication, all had symptoms at regular intervals that were treated with bronchodilators. Details are presented in table 1.

The study was performed from January to July 1984. Because of the summer holidays, a longer follow-up was not feasible. All children lived in centrally heated houses built in the previous 25 years, and in none of their families were there domestic animals. Quilts and pillows were of synthetic material, and bedrooms were kept as dust free as possible. Pollen counts in May and in June 1984 were very low in the southwestern part of The Netherlands because of the rainy weather in the spring and early summer (i.e., mean number of grass pollen in May, 26/m^3 of air, and in June, 282/m^3).

4.3.2. *FEV_1*

Pulmonary function was measured by means of flow-volume curves with a Fenyves device (Fenyves and Gut, Basel, Switzerland) with computerized data processing. FEV_1 was used as the index of bronchial caliber. Each measurement consisted of three flow-volume curves; the highest value of FEV_1 was obtained for data analysis.

Table 1. **Characteristics of patients**

	Sex	Age (year)	Baseline lung function: FVC (%)	Baseline lung function: FEV_1 (%)	Baseline lung function: FEV_1/VC (%)	Baseline lung function: FEV_1 reversibility (%)*	PD_{20} (μg)	Allergen specific IgE in serum#
Budesonide								
1	F	7	97	104	90	1	45	3+++
2	M	7	127	126	80	6	33	1+++ 3++
3	F	9	81	83	86	10	37	1++++ 3+
4	M	9	79	80	70	7	24	1+++
5	F	10	100	95	81	8	37	1+++++ 2++++ 3++++
6	M	11	80	86	85	1	68	1++++++
7	F	12	115	109	80	5	5	1+ 2++ 3+++
8	M	12	91	93	81	10	88	1+++ 2+++++
9	M	13	95	88	73	8	16	1++++ 2++++
10	F	13	86	89	90	10	68	1+++++ 2+++ 3++++
11	M	14	89	100	90	-3	58	1+++++ 2++++ 3+++
12	M	16	99	90	72	16	19	1++++ 2+++++ 3+++
Terbutaline								
13	M	7	88	91	81	7	47	1+++++ 3+++
14	M	12	99	89	72	5	54	1+++++
15	M	12	100	110	88	3	58	1++++ 2+++++ 3+++
16	M	13	90	86	74	8	28	1+ 2+++ 3+++++
17	M	14	84	87	83	0	44	1+++++ 2+
18	F	15	124	102	71	13	24	1+++ 2+
19	F	16	128	113	75	7	66	1+++++ 2+++++

* Percent increase from baseline after 0.5 mg of inhaled terbutaline.
\# 1, house dust mite; 2, mixed pollen; and 3, mixed animal dander.

4.3.3. *Methacholine challenge*

BR was measured by inhalation of methacholine in increasing dosages according to a standard protocol[7]. Methacholine was nebulized with a deVilbiss (deVilbiss Co., Somerset, Pa., USA) 617 nebulizer and a Rosenthal-French (The Johns Hopkins University, Baltimore, Md., USA) dosimeter.
Inhaled doses were doubled from 10 μg of methacholine up to 1280 μg as a maximum dose. The effect of each dose was determined by measuring FEV$_1$ 5 minutes after each administration. Tests were performed between 9 A.M. and 3 P.M. With this technique, we found in our laboratory a mean ± SD of the ^{10}log PD_{20} (in μg) in healthy children without current or past history of respiratory disease of 2.93 ± 0.25 corresponding to a mean PD_{20} of 850 μg. All patients had at least two BR determinations registered before the value that formed the basis for patient selection. Previous ^{10}log PD_{20} values were within plus or minus 0.50 of the ^{10}log PD_{20}, which was used for patient selection, determined in the 3 weeks before the study. It was not possible to perform the test in duplicate; however, the within-subject variability on two consecutive days of the provocative technique is low.[8]
During the study, BR was only determined if the baseline FEV$_1$ was at least 90% of the lowest prestudy value. PD_{20} was calculated by linear interpolation from a ^{10}log PD_{20} dose-response curve. All medication was stopped 12 hours before each measurement of log FEV$_1$ and BR.

4.3.4. *Symptoms*

Cough, wheezing, and asthmatic attacks, as well as medication, comedication, morning peak flow, and possible side effects were registered daily on a diary card.

4.3.5. *Design*

Patients were randomized double-blind with a computerized random number generator in two parallel groups. Twelve patients were allocated to treatment with budesonide (Pulmicort; Astra Pharmaceuticals, Rijswijk, The Netherlands) in a dose of 0.2 mg, and seven patients to a treatment with terbutaline (Bricanyl; Astra Pharmaceuticals) in a dose of 0.5 mg, both administered three times daily by a

metered-dose inhaler. We decided to use an unequal group size because of earlier observations on the effect of beta$_2$-agonists on BR that indicated that such an effect would be unlikely.[9] Since no prediction could be made on the effect of budesonide, we wanted to have more patients receiving the corticosteroid than receiving the beta$_2$-agonist.

The metered-dose inhalation technique was checked before the start of the study and at every visit to the doctor. Patients were advised to rinse their mouth and throat after drug inhalation. The only comedication allowed during the study was doses of 0.25 mg of terbutaline by regular metered-dose inhaler, to be taken on demand. Patient compliance on use of the inhaler with the study medication was checked by weighing the canisters at every visit. BR and pulmonary function were measured before and monthly during the study by the same technician in the pulmonary function laboratory. At the same time, the patients were observed by one of us. (EvE-Z). After completion of the study, a short adrenocorticotropic hormone test[10] was performed on 11 children treated with budesonide and on six children treated with terbutaline to check adrenaline function.

4.3.6. *Statistical methods*

PD_{20} is presented in the text in micrograms, but ^{10}log transformations were applied to the PD_{20} values before statistical evaluation. FEV_1 was expressed in percent predicted. Symptoms were expressed as the number of days per week with cough, wheezing, or breathlessness; ^{10}log PD_{20} was evaluated by analysis of covariance (general linear model) with treatments, patients, and time as independent factors.[11]

Linear regression was used to compare individual changes in FEV_1 to changes in ^{10}logPD_{20} with respect to baseline, to compare changes in ^{10}log PD_{20} to ^{10}log PD_{20} at entry into the study, and for the relation between ^{10}log PD_{20} and duration of treatment.

Student's t test for unpaired data was used to compare the two treatment groups at entry in the study and used in the budesonide group to compare the responders to the nonresponders. Student's t test for paired data was used to analyze changes within a treatment group. Two sided p values of <0.05 were regarded as statistically significant.

Patients were regarded as having highly increased BR if PD_{20} was below 150 μg (3 SDs below the predicted mean) and having slightly increased BR if PD_{20} was between 150 and 270 μg (3 to 2 SDs below the predicted mean) (see 4.3.3.).

4.4. Results

4.4.1. *Withdrawal*

In the terbutaline-group, patient No. 19 was withdrawn after 3 months because of a severe asthma attack for which hospital admission was necessary.

4.4.2. *FEV_1*

There was a small difference in effect of treatment on FEV_1. Mean FEV_1 increased by 8% with budesonide but remained approximately unchanged with terbutaline (-1%). Mean FEV_1 improved >10% of baseline in three patients receiving budesonide (Nos. 6, 11 and 12) and in one patient receiving terbutaline (No.15).

4.4.3. *PD_{20}*

Individual PD_{20} values are presented in table 2. The two treatment groups were comparable with respect to age, sex, PD_{20}, and baseline lung function at entry into the study. Mean PD_{20} increased with budesonide during the study and decreased slightly with terbutaline. The difference between treatments was significant ($p < 0.05$). The increase was considerable both in the first and second month of treatment with budesonide, but BR improved only slightly during the four months thereafter. There was a statistically significant interaction of treatment and time on $^{10}\log PD_{20}$ ($p < 0.001$). This means that the $^{10}\log PD_{20}$ versus time curve demonstrated a different slope for the two treatments (Fig. 1).
There was no significant relation between individual concomitant changes in FEV_1 and $^{10}\log PD_{20}$. PD_{20} increased in all patients but one while they were receiving budesonide. This increase in seven patients was significant, and mean PD_{20} increased more than fourfold in four patients. In four terbutaline-treated patients, PD_{20} decreased, significantly in two of these patients (nos. 14 and 18), and in none was there an increase in PD_{20}. No relationship existed between the degree of increase or decrease in PD_{20} and baseline PD_{20}. Patients receiving budesonide in whom PD_{20} increased were in no respect different from patients in whom there was no inconsistent increase.

Table 2. PD_{20} methacholine in micrograms before and during treatment*

	Patient	Entry	Month 1	2	3	4	5	6
Budesonide	1	45	69	140	500	160	105	75
	2	33	33	-	60	33	32	30
	3	37	64	67	23	35	48	30
	4	24	19	10	15	17	13	26
	5¶	37	46	60	105	178	145	135
	6	68	43	136	80	63	125	90
	7¶	5	22	25	48	67	235	81
	8¶	88	175	640	540	700	670	880
	9¶	16	55	46	54	38	48	145
	10¶	68	320	190	640	220	320	220
	11	58	34	115	87	150	43	94
	12¶	19	20	57	60	115	185	290
Mean ^{10}log PD_{20}		1.520	1.700	1.895	1.967	1.948	1.989	2.010
SD		0.345	0.370	0.476	0.525	0.449	0.481	0.445
Geometric mean PD_{20} (μg)		33	50	79	93	89	97	102
Terbutaline	13	47	23	11	17	29	13	25
	14¶	54	43	54	28	17	13	34
	15	58	40	190	80	27	38	80
	16	28	10	17	48	20	§	21
	17	44	26	35	40	30	70	35
	18¶	24	15	13	10	11	§	7
	19	66	14	#	16	§	§	§
Mean ^{10}log PD_{20}		1.637	1.334	1.490	1.438	1.324	1.413	1.424
SD		0.164	0.238	0.468	0.314	0.169	0.362	0.347
Geometric mean PD_{20} (μg)		43	22	31	27	21	26	27

* Normal PD_{20} > 270 μg; slightly decreased PD_{20} 150-270 μg; highly decreased PD_{20} < 150 μg.
¶ Significant correlation between ^{10}log PD_{20} and duration of treatment.
\# Responsive on saline.
§ Not determined.

4.4.4. *Symptoms*

Symptoms of asthma were few throughout the study. The average number of days per week with symptoms was 0.56 with budesonide and 0.95 with terbutaline (difference not significant). Hence, patients in both groups had a favorable clinical course, except for the one subject receiving terbutaline, who was withdrawn. Few patients used the extra terbutaline inhaler.

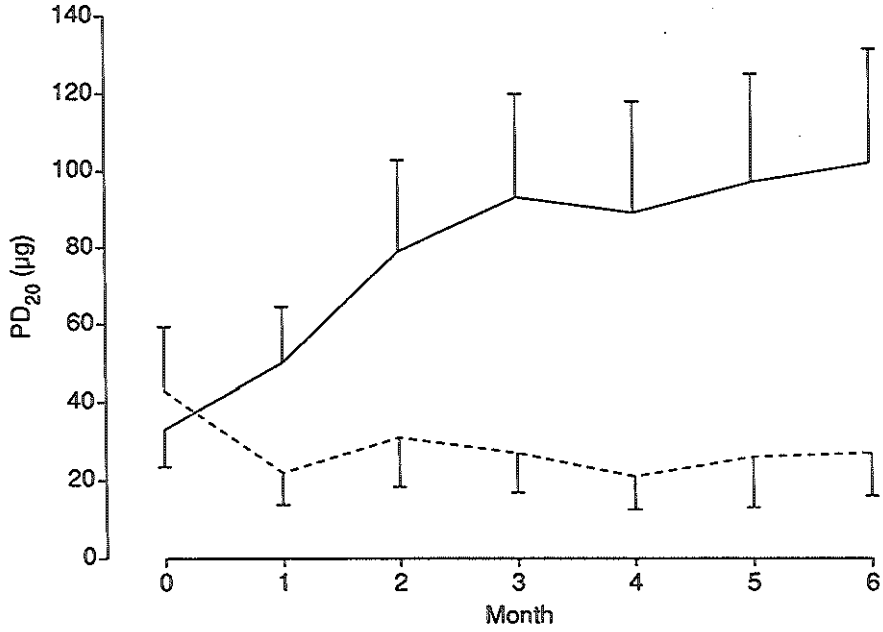

Figure 1. Mean ^{10}log PD_{20} methacholine (SEM) during treatment with budesonide (solid line) or terbutaline (dashed line).

4.4.5. *Adverse effects*

No serious side effects were observed. The increase in baseline plasma cortisol after adrenocorticotropic hormone was within the normal range in all patients tested, and there was no difference between the two treatment groups.

4.5. Discussion

In periods with increased BR, patients with asthma are more sensitive than usual to bronchoconstricting environmental exposures that in turn enhance responsiveness.[12] This mutual influence may cause a vicious circle, resulting in

symptoms that are not necessarily severe but long-lasting and frequently recurring. Airway inflammation appears to be an important contributor in this process.[3] Persistent BR is sometimes considered a risk factor for adult chronic airway disease,[13] and it is assumed that the prevention or suppression of bronchial inflammation may lead to a decrease of BR and, hence, to a limitation of airway obstruction and reduction of hyperinflation.[14]

Studies on the effect of anti-inflammatory treatment on BR are scarce. In this study, we tested the hypothesis that treatment with inhaled anti-inflammatory steroids but not with $beta_2$-agonists, which have no anti-inflammatory effect, would reduce BR. An ideal design would have been double-blind, placebo-controlled, and cross-over. A placebo-controlled study design was not feasible because of the long-term duration. Although the treatment was originally designed as double blind, the key to the treatment code became known to the investigators because of the different immediate effects. This was not considered a drawback because FEV_1 and PD_{20} are objective data. In view of the fact that most of our patients were highly atopic to house dust mite, we decided to perform the study in the first 6 months of the year in order to avoid the autumn season in which symptoms of house mite atopy are most prominent. We therefore decided to make a parallel instead of cross-over study design. Although many patients were atopic to pollen, the rainy weather in spring and early summer kept the number of pollen in the air low and, subsequently, symptoms of pollen atopy were at a low level.

PD_{20} increased in all but one of our patients receiving inhaled steroids with mild asthma but severe BR. The increase was significant in seven patients of whom two (Nos. 8 and 10) reached the level at which no or only a mild reaction to allergen inhalation or exercise was observed in previous studies.[12,15] Improvement was less spectacular in the other four patients or inconsistent.

It was not feasible to determine BR in duplicate or triplicate within a short period before the study. However, FEV_1 as well as BR had been found to be in the same order as baseline on earlier occasions. We therefore considered the single baseline measurements as representative. This is supported by the findings on FEV_1 and in the nonresponders on PD_{20}. Investigations of Clarke[16] and Ryan et al.[17] also indicate that treatment with inhaled steroids may reduce BR. Easton,[18] however, found no such effect of 4 months of treatment with BDP. This may have been due to the fact that his data were not logarithmically transformed before calculations. The effect found by Ryan et al., studying treatment with 0.4 mg of BDP per day for four weeks in 10 adults with asthma, was much smaller than the effect we found with budesonide. After adopting our criterion for responders to their data, only one of

their 10 patients receiving BDP had a significant decrease in BR. Hegardt et al.[19] recently presented a double-blind, placebo-controlled study in which mean PD_{20} histamine fell to 80% of baseline after 5 weeks of treatment with as high as 6 mg of terbutaline daily.

Although inhaled $beta_2$-agonists will shift the dose-response curve of histamine and methacholine to the right,[20] this effect does not last more than a few hours.[21] Inhaled corticosteroids can be considered to have an anti-inflammatory effect. They also inhibit mediator release, modify tissue responses to mediators, suppress responses to airway constrictors, and influence contractile properties of airway smooth muscle.[22]

Beta-agonists relax smooth muscle but are not, even in large doses, anti-inflammatory.[5] They also diminish mediator release, responses to airway constrictors, and smooth muscle contractions.[23]

In this study, we tested the hypothesis that an anti-inflammatory but not a smooth muscle relaxing non-anti-inflammatory agent would reduce BR. Since the degree of BR does not only depend on bronchial inflammation but also on the degree of bronchial smooth muscle contraction,[9] we studied the patients with chronic BR and mild symptoms in whom bronchoconstriction was not a predominant characteristic. We assumed that in these patients chronic inflammation of the airway wall might be an important factor determining BR. This assumption could not be proven, since bronchial biopsies or washings could not be performed. However, our finding that BR dropped in the patients treated with the steroid but not in the $beta_2$-agonist-treated children supports this assumption, although other steroid effects can not be excluded.

Improvement of PD_{20} could not be attributed to improvement in bronchial caliber because no correlation existed between the change in PD_{20} and the change in FEV_1. Of the four patients in whom the FEV_1 increased 10% or more, only one patient had a significant increase in PD_{20}. This is not unexpected because only subjects with a consistent and highly increased BR but with minimal bronchoconstriction were studied. The difference between the treatment regimens could have been due to an insufficient dosing of terbutaline. However, this possibility was excluded after examining in seven children the immediate effect of 0.5 mg of inhaled terbutaline on the dose-response curve of methacholine after the last PD_{20} determination in the study. Terbutaline caused a shift to the right in all subjects with an increase in PD_{20}, indicative of the immediate protective effect of this dose on methacholine-induced bronchoconstriction.

We have found a drop in PD_{20} compared to baseline in five patients receiving

terbutaline. This can neither be explained by the measurement technique that did not differ from that applied before the study nor by a difference in FEV_1 from baseline because FEV_1 during terbutaline treatment did not change. However, terbutaline is known to reduce receptor density as well as receptor coupling from the adenylatecyclase system.[24] The resulting receptor hyporesponsiveness may increase the bronchial reaction to methacholine.[25] The reason why some patients receiving inhaled steroids did not respond, whereas others did, is not clear. Nonresponders were not different from responders with respect to baseline BR, airflow obstruction, atopy, control of symptoms, age, duration, and severity of asthma or environmental conditions such as housing. Regular exposures to allergens to which they were sensitive might explain an insignificant or inconsistent responsiveness to budesonide and the drop in PD_{20} in some patients receiving terbutaline. Although it was impossible to record incidental exposures, continuous contact with significant amounts of house dust mites or animal danders was unlikely. They all lived in relatively newly built, centrally heated houses and had synthetic bed materials that make it likely that the allergen load caused by house dust mites was limited.[26] None of the patients was continuously exposed to animals at home or at school. The same was true for the children receiving terbutaline and children receiving budesonide who responded. Exposure to pollen was low in the year of the study. Also, the fact that only one of the nonresponders to budesonide and two of the patients receiving terbutaline who completed the study have a significant atopy to pollen excludes pollen exposure as a cause of lack or inconsistency of responsiveness; neither could these findings, according to the diary cards, be attributed to symptomatic viral infections. It can not be excluded that the effect on PD_{20} is dose related and that the nonresponders would have improved on a larger dose of budesonide. The budesonide dose that was applied had no side effects such as suppression of the adrenal function, hoarseness, or clinically apparent pharyngeal candidiasis.

We conclude that in children with asthma, treatment with inhaled corticosteroids may decrease persistent BR. It would be worthwhile to confirm our findings in a study on a larger number of patients.

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Chapter V

Effects of 22 months treatment with inhaled corticosteroids and/or beta$_2$-agonists on lung function, airway responsiveness and symptoms in children with asthma

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5.1. Summary

In a double blind multicenter clinical study, 116 children with asthma were randomly assigned to treatment with an inhaled $beta_2$-agonist (salbutamol 0.2 mg) plus an inhaled corticosteroid (budesonide 0.2 mg) t.i.d. (BA+CS) or to an inhaled $beta_2$-agonist (salbutamol 0.2 mg) plus a placebo t.i.d. (BA+PL).

After a median follow-up time of 22 months, 26 patients on BA+PL (45%) had withdrawn from randomized treatment, mainly due to asthma symptoms, compared with 3 withdrawals in the patients on BA+CS ($p<0.0001$). The forced expiratory volume in 1 second (FEV_1), expressed as a % of the predicted value for age, sex and height, showed an absolute increase of 7.0% after 2 months on BA+CS compared with a decrease of 4.0% after 2 months on BA+PL. This 11% difference in % predicted FEV_1 (95% confidence interval 7-15%, $p<0.0001$) was then maintained after a median follow-up period of 22 months. Post-bronchodilator FEV_1 showed an absolute increase of 3.7% predicted within 2 months in patients on BA+CS and an absolute decrease of 1.1% predicted in children on BA+PL ($p=0.0005$). Thereafter this difference between the two treatment groups was maintained. Average peak expiratory flow (PEF) increased from baseline by 36.6 l/min in the BA+CS group compared with 3.7 l/min in the BA+PL group ($p=0.003$). This difference then remained for the median follow-up time of 22 months. Mean airway responsiveness expressed as the provocative dose of histamine required to give a 20% fall in FEV_1 (PD_{20}) increased from baseline to 4 months by 0.98 doubling doses in children on BA+CS compared with a decrease of 0.42 doubling doses in patients on BA+PL. This represents a difference of 1.4 doubling dose (95% confidence interval 0.77 to 2.02; $p<0.0001$), which became even greater with further follow-up and did not reach a plateau after the median follow-up period of 22 months. PEF-variability expressed as the average standard deviation of daily measurements was reduced by 5.9 l/min within 2 months in patients on BA+CS (nearly one-quarter in relative terms) compared with an increase of 1.6 l/min in those on BA+PL ($p=0.015$). Thereafter the difference was maintained. After 2 months of treatment the median number of days with symptoms remained 3 days per two-week period in the BA+PL group and decreased to 2 days in the BA+CS group. This difference increased to 3 days versus 1 day at 12 months ($p=0.016$) and 4 days versus 0 day after 22 months ($p=0.25$). No serious side effects have been reported in either treatment group. This study provides strong evidence that inhaled corticosteroids are important in the long-term treatment for childhood asthma.

5.2. Introduction

In asthma inflammation is considered to be an important determinant of symptoms and other indicators of disease activity.[1] Several studies show the presence of an inflammatory process in the airways of asthmatics, even of patients with mild symptoms.[2-4] This is characterized by an increased number of inflammatory cells (mast cells, monocytes and activated eosinophils) in the submucosa, damage of the airway epithelium, increased vascular permeability and mucosal edema[1]. It is uncertain whether other features of asthma like smooth muscle hypertrophy, a change in airway wall mechanics, an increased mucosal gland mass and thickening of the basement membrane are also caused by chronic airway inflammation. The inflammatory process results in symptoms of asthma, reversible bronchoconstriction, an increased peak flow variability within and between days and airway hyperresponsiveness. Both cromoglycate and inhaled corticosteroids have an anti-inflammatory effect in asthma .[5,6] The National Asthma Education Program Expert Panel Report from the National Heart, Lung and Blood Institute is now recommending inhaled corticosteroids as first line maintenance therapy for asthma in adults;[7] the efficacy of chronic inhaled steroids as first line therapy for children with asthma has not been established until now. According to consensus reports about the treatment of asthma in children cromoglycate will be the drug of first choice in most children who need maintenance treatment.[8,9] However, cromoglycate will often inhibit symptoms incompletely and does not diminish airway hyperresponsiveness.[10] Various short-term clinical studies have shown that inhaled corticosteroids are highly effective in the reduction of symptoms and they may diminish airway hyperresponsiveness[10]. A similar result is seen in the few long-term studies with inhaled corticosteroids.[10-12] At present, $beta_2$-agonists are drugs of choice to reduce bronchoconstriction. Short-acting inhaled $beta_2$-agonists cause an acute protection against bronchoconstricting agents which lasts for about 3 hours.[13] However, long-term treatment with a short-acting beta-agonist does not diminish airway responsiveness[10] and has been associated with a deterioration in symptoms and airway caliber.[14,15]

This randomized double-blind multicenter study was designed to compare the long-term effect in children of regular bronchodilator plus antiinflammatory treatment using inhaled corticosteroids with regular bronchodilator treatment alone. We report here the course of airway caliber, bronchodilator response, airway responsiveness and symptoms over a median follow-up time of 22 months.

5.3. Methods

5.3.1. *Patients*

One hundred and sixteen children aged 7 to 16 years with asthma were selected from the outpatient clinics of two university children's hospitals and one general children's hospital. Inclusion criteria required the following lung function measurements: Forced expiratory volume in one second (FEV_1) 55-90% of predicted and/or the ratio FEV_1/forced vital capacity (FVC) 50-75%; an increase in FEV_1 after 0.2mg salbutamol of $\geq$15% of baseline; the provocative dose of histamine which causes a 20% fall in FEV_1 (PD_{20} histamine) $\leq$150 μg (this being more than two standard deviations below the mean value in healthy children[16]). The study was approved by the medical ethics committees of the participating centers. Informed consent was obtained from all participants and their parents.

5.3.2. *Design*

The study commenced with a run-in period of 2 to 4 weeks during which the only medication allowed consisted of inhaled $beta_2$-agonist on demand. At the end of this period, patients were randomly allocated in a double-blind manner to one of two treatment arms by calling an independent telephone center. Randomization was performed with stratification by sex, age, center and prior use of inhaled corticosteroid and/or cromoglycate using a computerized minimization method.[17] Using this method a patient is allocated to a treatment so as to minimize any imbalance between the treatment groups for each stratum. Treatment consisted of inhaled $beta_2$-agonist (salbutamol 0.2 mg) plus inhaled corticosteroid (budesonide 0.2 mg) t.i.d. (BA+CS) or inhaled $beta_2$-agonist (salbutamol 0.2 mg) plus placebo t.i.d. (BA+PL). Drugs were supplied in identical canisters so that both patient and doctors were blinded to a patient's treatment allocation. Additional bronchodilator medication was supplied in a different outfit (fenoterol inhaletten, a dry powder device) to be taken on demand at a maximum of 0.2 mg q.i.d. No other pulmonary medication was allowed. Before entry into the study any patients using inhaled corticosteroid or disodium cromoglycate had it tapered off (inhaled corticosteroids were reduced by 50-100 μg per week) and stopped 2 weeks before the first baseline measurements. Theophylline was not used within 48 hours of each baseline test and was not used at all after treatment allocation. Primary endpoints for this study were airway caliber measured as FEV_1 and airway

responsiveness expressed as sensitivity to histamine, i.e. the dose of histamine after which FEV, dropped 20 percent of baseline (PD_{20}). Peak expiratory flow (PEF) prior to medication both in early morning and late afternoon was also used as a measure of airway caliber and its day-to-day variability as a measure of airway responsiveness. Bronchodilator response was measured both as FEV_1 20 minutes after 0.8 mg salbutamol and as PEF 10 minutes after 0.2 mg salbutamol. All measurements were performed during clinically stable periods. Clinical endpoints were assessed using diary records of symptoms and additional $beta_2$-agonist usage, exacerbations for which prednisolone was prescribed, absence from school and hospitalization because of asthma symptoms.

In the baseline period, two separate visits to the clinic were necessary for measurements of PD_{20} histamine and bronchodilator response of FEV_1. The mean FEV_1 from these two visits was used as the baseline FEV_1. Thereafter, patients visited the outpatient clinic every 2 months and had their FEV, measured. Inhaled medication was stopped 8 hours prior to each test. At alternate visits bronchodilator response of FEV_1 (at 2, 6, 10 months etc.) and PD_{20} histamine (at 4, 8, 12 months etc.) were assessed. When an exacerbation occurred, a short course of prednisolone was prescribed (starting at 30 mg on the first day and tapering off to 0 mg in one week according to a scheme that depended on body weight). If prednisolone was taken within 4 weeks of a scheduled study visit to the clinic, then this visit was postponed until a 4-week period without oral steroid had elapsed.

During the two-week period prior to each visit, patients kept diaries of their usage of extra $beta_2$-agonist and recorded symptoms of asthma. In addition they measured their PEF using a mini-Wright peak flow meter at home on three occasions each day: in the morning within half an hour of rising prior to bronchodilation, 10 minutes after bronchodilation with 0.2 mg salbutamol and prior to bronchodilation in late afternoon. Each measurement consisted of 3 attempts, the highest value was recorded.

All data were collected and checked by the coordinating center in Rotterdam to ensure completeness and to prevent bias due to local procedures. Interim analyses of the study data were made by an independent statistical center every six months and reviewed by a data-monitoring committee. Investigators were kept blinded of the results of the interim analyses. At a meeting in November 1989 the data-monitoring committee recommended that, in the light of the interim results, all patients who were still on randomized BA+PL therapy be transferred to BA+CS at the next scheduled follow-up visit. Therefore, the time on randomized treatment ranged from 10 to 28 months (median 22 months). Consequently, at each patient's next scheduled visit, those patients still receiving BA+PL stopped that regimen and started the BA+CS

regimen. This was obtained in a double-blind way by stopping the original medication in both groups and starting with BA+CS in all patients. All these patients are now being followed up until the planned completion of the study after three years on treatment.

5.3.3. *Protocols*

FEV_1 was measured according to the ECCS recommendations[18] by water sealed or dry rolling seal spirometer or pneumotachograph. The largest value from an envelope curve consisting of 3-5 attempts was recorded.[19] Reference values of Zapletal were used.[20] Postbronchodilator FEV_1 was measured with 0.8 mg $beta_2$-agonist in order to obtain maximal bronchodilation.[21] Salbutamol was administered using a metered dose inhaler with a spacer (Volumatic®). One puff contained 0.2 mg salbutamol (the same dosage per puff as used for treatment in this study). While inspiring slowly from functional residual capacity to total lung capacity, salbutamol was inhaled immediately after actuation. Subsequently, each breath was held for about 10 seconds before expiration. This was done 4 times and FEV_1 was recorded 20 minutes after the last dose of salbutamol. PD_{20} histamine was measured by inhalation of histamine diphosphate in doubling dosages according to a standardized protocol.[22] Histamine solutions were stored at 4°C and nebulised at room temperature. Each concentration of histamine was nebulised with a separate deVilbiss 646 nebulizer attached to a Rosenthal-French dosimeter. Output of nebulizers was measured before the start of the study and checked centrally once a year in the pediatric lung function laboratory in Rotterdam[23] and nebulizers with an output variability of $\geq$ 10 % were replaced. All parts of each nebulizer were marked with waterproof paint to prevent interchanging. As histamine salt crystal precipitations may reduce nebulizer output by almost 50%, nebulizers were cleaned after each measurement to prevent precipitations on the orifice.[23] Preparation of histamine solutions was standardized in all centers. Inhaled doses of histamine were doubled from 2.5 µg up to 640 µg as a maximum. The effect of each dose was determined by measuring FEV_1 3 minutes after each administration. PD_{20} histamine was calculated by a computer programme which used linear interpolation. Airway responsiveness was only measured if FEV_1 before histamine provocation was $\geq$ 60% of predicted. All centers used written guidelines for measurements and technicians were trained in the methods of measurements. Site visits were made once a year to control the equipment and the methods used.

5.3.4. *Statistical methods*

Time to withdrawal from randomized treatment was analyzed using life-table methods and the logrank test.[24] Comparisons of FEV_1, the logarithm of PD_{20} and average PEF levels during the two week assessment periods were made using t-tests. Numbers of patients using extra $beta_2$-agonist medication were compared using Fisher's exact test. Distributions of days with symptoms during the two-week diary periods were compared using the Mann-Whitney test. For the morning PEF measurement prior to bronchodilation, variability for each patient was expressed as the standard deviation of the daily measurement prior to the clinic visit. Similar measures of day-to-day variability were then obtained for the morning PEF measurements after bronchodilation and the afternoon PEF measurements. Each measure of day-to-day variability was compared between treatment groups using the Mann Whitney test. For the few patients failing to complete their diary for more than 7 days in any two-week period, that assessment was not included in analyses. Otherwise, when there were missing days in the record, pro rata adjustment was made to give a two-week assessment. All reported p-values are for two-sided tests and for simplicity of presentation are without formal adjustment for multiple comparisons over time. In all cases, use of the Bonferroni adjustment procedure[25] would not change the statistical conclusions based on such observed small p-values. Changes in p-values over time should be interpreted with care; in particular, increases can be attributable to the smaller number of patients followed for longer periods.

5.4. **Results**

5.4.1. *Baseline Comparison*

Between October 1987 and April 1989, 116 patients were randomized into the study. Patient characteristics at randomization were similar (table 1) except for a slightly poorer mean FEV, % predicted in the BA+CS group compared with the BA+PL group which is well within chance variation.

Table 1. **Baseline characteristics by treatment group**

Treatment group*	BA+PL (n=58)	BA+CS (n=58)
Sex: no. (%) male	44 (76%)	42 (72%)
Age (years): mean (s.d.)	10.9 (1.9)	11.0 (1.9)
Prior use of inhaled corticosteroids: no. (%)	32 (55%)	29 (50%)
Prior use of cromoglycate: no. (%)	28 (48%)	28 (48%)
FEV_1 % predicted: mean (s.d.)	78.7% (12.0%)	75.7% (10.8%)
Post-bronchodilation FEV_1 % predicted: mean (s.d.)	95.5% (11.3%)	93.6% (11.4%)
Morning PEF l/min: mean (s.d.)	278 (63)	287 (73)
PD_{20} µg histamine: median (quartiles)	21.5 (10, 49)	21.5 (8, 38)
Days in two weeks with symptoms: median (quartiles)	4 (1, 9)	4 (1, 9)

* BA+PL: beta-agonist plus placebo
BA+CS: beta-agonist plus corticosteroid

5.4.2. *Withdrawals from the study*

The length of follow-up from randomization to the termination of the comparative part of the study ranged from 10 to 28 months. Twenty-nine patients withdrew from the study during this time: 26 of these received BA+PL and 3 received BA+CS. The main reason for withdrawal was an increase in asthma symptoms (24 on BA+PL, 1 on BA+CS). Figure 1 shows the estimated percentages withdrawing by each visit by treatment group obtained using the life-table method[24] to allow for the variable follow-up. The rate of withdrawals from BA+PL appears continuous over time and the difference compared with the BA+CS group is highly significant ($P<0.0001$). As only

10 patients randomized to receive BA+PL have been followed on-treatment for longer than 22 months, the on-treatment analyses presented are restricted to this period.

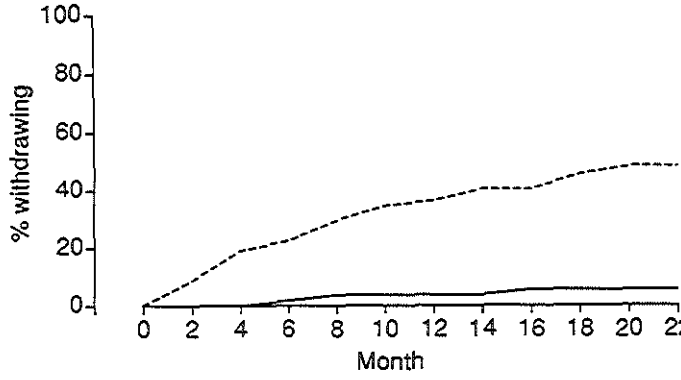

Figure 1. Withdrawals adjusted for variable follow-up during treatment with beta$_2$-agonist + inhaled corticosteroid (solid line) or with beta$_2$-agonist + placebo (dashed line).

It is important to appreciate how the differential withdrawal rate might affect the estimation of treatment differences. Table 2 shows a comparison, for the BA+PL patients, of characteristics at randomization for those patients not withdrawing from the study compared with those patients withdrawing from randomized treatment. This shows that patients in the BA+PL group who withdrew tended to have poorer airway caliber, greater histamine sensitivity and more frequent symptoms than those staying on the study treatment. The percentage that was treated with inhaled corticosteroids before entry into this study was slightly higher, and the percentage that used cromoglycate before the study was slightly lower than in the non-withdrawals. Further analysis of the patients who withdrew showed that they also tended to have greater deteriorations in lung function. For example, the mean decline in FEV$_1$ during the first 2 months was 3.5% predicted amongst patients still receiving BA+PL at 12 months compared with a mean decline of 7.0% predicted for those who withdrew between 2 and 12 months from randomization. Therefore, the following on-treatment comparisons are likely to produce conservative estimates of the differences between the treatment arms and hence of the addition of corticosteroids to bronchodilator therapy.

5.4.3. *Airway caliber and bronchodilator response*

5.4.3.1. *FEV$_1$*

At the first follow-up visit (2 months), FEV$_1$ % predicted shows a mean absolute increase of 7.0% in BA+CS and a mean reduction of 4.0% in BA+PL. This mean

treatment difference of 11.0% (95% confidence interval: 7.1% to 14.9%, p<0.0001) is then maintained at all subsequent visits up to 22 months. For instance, at 12 months the mean difference in FEV, % predicted is 14.1% (95% confidence interval: 9.1% to 19.1%, p<0.0001) and at 22 months the mean difference in FEV, % predicted is 14.0% (95% confidence interval: 5.9% to 22.1%, p = 0.0017). The same pattern emerges in looking at the percentages of patients with improved FEV, % predicted (relative to baseline) over time and at the percentages that reach a level which is within the normal range (>90% predicted) (table 3). The majority of patients on BA+PL have some deterioration in FEV, % predicted while at least three-quarters of patients on BA+CS show an improvement. 42% reached a level of >90%. The decline in the numbers of patients followed-up reflects both the staggered times of entry to the study and withdrawals from randomized treatment.

Figure 2 shows the mean levels of FEV_1 before and after bronchodilation measured at alternate visits. For FEV_1 after bronchodilation, there is an absolute increase of 3.7% predicted within 2 months on BA+CS and an absolute decrease of 1.1% predicted on BA+PL (p=0.0005). There is then no significant trend in mean level so that the

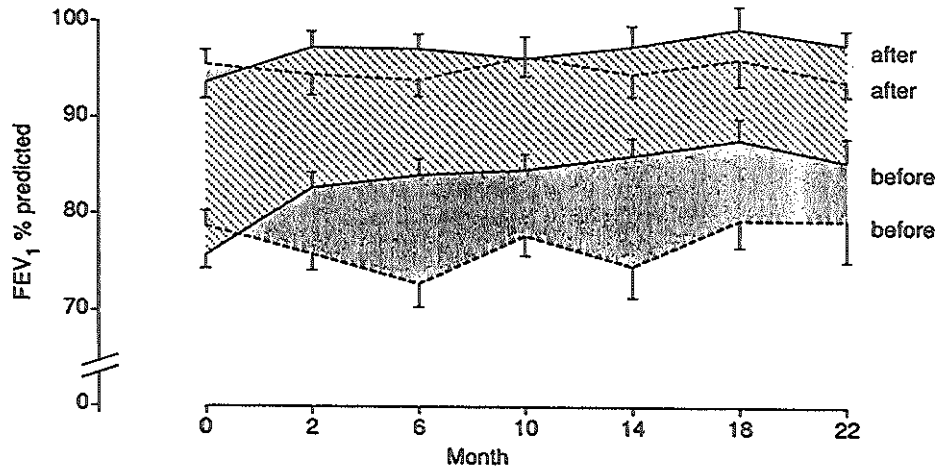

Figure 2. FEV_1% predicted (SEM) before and after 0.8 mg salbutamol during treatment with $beta_2$-agonist + inhaled corticosteroid (solid line) or with $beta_2$-agonist + placebo (dashed line).

difference between the two treatment groups is maintained. It is important to notice that even after a median follow-up period of 22 months there is a significant improvement of FEV_1 after administration of a bronchodilator.

Table 2. **Baseline characteristics of beta-agonist+placebo patients according to whether they subsequently withdrew or not**

	Non-withdrawals (n=32)	Withdrawals (n=26)
Sex: no. (%) male	24 (75%)	20 (77%)
Age: mean (s.d.)	10.8 (1.9)	11.0 (1.9)
Prior use of inhaled corticosteroids: no. (%)	16 (50%)	16 (62%)
Prior use of cromoglycate: no. (%)	17 (53%)	11 (42%)
FEV_1 % predicted: mean (s.d.)	81.6% (12.1%)	75.3% (11.1%)
Post-bronchodilation FEV_1 % predicted: mean (s.d.)	97.0% (12.3%)	93.7% (9.8%)
Morning PEF l/min: mean (s.d.)	279 (59)	276 (68)
PD_{20} μg histamine: median (quartiles)	24.5 (14, 53)	15.5 (6, 29)
Days in two weeks with symptoms: median (quartiles)	3 (1, 7)	6 (2, 11)

Table 3. **Individual patient change in FEV_1 and PD_{20}: percentages reaching normal levels and percentages showing improvement from baseline by treatment group**

Months	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**
BA+CS*:												
no. of patients	58	58	58	57	56	56	55	49	42	36	32	29
% improved FEV_1	--	79	86	82	93	80	88	93	89	91	93	92
% FEV_1 > 90% predicted	5	29	33	35	44	34	43	43	47	50	46	42
% improved PD_{20}	--		77		84		82		87		100	
% PD_{20} > 150 μg	2		9		9		18		24		26	
BA+PL*:												
no. of patients	58	53	47	45	41	38	34	28	25	20	17	17
% improved FEV_1	--	42	30	31	42	31	46	31	42	26	37	57
% FEV_1 > 90% predicted	15	12	9	13	25	13	21	19	17	21	19	29
% improved PD_{20}	--		34		30		37		48		37	
% PD_{20} > 150 μg	0		0		3		4		0		6	

* BA+PL: beta-agonist plus placebo
BA+CS: beta-agonist plus corticosteroid

5.4.3.2. *Peak expiratory flow*

Changes in PEF are shown in figure 3 for the morning measurements, before and after bronchodilation. Without bronchodilation, there is an acute effect of corticosteroids in raising PEF which is established within two months of the start of treatment: average increase from baseline of 36.6 l/min in the BA+CS group compared with 3.7 l/min in the BA+PL group (p=0.0030). This difference then remains for the 22 months of follow-up though both groups show continuing but weak upward trends which are attributable to the childrens' growth.[26] Both treatment arms show average differences of 20 to 25 l/min between morning and afternoon measurements throughout the period so that the same pattern of change with time is observed for the afternoon PEF as for the morning level. Thus the magnitude of the benefit of corticosteroid treatment is not influenced by the time of day.

Additional bronchodilation shows the same pattern on PEF (figure 3) as on FEV_1 (figure 2): There are still gains in PEF in the BA+CS group though the difference between levels achieved with and without bronchodilation is smaller in the BA+CS group compared with the BA+PL group. However, unlike FEV_1, the effect of BA+CS is to increase prebronchodilator PEF to a similar level as patients on BA+PL obtain after bronchodilation.

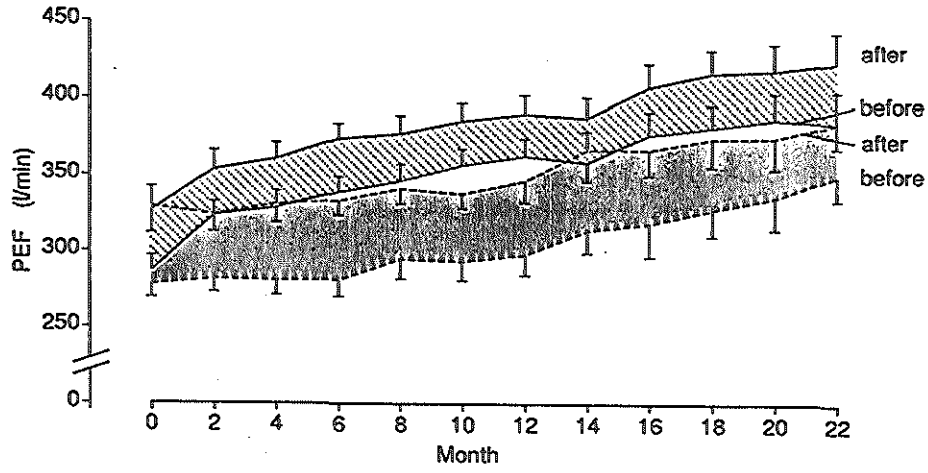

Figure 3. Morning PEF (l/min, SE) before and after 0.2 mg salbutamol during treatment with $beta_2$-agonist + inhaled corticosteroid (solid line) or with $beta_2$-agonist + placebo (dashed line).

5.4.4. *Airway responsiveness*

5.4.4.1. *PD_{20} to histamine*

Figure 4 shows the effect of treatment on airway responsiveness. In the BA+CS group, there is an average increase of 0.98 (doubling) dose steps between baseline and 4 months compared with a 0.42 decrease in the BA+PL group. This difference of 1.40 dose steps is highly significant ($p<0.0001$, 95% confidence interval: 0.77 to 2.02). After 4 months, the difference increases further without reaching a plateau during the ensuing follow-up. This is also reflected by the increasing percentage of patients with time who reach levels that can be considered as within the normal range (>150 µg histamine) (table 3). After a median follow-up period of 22 months the mean PD_{20} value is 80 µg in patients on BA+CS.

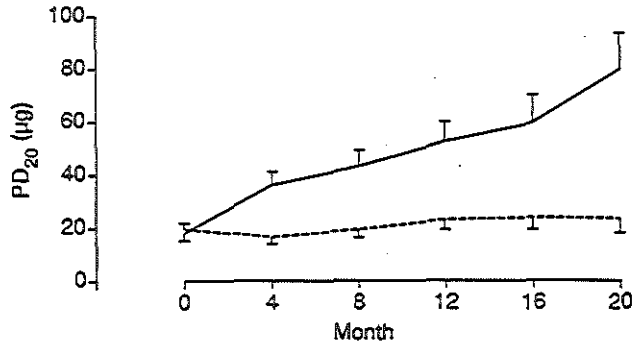

Figure 4. Geometric mean PD_{20} histamine (SEM) during treatment with $beta_2$-agonist + inhaled corticosteroid (solid line) or with $beta_2$-agonist + placebo (dashed line).

5.4.4.2. *Variability in peak expiratory flow rate*

Figure 5 shows the change with time in the day-to-day variability of the morning PEF before bronchodilation during the two week measurement periods. In the BA+CS group, the average standard deviation of measurements is reduced by 5.9 l/min within 2 months (nearly one-quarter in relative terms) compared with an increase of 1.6 l/min in the BA+PL group ($p = 0.015$). Thereafter the difference between treatment groups is maintained. The reduction in effect beyond 16 months suggested in figure 5 should be interpreted with caution because of the small numbers of patients followed for that long. Similar patterns of change are seen for the morning PEF after bronchodilation, the afternoon PEF and the difference between morning and afternoon measurements. Thus the addition of corticosteroid to $beta_2$-agonist benefits patients both by increasing

PEF levels and by decreasing day-to-day variation which, together, imply a much reduced probability of days with very poor PEF and also of greater stability in airway caliber.

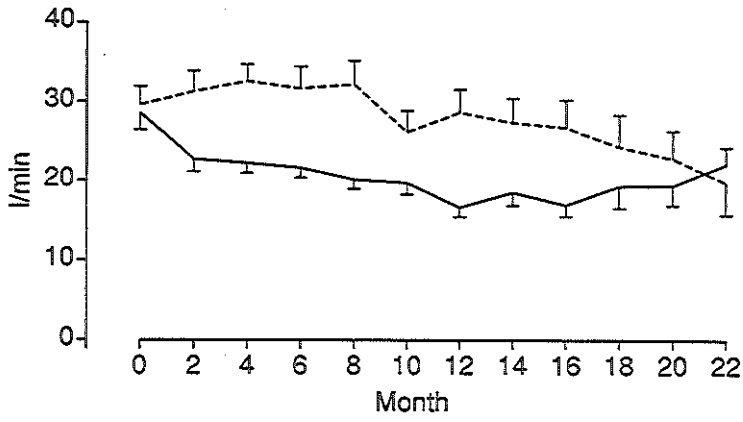

Figure 5. Standard deviation between days in morning PEF (l/min, SEM) before bronchodilation during treatment with beta$_2$-agonist + inhaled cortocosteroid (solid line) or with beta$_2$-agonist + placebo (dashed line)

5.4.5. Clinical signs

5.4.5.1. *Symptoms*

The number of days during the 14-day period before a clinical visit on which a patient recorded any symptoms is used as a measure of symptom burden. Figure 6 shows the percentages of patients by the number of days affected. In the BA+PL group 20-25% of patients have 8 or more days with symptoms at each visit. This compares with a decline to about 10% of patients receiving BA+CS. A similar effect is seen for the percentage of patients that was symptom free during the two weeks before each visit: 32% in the BA+PL group at 12 months increasing to 47% at 22 months compared with 49% in the BA+CS group at 12 months and 61% at 22 months. After 2 months of treatment the median number of days with symptoms is 3 days in the BA+PL group and 2 days in the BA+CS group. This difference increases to 3 days versus 1 day at 12 months ($p=0.016$) and to 4 days in the BA+PL-group and 0 days in the BA+CS-group after 22 months ($p=0.25$). The latter lack of significance needs to be interpreted with caution because of the small numbers.

5.4.5.2. *On-demand use of extra beta$_2$-agonist medication*

At baseline 49% in the BA+CS group and 52% of patients in the BA+PL group report no use of the additional beta$_2$-agonist medication during the 14 days prior to the clinic visit. There is little change in either group by the 2-month visit but at 12 months 66% of BA+CS patients report no use compared with 52% of BA+PL patients. At 22 months these percentages are 71 and 67 respectively. These differences are not statistically significant ($p = 0.26$) though they are consistent with the findings for other endpoints.

5.4.5.3. *Exacerbations and hospitalizations*

Twenty-eight patients (48% and including a total of 45 episodes) on BA+PL suffered exacerbations for which prednisolone was prescribed compared with 8 (14% and including a total of 14 episodes) on BA+CS ($p<0.001$). Adjustment for the different lengths of follow-up on randomized treatment indicates a difference in the rate of episodes per person-year between the BA+PL group (0.71) and the BA+CS group (0.15).

Three patients were hospitalized whilst receiving randomized treatment because of the

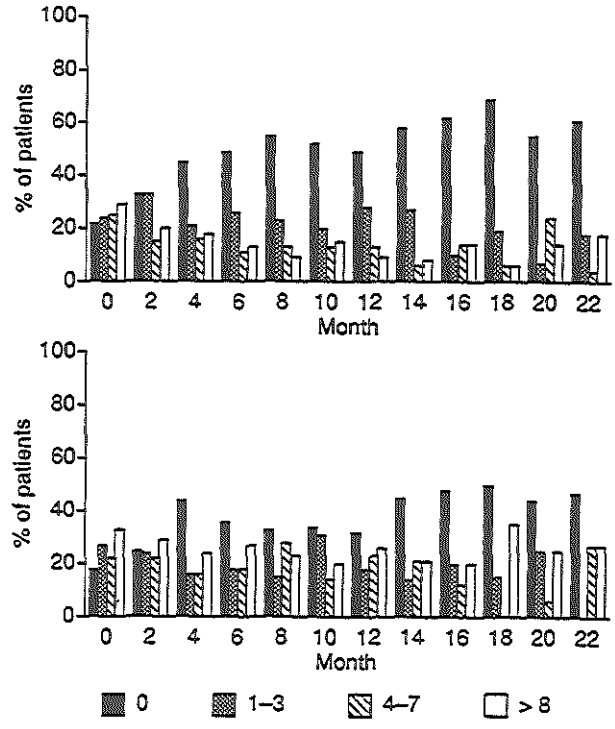

Figure 6. Days with symptoms per two weeks, % of patients during treatment with beta$_2$-agonist + inhaled corticosteroid (upper panel) or with beta$_2$-agonist + placebo (lower panel).

5.4.5.4. *Absence from school*

Absence from school due to asthma symptoms was recorded throughout the study. Whilst receiving randomized treatment, 23 patients (47%) in the BA+PL group had no absences compared with 32 (62%) in the BA+CS group. Five children in the BA+PL group averaged more than one day per month compared with three in the BA+CS group. Comparison of the absence rates adjusted for time on-treatment shows a lower rate for the BA+CS patients which is of marginal significance (p=0.07).

5.4.5.5. *Adverse events*

Thirty-four patients reported adverse events (table 4): 18 on BA+PL (33 events in total) and 16 on BA+CS (31 events in total). All adverse events are minor and none was a reason for stopping randomized treatment. No abnormal growth in height was reported for any child during the study. Mean height (S.E.) in cm was 153.6 (2.0) for BA+CS, 151.3 (2.2) for BA+PL at 12 months (p=0.44) and 159.8 (3.0) for BA+CS, 156.2 (3.3) for BA+PL at 22 months follow-up (p=0.43).

Table 4. **Adverse events reported**

Adverse event	Frequency	
	BA+PL	BA+CS*
Tremble	2	7
Agitation	0	2
Headache	4	4
Nausea	1	2
Vomiting	1	0
Sleepiness	0	1
Unpleasant taste	1	0
Irritated throat	2	1
Hoarse voice	0	3
Cough	20	8
Wheeziness	1	0
Sneezing	0	1
Nose bleeding	0	1
Increased appetite	0	1
Increased weight	1	0

* BA+PL: beta-agonist plus placebo
* BA+CS: beta-agonist plus corticosteroid

5.4.6. *Subgroup analysis*

Further subgroup analyses of treatment differences in all endpoints were undertaken for patients stratified by sex, previous corticosteroid use, previous use of cromoglycate and baseline lung function. These did not reveal any particular type of patient that was more (or less) prone to benefit from BA+CS.

5.5. Discussion

This is the first long-term randomized intervention study that assesses the effect of adding inhaled corticosteroid to beta$_2$-agonist therapy in children with asthma. Endpoints were airway caliber, bronchodilator response, airway responsiveness and symptoms. All endpoints improved in the group that received inhaled corticosteroid. In these patients stabilization of airway caliber and bronchodilator response occurred after 2 months, and of symptoms after 8 months, whereas airway responsiveness improved continuously over the whole period. In patients on beta$_2$-agonist only there was a high rate of withdrawals due to an increase in symptoms and there was no improvement in any endpoint.

For this study, only children with asthma who needed daily medication were selected. It took 18 months to include all 116 children, which was mainly caused by the high percentage of eligible patients (over 50 %) who refused participation. The main reason for refusal was the foreseen long duration of the study. Of the patients who agreed to participate 33 withdrew before randomization mainly because of worsening of their asthma due to tapering off their inhaled corticosteroids or cromoglycate. This underlines the difficulty in enrolling a large number of patients into a long-term intervention study such as this one.

Results from long-term studies are only reliable when methods for measurements and equipment remain unchanged over time. We therefore put maximal effort into standardization of lung function measurements. Lung function technicians adhered to a strict protocol and had their techniques checked on regular occasions. Furthermore, a committee of lung physiologists visited each site once a year to ensure the standardization of techniques.

For this study we decided to use the position of the dose-response curve (PD_{20}) as the only measure of airway responsiveness. Using methacholine instead of histamine would have enabled us to also measure a response plateau.[27] However, when we planned the study this technique had not yet been used in children so that we did not consider it justified to perform plateau measurements in a large multicenter study.

The median follow-up period on randomized treatment after which data were analysed was 22 months. One of the most striking effects found was the high rate of withdrawals due to symptoms amongst patients on beta$_2$-agonist only. For ethical reasons it was therefore decided to stop the study. Half of the patients on beta$_2$-agonist only were still on randomized treatment at that time. This high rate of withdrawals seems to be partly attributable to tapering off inhaled corticosteroids shortly before the start of the study. We might have had less drop-outs if the washout period had been longer, but that would have raised practical problems. For most patients who were on inhaled corticosteroids before the start of the study it took 6-12 weeks before wash-out had been completed. As mentioned above, 33 patients withdrew before randomization (before the first baseline visit or between the two baseline visits) because of worsening of asthma during or after tapering off inhaled corticosteroids. It would have been ideal to include only newly referred patients with asthma who were not on inhaled corticosteroids, like Haahtela et al.[12] did, but we were not able to enroll enough new patients within the predefined entry period. Amongst patients randomized to receive beta$_2$-agonist only and who subsequently withdrew, baseline lung function was poorer, they were more hyperresponsive and had more symptoms compared with those who remained on-treatment throughout. Patients on beta$_2$-agonist only who dropped out also showed a deterioration in lung function that was more pronounced than in patients who remained on randomized treatment. This makes it likely that differences between the two treatment groups would have been larger if patients who dropped out had remained in the study.

The endpoints taken were considered relevant as indicators of different aspects of disease activity.[28,29] All endpoints showed an improvement over time in patients on beta$_2$-agonist plus inhaled corticosteroid. However, the time needed to reach a plateau differed for the various endpoints: Stabilization of airway caliber and bronchodilator response occurred after 2 months on randomized treatment, whereas this took about 8 months for PEF-variability, reported symptoms and the use of additional beta$_2$-agonist. The only measurement that did not appear to reach a plateau after a median follow-up time of 22 months was histamine responsiveness. The average level reached after such a long period was not within the normal range although about a quarter of the patients did reach values within -2 SD of the mean value for non-asthmatic children. This finding strongly suggests that even after a year of treatment, a further improvement in airway responsiveness occurs. This is likely to be of clinical significance because many studies have shown that, within a group of subjects, levels of bronchial responsiveness are related to the severity of asthma symptoms, although this may not always be the case within subjects.[30,31] Within each group a number of individual

patients did normalize after the median follow-up period of 22 months: 26% of the patients on $beta_2$-agonist plus inhaled corticosteroid reached a PD_{20} within the normal range versus 6% on $beta_2$-agonist only. FEV_1 came within the normal range in 42% of patients on $beta_2$-agonist plus inhaled corticosteroid and in 29% of those on $beta_2$-agonist only.

In our design we chose for a comparison between daily treatment with inhaled $beta_2$-agonist and a combination of inhaled $beta_2$-agonist plus inhaled corticosteroid in order to obtain optimal intervention directed against factors which are predictive for long-term outcome, i.e. airway caliber and airway hyperresponsiveness[29] during the whole study period. Both regimens employed commonly used doses. Recent shorter-term studies suggest that $beta_2$-agonist on demand is preferable to regular beta-agonist because of an increase in asthma symptoms[14] and an accelerated decline in lung function in adult patients on daily beta-agonist treatment[15]. Also Haahtela and coworkers recently found a somewhat faster decline in FEV_1 in patients who were regularly treated with $beta_2$-agonist than in patients who received inhaled corticosteroid, although the difference between the groups was not significant[12]. In our study, patients who were randomized to receive $beta_2$-agonist only and remained in the study showed a decrease in airway caliber which was slight and occurred in the first 6 months; after about 8 months airway caliber stabilized. However, the decrease in airway caliber was not associated with an increase in symptoms or an increase in the use of additional $beta_2$-agonist. The reduction in airway caliber was therefore apparently not of clinical relevance. In the patients on $beta_2$-agonist only who subsequently withdrew airway caliber deteriorated to the same extent whereas their initial FEV_1 before randomization was lower than in the group on $beta_2$-agonist only who remained in the study. It is likely that the main reason for this decrease in airway caliber could be the withdrawal of inhaled corticosteroids before entry, but we cannot exclude that other factors have also played a role. The bronchodilator response remained unchanged in both subgroups, which indicates that tachyphylaxis to $beta_2$-agonists is unlikely. This conforms to the findings of Van Schayck and coworkers.[32]

In a number of studies a deterioration of histamine or methacholine responsiveness was observed during treatment with $beta_2$-agonists for weeks or months.[32-34] However, this was not the case in another study.[35] There was a slight deterioration of airway responsiveness in the patients on $beta_2$-agonist only in the first 4 months of follow-up, but mean histamine responsiveness improved again thereafter. This could well be due to the fact that the most hyperresponsive and symptomatic patients had withdrawn by that time.

In the $beta_2$-agonist only group symptoms were unchanged. Here again we have to

emphasize that only data are presented of the patients who did not withdraw. As the major reason of withdrawal on beta$_2$-agonist only was an increase in symptoms, the on-treatment analyses underestimate the likely difference between treatment groups.

Airway caliber might influence the degree of airway hyperresponsiveness as has been extensively discussed.[30,36,37] Yan and coworkers could not find a relation between these two, while Ramsdale and coworkers found that airway obstruction could explain about 35% of the response to methacholine in asthmatic patients.[36,37] Analysis of individual changes in lung function after bronchial provocation tests did not show a correlation with the degree of BHR.[30] In our patients the improvement in PD_{20} histamine occurred independently of airway caliber after about 2 months of randomized treatment. FEV$_1$ increased mainly during the first two months, while airway hyperresponsiveness diminished steadily during the median follow-up period of 22 months.
Airway responsiveness and PEF variability are both considered to reflect asthma severity. According to some authors[28,38] they are interrelated. We did not find such a relationship in children on beta$_2$-agonist plus corticosteroid: whereas PD_{20}histamine improved steadily over the whole study period, PEF variability decreased in the first 2 months but remained unchanged thereafter. We suggest that PD_{20}histamine and PEF are different indicators of the "twitchiness" of airways.
It remains to be determined how the combined effect of inhaled beta$_2$-agonist plus inhaled corticosteroid is compared to that of inhaled corticosteroid alone. In our study bronchodilation remained to have an effect on FEV$_1$ and PEF in the patients on beta$_2$-agonist plus inhaled corticosteroid. However, Sears and coworkers[14] suggest that even in patients who are on a maintenance treatment with inhaled corticosteroid an on-demand treatment with beta$_2$-agonist is preferable to additional maintenance therapy with these drugs. This issue is the subject of considerable debate and further studies are needed to resolve it.

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Chapter VI

Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide). Can it be reached?

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Submitted.

6.1. Summary

In a previous study we showed that long-term (median follow-up 22 months) treatment with inhaled corticosteroid plus beta$_2$-agonist improves both symptoms, airway caliber and airway responsiveness in children with asthma compared with beta$_2$-agonist alone. We found that on treatment with inhaled corticosteroid plus beta$_2$-agonist airway caliber did not further improve after 4 months, whereas PD_{20} histamine showed gradual improvement without reaching an apparent plateau. In this study we followed the children who received inhaled corticosteroid plus beta$_2$-agonist further up until 28-36 months and considered whether they achieved remission of their asthma. Remission was defined as being symptom free during any 8 month period. Of the 58 children originally randomized to receive salbutamol 0.2 mg plus budesonide 0.2 mg t.i.d. five children withdrew: 3 due to lack of motivation, 1 due to psychological reasons and 1 due to a deterioration of asthma. One patient was hospitalized because of an asthma exacerbation. Airway caliber showed no improvement after 4 months up to 36 months. Geometric mean PD_{20} histamine stabilised after 20 months at 2.1 doubling doses above baseline but at a subnormal level of 80 ug. Symptoms improved during the first 18 months and may be improving further but slowly after 18 months up to 36 months. Thirty-five patients (60%) achieved a period of remission at some time during the 28-36 months of treatment. However, 23 (66%) of these had a relapse. These results suggest that long-term treatment with inhaled corticosteroid improves both clinical signs, airway caliber and airway responsiveness but brings only a minority of the patients into a long-lasting remission.

6.2. Introduction

Asthma is a chronic inflammatory disease[1-4] which is characterized by symptoms of airway obstruction such as cough, wheezing and breathlessness. Currently the most effective drug is inhaled corticosteroid, probably because of antiinflammatory properties[5,6]. Inhaled corticosteroids diminish symptoms, the need for additional beta$_2$-agonists[7-9] and peak expiratory flow (PEF) variability[9]; they improve airway caliber[7-9], and after long-term use they decrease airway responsiveness to histamine and methacholine[7-10]. One study suggests that long-term antiinflammatory treatment may even induce remission[7]. According to international consensus reports, the primary aim of asthma treatment should be the total relief of symptoms [11,12]. It

remains to be determined if normalization of airway responsiveness should also be an aim of treatment, as this is one of the known risk factors for symptomatic asthma in adulthood[13]. In an earlier paper we described results of a double-blind randomized multicenter intervention study in children with moderate asthma in which the effects of treatment either with a beta$_2$-agonist plus an inhaled corticosteroid or with a beta$_2$-agonist alone were compared on airway caliber, bronchodilator response, airway responsiveness to histamine and symptoms[9]. After a median follow-up period of 22 months this study was stopped because of the high rate of withdrawals mainly due to an increase of symptoms in the group of patients receiving beta$_2$-agonist only. At that time a significant improvement in lung function and airway responsiveness and a reduction in symptoms were observed in patients receiving inhaled corticosteroid. However, it was not clear if the full benefits of inhaled corticosteroid had been obtained by this time. Therefore patients on beta$_2$-agonist plus inhaled corticosteroid continued their treatment, being followed up for 28 to 36 months to assess the important question as to whether remission might be reached. We report here the results of this extended follow-up.

6.3. Methods

6.3.1. *Patients*

Fifty-eight children aged 7 to 16 years with moderate asthma were selected from the outpatient clinics of two university children's hospitals and one general children's hospital. All children belonged to the group that had been randomized to treatment with beta$_2$-agonist plus inhaled corticosteroid in the 22-month comparative study[9]. Inclusion criteria were symptomatic asthma, forced expiratory volume in one second (FEV_1) 55-90% of predicted and/or the ratio FEV_1/forced vital capacity (FVC) 50-75%; provocative dose of histamine which causes a 20% fall in FEV_1 (PD_{20} histamine) $\leq$150 μg (more than two standard deviations below the mean value in healthy children[14]). The study was approved by the medical ethics committees of the three centers. All children and their parents gave their informed consent.

6.3.2. *Design*

Details of methods and protocols are described in our previous publication, so we

give only a brief summary here[9]. Clinical endpoints for this study were symptom scores and additional $beta_2$-agonist usage, exacerbations for which prednisolone was prescribed, absence from school because of asthma symptoms and hospitalization. During the two-week period prior to each visit, patients kept diaries of additional $beta_2$-agonist use and recorded whether symptoms of asthma were experienced. In addition they measured their PEF using a mini-Wright peak flow meter at home on three occasions each day: in the morning within half an hour of rising and prior to bronchodilation, 10 minutes after bronchodilation with 0.2 mg salbutamol and prior to bronchodilation in the late afternoon. Each measurement consisted of 3 attempts, the highest value was recorded. The number of days on which symptoms were recorded during the 14-day period prior to a clinic visit was used as a measure of symptom burden. Functional endpoints were airway caliber measured as FEV_1 and airway responsiveness expressed as PD_{20} to histamine. PEF prior to medication both in early morning and late afternoon was also used as a measure of airway caliber and both its day-to-day variability and its within-day variability as additional measures of airway responsiveness. Bronchodilator response was measured both as FEV, 20 minutes after 0.8 mg salbutamol and as PEF 10 minutes after 0.2 mg salbutamol. The coordinating center in Rotterdam collected and checked all data to ensure completeness and to prevent bias due to local procedures.
The study had to be terminated on October 1, 1990 because the financial support stopped at that time. Thus, not all patients could be followed for the period of 3 years originally planned. The range of follow-up was 28-36 months.

6.3.3. *Definition of remission*

We defined remission in terms of symptomatic outcome during any eight-month period preceding a clinic visit. A patient was said to be in remission if he reported no symptoms and used no additional $beta_2$-agonist during any of the two-week periods prior to each of the four clinic visits during the eight-month period, and also took no prednisolone course and had no hospitalization nor absence from school due to asthma during the same period. The choice of an eight-month period is arbitrary but was motivated by the fact that the group behaviour of the study cohort appeared to be stable after 20 months and up until the minimum length of follow-up of 28 months, a period spanning 8 months.

6.3.4. *Statistical methods*

To assess whether FEV_1, PD_{20} and PEF were changing after at least 18 months on treatment, a linear regression line was fitted to each subject's sequence of measurements from the visits at and after 18 months. A t-test was then used to test whether the average rate of change across subjects (the average slope of the individual regressions) was significantly different from zero. To assess whether the rate of reporting of symptoms and of additional $beta_2$-agonist was changing after 18 months, a simple linear regression was fitted to the rates at each visit and the hypothesis that the slope of this regression is zero was tested.
Fisher's exact test, the two-sample t-test or the Wilcoxon test were used to assess for factors that were predictive of remission. For all tests undertaken, p-values are only reported when smaller than 0.2.

6.4. Results

6.4.1. *Baseline characteristics and length of follow-up*

Between October 1987 and April 1989, 58 patients were randomized to receive $beta_2$-agonist plus inhaled corticosteroid in the comparative study[9]. Table 1 shows their baseline characteristics. All patients who had not withdrawn from this treatment continued their medication until October 1991 or until 36 months after randomization if this was earlier. Five patients withdrew from treatment. Three withdrawals occurred during the blinded comparative phase (after 4, 6 and 14 months, respectively), one because of many asthma symptoms, one because of social reasons (lack of motivation) and one because of psychological reasons. Two patients withdrew during the open phase (at 22 and 24 months, respectively), both due to lack of motivation. All the remaining 53 patients completed a minimum of 28 months of treatment with BA+CS; 50 completed 30 months and 34 finished 36 months.

6.4.2. *Clinical signs*

The number of days on which symptoms were recorded during the 14-day period prior to a clinic visit is used as a measure of symptom burden. The number of days

Table 1. **Characteristics at baseline for the whole group and by whether patients were in remission or not at the end of follow-up.**

	Whole group (n = 58)	No remission (n = 38)	Remission (n = 20)	p-value
Sex: no. (%) male	42 (72%)	25 (66%)	17 (85%)	0.22
Age (years): mean (s.d.)	11.0 (1.9)	11.1 (2.0)	10.9 (1.8)	0.77
Prior use of inhaled corticosteroids: no. (%)	29 (50%)	21 (55%)	8 (40%)	0.41
Prior use of cromoglycate: no. (%)	28 (48%)	20 (53%)	8 (40%)	0.42
FEV_1 % predicted: mean (s.d.)	75.7% (10.8%)	75.6 (9.7%)	75.8 (12.8%)	0.95
Post-bronchodilation FEV_1 % predicted: mean (s.d.)	93.6% (11.4%)	93.7% (9.1%)	93.5% (15.2%)	0.95
Morning PEF l/min: mean (s.d.)	287 (73)	291 (78)	280 (59)	0.60
PD_{20} µg histamine: median (quartiles)	21.5 (8, 38)	21 (9,35)	25.5 (8,45)	0.36
Days in two weeks with symptoms: median (quartiles)	4 (1, 9)	6 (2,9)	2.5 (0.4)	0.045

on which symptoms were recorded during the 14-day period prior to a clinic visit is used as a measure of symptom burden. Figure 1 shows the percentages of patients by the number of days affected. Symptom burden decreased over time during the first 18 months. Thereafter, about one-third of patients reported symptoms on at least one day during the two-week period prior to each clinic

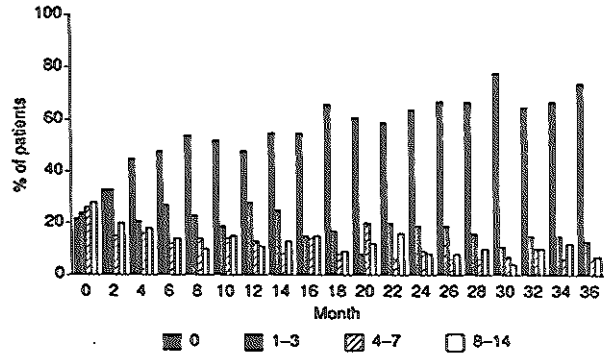

Figure 1. Days with symptoms per two weeks, % of patients.

visit though there was evidence of marginal significance (p=0.06) that this rate may still be decreasing slowly during the period between 18 and 36 months.
At baseline, 50% of patients reported no use of additional beta$_2$-agonist during the 14 days prior to the clinic visit. By 18 months 83% of patients reported no use. There was no further improvement between 18 and 36 months.
Fourteen of the 58 patients (24%) received a total of 26 courses of prednisolone. This represents an average of 0.17 courses per patient-year of treatment. This rate was fairly constant from year to year: 0.16 in the first year, 0.20 in the second and 0.13 in the third.
One patient was hospitalized because of severe asthma symptoms for 7 days after 18 months of treatment.
Twenty-five patients (43%) had absences from school due to asthma. The total days absent was 293 representing 1.9 days per patient-year of treatment with BA+CS. This remained stable from year to year: 1.8 in the first year, 2.0 in the second and 1.5 in the third.

6.4.3. *Airway caliber and bronchodilator response*

Average pre-bronchodilation FEV$_1$ had increased from 76% predicted at baseline to 86% at 4 months and did not improve further over time. Average post-bronchodilator FEV$_1$ showed an increase from 94% at baseline to 97% by 2 months after which it remained stable during the complete follow-up period.
There was an acute effect of treatment in raising PEF by 36.6 l/min between baseline and 2 months. Thereafter, the upward trend in both pre- and post-bronchodilation PEF (averaging 12.3 l/min per year between 18 and 36 months, p=0.03) was similar to that expected due to the children's growth[15]. The average evening PEF (before bronchodilation) showed the same trend, being consistently between the pre- and post-bronchodilation morning levels.

6.4.4. *Airway responsiveness*

The geometric mean PD_{20} histamine increased steadily over time and reached a plateau at a subnormal level (just below 80 ug) between 20 and 24 months (mean increase 2.1 doubling doses (DD))(figure 2).
The average standard deviation in day-to-day variability of the morning PEF before

bronchodilation was reduced by 5.9 l/min within 2 months and stabilized after 8 months at a level of about 20 l/min which was maintained until the end of the study period. Average change in PEF between morning and afternoon measurements declined from 24.7 l/min at baseline to a level of about 18 l/min after 8 months and was maintained thereafter.

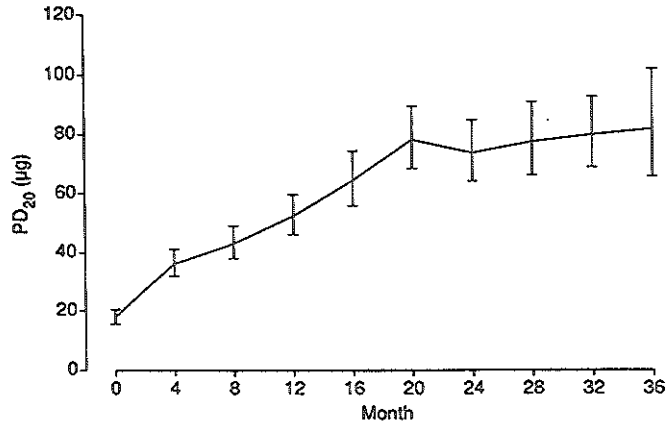

Figure 2. PD_{20} histamine geometric mean (SE).

6.4.5. *Remission*

Of the 58 patients, 35 (60%) experienced at least one period of symptomatic remission. The number of patients in remission increased with time on treatment. For instance, in the first 8 months of the study 7 patients (12%) were in remission; in the eight-month periods prior to the visits at 20, 28 and 36 months, the numbers in remission were 10 (17%), 15 (26%) and 13 (34% of those completing 36 months), respectively. However, the majority of patients achieving remission (23 of 35, 66%) had a relapse during the three years of follow-up.

At the end of the study's follow-up, 20 patients (35%) were in remission. Of these, only 8 also had normal PD_{20} histamine (>150 µg) and FEV_1 (>90% predicted) at the clinic visits during the previous eight months; 3 other patients had one, but not both, levels of PD_{20} histamine or FEV_1 % predicted within the normal range at each visit. Comparison of baseline characteristics for patients in remission at the end of follow-up versus those who were not showed that the former group reported significantly fewer days with symptoms at baseline (Table 1). FEV_1 % predicted and post-bronchodilator response showed no predictive value and there is little difference in average PD_{20} histamine or PEF levels.

6.4.6. *Adverse events*

Twenty patients mentioned side effects. All these side effects were minor (e.g. cough, hoarseness, trembleness) and were never a reason to stop study medication. Increase in height was not different from a healthy reference group which was followed up longitudinally.[16]

6.5. Discussion

This study describes the results of 28-36 months treatment with inhaled corticosteroid plus inhaled beta$_2$-agonist on asthma in children. Average levels of all lung function measurements had stabilized after 20 months of treatment but at levels outside of the normal range. Symptom burden reduced markedly over the first few months of treatment but there may still be a continued slow improvement beyond 18 months as evidenced, in particular, by the increasing numbers achieving symptomatic remission. In general, individual patients improved with treatment: the majority showed a decrease in symptoms, PD_{20} levels were improved in all but four patients and FEV_1 in all but five patients. Also, we did not find any pre-treatment characteristics which identify a subgroup of patients for whom the treatment had no benefit.

This is the first study in children to have considered whether a long-lasting remission from asthma can be induced by drug treatment. Although it has been shown that about half of children will reach a remission spontaneously in puberty or adolescence[13,17-20] (especially amongst those with mild asthma), it is an important question as children with more severe asthma are more likely to suffer from asthma in adulthood.[18-20] We arbitrarily defined a symptomatic remission as a period of 8 months without any symptoms reported. During 28-36 months of treatment with inhaled corticosteroid plus beta$_2$-agonist, 35 patients (60%) achieved a remission, though fewer (20, 35%) were in remission at the end of the study and only about half of these had normal levels of PD_{20} histamine and FEV_1 % predicted at that time. Lower symptom burden at baseline seemed to be the only predictor of patients likely to reach remission; notably measures of lung function had no predictive value. It is unlikely that the patients reaching remission had a spontaneous recovery since they had moderate asthma which required maintenance treatment for a long period before entering the study. Also, comparison of the number of remissions during the comparative phase of the study showed that only 7 patients (12%) of those taking

beta$_2$-agonist alone achieved remission compared with 20 (34%) of those taking corticosteroid plus beta$_2$-agonist. However, our results also showed that two-thirds of patients achieving a symptomatic remission suffered a relapse. Thus, although 28-36 months of treatment with inhaled corticosteroid improves both symptoms and objective measures of lung function, it does not cure asthma. This is underlined by our findings in a subsequent study in which cessation of inhaled corticosteroid from our cohort was followed by a rapid increase in symptoms and a deterioration in lung function (Waalkens et al., personal communication).

Recently Juniper et al.[7] investigated the effect of long-term therapy with inhaled corticosteroid on remission rate in adults. In this double-blind randomized study 16 adult patients with mild asthma received 200 μg budesonide twice daily, 16 others received placebo. The steroid group showed an improvement in symptoms, which was maximal after 9 months on treatment. In 8 patients airway responsiveness reached a plateau after 6 to 12 months on budesonide. Nevertheless, mean PC_{20} methacholine had increased by 2 DD but had not yet stabilised after 12 months on this treatment. Five patients on budesonide (31%) reached a value of PC_{20} methacholine within the normal range (>8 mg/ml). They were among those who became symptom free and no longer needed additional bronchodilator. Our results differ in some respect from those of Juniper et al.[7]. We found that mean PD_{20} histamine stabilized after 20 months; this difference might be explained by the longer duration of treatment and the higher dose of inhaled corticosteroid taken. Findings regarding normalization in airway responsiveness were similar: even after 36 months on inhaled corticosteroid airway responsiveness remained abnormal in the majority of patients.

Haahtela et al.[8] studied 103 newly detected adults in a 22-months study, and randomized them to budesonide 600 μg b.i.d or terbutaline 375 μg b.i.d.. In the patients on budesonide, symptoms and the need for additional bronchodilator decreased within one week after randomization, but did not diminish thereafter. They did not provide data on the rate of patients who became symptom free after such a long treatment period. PC_{15} histamine showed a marked increase within 6 weeks on budesonide and a further increase at a slower rate thereafter. At 16 months a plateau was reached at a mean value of 1.6 DD above baseline. Since Haahtela et al.[8] used a different method to assess airway responsiveness, the results are not fully comparable. However, the shift of PC_{15} (1.6 DD after 1 year) is in the same range as the shift of PC_{20} in the study of Juniper et al.[7] (2 DD after 1 year) and of PD_{20} in our study (2.1 DD after 20 months).

These findings suggest that the underlying inflammatory process is still present

after years on inhaled corticosteroid. Little is known about the effect of inhaled corticosteroid on airway morphology in asthma. The only long-term study is that of Lundgren et al.[21], who found that in 6 adult patients with severe intrinsic asthma after 10 years treatment with 200-1600 μg inhaled corticosteroid per day the number of inflammatory cells had normalised and epithelial damage had decreased although PC_{20} to methacholine was still highly abnormal. The short-term study of Djukanovic et al.[5] showed a clinical improvement together with a significant reduction of inflammatory cells, whereas the extent of mast cell and eosinophil degranulation did not change over time during 6 weeks on 1000-2000 μg beclomethasone dipropionate. Jeffery et al.[22] found both a decrease in the number of mast cells and eosinophils and of eosinophil degranulation to normal levels in bronchial biopsies of patients after 4 weeks on budesonide, whereas the reticular basement membrane remained thickened. Laitinen et al.[9] showed that treatment with 600 μg budesonide during 12 weeks resulted in a decrease in the number of mast cells, eosinophils, neutrophils, lymphocytes and macrophages in the airway epithelium, obtained by bronchial biopsies.

What do these findings imply for clinical practice? It seems that long-term follow-up of airway caliber, bronchodilator response and airway responsiveness are not useful guides for a strategy in the treatment of asthma, once inhaled corticosteroid has been started. Almost all patients will improve, both in symptoms and in objective measurements, independent of the level from where they start. A normalization of airway responsiveness will only occur in a minority of patients. Treatment should therefore be aimed at a symptomatic remission, i.e. normalization in symptoms, without need for additional bronchodilator. This is in agreement with recently published consensus reports on the treatment of asthma, in which a normalisation of airway sensitivity was not stated as an aim to achieve.[11,12] In our study all patients used both inhaled corticosteroid and $beta_2$-agonist on a regular basis. It is uncertain to what extent a normalisation in symptoms can be reached with inhaled corticosteroid as the only regular drug taken. Therefore, new long-term studies in which the effect of regular treatment with inhaled corticosteroid only is compared with inhaled corticosteroid plus $beta_2$-agonist are needed to answer this question.

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Chapter VII

Cessation of long-term treatment with inhaled corticosteroid (budesonide) in children with asthma results in rapid deterioration.

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Submitted.

7.1. Summary

Inhaled corticosteroid has been shown to be effective in the management of asthma. However, there is a lack of studies that assess the effect of cessation after long-term treatment with inhaled corticosteroid. This question was addressed in 28 children with stable asthma, aged 11-18 yr, who had completed 28-36 months of treatment with inhaled corticosteroid (budesonide 200 μg t.i.d.) and inhaled $beta_2$-agonist (salbutamol 200 μg t.i.d.). The children were randomized in a 1:2 ratio in a double-blind study either to continue budesonide (n=8) during a period of 6 months or to decrease the dose of budesonide (n=20) within 2 months, followed by placebo for 4 months. Treatment with salbutamol 600μg daily was continued in both groups. Eight children from the tapering-off group were withdrawn, mainly due to symptoms of asthma, compared with none in the continuous treatment group. Five patients in the tapering off group experienced exacerbations for which prednisolone was given, compared with none in the continuous treatment group. After tapering-off, symptoms of asthma and additional bronchodilator use increased, and both FEV, % predicted and PD_{20}-histamine (provocation dose of histamine causing a 20% fall in FEV_1) rapidly decreased, whereas these all remained unchanged in the group that continued treatment with inhaled corticosteroid. From this study we conclude that long-term treatment with 600μg budesonide daily suppresses underlying mechanisms of asthma, but does not cure the disease.

7.2. Introduction

It is now generally accepted that asthma is a chronic inflammatory disease characterized by epithelial shedding, increased vascular permeability, mucosal edema and an increased number of inflammatory cells in the airway wall.[1] Airway hyperresponsiveness, one of the main features of asthma,[2] is thought to be a consequence of the underlying airway inflammation.[3]

A number of long-term studies (12-22 months) in children and adults with asthma have shown that regular treatment with inhaled corticosteroid leads to a decrease of asthma symptoms, bronchodilator use and the number of asthma exacerbations as well as a reduction in airway responsiveness and an increase in airway caliber.[4-7] After treatment with inhaled corticosteroid the number of eosinophils and mast cells,[8-11] as well as eosinophil degranulation decreased.[9] Recently it has been shown

that a decreased number of inflammatory cells in the airways was related to clinical improvement of asthma, suggesting a direct association between both.[10]
Short-term studies in adults in which inhaled corticosteroid was administered for 4-10 weeks suggest that airway responsiveness and airway caliber deteriorate within weeks after stopping steroid treatment.[12-16] However, one long-term study in adults has shown that reduced airway responsiveness and a diminished need for bronchodilators following 1 year of treatment with budesonide persisted for at least 3 months after abrupt discontinuation.[17]
In this study, we assessed the effect of the cessation of inhaled corticosteroid on airway caliber, airway responsiveness, symptoms and additional bronchodilator use in children with stable asthma, who have been treated for 28-36 months with a daily dose of 600 μg budesonide.

7.3. Methods

7.3.1. *Patients*

All 31 asthmatic children who participated in a long-term multicenter study,[7,18] and who had completed 28-36 months of treatment with daily budesonide (200 μg t.i.d.) and salbutamol (200 μg t.i.d.) on July 1, 1991, were asked to participate. Three patients were unwilling because they wanted to continue their medication. Criteria for entering the long-term intervention study were a forced expiratory volume in one second (FEV_1) between 55-90% of predicted and/or a FEV_1/forced vital capacity (FVC) ratio between 50-75%, as well as the provocative dose of histamine causing a 20% fall in FEV_1 (PD_{20} histamine) less than 150 μg (this being more than two standard deviations below the mean value in healthy children[19]).
Informed consent was obtained from both children and parents. The study was approved by the Medical Ethics Committees of the participating centers.

7.3.2. *Measurements*

Spirometry was carried out at least 8 hours after the last drug administration and was performed at the same time of the day in each individual. FEV_1 was measured according to the recommendations of the European Community for Coal and Steel[20] by water-sealed or dry rolling spirometer or pneumotachograph. The largest value

of 3-5 attempts was recorded. These were sex and height adjusted to give values as % predicted using the reference values of Zapletal.[21]
Airway responsiveness was measured by inhalation of histamine diphosphate in increasing dosages according to a standardized protocol.[22] Histamine was nebulized with a deVilbiss 646 nebulizer and a Rosenthal-French dosimeter. Inhaled doses were doubled in five-minute intervals from 2.5 up to 640 μg as the maximum. The effect was determined by measuring FEV_1 3 minutes after each histamine administration. PD_{20} histamine was calculated using linear interpolation of log-transformed data.
Children kept a diary for 14 consecutive days prior to each visit to record both symptoms and the use of additional $beta_2$-agonist. During the same periods, they also recorded their peak expiratory flow (PEF) using a mini Wright peak flow meter. All children received standard instruction in the use of peakflow meters. PEF was recorded in the morning (immediately after rising) before and 10 minutes after medication, and in the evening (before the evening meal) before taking any treatment, as the best of three performances.
All data were collected and checked by the coordinating center in Rotterdam to ensure completeness and to prevent bias due to local procedures.

7.3.3. *Design*

The study was a multicenter, double-blind, randomized comparison of two parallel treatment groups for a period of 6 months. It was designed to follow immediately after completion of 28-36 months of treatment with salbutamol and budesonide.[7,16] At their final visit in the long-term study the children were randomized either to continue taking 600 μg budesonide daily or to decrease the dose of budesonide gradually. The cessation schedule in the tapering-off group consisted of 100 μg t.i.d. budesonide in the first and 50 μg t.i.d. in the second month; during the following 4 months a placebo was administered. Treatment with salbutamol 600 μg daily was continued in both groups. When needed, up to four 200 μg doses of fenoterol inhaletten, a dry powder device, were allowed per day.
Randomization was performed by calling an independent telephone office with stratification by age, sex and participating center using the minimization procedure.[23] Because of stability in symptoms, FEV_1%pred and PD_{20} achieved during long-term treatment with budesonide,[7] and because more withdrawals were expected to occur in the tapering-off group, we aimed to allocate twice as many

patients to the tapering-off group as to the continuous treatment group. It has been shown that the power of a study is only marginally reduced by randomization in a 2:1 ratio.[23]

Clinical endpoints were symptoms, the use of additional $beta_2$-agonist, exacerbations for which prednisolone was prescribed and absence from school and hospitalization due to asthma. Functional endpoints were airway caliber (FEV,%pred and PEF) and the level of airway responsiveness (PD_{20} histamine and day to day PEF variation). All measurements were performed in a clinically stable period. When an exacerbation occurred, a short course of prednisolone was prescribed (starting with 30 mg on the first day and diminishing to 0 mg in one week according to a scheme that depended on body weight). If prednisolone had been taken within 4 weeks of a scheduled study visit to the clinic, this visit was postponed until a 4-week period without oral steroid had elapsed. Children visited the outpatient department after 1,2,3,4, and 6 months.

7.3.4. *Statistical methods*

Numbers of withdrawals, patients reporting symptoms and additional $beta_2$-agonist use were compared using Fisher's exact test. Repeated measures of the continuous covariates (FEV_1, PD_{20} and PEF) were analysed using an area under the curve summary statistic method.[24] The area under the curve is the area bounded above by a line joining an individual's successive measurements and bounded below by their baseline level. These areas were then compared between treatment groups using t-tests. To improve precision in ascertaining baseline level, the mean of measurements taken at randomization in this study and those taken during the 6 months previous to randomization was used. During the latter period, all patients received the combination of salbutamol plus budesonide and the group behaviour was stable over this period. Measuring the area relative to each individual's baseline level adjusts for differences in the average baseline level between treatment groups. All p-values are for two-sided tests of significance.

7.4. Results

7.4.1. *Baseline characteristics*

Table 1 shows baseline characteristics of the 28 subjects randomized by treatment group. Age ranged between 11 and 18 years. Because of the small group sizes, there are some differences between the two groups, though these are consistent with what is expected due to random variability.

7.4.2. *Withdrawals*

Eight patients withdrew from their assigned treatment. All were in the tapering-off group; 2 after 2 months, 1 after 3 months and 5 after 4 months. Seven withdrawals were attributable to asthma symptoms and 1 to lack of motivation. The difference in rate of withdrawals between both groups was of marginal significance ($p=0.063$). Exclusion of these children from the analyses might have introduced bias; therefore they were included in the analyses up to the visit at which they were withdrawn.

7.4.3. *Clinical signs*

Of the 20 patients in the tapering-off group, 19 (95%) reported symptoms during the two-week period prior to at least one of the clinic visits following randomization. The only patient not to report symptoms was the patient who withdrew after 2 months due to lack of motivation. This compares with just 2 (25%) patients in the continuous treatment group; this difference is highly significant ($p<0.001$). The median number of days out of 14 on which symptoms were recorded in the tapering-off group tended to increase with time: 1 at one month; 1, 2, 0 at months 2, 3 and 4, respectively; and 2 at 6 months. In the continuous treatment group the median number of days with symptoms remained 0 at all time points.
Similar results were obtained for the number of patients using additional $beta_2$-agonist during the study period: 16 (80%) in the tapering-off group compared with 1 (13%) in the continuous treatment group ($p=0.002$). Four patients in the tapering-off group required one course of prednisolone and 1 patient 2 because of a severe exacerbation compared with none in the continuous treatment group ($p=0.28$). In the tapering-off group 1 child was absent from school for 10 days because of

Table 1. **Baseline characteristics by treatment group**

	CONTINUOUS (n=8)	TAPERING-OFF (n=20)
Sex: no. (%) male	6 (75%)	17 (85%)
Age in years: mean (sd)	13.6 (1.6)	14.6 (2.0)
Symptoms: no. (%) reporting none	6 (75%)	14 (82%)*
Additional beta$_2$-agonist use: no. (%) reporting none	7 (88%)	14 (82%)*

	pretreatment#	baseline@	pretreatment	baseline
FEV_1 % predicted: mean (sd)	72.0 (14.0)	79.8 (14.8)	78.3 (10.6)	89.6 (10.4)
PD_{20} histamine: median (quartiles) µg	9 (4,34)	87 (46,138)	21 (11,40)	116 (38,187)
PEF: morning before bronchodilation l/min mean (sd)	273 (53)	407 (131)	315 (77)	422 (76)
PEF: morning after bronchodilation l/min mean (sd)	331 (58)	439 (129)	356 (110)	452 (78)
PEF: afternoon before bronchodilation l/min mean (sd)	307 (60)	437 (118)	346 (98)	438 (83)
PEF: day-to-day variability for morning PEF before bronchodilation l/min mean (sd)	27.8 (15.3)	20.9 (9.3)	29.0 (14.0)	24.8 (24.9)

* = only 17 patients completed diaries at baseline

\# pretreatment = prior to the 28-36 months of treatment with inhaled corticosteroid and inhaled beta$_2$-agonist

@ baseline = entry into this study; FEV_1 % predicted, PD_{20} histamine, PEF and PEF variability values are averaged over a 6 months' period from visits prior to randomization

asthma symptoms. No such absences occurred in the continuous treatment group. None of the children was hospitalized.

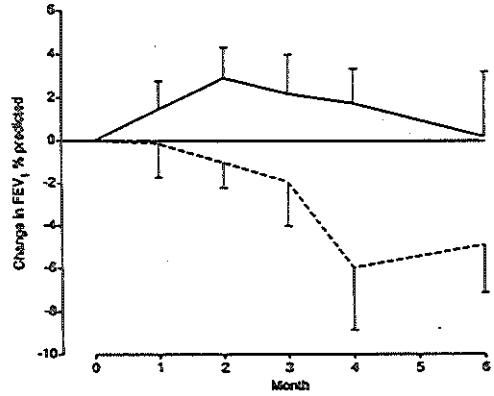

Figure 1. Mean change from baseline of FEV_1 % predicted (SEM) during continuous treatment with budesonide and salbutamol (solid line) or cessation of budesonide and continuous salbutamol (dashed line).

7.4.4. *Airway caliber*

The mean change in FEV_1%predicted from baseline by time is shown in Figure 1. The difference between the two groups is of marginal significance (p=0.075). The average change amongst patients completing the 6 months period was a decline of 4.9% predicted in the tapering-off group compared to a gain of 0.2% predicted in the continuous treatment group. The level observed in the latter was not significantly different from that found before starting the 28-36 months of treatment (p=0.31).

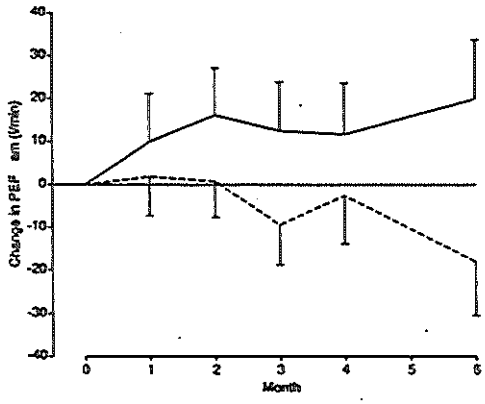

Figure 2. Mean change from baseline of morning PEF (SEM) before bronchodilation during continuous treatment with budesonide + salbutamol (solid line) or cessation of budesonide + continuous salbutamol (dashed line).

For PEF a similar pattern of changes by treatment group was seen for both the morning (Figure 2) and afternoon measurements before bronchodilation. Both showed average increases by 6 months in the tapering-off group and declines in the continuous treatment group. However the difference between the two groups is not statistically significant for either time of day (p=0.27 for the morning PEF, p=0.61 for the afternoon PEF). The PEF after bronchodilation in the morning showed a similar insignificant difference between treatment groups (p=0.57).

7.4.5. *Airway responsiveness*

The results for the PD_{20} histamine are shown in figure 3. The difference between the two treatment groups is statistically significant (p=0.043) with patients on tapering-off showing an average decline of 1.61 doubling doses between baseline and 6 months compared with 0.34 doubling doses after continuous treatment. In the tapering-off group PD_{20} histamine at the end of the 6 months period did not differ significantly from the level prior to the 28-36 months of treatment with budesonide (p= 0.16).

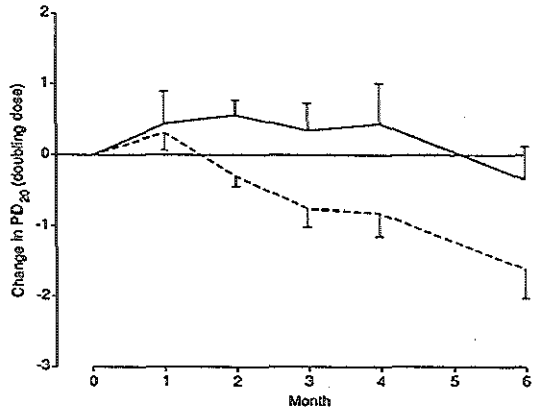

Figure 3. Mean change from baseline of $PD_{20}FEV_1$ histamine (SEM) during continuous treatment with budesonide and salbutamol (solid line) or cessation of budesonide and continuous salbutamol (dashed line).

For the day-to-day variability in PEF there were no significant differences between treatment groups though the results were consistent with the other findings.

7.5. Discussion

This is the first randomized controlled study of the effects of stopping inhaled corticosteroids following long-term (28-36 months) treatment in children with asthma. Our results show that there is a rapid increase in symptoms, additional bronchodilator use and airway responsiveness and a decrease in airway caliber after cessation of inhaled corticosteroid.

The selection of the patients in this study needs some consideration. Of the 58

patients who originally started treatment with inhaled corticosteroid plus inhaled beta$_2$-agonist, 53 completed the study with a follow-up of between 28-36 months. Five children withdrew from treatment before the study closed: only 1 was due to asthma symptoms; the other 4 were due to lack of motivation.[18] The 31 children who had finished at least 28 months of follow-up on July 1, 1991 were asked to participate and most of them (28) agreed. They can therefore be considered to be representative of all children on inhaled corticosteroid and inhaled beta$_2$-agonist from the beginning.

Inhaled corticosteroid has been shown to be effective in the management of asthma. However, Kraan et al.[12] found that airway caliber and airway responsiveness returned to pretreatment values within 2 weeks after abrupt discontinuation following 8 weeks of treatment with 800μg budesonide. Vathenen et al.[13] showed that both FEV$_1$ and PD$_{20}$ declined and PD$_{20}$ returned to pretreatment values within 1 week of discontinuation following 6 weeks treatment with 800 μg budesonide daily. Similar findings were shown in a study by Bel et al..[14] They treated adults with 800μg budesonide daily for 4 weeks and found that both PC$_{20}$ and FEV$_1$ decreased, and that the maximal degree of airway narrowing to methacholine increased to pretreatment levels 2 weeks after cessation. Recently, Magnussen et al.[25] found a decrease in FEV$_1$ % predicted and PC$_{20}$ in adults with stable asthma within 6 weeks of cessation after a treatment period for at least 6 months with 2000μg beclomethasone daily. These results are in contrast with the findings of a study in mild asthmatic adults by Juniper et al.[17] who discontinued treatment acutely after 1 year on 400μg budesonide. They found that the improvement in airway responsiveness and the reduced need for bronchodilator persisted for more than 3 months, although symptoms of asthma tended to recur within 3 months. Furthermore, they found no decrease in lung function, which was not surprising since FEV$_1$ % predicted before treatment with budesonide was almost normal (90%).[4,5] Our study does not confirm the results of Juniper et al..[17] This might be explained in part by differences in patient characteristics and study design. We investigated children with moderately severe asthma who had been treated with budesonide for 28-36 months, in whom symptoms and additional use of bronchodilator had diminished, airway caliber and airway responsiveness had improved and had become stable.[7] Furthermore, we gradually reduced and stopped budesonide and observed the effect over a longer period.

The primary objective of treatment of asthma is to achieve and maintain control of symptoms, and to prevent severe exacerbations.[26,27] The children in the present study approached that situation; they were stable for symptoms, airway caliber and

airway responsiveness for at least 6 months.[16] Both children and parents raised the question as to whether maintenance medication with inhaled corticosteroid could be diminished or stopped. Our results suggest that the improvement after long-term treatment with inhaled corticosteroid on symptoms, airway caliber and airway responsiveness will rapidly disappear after discontinuation. Children who were in a complete remission, defined as being symptom free and having a normal PD_{20} histamine ($PD_{20} \geq 150 \mu g$) during the 6 months prior to entry into this study, appeared to decline as rapidly as those who had not normalized on inhaled steroids. This confirms the findings in the previous treatment period[7,18] that the effect of 600μg inhaled corticosteroid daily for 28-36 months in children with moderate asthma, is only restricted to the treatment period and that inhaled corticosteroid does not cure asthma.

A critical question that emerges is: what is the risk benefit ratio of long-term treatment with inhaled corticosteroid in children? It has been shown that systemic side effects of inhaled corticosteroid, such as suppresion of the hypothalamic-pituitary-adrenal axis and growth retardation may occur.[28] Recently it has been shown that long-term treatment with 400-600 μg budesonide does not inhibit growth in asthmatic adolescents (PJFM Merkus, personal communication). A review of several studies suggests that clinically important side effects are rare with doses of 800 μg/day or less.[29] As the effect of inhaled corticosteroid is dose related,[30,31] long-term treatment with higher doses might lead to more patients coming into remission, and might maintain remission for a longer period after cessation. However, the risk of side effects of inhaled corticosteroid is increased with doses over 600-800μg daily,[28,29] which contraindicates a higher dose than we administered.

The results of our study do not imply that treatment with inhaled corticosteroid should be maintained permanently in children with moderate asthma, since it has been shown that asthma can be outgrown spontaneously in some patients.[32,33] Furthermore in adults with stable asthma it has been shown that PD_{20}, FEV_1 and PEF remained at similar levels after reducing the dose of inhaled corticosteroid.[31] Thus, once control of asthma is sustained for several months, a careful reduction in therapy might be considered. One should aim to identify the minimum therapy required to maintain control, i.e. a situation with minimal symptoms, no exacerbations, and minimal use of extra bronchodilator, a diurnal PEF variation < 20%, and a (near) normal PEF.[27]

We conclude that treatment with inhaled corticosteroid does not cure asthma and that its therapeutic effect disappears rapidly after discontinuation.

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Chapter VIII

Summary and conclusions

8.1 Summary

The subject of this thesis is the effect of long-term pharmaceutical intervention on childhood asthma. The central part of it consists of a long-term study performed in 3 centers for pediatric respiratory disease. This study was sponsored by the Dutch government as part of the so-called "Stimulation Program", a program which supports clinical research in selected areas with a high burden of costs.
The principles underlying the study were

1) Asthma is a chronic inflammatory airway disease for which maintenance treatment is needed in many patients.
2) Childhood asthma often remains into adulthood and may ultimately develop into chronic obstructive lung disease.
3) The hypothesis that long-term outcome of childhood asthma can be modified by drug treatment.
4) Treatment that suppresses inflammation, possibly with bronchodilation, is the therapy of choice.

Chapter I gives a general introduction. Asthma is a disease with a genetic predisposition. The expression of symptoms depends on exposure to allergens, respiratory viruses and other triggers like exercise, cigarette smoke, other irritants and air pollutants. Asthma is characterized by airway wall inflammation, increased microvascular permeability, mucosal edema and epithelial damage. Important inflammatory cells are activated eosinophils, mastcells, macrophages and T-lymphocytes. In children asthma symptoms are cough, wheeziness, breathlessness and sputum production. Nocturnal dyspnea and exercise-induced breathlessness are commonly seen. Bronchoconstriction is intermittently or permanently present. Another characteristic of asthma is airway hyperresponsiveness (AHR), i.e. an increased tendency of airways to constrict after exposure to a number of pharmacological and physical stimuli. This can be seen as an increased sensitivity of airways to contractile stimuli and an increased maximal response plateau. Airway inflammation is an important determinant in AHR but by no means the only one. Although symptoms and AHR appear to be related in studies on groups of patients, this is not necessarily true within individuals. Several studies have shown that about 50% of children with asthma are asymptomatic in adult life. Both airway caliber and the degree of AHR (sensitivity) in children predict the likelihood of having symptoms in adulthood.
In many patients with asthma drug treatment cannot be avoided. The effects of the currently used asthma drugs are summarized.

In the last part of chapter I the background, the organisation and the aims of the long-term study are outlined, with special attention to standardization of methods.

Chapter II gives a literature review on the effect of asthma drugs on airway responsiveness to histamine and methacholine. Short-acting $beta_2$-agonists give acute protection which lasts only for a couple of hours. Long-term administration does not improve AHR. Long-acting $beta_2$-agonists have an acute protective effect which lasts for at least 24 hours. No data are available on their effect on AHR after long-term administration. Other bronchodilators such as anticholinergic drugs and methylxanthines exert either no protection or only a small acute short-lasting protection, but do not change AHR when administered for weeks or months. The same is true for H_1 antihistamines.

Results of treatment with disodium cromoglycate (DSCG), which also has anti-inflammatory properties, are disappointing. DSCG protects against the transient increase in AHR after contact with allergen but offers no acute protection against histamine or methacholine challenge. Long-term administration of DSCG does not modulate AHR. The limited data on a related drug nedocromil sodium (NSO) are not conclusive on its effect on AHR.

Treatment with inhaled corticosteroids, which have an anti-inflammatory action, results in a modest decrease in AHR. Two studies suggest that this effect disappears within months of cessation though one other study did not find a change within 3 months of stopping inhaled corticosteroid. Further studies are needed to establish the influence of dose and duration of treatment in relation to the severity of asthma.

In **Chapter III** results of a study on the acute effect of a single dose inhaled corticosteroid on methacholine sensitivity are presented. In this double-blind placebo-controlled cross-over study 0.8 mg budesonide caused a marginal decrease in methacholine responsiveness compared to placebo 5 hours after treatment though this was not statistically significant. From this and other studies it is unlikely that measurement of airway responsiveness is influenced significantly by the last dose of inhaled corticosteroid taken prior to the test. It was concluded that measurements of histamine or methacholine sensitivity during long-term intervention studies with inhaled corticosteroid are reliable as indicators of airway twitchiness.

Chapter IV contains the results of a six-month study in 19 children with asthma in whom the effect of 0.2 mg budesonide t.i.d. was compared with that of 0.5 mg terbutaline t.i.d. on airway caliber (forced expiratory volume in 1 s, FEV_1), airway responsiveness (PD_{20} methacholine) and symptoms. Only mildly asthmatic children were included in this study: they had an $FEV_1 \geq 80\%$ of predicted and an increase in FEV_1 after 0.5 mg terbutaline <20%. FEV_1 increased slightly in the patients on

budesonide and did not change on terbutaline. Mean PD_{20} methacholine increased by 1.5 doubling doses after 3 months in the budesonide group and stabilized thereafter, whereas it decreased by 0.8 doubling dose in the terbutaline group. Symptom scores were low and were not different between the two groups. It was concluded that long-term treatment with inhaled corticosteroid but not with $beta_2$-agonist improves both airway caliber and airway responsiveness to methacholine.

In **Chapter V** the first part of the long-term multicenter study is described. 116 children were randomized to receive either 0.2 mg salbutamol plus 0.2 mg budesonide t.i.d. or 0.2 mg salbutamol plus placebo t.i.d.. All children had moderate asthma with an FEV_1 of 55-90% of predicted and/or the ratio FEV_1/forced vital capacity in the range 50-75%, an increase in FEV_1 after 0.2mg salbutamol of $\geq$15% of baseline and a provocative dose of histamine which causes a 20% fall in FEV_1 (PD_{20} histamine) of $\leq$150 μg. Endpoints were symptoms, including symptom scores, use of additional $beta_2$-agonist, temporary prednisolone, absence from school and hospital admission because of asthma, airway caliber expressed as FEV, and peak expiratory flow (PEF), bronchodilator response both as postbronchodilator FEV_1 and as postbronchodilator PEF, and airway responsiveness (sensitivity) expressed as PD_{20} histamine and as between day PEF variation. There was a remarkably high rate of withdrawal amongst children who were treated with $beta_2$-agonist plus placebo (26 out of 58), mainly because of worsening asthma symptoms, compared to only 3 withdrawals out of 58 in the steroid group, of which only one occurred because of symptoms. This was a major reason for stopping this part of the study after a median follow-up period of 22 months. All endpoints improved in the group who received corticosteroid plus $beta_2$-agonist and remained unchanged in the $beta_2$-agonist group. The period after which endpoints had stabilized in the children on inhaled corticosteroid differed for the various endpoints. For both pre- and postbronchodilator FEV_1 and PEF this took 2 months, for PEF variation 8 months, for symptoms about 18 months, whereas PD_{20} histamine did not show a plateau at a median follow-up time of 22 months. The conclusion of this part of the study was that the addition of inhaled corticosteroid to an inhaled $beta_2$-agonist improves both clinical and physiological endpoints and that inhaled corticosteroid has an important place in the long-term therapy of children with asthma.

Chapter VI presents the second part of the long-term study which includes an extended follow-up for 28-36 months of the children on inhaled corticosteroid plus $beta_2$-agonist. In this chapter the further development of endpoints and the remission rate are described. A remission was arbitrarily defined as being symptom free during any 8 months period. Symptoms tended to improve during the whole follow-

up period, the other endpoints except PD_{20} histamine remained stable. PD_{20} histamine showed a plateau after 20 months, although at a subnormal level. Thirty-five patients (60%) achieved a period of remission at some time during the 28-36 months of treatment. However, 23 (66%) of these had a relapse. It was concluded that long-term treatment with inhaled corticosteroid improves both clinical signs, airway caliber and airway responsiveness but brings only a minority of the patients into a long-lasting remission.

In **Chapter VII** the third part of the long-term study is described. In this study the effect of cessation of long-term administration of inhaled corticosteroid on FEV_1, PD_{20} histamine and symptoms was investigated. The first 28 children who had completed a follow-up of at least 28 months on inhaled corticosteroid plus $beta_2$-agonist on July 1, 1991 were allocated in a 1:2 ratio to receive in a double-blind manner either a regimen in which inhaled corticosteroid plus $beta_2$-agonist were continued or a regimen in which inhaled corticosteroid was discontinued after a tapering-off period of 2 months. Symptoms, FEV_1 and PD_{20} were measured monthly for 6 months. In the group in which inhaled corticosteroid was stopped 8 out of 21 patients withdrew because of symptoms, FEV_1 and PD_{20} histamine decreased. In the children who continued treatment no withdrawals occurred, and FEV_1 and PD_{20} histamine remained stable. It was concluded that cessation of long-term administration of inhaled corticosteroid leads to a rapid deterioration of both clinical and physiological endpoints.

8.2 Conclusions

Asthma is now considered as a chronic inflammatory disease. Therefore antiinflammatory drugs have an important place in asthma treatment. Both cromones and corticosteroids have antiinflammatory properties, but corticosteroid is the most potent. Among the corticosteroid drugs the inhaled form is preferable because it is safe in the generally recommended doses. It is the only drug known to diminish AHR. This seems important for long-term therapeutic strategies in asthma as AHR is one of the indicators of disease activity and a predictor for the long-term outcome of childhood asthma. The magnitude of the effect of inhaled corticosteroid on AHR is dose and time related and a single dose does not change AHR significantly.

We showed that all indicators of asthma in children can be modulated with antiinflammatory intervention with inhaled corticosteroid. We learned that the time to reach stabilisation was different for symptoms, airway caliber, airway responsive-

ness to histamine and peak flow variation between days. This implies that these indicators give different information on the disease activity. Only a minority of the children was in a long-lasting complete or symptomatic remission at the end of follow-up on 0.6 mg inhaled corticosteroid. This indicates that even after three years of treatment with inhaled corticosteroid the underlying mechanisms are still present. This was confirmed by the finding that after cessation of inhaled corticosteroid symptoms returned rapidly, and that deterioration of both airway caliber and AHR occurred.

We conclude that daily use of inhaled corticosteroid improves clinical and physiological indices of asthma in children, but does not cure the disease, and that in most patients with moderate to severe asthma very long-term steroid treatment is needed.

8.3 Directions for future research

Theoretically complete suppression of airway inflammation should lead to a decreased bronchoconstriction and hence a decrease in symptoms of asthma. However, our data show that even after 28-36 months on inhaled corticosteroid plus short-acting $beta_2$-agonist some 30% of the patients are not yet symptom free. This indicates that inhaled corticosteroids are insufficiently potent to abolish symptoms in a substantial part of patients with moderately severe asthma. In the light of a recent publication that suggests a harmful effect of maintenance treatment with short-acting $beta_2$-agonists on symptoms of asthma it is important to assess the additional effect of a short-acting $beta_2$-agonist to a moderate dose of inhaled corticosteroid. The same question is valid with regard to long-acting $beta_2$-agonists.

Long-acting $beta_2$-agonists might well be preferable to short-acting $beta_2$-agonists, as they provide a continuous bronchodilation during the whole 24h period.

A minority of children needs more than 0.8 mg inhaled corticosteroid daily to control their asthma and may therefore suffer from unwanted side effects. An alternative for treatment with a high dose of inhaled corticosteroid might be a combination of a lower dose with a long-acting $beta_2$-agonist. Taken this into account the following studies are warranted:

1. Short-acting $beta_2$-agonist plus inhaled corticosteroid versus inhaled corticosteroid only.

2. Long-acting $beta_2$-agonist plus inhaled corticosteroid versus inhaled corticosteroid only.
3. Long-acting $beta_2$-agonist plus inhaled corticosteroid $\leq$800 µg versus high dose (>800 µg) inhaled corticosteroid.

Much has been written about possible side effects of long-term treatment with inhaled corticosteroid in childhood asthma. Doses up to 800 µg daily are safe with regard to growth of height and adrenal function. In adults it has been shown that bone density may decrease under this therapy.[1] No such data are available in children. However, long-term assessment of bone density in children is difficult because of growth and furthermore normal data are lacking.

In the international consensus reports on asthma therapy in children cromoglycate is suggested as the drug of first choice for maintenance treatment in children with moderately severe asthma. Both sodium cromoglycate and nedocromil sodium have antiinflammatory properties, but they do not diminish AHR. The safety of cromones makes these drugs attractive for long-term treatment of children, but based on the lack of effect on AHR, it might well be that the long-term outcome of children treated with cromones is less favorable than of children treated with inhaled corticosteroid. Clinicians know that there is a subgroup of children with asthma who are well controlled on cromoglycate, but it remains to be determined in which type of patients the effect of cromones is similar to that of inhaled corticosteroid. Long-term studies in children with moderately severe asthma are needed in which the effect of a cromone with that of inhaled corticosteroid on both clinical and physiological endpoints is compared. Little is known about the combination of a cromone with an inhaled corticosteroid. Some studies suggest a steroid-sparing effect of DSCG[2-4], whereas more recent studies did not show such an effect[5,6]. More studies in children are warranted.

As inflammation has a central place in asthma it is attractive to look at local effects in the bronchial tree of therapeutic intervention. Bronchial lavage content and bronchial biopsies give important information on the effect of interventions at the cellular level. In relating these findings to symptoms and physiological measurements, processes underlying clinical phenomena are becoming more obvious. Such studies have recently started in adults. They are more difficult to carry out in children. Although we know little about mild and beginning stages of asthma, it remains to be determined whether they are also warranted in children.

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SAMENVATTING

Dit proefschrift beschrijft het effect van langdurige interventie met geneesmiddelen op astma bij kinderen. Het centrale deel bestaat uit een langdurig interventie onderzoek dat is uitgevoerd in 3 centra voor kinderlongziekten. Dit onderzoek werd financieel mogelijk gemaakt door een subsidie van het Ministerie van Onderwijs en Wetenschappen als onderdeel van het zogenaamde "Stimuleringsprogramma", een programma dat klinisch onderzoek stimuleert in een aantal gebieden van de gezondheidszorg die hoge kosten met zich meebrengen.
Uitgangspunten voor dit onderzoek waren:

1) Astma is een chronische ontstekingsziekte van de luchtwegen waarvoor veel patiënten een onderhoudsbehandeling nodig hebben.
2) Astma op de kinderleeftijd mondt vaak uit in astma op volwassen leeftijd, en een aantal patiënten ontwikkelt uiteindelijk een chronisch obstructieve longziekte.
3) De hypothese dat het lange-termijnsbeloop van astma op de kinderleeftijd in gunstige zin kan worden gewijzigd door behandeling met medicijnen.
4) Behandeling met ontstekingsremmende middelen, mogelijk samen met bronchusverwijdende middelen, is de eerste keus.

Hoofdstuk I geeft een algemene inleiding. Astma is een ziekte met een erfelijke predispositie. De expressie van symptomen hangt af van blootstelling aan allergenen, luchtwegvirussen en andere uitlokkende factoren zoals inspanning, sigaretterook, andere prikkelende stoffen en luchtverontreiniging. Astma wordt gekenmerkt door ontsteking van de luchtwegwand, een toegenomen doorlaatbaarheid van de kleine bloedvaten in de luchtwegwand, oedeem van de mucosa en epitheelbeschadiging. Belangrijke ontstekingscellen zijn eosinofiele granulocyten, mestcellen, macrofagen en T-lymfocyten. Symptomen van astma op de kinderleeftijd zijn hoesten, piepen, kortademigheid en sputumproductie. Nachtelijke kortademigheid en inspanningsbenauwdheid treden vaak op. Een ander kenmerk van astma is bronchiale hyperreactiviteit, d.w.z. een versterkte neiging van luchtwegen om te vernauwen na blootstelling aan een aantal farmacologische en fysische prikkels. Dit kan zich uiten als een verhoogde

gevoeligheid van luchtwegen voor deze prikkels (sensitiviteit) en als een verhoogd maximaal respons plateau. Ontsteking van de luchtwegen is een belangrijke factor voor bronchiale hyperreactiviteit maar niet de enige. Hoewel symptomen en bronchiale hyperreactiveit bij onderzoek van groepen patiënten met elkaar correleren is dit niet zonder meer waar voor individuen. Verschillende onderzoekingen hebben aangetoond dat ongeveer 50% van de kinderen met astma symptoomvrij is op de volwassen leeftijd. Zowel luchtwegdiameter als de mate van luchtweg prikkelbaarheid (gevoeligheid) voorspellen de kans op het hebben van symptomen op de volwassen leeftijd.
Veel patiënten hebben een behandeling met medicijnen nodig. De effecten van de gangbare astmamedicijnen worden samengevat.
In het laatste gedeelte van hoofdstuk I worden de achtergronden, organisatie en doeleinden van het langdurige onderzoek besproken, met speciale aandacht voor standaardisatie methoden.
Hoofdstuk II geeft een literatuur overzicht van het effect van astma medicamenten op luchtweggevoeligheid voor histamine en methacholine. Kortwerkende $beta_2$-mimetica geven een acute bescherming die slechts enkele uren duurt. Langdurige toediening verbetert de luchtweggevoeligheid niet. Langwerkende $beta_2$-mimetica hebben een acuut beschermend effect dat tenminste 24 uur aanhoudt. Gegevens over hun effect na langdurige toediening ontbreken. Andere bronchusverwijdende middelen zoals anticholinergica en methylxanthines hebben of geen of slechts een gering kortdurend beschermend effect, maar veranderen de luchtweggevoeligheid niet na toediening gedurende weken tot maanden. Hetzelfde geldt voor H_1 antihistamines.
Resultaten van behandeling met disodium cromoglicaat (DSCG), dat ook ontstekingsremmende eigenschappen heeft, zijn teleurstellend. DSCG beschermt tegen de voorbijgaande stijging in luchtweggevoeligheid na expositie aan een allergeen maar geeft geen acute bescherming tegen histamine of methacholine. Langdurige toediening van DSCG verandert de luchtweggevoeligheid niet. De beperkte gegevens over een verwant medicament nedocromil sodium (NSO) geven geen uitsluitsel over het effect ervan op luchtweggevoeligheid.
Behandeling met inhalatie corticosteroïden, die ontstekingsremmend werken, leidt tot een matige afname van de luchtweggevoeligheid. Twee onderzoeken suggereren dat dit effect binnen enkele maanden na stoppen verdwijnt hoewel in een ander onderzoek geen verandering werd vastgesteld binnen 3 maanden na stoppen van inhalatie corticosteroïd. Er is meer onderzoek nodig naar het effect van dosis en duur van behandeling in relatie tot de ernst van het astma.

In **Hoofdstuk III** worden de resultaten gepresenteerd van een onderzoek naar het acuut effect van een eenmalige dosis inhalatie corticosteroïd op de methacholine gevoeligheid. In dit dubbelblind placebo-gecontroleerd cross-over onderzoek bewerkstelligde 0.8 mg budesonide vergeleken met placebo een marginale daling in de methacholine gevoeligheid 5 uur na toediening ofschoon het verschil niet statistisch significant was. Uit dit en ander onderzoek lijkt het onwaarschijnlijk dat de meting van luchtweggevoeligheid significant wordt beïnvloed door de laatste dosis inhalatie corticosteroïd die werd genomen vóór de test. Er werd geconcludeerd dat histamine- en methacholine gevoeligheid tijdens langdurige interventie geschikt zijn als indicator voor luchtweg instabiliteit.

Hoofdstuk IV bevat de resultaten van een onderzoek gedurende 6 maanden bij 19 kinderen met astma bij wie het effect van 0.2 mg budesonide 3dd werd vergeleken met dat van 0.5 mg terbutaline 3dd op luchtwegdiameter (forced expiratory flow in 1 s, FEV_1), luchtweggevoeligheid (PD_{20} methacholine) en symptomen. Alleen kinderen met mild astma werden in dit onderzoek opgenomen: ze hadden een FEV_1 $\geq$80% van de voorspelde waarde en een toename van FEV_1 na 0.5 mg terbutaline van $\leq$20%. Het FEV_1 nam iets toe bij de patiënten die budesonide kregen en veranderde niet onder terbutaline. De gemiddelde PD_{20} methacholine nam toe met 1.5 verdubbelingsdosis in de budesonide groep, terwijl deze met 0.8 verdubbelingsdosis daalde in de terbutaline groep. De symptoomscores waren laag en verschilden niet tussen de twee groepen. Er werd geconcludeerd dat langdurige behandeling met inhalatie corticosteroïd maar niet met een kortwerkend $beta_2$-mimeticum zowel de luchtwegdiameter als de luchtweggevoeligheid voor methacholine verbetert.

In **Hoofdstuk V** wordt het eerste deel van het langdurig multicentrisch onderzoek beschreven. 116 kinderen kregen volgens toeval 0.2 mg salbutamol plus 0.2 mg budesonide 3dd of 0.2 mg salbutamol plus placebo 3dd. Alle kinderen hadden matig astma met een FEV_1 van 55-90% van de voorspelde waarde en/of een ratio FEV_1/geforceerde vitale capaciteit van 50-75%, een toename van FEV_1 na 0.2 mg salbutamol $\geq$15% van de uitgangswaarde en een provocatie dosis histamine die 20% daling van FEV_1 veroorzaakt (PD_{20} histamine) van $\leq$150 µg. Eindpunten waren symptomen, bestaande uit symptoomscores, gebruik van extra $beta_2$-mimetica en tijdelijk prednisolon, schoolverzuim en ziekenhuisopname i.v.m. astma, luchtwegdiameter uitgedrukt als FEV_1 en peak expiratory flow (PEF), respons op bronchusverwijder zowel als FEV_1 en als PEF na bronchusverwijding, en luchtweg prikkelbaarheid (gevoeligheid) uitgedrukt als PD_{20} histamine en als PEF variatie tussen dagen. Er was een opvallend hoog aantal uitvallers in de groep kinderen

die met beta$_2$-mimeticum plus placebo werden behandeld (26 van de 58), voornamelijk vanwege een toename van astma symptomen, vergeleken met slechts 3 uitvallers uit een groep van 58 die corticosteroïd kreeg, en van wie slechts 1 uitviel i.v.m symptomen. Dit was de belangrijkste reden om dit gedeelte van het onderzoek te stoppen na een mediane studieduur van 22 maanden. Alle eindpunten verbeterden in de groep die corticosteroïd plus beta$_2$-mimeticum ontving en bleven onveranderd in de beta$_2$-mimeticumgroep. De periode die verstreek voordat eindpunten gestabiliseerd waren in de groep die corticosteroïd kreeg verschilde voor de verschillende eindpunten. Voor FEV$_1$ en PEF voor en na bronchusverwijder duurde dit 2 maanden, voor PEF variatie 8 maanden, voor symptomen ongeveer 18 maanden, terwijl PD_{20} histamine nog geen plateau had bereikt na een mediane studieduur van 22 maanden. De conclusie van dit gedeelte van het onderzoek was dat de toevoeging van inhalatie corticosteroïd aan een inhalatie beta$_2$-mimeticum zowel klinische als fysiologische eindpunten verbetert en dat inhalatie corticosteroïd een belangrijke plaats heeft in de langdurige behandeling van kinderen met astma.

Hoofdstuk VI beschijft het tweede deel van het langdurige onderzoek waarin de kinderen die met inhalatie corticosteroïd plus beta$_2$-mimieticum werden behandeld gedurende 28-36 maanden werden vervolgd. In dit hoofdstuk worden het verdere verloop van de eindpunten en het voorkomen van remissie beschreven. Een remissie werd arbitrair gedefinieerd als een symptoomvrije periode gedurende 8 maanden tijdens follow-up. Symptomen toonden een lichte neiging tot verbetering gedurende de gehele follow-up, de overige eindpunten behalve PD_{20} histamine bleven stabiel. PD_{20} histamine toonde een plateau vanaf 20 maanden, hoewel op een subnormaal niveau. Vijf en dertig patiënten (60%) bereikten een periode van remissie tijdens de 28-36 maanden onderzoeksperiode, maar bij 23 van hen (66%) werd deze gevolgd door een relapse. Er werd geconcludeerd dat langdurige behandeling met inhalatie corticosteroïd zowel klinische eindpunten als luchtwegdiameter en luchtweg prikkelbaarheid verbetert maar slechts een minderheid van de patiënten in een langdurige remissie brengt.

In **Hoofdstuk VII** wordt het derde deel beschreven. In dit onderzoek werd het effect van het stoppen van inhalatie corticosteroïd na langdurige behandeling op FEV_1, PD_{20} histamine en symptomen bestudeerd. De eerste 28 kinderen die op 1 juli 1991 een studieduur van tenminste 28 maanden met inhalatie corticosteroïd plus beta$_2$-mimeticum hadden werden in een 1:2 verhouding dubbelblind gerandomiseerd voor continuering van deze behandeling of voor geleidelijk verminderen gedurende 2 maanden en daarna stoppen van uitsluitend het inhalatie

corticosteroïd. Symptomen, FEV_1 en PD_{20} werden iedere maand gemeten gedurende 6 maanden. In de groep waarin inhalatie corticosteroïd werd gestaakt vielen 8 van de 21 patiënten uit vanwege symptomen, FEV_1 en PD_{20} namen af. In de groep waar corticosteroïd werd gecontinueerd trad geen uitval op en FEV_1 en PD_{20} histamine bleven stabiel. Er werd geconcludeerd dat het stoppen van inhalatie corticosteroïd na langdurig gebruik leidt tot een snelle verslechtering van zowel klinische als fysiologische eindpunten.

Conclusies

Astma is een chronische ontstekingsziekte. Derhalve hebben ontstekingsremmende geneesmiddelen een belangrijke plaats in de behandeling van astma. Zowel cromonen als corticosteroïden hebben ontstekingsremmende eigenschappen, maar corticosteroïden zijn het meest potent. Van de verschillende toedieningsvormen corticosteroïd heeft de inhalatievorm de voorkeur omdat deze veilig is in de gebruikelijke doseringen. Corticosteroïd is het enige medicament dat luchtweggevoeligheid vermindert. Dit lijkt belangrijk voor lange-termijn strategie bij de behandeling van astma, aangezien luchtweggevoeligheid een van de indicatoren van ziekte-activiteit is en een voorspellende waarde heeft voor het lange-termijns verloop bij kinderen. De sterkte van het effect van inhalatie corticosteroïd op luchtweggevoeligheid is afhankelijk van de dosis en de duur van behandeling, en een eenmalige dosering verandert de luchtweggevoeligheid niet significant.
Wij toonden aan dat alle indicatoren van astma bij kinderen gemoduleerd kunnen worden met ontstekingsremmende interventie met inhalatie corticosteroïd. De tijd die nodig is om te stabiliseren is verschillend voor symptomen, luchtwegdiameter, luchtweggevoeligheid voor histamine en piekstroom variatie tussen dagen. Dit betekent dat deze indicatoren verschillende informatie over de ziekte-activiteit verschaffen. Slechts een minderheid van de kinderen was in een langdurige complete of symptomatische remissie aan het eind van de follow-up. Dit betekent dat zelfs na 2.5-3 jaar behandeling met inhalatie corticosteroïd de onderliggende mechanismen nog actief zijn. Dit werd bevestigd door de bevinding dat na stoppen van inhalatie corticosteroïd de symptomen snel terugkeerden, en dat een verslechtering optrad van zowel luchtwegdiameter als luchtweggevoeligheid.
We concludeerden dat het dagelijks gebruik van inhalatie corticosteroïd klinische en fysiologische indicatoren van astma bij kinderen verbetert, maar de ziekte niet

geneest, en dat bij de meeste patiënten met matig tot ernstig astma zeer langdurige behandeling met inhalatie corticosteroïden noodzakelijk lijkt te zijn.

Richtlijnen voor toekomstig onderzoek

Theoretisch zou volledige onderdrukking van luchtwegontsteking moeten leiden tot afname van de luchtwegvernauwing en dientengevolge afname van symptomen van astma. Onze gegevens laten echter zien dat zelfs na 28-36 maanden behandeling met inhalatie corticosteroïd plus kortwerkend $beta_2$-mimeticum ongeveer 30% van de patiënten nog niet symptoomvrij is. Dit geeft aan dat inhalatie corticosteroïden onvoldoende in staat zijn de symptomen te doen verdwijnen in een aanzienlijk deel van de patiënten met matig ernstig astma. In het kader van een recente publicatie die een nadelig effect van een onderhoudsbehandeling met kortwerkende $beta_2$-mimetica op symptomen van astma suggereert is het belangrijk na te gaan wat het additionele effect van een kortwerkend $beta_2$-mimeticum naast een matige dosis inhalatie corticosteroïd is. Hetzelfde geldt voor de langwerkende $beta_2$-mimetica. Langwerkende $beta_2$-mimetica zouden wel eens de voorkeur kunnen hebben boven kortwerkende $beta_2$-mimetica, aangezien bij een toedieningsfrequentie van 2dd een continue luchtwegverwijding gedurende 24 uur bestaat.

Een minderheid van de kinderen heeft meer dan 0.8 mg inhalatie corticosteroïd per dag nodig om hun astma onder controle te houden en kan daarom wellicht ongewenste bijwerkingen krijgen. Een alternatief voor behandeling met een hoge dosis inhalatie corticosteroïd zou een combinatie van een lagere dosis met een langwerkend $beta_2$-mimeticum kunnen zijn. Hiervan uitgaande zijn de volgende onderzoeken gewenst:

1. Kortwerkend $beta_2$-mimeticum plus inhalatie corticosteroïd versus inhalatie corticosteroïd alleen.
2. Langwerkend $beta_2$-mimeticum plus inhalatie corticosteroïd versus inhalatie corticosteroïd alleen.
3. Langwerkend $beta_2$-mimeticum plus inhalatie corticosteroïd $\leq$800 µg versus hoge dosis (>800 µg) inhalatie corticosteroïd.

Er is veel geschreven over mogelijke bijwerkingen van langdurige behandeling met inhalatie corticosteroïd bij kinderen met astma. Doseringen tot maximaal 800 µg zijn veilig wat betreft lengtegroei en bijnierfunctie. Bij volwassenen is aangetoond dat de botdichtheid kan afnemen onder deze behandeling.[1] Dergelijke gegevens zijn niet

beschikbaar over kinderen. De bepaling van botdichtheid bij groeiende individuen is echter moeilijk en normaalwaarden ontbreken.

In de internationale consensusrapporten over de behandeling van astma bij kinderen wordt cromoglicaat genoemd als medicament van eerste keus voor onderhoudsbehandeling bij matig ernstig astma. Zowel cromoglicaat als nedocromil hebben ontstekingsremmende eigenschappen, maar zij verminderen de luchtweggevoeligheid niet. De veiligheid van cromonen maakt deze geneesmiddelen aantrekkelijk voor langdurige behandeling van kinderen, maar uitgaand van het ontbreken van een effect op de luchtweggevoeligheid zou het lange-termijns beloop van kinderen die hiermee behandeld worden minder gunstig kunnen zijn dan van kinderen die behandeld worden met inhalatie corticosteroïd. Het is klinici bekend dat een subgroep kinderen bestaat die goed ingesteld zijn met cromoglicaat, maar uitgezocht moet nog worden bij welke kinderen het effect van cromonen gelijk is aan dat van inhalatie corticosteroïd. Langdurig onderzoek bij kinderen met matig ernstig astma waarin het effect van een cromoon met dat van een inhalatie corticosteroïd wordt vergeleken op zowel klinische als fysiologische eindpunten is gewenst. Er is weinig bekend over de combinatie van een cromoon met een inhalatie corticosteroïd. Sommige onderzoeken suggereren een steroidsparend effect van DSCG[2-4], terwijl recentere onderzoeken een dergelijk effect niet vonden.[5,6] Dit vergt meer onderzoek bij kinderen.

Aangezien ontsteking een centrale plaats heeft bij astma is het aantrekkelijk te kijken naar locale effecten van therapeutische interventie in de bronchiaalboom. Bronchiaallavage en bronchusbiopsieën geven belangrijke informatie over het effect van interventies op cellulair niveau. Door verband te leggen tussen deze bevindingen en symptomen en fysiologische metingen, worden processen die ten grondslag liggen aan klinische verschijnselen duidelijker. Dergelijke onderzoeken zijn recent gestart bij volwassenen. Ze zijn moeilijker uitvoerbaar bij kinderen. Hoewel we weinig weten over milde en beginnende vormen van astma, moet nog worden uitgemaakt of deze onderzoeken eveneens gewenst zijn bij kinderen.

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ABBREVIATIONS

AHR	Airway hyperresponsiveness
BA	$Beta_2$-agonist
BAL	Bronchoalveolar lavage
BID	Twice daily
BHR	Bronchial hyperresponsiveness
BR	Bronchial responsiveness
CS	Inhaled corticosteroid
DD	Doubling dose
DSCG	Disodium cromoglycate
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
NS	Not stated
NSO	Nedocromil sodium
PEF	Peak expiratory flow
$PC_{20}FEV_1$ (PC)	Provocation concentration which causes 20% decrease of FEV_1
$PD_{20}FEV_1$ (PD)	Provocation dose which causes 20% decrease of FEV_1
PL	Placebo
SD	Standard deviation
SEM	Standard error of the mean
SGAW	Specific airway conductance
TID	Three times daily
QID	Four times daily

MANUSCRIPTS ON SIDE-PROJECTS

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CURRICULUM VITAE

De schrijfster van dit proefschrift werd geboren op 7 november 1953 te Leiden. In 1972 behaalde zij het eindexamen gymnasium beta aan het Agneslyceum te Leiden. In dat jaar begon zij de studie geneeskunde aan de Medische Faculteit van de Rijksuniversiteit Leiden. Zij slaagde in 1976 voor het doctoraal examen en in 1979 voor het artsexamen. Van 1979 tot 1983 specialiseerde zij zich in de kindergeneeskunde in het Academisch Ziekenhuis Leiden (opleider prof. dr. L.J. Dooren). In 1983 was zij gedurende 8 maanden vervangend kinderarts op de polikliniek algemene kindergeneeskunde en in de kliniek van het Sophia Kinderziekenhuis te Rotterdam (hoofd prof. dr. H.K. A. Visser). In dit ziekenhuis was zij vanaf eind 1983 gedurende 10 maanden werkzaam op de afdeling kinderlongziekten, gevolgd door een jaar op de afdeling kindercardiologie en een jaar op de afdelingen zuigelingen en kleuters als chef de clinique. Vanaf 1986 is zij werkzaam op de afdeling kinderlongziekten, waar zij door prof. dr. K.F. Kerrebijn werd opgeleid tot kinderlongarts. Op deze afdeling bewerkte zij de gegevens voor dit proefschrift.
De schrijfster is getrouwd met Hans van Essen.