SALMETEROL IN THE TREATMENT OF CHILDHOOD ASTHMA

Salmeterol in de behandeling van astma bij kinderen

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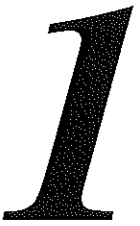
Contents

Chapter One

General introduction



General Introduction

1.1 Clinical aspects of asthma

Asthma is the most common chronic disease of childhood. Although mortality rates in the Netherlands and other Western European countries are low, asthma causes a great deal of morbidity and school absence.[1] Incidence rates in our country are about 10% and recent epidemiologic studies show an increase especially in the young age group.[2,3] Despite the availability of several classes of effective and safe anti-asthma drugs, so far childhood asthma can not be cured.[4] However, there is no doubt that medical treatment may result in appreciable clinical improvement.[5] With medical intervention, it is hardly possible to address the natural history of asthma from childhood to adulthood. The longest prospective ongoing study in Melbourne, following a cohort of 249 subjects from 7 to 35 years now revealed that 29% of those with wheeze at age 7 still had symptoms at the age of 35 years.[6] Follow-up studies in our country by Gerritsen[7] and Roorda[8] showed 43%, respectively 76% of their populations still having respiratory symptoms in adulthood. Airway caliber and the degree of airway responsiveness during childhood may be predictors of the outcome of childhood asthma.[7,8]

The last decade several consensus reports have focused on the treatment of childhood asthma.[9,10,11,12,13] Asthma is now generally regarded as an inflammatory disease and the cornerstone of treatment constitutes of prophylactic use of inhaled corticosteroids. Although airway hyperresponsiveness is not identical to airway inflammation, airway hyperresponsiveness is considered as the functional abnormality related to the inflammatory process of the disease.[14,15,16,17] As measurements of airway inflammation, such as bronchial biopsies and broncho-alveolar lavage studies, are unethical to perform in children for effect measurements of medical interventions, measurements of airway responsiveness and airway caliber (FEV_1) are now besides symptom scores and peak flow measurements regarded as valuable outcome parameters.[18] Studies in children as well as adults have shown that inhaled corticosteroids may improve asthma symptoms, airway caliber as well as airway responsiveness, although the majority of patients does not become symptom free and still needs the use of short-acting β_2-agonists for symptom relief.[5,19,20,21] Furthermore, stopping inhaled corticosteroids after 1 to 3 years results in reoccurrence of symptoms and lung function abnormalities.[22,23,24] Two long-acting β_2-agonists, salmeterol and formoterol, recently became available. Their long duration of action may result in effective symptom relief, however it is not yet clear whether these drugs also effect the inflammatory process. Their place in the treatment of asthma should be further elucidated.

This thesis deals with the effect of salmeterol in a single dose and during long-term treatment in children with asthma.

1.2 Pathophysiology of asthma

The pathophysiologic concept of asthma has changed during the past decades from smooth muscle contraction as the essential mechanism[25] to chronic inflammation. Activated cells, especially mast cells and eosinophils, play a role and T-lymphocytes are regarded to regulate the process.[11 12 26] Until recently most information was based on autopsy material from patients dying in status asthmaticus. Mucus plugging of airways, basement membrane thickening, disruption of airway epithelium (shedding), smooth muscle hypertrophy and infiltration of the airway wall by inflammatory cells, especially eosinophils have long been recognized in fatal cases.[27 28 29] Endobronchial biopsies in adult asthmatics have confirmed that extensive inflammatory changes are also present in non-fatal asthma and even in mild asthmatics.[30 31 32] Infiltration of the lamina propria by inflammatory cells, especially eosinophils, mast cells and lymphocytes, epithelial shedding and collagen deposition beneath the basement membrane are common findings. Due to ethical difficulties in using invasive techniques as bronchial biopsies and broncho-alveolar lavages, only limited studies in children have been performed.[33] As in adults, in children with asthma increased numbers of eosinophils and increased levels of activation markers, such as eosinophilic cationic protein and mast cell tryptase have been found in alveolar lavage fluid.[34 35]

Although the underlying immunological mechanisms are not yet fully understood, activated T-lymphocytes, by producing cytokines, seem to orchestrate the chronic inflammation in atopic asthma.[36] Antigens are presented to T-lymphocytes by antigen presenting cells, especially dendritic cells and T-cells are stimulated, resulting in cytokine production. Two patterns of cytokine production have been distinguished. Th-1 cells synthesize IL-2, interferon-gamma (IFN-γ) and granulocyte macrophage-colony stimulating factor (GM-CSF), whereas Th-2 cells synthesize IL-3, IL-4, IL-5 and IL-10.[36 37] In asthma there is evidence for a T-cell response switch to the Th-2 phenotype.[38] IL-4 causes an isotype switch of the B-cells to produce IgE.[39] IL-5 causes recruitment and activation of eosinophils.[40] Activation of eosinophils results in the secretion of several toxic proteins such as major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophilic peroxidase (EPO), which are toxic to the airway epithelium, causing epithelial shedding.[41] Furthermore, eosinophils are able to secrete lipid mediators (e.g. prostaglandins, platelet activating factor, leukotriens), oxygen radicals, neuropeptides (substance P, vasoactive intestinal peptide) and cytokines (IL-3, IL-5, GM-CSF and tumor growth factor-β_1).[42] This activation process of eosinophils is now regarded as the basis of the eosinophilic inflammation in asthma.[43]

1.3 Definition of asthma in childhood

The knowledge that inflammatory processes underlie the clinical expression of asthma, forced to a new operational definition of asthma in the National Heart, Lung, and Blood Institute consensus report.[12] "Asthma is a chronic inflammatory disorder of the airways in which many cells 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, and causes an associated increase in airway responsiveness to a variety of stimuli". In daily clinical practice, especially in children, the diagnosis of asthma will still be based on the clinical characteristics of symptoms of wheeze, dyspnea and cough in combination with reversible airway obstruction and airway responsiveness.[10 44] Especially in infants and young children no clear distinction can be made between asthma and wheezing-associated lower respiratory illnesses.[45] Martinez et al. recently described two distinct manifestations of infant wheezing and suggested that early-onset asthma is associated with increased prevalence of allergic markers, bronchial hyperresponsiveness and deterioration in lung function during the first 6 years of life, while transient wheeze was associated with a small airway diameter at birth and maternal smoking during pregnancy.[46]

1.4 Treatment of asthma: consensus reports

Several international consensus reports have focused on the management of asthma in adults[11 12 13] as well as children[9 10]. Apart from medication regimens these reports promote allergen avoidance in allergic patients, control of air pollution especially indoor (such as tobacco smoke) and avoidance of drugs which may aggravate asthma (such as aspirins). Drug therapy is based on a stepwise approach according to the severity of the disease. Severity of the disease may be assessed by taking into account the frequency of symptoms, nocturnal symptoms, peak flow variability and use of bronchodilators. All consensus reports aim to establish control of asthma in terms of minimal possible symptoms, preferably no exacerbations, minimal need for additional bronchodilators, minimal or no peak flow variability and normal or best personal lung function. This should be achieved with no or minimal adverse effects from medication. Asthma management plans, which include an important role for the patients self-management, are part of the consensus reports. All consensus reports focus on the inflammatory process which underlies the disease and advocate anti-inflammatory treatment at least for moderate and severe asthma. Whereas in most adult reports inhaled corticosteroids are the first prophylactic treatment, consensus reports on childhood asthma do make room for cromoglycate in mild to moderate asthma. Comparing the different consensus

reports at the more severe end of the asthma spectrum, there is no evident consensus on the place of long-acting $ß_2$-agonists. So far, it is not clear whether an increase in inhaled corticosteroids, addition of long-acting $ß_2$-agonists or even introduction of theophyllines is the first step after a moderate dose of inhaled corticosteroid fails to control asthma.

1.5 Treatment of asthma: inhaled corticosteroids and $ß_2$-agonists

Inhaled corticosteroids are regarded as the most effective anti-inflammatory drugs for the treatment of asthma.[47] Inhaled short-acting $ß_2$-agonists are the most effective bronchodilators for short-term relief of symptoms.[48 49] Both are described in more detail. As this thesis focusses on salmeterol, a long-acting $ß_2$-agonist, pharmacology and clinical effects of these class of drugs will also be summarized here.

1.5.1 Inhaled corticosteroids

PHARMACOLOGY

Glucocorticosteroids diffuse into the cell and bind to the glucocorticoid receptor within the cytoplasm. Activation of the receptor results in dissociation of heat shock proteins and translocation of the resulting complex into the nucleus, where it binds directly to glucocorticoid responsive elements on DNA in the promoter region of steroid sensitive genes.[50] Depending on the gene and the cell type, activation of the glucocorticoid responsive elements can cause up-regulation or down-regulation.[50 51] One example of downregulation is the blocking effect of glucocorticosteroids on the transcription of several cytokines.[47 52] Alternatively, the steroid receptor complex may directly bind with pro-inflammatory transcription factors as activating protein-1 (AP-1) and nuclear factor-κB (NF-κB) and thereby having a modulating effect on gene transcription.[53] Glucocorticoids have suppressive effects on inflammatory cells as eosinophils and mast cells, probably partly by reducing their survival.[47 54] Glucocorticosteroids reduce the cytokine production by T-lymphocytes as well as airway epithelial cells.[55] Furthermore they may inhibit plasma exudation[56] and mucus secretion.[57]

CLINICAL ASPECTS

Although clinical effects on airway responsiveness[58] and the late asthmatic reaction after allergen challenge[59] are measurable after a single dose of an inhaled corticosteroid, from a clinical point of view effects during long-term treatment are more interesting. Several long term studies, ranging from 1 to 2.5 years, have revealed positive effects on symptoms, exacerbations, peak flow rates, airway caliber and airway responsiveness with twice daily dosing of either beclomethasone or budesonide.[5 19 20 21]

However, the time course for the effect parameters is different, whereby symptoms and peak flow rates improve more rapidly than airway responsiveness. For airway responsiveness to histamine a plateau seemed to be reached only after 22 months of treatment with budesonide.[4] Prolonged treatment with inhaled corticosteroids also reduces airway responsiveness to other bronchoconstricting stimuli than histamine, such as exercise,[60] dry air hyperventilation,[61] methacholine,[62] metabisulfite[63], the late asthmatic reaction to allergen[60] and the allergen-induced increase in airway responsiveness.[64] Not only the position of the dose response curve is influenced by inhaled corticosteroids, but also the maximum degree of airway narrowing.[65] Airway inflammation is modified, as has been shown by bronchial biopsy studies which show a reduction in the number of inflammatory cells as well as in the activation of these cells after treatment with inhaled corticosteroids for several months.[66] [67] Despite clinical improvement, even long-term use of inhaled corticosteroid therapy does not cure the disease, in that most of the patients do not become symptom free and the majority keeps lung function abnormalities.[4] Furthermore, stopping or reducing inhaled corticosteroid treatment rapidly reverses symptoms and increases airway responsiveness.[22] [23] [24] Side effects on the hypophyseal-pituitary-adrenal axis, on growth and on bone density are considered unlikely when daily doses of inhaled corticosteroids are below 400-800 μg, but individual susceptibility may vary.[68]

Inhaled corticosteroids are now recommended as prophylactic treatment in adult asthma, which is not well controlled with a β_2-agonist on demand.[11] [12] [13] In children these are recommended when prophylactic therapy with cromoglycate fails or in case of severe symptoms.[9] [10]

1.5.2 Short-acting β_2-agonists

PHARMACOLOGY

Selective β_2-agonists, as salbutamol, terbutaline and fenoterol bind to the β_2-receptors in airway smooth muscle, consisting of seven transmembrane α-helices with a binding cleft between them. Binding to the receptor results in activation of the α-unit of the receptor-associated stimulating G-protein. Activation of this alpha-unit in turn activates the cell surface-associated enzyme adenylyl cyclase (AC), which results in conversion of ATP to cyclic 3'-5'-adenosine monophosphate (cAMP). cAMP activates protein kinase A (PKA), which in airway smooth muscle inhibits myosin light chain phosphorylation, inhibits phosphoinositide hydrolysis and promotes Ca^{2+}/Na^{+} exchange, thus resulting in a fall in intracellular Ca^{2+}.[69] This leads to relaxation of airway smooth muscle. β_2-agonists, already in low doses, may also relax airway smooth muscle independent of cAMP via direct coupling between the receptor-activated stimulating G

protein and a large conductance Ca^{2+}-activated potassium channel, leading to cell hyperpolarization.[69] Furthermore β_2-agonists may influence gene transcription through elevation of cAMP and activation of PKA, which mediates phosphorylation of cAMP responsive element binding protein (CREB) within the nucleus. CREB binds to a cAMP response element (CRE) in the upstream promotor region of a responsive gene and in this way short term exposure to a β_2-agonist may exert positive feedback on the transcription of the β_2-receptor gene.[69 70] Long-term exposure to β_2-agonists however results in reduced gene transcription and is associated with a reduction in CREB activity.[71] Apart from the effects on airway smooth muscle, short-acting β_2-agonists have also an effect on mast cells, by reducing the release of mediators[72] and may inhibit microvascular leakage.[73]

CLINICAL ASPECTS

After a single dose short-acting β_2-agonists result in bronchodilatation up to 4-6 hours after inhalation. They also protect against bronchoconstricting stimuli as methacholine, histamine, exercise, hyperventilation with dry cold air and inhibit the early asthmatic reaction after allergen challenge.[74 75] Recently, also an inhibition of the late asthmatic reaction was found with a higher dose of salbutamol.[76] Although salbutamol results in a rightward shift of the dose-response curve to methacholine, it was found that with higher doses of methacholine this protection is overcome and there is an even steeper fall in lung function.[77] The protective effects of short-acting β_2-agonists may be due to functional antagonism on airway smooth muscle. However, some evidence exists that separate mechanisms may be involved.[78]

Chronic continuous use of β_2-agonists has been related to worsening of asthma control[79] and to a reduction in lung function,[80] compared to the use of β_2-agonists on demand. Also an association has been made between the use of inhaled β_2-agonists and asthma mortality rates in several epidemiological studies.[81 82 83 84 85] An unresolvable problem of these studies is the confounding by severity of the disease.[86] Furthermore, despite the increase in sales of β_2-agonists, no increase in asthma mortality was found in the past two decades in several European countries.[87] After cessation of treatment with a short-acting β_2-agonist for several weeks a rebound increase in airway responsiveness and fall in FEV_1 was noted, lasting up to 59 hours.[88 89] Regular use of inhaled β_2-agonists induces tolerance to the bronchoprotective effects of several stimuli as histamine, methacholine, AMP, allergen and exercise[90], while tolerance to the bronchodilator effect has not been convincingly established so far and seems not to be of clinical importance.[90 91] Loss of protection is greater for AMP than for methacholine, suggesting that β_2-receptors on mast cells may be more susceptible to

tolerance than β_2-receptors on airway smooth muscle.[92] Corticosteroids do not prevent the development of tolerance.[93] In addition to tolerance to the bronchoprotective effects, it was found that after regular use of salbutamol for 1-2 weeks the early as well as late response to allergen was increased, as well as the allergen-induced airway responsiveness.[94] [95] The clinical relevance of the development of tolerance remains unclear, but theoretically the enhanced response to allergen may result in increased airway inflammation. This may explain the increased numbers of eosinophils found in bronchial biopsies after regular treatment with salbutamol.[96]
Short-acting β_2-agonists are now advised as rescue medication in all steps of the asthma treatment plan in the consensus reports.[9] [10] [11] [12] [13]

1.5.3 Long-acting β_2-agonists

PHARMACOLOGY

Two long-acting β_2-agonists are now available, salmeterol and formoterol. Their long duration of action distinguishes these drugs from short-acting β_2-agonists. Both drugs are selective β_2-agonists.[97] Moreover, salmeterol was found to reassert its relaxing effect on airway smooth muscle after adding a ß-antagonist and subsequently wash-out of this antagonist.[98] The underlying mechanism of this action has been hypothesized as the result of the binding of the inactive long aliphatic tail to an "exosite" near or in the β_2-receptor.[97] Recently the underlying mechanism for the long duration of action was explained in the so called "plasmalemma diffusion microkinetic model".[99] According to this model a moderate (formoterol) and highly (salmeterol) lipophilic molecule may approach the β_2-receptor in airway smooth muscle by entering the plasmalemma lipid bilayer and once having partitioned into the bilayer, remain available to interact with the β_2-adrenoceptor active site. This model may also explain the difference between formoterol and salmeterol in onset of action; formoterol having a more rapid onset of bronchodilator effect.[100] The faster onset of formoterol is based on the moderate lipophilicity, making it possible also to rapidly diffuse to the active site of the β_2-receptor by the aqueous biphase.[99] The partitioning of salmeterol as well as formoterol has been demonstrated in vitro.[99] [101] Apart from their effects on airway smooth muscle, salmeterol and formoterol are able to inhibit the release of mediators from mast cells, such as histamine, prostaglandins and leukotriens.[97] [102] Furthermore, inhibition of vascular permeability and inhibition of activation of eosinophils and neutrophils have been found in animal models and human cells in vitro.[97] [103] These effects are β_2-receptor mediated. Salmeterol also has inhibitory effects on the release of thromboxane B_2 from human airway macrophages and blood monocytes in vitro; these effects are not or only partially mediated by β_2-receptors and are probably due to the lipophilic

properties of the molecule.[104] So far, no inhibition of T-lymphocytes could be detected, as was measured by the serum level of the soluble interleukin-2 receptor after two weeks of treatment with salmeterol.[105]

CLINICAL ASPECTS

The clinical aspects of salmeterol and formoterol are described in more detail in chapter 3.

Single doses of salmeterol as well as formoterol give prolonged bronchodilation, up to 12 hours after inhalation.[106] [107] Protection against various bronchoconstricting stimuli as methacholine,[108] histamine,[109] exercise[110] [111] and hyperventilation with dry cold air[112] is also prolonged compared to short-acting β_2-agonists. Salmeterol as well as formoterol are able to block the early and partially the late asthmatic reaction to allergen.[113] [114] [115] These blocking effects are most likely due to functional antagonism on airway smooth muscle. Used on a twice daily base for several weeks to months, compared to placebo or salbutamol, these drugs result in better symptom control and higher peak flow rates.[116] [117] [118] No deterioration of asthma or increase in exacerbations has been noted during long-term treatment.[119] A surveillance study could not reveal an increased number of deaths.[120] Tolerance to the protective effect to methacholine,[121] exercise[122] and allergen[123] has been described and seems to develop within several days after starting therapy.[124] However, there remains a residual protective effect during treatment.[125] As with short-acting β_2-agonists, corticosteroids do not prevent the development of tolerance.[126] [127] [128] So far, no rebound increase in airway responsiveness has been noted after cessation of treatment.[121] [129] Long-acting β_2-agonists should not be considered as anti-inflammatory drugs. Eight weeks of treatment with salmeterol did not affect the total cell count, cellular profile, tryptase or albumine concentrations in bronchoalveolar lavage fluids.[130] Consensus reports place these drugs after the introduction of inhaled corticosteroids.[9] [10] [11] [12] [13] Recently two studies in adult asthmatic patients revealed better symptom control and peak flow rates with the addition of salmeterol to a moderate-high dose of an inhaled corticosteroid compared to doubling the dose of an inhaled corticosteroid.[131] [132]

1.5.4 Interactions between corticosteroids and β_2-agonists

Glucocorticoids are able to upregulate β_2-receptors and prevent down-regulation of β_2-receptors in airway smooth muscle and other cell types, probably by receptor complex binding to a glucocorticoid responsive element within the promotor region of the β_2-receptor gene.[70] [133] In rat lung salbutamol decreased the binding of the glucocorticosteroid to the glucocorticosteroid responsive element, due to an interaction between the

glucocorticoid receptor and CREB.[134] High doses of β_2-agonists are able to activate CREB and so inhibit the binding of an activated glucocorticosteroid receptor and have functional anti-glucocorticoid activity.[70]
Clinically there is no evidence for preventing down-regulation of β_2-receptors by inhaled corticosteroids; in contrast, there is now evidence that inhaled corticosteroids are not able to protect against tolerance to the ß-agonist effect on bronchoconstricting stimuli.[126] [127] [128] It was shown that oral terbutaline caused desensitization of β_2-receptors in human alveolar macrophages, which was not influenced by either inhaled or oral corticosteroid treatment.[135] One study has suggested a negative effect of continuous administration of β_2-agonists on the protection by inhaled corticosteroid. After treatment with terbutaline and budesonide in combination less protection against allergen induced bronchoconstriction was found compared to budesonide treatment alone.[136] A recent study from the same department, however, could not confirm these results for the protection against histamine and adenosine monophosphate induced bronchoconstriction.[137]

1.6 Aims of the study

The aim of our studies was to investigate the effect of salmeterol after a single dose and during prolonged treatment on several outcome parameters of asthma in children. Primary outcome parameters were airway caliber, as measured by FEV_1 and airway responsiveness, as measured by PD_{20} methacholine. In the long term studies secondary outcome parameters were symptom scores, peak flow rates, use of additional short-acting β_2-agonists and exacerbations of asthma.

Chapter 2 gives a review of the literature on the effects of short- and long-acting β_2-agonists on airway responsiveness.

Chapter 3 describes in more detail the clinical effects of long-acting β_2-agonists.

In chapter 4 the bronchodilator and bronchoprotective effect against methacholine induced bronchial obstruction of a single dose of salmeterol are compared to placebo in children with mild to moderate asthma.

In chapter 5 the bronchodilator and bronchoprotective effect of salmeterol after a single dose and after 4 months treatment are compared with salbutamol in children with mild to moderate asthma, who were not on treatment with inhaled corticosteroids.

In chapter 6 one year treatment with twice daily salmeterol is compared to treatment with a moderate dose of beclomethasone (400 μg daily) in children with mild to moderate asthma, who were not already treated with inhaled corticosteroids.

In chapter 7 the addition of salmeterol to a moderate dose of beclomethasone (400 μg daily) is compared to beclomethasone alone in the same dose and to a doubling dose of beclomethasone in children with mild to moderate asthma, who were already on treatment with an inhaled corticosteroid

Chapter 8 gives a summary on previous chapters and a general discussion.

1.7 References

1. Lenney W, Wells NEJ, O'Neill BA. The burden of paediatric asthma. *Eur Respir Rev* 1994;4:49-62.
2. Stuurgroep Toekomstscenario's Gezondheidszorg. Chronische ziekten in het jaar 2005. Deel 2. *Scenario's over CARA 1990-2005*. Houten/Antwerpen: Bohn, Stafleu van Loghum, 1990.
3. Wever-Hess J, Wever AMJ, Yntema JL. Mortality and morbidity from respiratory diseases in childhood in the Netherlands, 1980-1987. *Eur Respir J* 1991;4:429-33.
4. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Kerrebijn KF and the Dutch CNSLD Study Group. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide). Can it be achieved? *Eur Respir J* 1994;7:63-8.
5. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF and the Dutch CNSLD Study Group. Effects of 22 months' treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness and symptoms in children with asthma. *Am Rev Respir Dis* 1992;146:547-54.
6. Oswald H, Phelan PD, Lanigan A, Hibbert M, Bowes G, Olinsky A. Outcome of childhood asthma in mid-adult life. *Br Med J* 1994;309:95-6.
7. Gerritsen J, Koëter GH, Postma DS, Schouten JP, Knol K. Prognosis of asthma from childhood to adulthood. *Am Rev Respir Dis* 1989;140:1325-30.
8. Roorda RJ, Gerritsen J, Van Aalderen WMC, Schouten JP, Veltman J, Weiss ST, Knol K. Risk factors for the persistence of respiratory symptoms in childhood asthma. *Am Rev Respir Dis* 1993;148:1490-5.
9. Warner JO, Götz M, Landau LI, Levison H, Milner AD, Pedersen S, Silverman M. Management of asthma: a consensus statement. *Arch Dis Child* 1989;64:1065-79.
10. Warner JO. Asthma: a follow up statement from an international paediatric asthma consensus group. *Arch Dis Child* 1992;67:240-8.
11. Guidelines for the diagnosis and management of asthma. National Heart, Lung and Blood Institute, National Asthma Education Program Expert Panel Report. *J Allergy Clin Immunol* 1991;88:425-534.
12. International consensus report on diagnosis and treatment of asthma. National Heart, Lung and Blood Institute, National Institutes of Health Bethesda. *Eur Respir J* 1992;5:601-41.
13. Guidelines on the management of asthma. Woodhead M, Editor. *Thorax* 1993;48:S1-24.
14. Chung KF. Role of inflammation in the hyperreactivity of the airways in asthma. *Thorax* 1986;41:657-62.
15. Drazen JM. Physiology. In: Holgate ST (ed). The role of inflammatory processes in airway responsiveness. *Blackwell Scientific publications*, Oxford 1989.

16. Chetta A, Foresi A, Del Donno M, Consigli GF, Bertorelli G, Pesci A, Barbee RA, Olivieri D. Bronchial responsiveness to distilled water and methacholine and its relationship to inflammation and remodelling of the airways in asthma. *Am J Respir Crit Care Med* 1996;**153**:910-7.
17. Sont JK, Van Krieken JHJM, Evertse CE, Hooijer R, Willems LNA, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996;**51**:496-502.
18. Tepper R, Sly P. Physiological outcomes. *Eur Respir J* 1996;**9**:12s-15s.
19. Juniper EF, Kline PA, Vanzieleghem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway responsiveness and clinical asthma in non-steroid-dependent asthmatics. *Am Rev Respir Dis* 1990;**142**:832-6.
20. Haahtela T, Järvinen M, Kava T, Kiviranti K, Koskinen S, Lehtonen K, Nikander K, Persson T, Reinikainen K, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R, Laitinen L. Comparison of a beta-2-agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. *N Engl J Med* 1991;**325**:388-92.
21. Kerstjens HAM, Brand PLP, Hughes MD, Robinson NJ, Postma DS, Sluiter HJ, Bleecker ER, Dekhuijzen PNR, De Jong PM, Mengelers HJJ, Overbeek SE, Schoonbrood DFME and the Dutch CNSLD Study Group. A comparison of bronchodilator therapy with or without inhaled corticosteroid therapy for obstructive airways disease. *N Engl J Med* 1992;**327**:1413-9.
22. Waalkens HJ, Van Essen-Zandvliet EEM, Hughes MD, Gerritsen J, Duiverman EJ, Knol K, Kerrebijn KF and the Dutch CNSLD Study Group. Cessation of long-term treatment with inhaled corticosteroid (budesonide) in children with asthma results in deterioration. *Am Rev Respir Dis* 1993;**148**:1252-7.
23. Haahtela T, Järvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Persson T, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R, Laitinen L. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N Engl J Med* 1994;**331**:700-5.
24. Juniper EF, Kline PA, Vanzieleghem MA, Hargreave FE. Reduction of budesonide after a year of increased use: a randomized controlled trial to evaluate whether improvements in airway responsiveness and clinical asthma are maintained. *J Allergy Clin Immunol* 1991;**87**:483-9.
25. Szentivanyi A. The beta adrenergic theory of the atopic abnormality in bronchial asthma. *J Allergy* 1968;**42**:203-32.
26. Djukanovic R, Roche WR, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH, Holgate ST. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;**142**:434-57.
27. Dunnill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. *J Clin Pathol* 1960;**13**:27-33.
28. Dunnill MS, Massarella GR, Anderson JA. A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis and emphysema. *Thorax* 1969;**24**:176-9.
29. Cutz E, Levison H, Cooper DM. Ultrastructure of airways in children with asthma. *Histopathology* 1978;**2**:407-21.
30. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;**140**:1745-53.
31. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;**139**:806-17.
32. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993;**147**:697-704.
33. Busse W, Banks-Schlegel SP, Larsen GL. Childhood- versus adult-onset asthma. NHLBI Workshop Summary. *Am J Respir Crit Care Med* 1995;**151**:1635-9.
34. Ferguson AC, Wong FWM. Bronchial hyperresponsiveness in asthmatic children: correlation with macrophages and eosinophils in broncholavage fluid. *Chest* 1989;**96**:988-91.

35. Ferguson AC, Whitelaw M, Brown H. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. *J Allergy Clin Immunol* 1992;**90**:609-13.
36. Barnes PJ. Cytokines as mediators of chronic asthma. *Am J Respir Crit Care Med* 1994;**150**:S42-49.
37. Mossman TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;**7**:145-73.
38. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant Th2-type bronchoalveolar lavage T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;**326**:298-304..
39. Romagniani S. Regulation and deregulation of human IgE synthesis. *Immunol Today* 1990;**11**:316-21.
40. Sanderson CJ. Interleukin-5, eosinophils and disease. *Blood* 1992;**79**:3101-9.
41. Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J Allergy Clin Immunol* 1988;**81**-:776-81.
42. Kroegel C, Virchow JC, Luttman W, Walker C, Warner JA. Pulmonary immune cells in health and disease; the eosinophil leucocyte (Part 1). *Eur Respir J* 1994;**7**:519-43.
43. Bousquet J, Chanez P, Lacoste JY, Barnéon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Lafontaine J, Godard P, Michel FB. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;**323**:1033-9.
44. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225-44.
45. Silverman M. Out of the mouths of babes and sucklings: lessons from early childhood asthma. *Thorax* 1993;**48**:1200-4.
46. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ and the group Health Medical Associates. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;**332**:133-8.
47. Barnes PJ. Inhaled glucocorticoids for asthma. *N Engl J Med* 1995;**332**:868-875.
48. Chaieb J, Belcher N, Rees PJ. Maximum achievable bronchodilatation in asthma. *Respir Med* 1989;**83**:497-502.
49. Barnes PJ. A new approach to the treatment of asthma. *N Engl J Med* 1989;**321**:1517-27.
50. Barnes PJ, Adcock I. Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol Sci* 1993;**14**:436-41.
51. Miesfeld RL. Molecular genetics of corticosteroid action. *Am Rev Respir Dis* 1990;**141(suppl)**-:S11-3.
52. Laforge ARKS, Sehgal PB. On the mechanism for efficient repression of the interleukin-6 promoter by glucocorticoids; enhancer, TATA box, and RNA start site (INR motif) occlusion. *Mol Cell Biol* 1990;**10**:5736-46.
53. Adcock IM, Shirasaki H, Gelder CM, Peters MJ, Brown CR, Barnes PJ. The effects of glucocorticosteroids on phorbol ester and cytokine stimulated transcription facor activation in human lung. *Life Sci* 1994;**55**:1147-53.
54. Schleimer RP. Effects of glucocorticosteroids on inflammatory cells relevant to their therapeutic applications in asthma. *Am Rev Respir Dis* 1990;**141**:S59-69.
55. Barnes PJ. Mechanisms of action of glucocorticoids in asthma. *Am J Respir Crit Care Med* 1996;**154**:S21-27.
56. Boschetto P, Rogers DF, Fabbri LM, Barnes PJ. Corticosteroid inhibition of airway microvascular leakage. *Am Rev Respir Dis* 1991;**143**:605-9.
57. Shimura S, Sasaki T, Ikeda K, Yamauchi K, Sasaki H, Takishima T. Direct inhibitory action of glucocorticoid on glycoconjugate secretion from airway submucosal glands. *Am Rev Respir Dis* 1990;**141**:1044-9.

58. Van Essen-Zandvliet EEM, Hop WCJ, De Jong H, Ferwerda A, Kerrebijn KF. Minor acute effect of an inhaled corticosteroid (budesonide) on bronchial hyperresponsiveness to methacholine in children with asthma. *Eur Respir J* 1993;6:383-86.
59. Cockcroft DW, Murdock KY. Comparative effect of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses and increased bronchial responsiveness to histamine. *J Allergy Clin Immunol* 1987;79-:734-40.
60. Waalkens HJ, Van Essen-Zandvliet EEM, Gerritsen J, Duiverman EJ, Kerrebijn KF, Knol K and the Dutch CNSLD Study Group. The effect of an inhaled corticosteroid (budesonide) on exercise-induced asthma in children. *Eur Respir J* 1993;6:652-6.
61. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise and eucapnic dry air hyperventilation in patients with asthma. *Thorax* 1991;**46**:811-6.
62. Bel EH, Timmers MC, Hermans JO, Dijkman JH, Sterk PJ. The long-term effects of nedocromil sodium and beclomethasone dipropionate on bronchial responsiveness to methacholine in non-atopic asthmatic subjects. *Am Rev Respir Dis* 1990;**141**:21-8.
63. O'Connor BJ, Ridge SM, Barnes PJ, Fuller RW. Greater effect of inhaled budesonide on adenosine 5'-monophosphate-induced than on metabisulfite-induced bronchoconstriction in asthma. *Am Rev Respir Dis* 1992;**146**:560-4.
64. De Baets FM, Goeteyn M, Kerrebijn KF. The effect of two months of treatment with inhaled budesonide on bronchial responsiveness to histamine and house-dust mite antigen in asthmatic children. *Am Rev Respir Dis* 1990;**142**:581-6.
65. Bel EH, Timmers MC, Zwinderman AH, Dijkman JH, Sterk PJ. The effect of inhaled corticosteroids on the maximal degree of airway narrowing to methacholine in asthmatic subjects. *Am Rev Respir Dis* 1991;**143**:109-13.
66. Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992;**145**:669-74.
67. Burke C, Power CK, Norris A, Condez A, Schmekel B, Poulter LW. Lung function and immunopathological changes after inhaled corticosteroid therapy in asthma. *Eur Respir J* 1992;5:73-9.
68. Barnes PJ, Pedersen S. Efficacy and safety of inhaled corticosteroids in asthma. *Am Rev Respir Dis* 1993;**148**:S21-6.
69. Barnes PJ. Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 1995;**152**:-838-60.
70. Adcock IM, Stevens DA, Barnes PJ. Interactions of glucocorticoids and beta-2-agonists. *Eur Respir J* 1996;**9**:160-8.
71. Nishikawa M, Mak JC, Shirasaki H, Barnes PJ. Differential downregulation of pulmonary $beta_1$- and $beta_2$-adrenoceptor messenger RNA with prolonged in vivo infusion of isoprenaline. *Eur J Pharmacol* 1993;**247**:131-8.
72. Peters SP, Schulman ES, Schleimer RP, Macglashan DW, Newball HH, Lichtenstein LM. Dispersed human lung mast cells. Pharmacological aspects and comparison with human lung tissue fragments. *Am Rev Respir Dis* 1982;**126**:1034-9.
73. Boschetto P, Roberts NM, Rogers DF, Barnes PJ. The effect of anti-asthma drugs on microvascular leakage in guinea pig airways. *Am Rev Respir Dis* 1989;**139**:416-21.
74. Kerrebijn KF. Beta agonists. In: Kaliner MA, Barnes PJ, Persson CGA, eds. *Asthma, it's pathology and treatment*. Marcel Dekker, New York. 1991:523-59.
75. Nelson HS. Beta-adrenergic bronchodilators. *N Engl J Med* 1995;**333**:499-505.
76. Twentyman OP, Finnerty JP, Holgate ST. The inhibitory effect of nebulized albuterol on the early and late asthmatic reactions and increase in airway responsiveness provoked by inhaled allergen in asthma. *Am Rev Respir Dis* 1991;**144**:782-7.

77. Bel EH, Zwinderman AH, Timmers MC, Dijkman JH, Sterk PJ. The protective effect of a beta-2-agonist against excessive airway narrowing in response to bronchoconstrictor stimuli in asthma and chronic obstructive lung disease. *Thorax* 1991;**46**:9-14.
78. Gustafsson B, Persson CGA. Effect of different bronchodilators on airway smooth muscle responsiveness to contractile agents. *Thorax* 1991;**46**:300-5.
79. Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery EM, Yates DM, Lucas MK, Herbison GP. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;**336**:1391-6.
80. Van Schayck CP, Dompeling E, Van Herwaarden CLA, Folgering H, Verbeek ALM, Van der Haagen HJM, Van Weel C. Bronchodilator treatment in moderate asthma or chronic bronchitis: continuous or on demand? A randomized controlled study. *Br Med J* 1991;**303**:1426-31.
81. Crane J, Pearce N, Flatt A, Jackson R, Ball M, Pearce N, Burgess C, Kwong T, Beasley R. Prescribed fenoterol and death from asthma in New Zealand, 1981-3; case-control study. *Lancet* 1989;**i**:917-22.
82. Pearce N, Grainger J, Atkinson M, Crane J, Burgess C, Culling C, Windom H, Beasley R. Case control study of prescribed fenoterol and death from asthma in New Zealand, 1977-81. *Thorax* 1990;**45**:170-5.
83. Grainger J, Woodman K, Pearce N, Crane J, Burgess C, Keane A, Beasley R. Prescribed fenoterol and death from asthma in New Zealand, 1981-87. *Thorax* 1991;**46**:105-11.
84. Pearce N, Beasley R, Crane J, Burgess C, Jackson R. End of the New Zealand asthma mortality epidemic. *Lancet* 1995;**345**:41-4.
85. Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, Boivin JF, McNutt M, Buist S, Rebuck AS. The use of beta-agonists and the risk of death and near death from asthma. *N Engl J Med* 1992;**326**:501-6.
86. Suissa S, Blais L, Ernst P. Patterns of increasing beta-agonist use and the risk of fatal or near-fatal asthma. *Eur Resp J* 1994;**7**:1602-9.
87. Löfdahl CG, Svedmyr N. Beta-agonists: still more friends than foes. *Eur Respir J* 1992;**5**:898-900.
88. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988;**i**:554-8.
89. Wahedna I, Wong CS, Wisniewski AFZ, Pavord ID, Tattersfield AE. Asthma control during and after cessation of regular beta-2-agonist treatment. *Am Rev Respir Dis* 1993;**148**:707-12.
90. Cockcroft DW, Swystun VA. Functional antagonism: tolerance produced by inhaled beta-2-agonists. *Thorax* 1996;**51**:1051-6.
91. Tattersfield AE. Tolerance to beta-agonists. *Bull Eur Physiopathol Respir* 1985;**21**:1-5s.
92. O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled beta-2-agonists in asthma. *N Engl J Med* 1992;**327**:1204-8.
93. Cockcroft DW, Swystun VA, Bhagat R. Interaction of inhaled beta-2-agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. *Am J Respir Crit Care Med* 1995;**152**:1485-9.
94. Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet* 1993;**342**:833-7.
95. Cockcroft DW, O'Byrne PM, Swystun VA, Bhagat R. Regular use of inhaled albuterol and the allergen-induced late asthmatic response. *J Allergy Clin Immunol* 1995;**96**:44-9.
96. Manolitsas ND, Wang J, Devalia JL, Trigg CJ, McAulay AE, Davies RJ. Regular albuterol, nedocromil sodium, and bronchial inflammation in asthma. *Am J Respir Crit Care Med* 1995;**151**:-1925-30.
97. Johnson M, Butchers PR, Coleman RA, Nials AT, Strong P, Sumner MJ, Vardey CJ, Whelan CJ. The pharmacology of salmeterol. *Life Sci* 1993;**52**:2131-43.
98. Ball DI, Brittain RT, Coleman RA, Denyer LH, Jack D, Johnson M, Lunts LHC, Nials AT, Sheldrick KE, Skidmore IF. Salmeterol, a novel, long-acting, beta-adrenoceptor agonist: characterization of pharmacological activity in vitro and in vivo. *Br J Pharmacol* 1991;**104**:665-671.

99. Anderson GP, Linden A, Rabe KF. Why are long-acting beta-adrenoceptor agonists long-acting? *Eur Respir J* 1994;**7**:569-78.
100. Van Noord JA, Smeets JJ, Raaijmakers JAM, Bommer AM, Maessen FPV. Salmeterol versus formoterol in patients with moderately severe asthma: onset and duration of action. *Eur Respir J* 1996;**9**:1684-8.
101. Rhodes DG, Newton R, Butler R, Herbette L. Equilibrium and kinetic studies of the interactions of salmeterol with membrane bilayers. *Mol Pharmacol* 1992;**42**:596-602.
102. Butchers PR, Vardey CJ, Johnson M. Salmeterol; a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Br J Pharmacol* 1991;**104**:672-6.
103. Whelan CJ, Johnson M. Inhibition by salmeterol of increased vascular permeability and granulocyte accumulation in guinea-pig lung and skin. *Br J Pharmacol* 1992;**105**:831-8.
104. Baker AJ, Palmer J, Johnson M, Fuller RW. Inhibitory actions of salmeterol on human airway macrophages and blood monocytes. *Eur J Pharmacol* 1994;**264**:301-6.
105. Lai CKW, Chan CHS, Ho SS, Hui ACF, Lai KN. Inhaled salmeterol and albuterol in asthmatic patients receiving high-dose inhaled corticosteroids. *Chest* 1995;**108**:36-40.
106. Ullman A, Svedmyr N. Salmeterol, a new long-acting inhaled beta-2-adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988;**43**:574-8.
107. Graff-Lonnevig V, Browaldh L. Twelve hours' bronchodilating effect of inhaled formoterol in children with asthma: a double-blind cross-over study versus salbutamol. *Clin Exp Allergy* 1990;**20**:429-32.
108. Derom EY, Pauwels RA, Van der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992;**89**:811-5.
109. Campos Gongora H, Wisniewski AFZ, Tattersfield AE. A single dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991;**144**:626-9.
110. Green GP, Price JF. Prevention of exercise-induced asthma by inhaled salmeterol xinafoate. *Arch Dis Child* 1992;**67**:1014-7.
111. Boner AL, Spezia E, Piovesan P, Chiocca E, Maiocchi G. Inhaled formoterol in the prevention of exercise-induced bronchoconstriction in asthmatic children. *Am J Respir Crit Care Med* 1994;**149**:935-9.
112. Malo JL, Ghezzo H, Trudeau C, L'Archévêque J, Cartier A. Salmeterol, a new inhaled beta-2-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *J Allergy Clin Immunol* 1992;**89**:567-74.
113. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;**336**:1338-42.
114. Weersink EJM, Aalbers R, Koëter GH, Kauffman HF, De Monchy JGR, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of salmeterol. *Am J Respir Crit Care Med* 1994;**150**:1262-7.
115. Palmqvist M, Balder B, Löwhagen O, Melander B, Svedmyr N, Wahlander L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting beta-2-agonist. *J Allergy Clin Immunol* 1992;**89**:844-9.
116. Britton MG, Earnshaw JS, Palmer JBD. Twelve month comparison of salmeterol with salbutamol in asthmatic patients. *Eur Respir J* 1992;**5**:1062-7.
117. Lundback B, Rawlinson DW, Palmer JBD. Twelve month comparison of salmeterol and salbutamol as dry powder formulations in asthmatic patients. *Thorax* 1993;**48**:148-53.
118. Lenney W, Pedersen S, Boner AC, Ebbutt A, Jenkins MM. Efficacy and safety of salmeterol in childhood asthma. *Eur J Paediatr* 1995;**154**:983-90.
119. Devoy MAB, Fuller RW, Palmer JBD. Are there any detrimental effects of the use of inhaled long-acting beta-2-agonists in the treatment of asthma? *Chest* 1995;**107**:1116-24.

120. Castle W, Fuller R, Hall J, Palmer J. Serevent nationwide surveillance study: comparison of salmeterol with salbutamol in asthmatic patients who require regular bronchodilator treatment. *Br Med J* 1993;**306**:1034-7.
121. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting beta-2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;**327**:1198-203.
122. Ramage L, Lipworth BJ, Ingram CG, Cree IA, Dhillon DP. Reduced protection against exercise-induced bronchoconstriction after chronic dosing with salmeterol. *Respir Med* 1994;**88**:363-8.
123. Giannini D, Carletti A, Dente FL, Bacci E, Bancalari L, Di Franco A, Vagaggini B, Paggiaro PL. Tolerance to salmeterol in allergen induced bronchoconstriction. *Am J Respir Crit Care Med* 1995;**151**:A39.
124. Bhagat R, Kalra S, Swystun VA, Cockcroft DW. Rapid onset of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1995;**108**:1235-9.
125. Cockcroft DW, Swystun VA. Functional antagonism: tolerance produced by inhaled beta-2-agonists. *Thorax* 1996;**51**:1051-6.
126. Kalra S, Swystun VA, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1996;**109**:953-6.
127. Booth H, Bish R, Walters J, Whitehead F, Walters EH. Salmeterol tachyphylaxis in steroid treated asthmatic subjects. *Thorax* 1996;**51**;1100-4.
128. Yates DH, Kharitonov SA, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled beta-2-agonist. *Am J Respir Crit Care Med* 1996;**154**:1603-7.
129. Yates DH, Sussman HS, Shaw MJ, Barnes PJ, Chung KF. Regular formoterol treatment in mild asthma: effect on bronchial responsiveness during and after treatment. *Am J Respir Crit Care Med* 1995;**152**:1170-4.
130. Gardiner PV, Ward C, Booth H, Allison A, Hendrick DJ, Walters EH. Effect of eight weeks of treatment with salmeterol on bronchoalveolar lavage inflammatory indices in asthmatics. *Am J Respir Crit Care Med* 1994;**150**:1006-11.
131. Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1994;**344**:219-24.
132. Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroid with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996;**153**:1481-8.
133. Mak JCW, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ. Protective effects of dexamethasone on isoproterenol-induced downregulation of pulmonary beta-2-receptors. *J Clin Invest* 1995;**9-6**:99-106.
134. Peters MJ, Adcock IM, Brown CR, Barnes PJ. Beta-adrenoceptor agonists interfere with glucocorticoid receptor DNA-binding in rat lung. *Eur J Pharmacol* 1995;**289**:275-81.
135. Hjemdahl P, Zetterlund A, Larsson K. Beta-2-agonist treatment reduces beta-2-sensitivity in alveolar macrophages despite corticosteroid treatment. *Am J Respir Crit Care Med* 1996;**153**:576-81.
136. Wong CS, Wahedna I, Pavord ID, Tattersfield AE. Effect of regular terbutaline and budesonide on bronchial reactivity to allergen challenge. *Am J Respir Crit Care Med* 1994;**150**:1268-73.
137. Wilding PJ, Clark MM, Osborne J, Bennett JA, Tattersfield AE. Effect of regular terbutaline on the airway response to inhaled budesonide. *Thorax* 1996;**51**:989-92.

Chapter Two

Effects of β_2-receptor agonists on airway responsiveness

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2

Effects of ß$_2$-receptor agonists on airway responsiveness

2.1 Summary

Airway hyper-responsiveness is one of the characteristics of asthma. It may be distinguished by airway hypersensitivity and an increase of the maximal response plateau. Short-acting β_2-agonists have an acute protective effect on airway sensitivity, which is shorter in duration than the bronchodilating effect, without affecting the maximal response plateau. Long-term treatment has no beneficial effect on airway responsiveness. A diminishment of the protection to methacholine- and histamine-induced airway obstruction and a rebound increase of this after cessation of continuous treatment have been reported.

Single doses of long-acting β_2-agonists give a prolonged protection against methacholine- and histamine-induced airway sensitivity of at least 12 hours. A small decrease in the maximal response plateau has been noted. During long-term treatment tolerance to the protecting effects develops, although significant protection remains and the bronchodilating effect is not influenced. So far, no rebound increase in airway sensitivity has been reported after cessation of continuous treatment.

2.2 The role of airway responsiveness and airway inflammation in asthma

Asthma is characterized by a variable degree of airway obstruction and airway hyper-responsiveness to several stimuli.[1] Although it seems likely that airway hyper-responsiveness and airway inflammation are closely related, the underlying mechanism remains unclear. Chronic airway inflammation is the result of epithelial tissue injury, extensive mediator release from inflammatory cells and nerve endings, increased vascular permeability, cell migration into the exudate and repair phenomena. The repair phase of the inflammatory response consists of basement membrane thickening, goblet and squamous cell metaplasia, connective tissue deposition and smooth muscle hypertrophy.[2] Biopsy studies in patients with stable asthma and airway hyper-responsiveness reveal chronic inflammatory changes of the airway wall.[3,4,5] Although a correlation was found between epithelial damage and the degree of airway responsiveness,[3,4] this could not be confirmed in a study comparing biopsies from asthma patients with airway hyper-responsiveness with biopsies from healthy individuals without airway hyper-responsiveness.[6]

Airway hyper-responsiveness can be distinguished in two components: airway hypersensitivity and excessive airway narrowing, which are probably the result of distinct underlying mechanisms.[7] It has been postulated that the maximal degree of airway narrowing determines the degree of obstruction and thereby the severity of symptoms. In patients with mild asthma there is a shift of the dose-response curve for methacholine to the left which reaches a plateau; moderate asthma is characterized by

a more pronounced leftward shift, but without a plateau, and the maximal degree of airway narrowing is higher than the plateau in patients with mild asthma (Figure 2.1).[8] There is a general relationship between the degree of airway sensitivity and the severity of asthma symptoms in groups of patients, but within subjects this relationship is rather weak.[9] For individual patients a direct correlation was found between the degree of airway sensitivity to a non-specific stimulus and the amount of allergen which could be tolerated.[10,11] The prognostic value of airway hyper-responsiveness was studied in adults as well as in children. In adults with chronic obstructive lung disease, airway sensitivity is associated with progressive loss of pulmonary function.[12] In children airway sensitivity is considered to be a risk factor for the outcome of childhood asthma into adulthood.[13] Treatment which aims at suppression of airway hyper-responsiveness seems therefore appropriate.

Airway responsiveness is usually measured to histamine or methacholine, using the

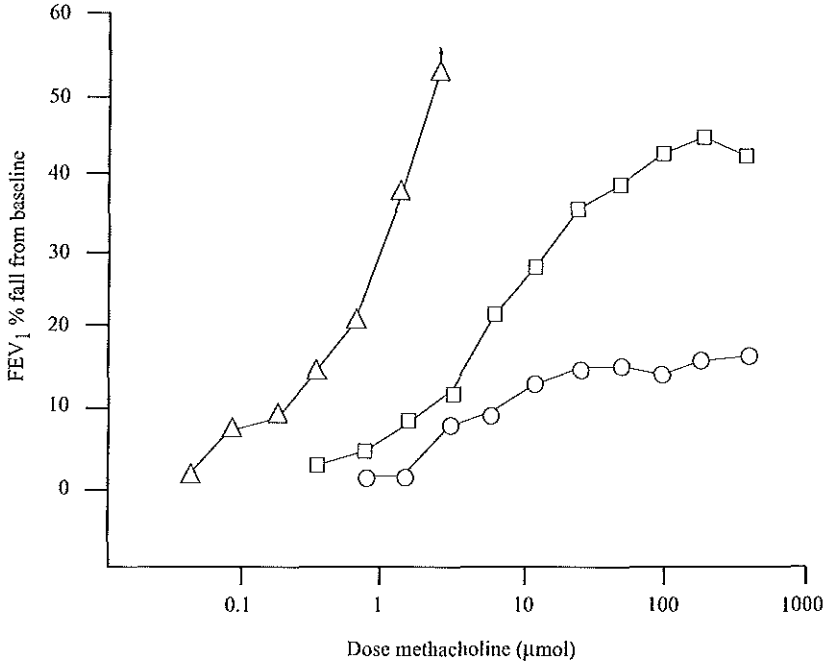

Figure 2.1
Dose-response curves to methacholine for normal, mild and moderate asthma patients. Moderate asthma (△); Mild asthma (□); Normal (○)

Table 2.1 Acute protection of short-acting β_2-agonists against airway sensitivity to histamine or methacholine.

Reference	Drug	Route	Dose (μg)	Interval (minutes)	Test	Mean change in PC_{20} or PD_{20} (DD)
Casterline et al (14)	salbutamol	IN	170	0	histamine	+3.6 (PD)
Cockcroft et al (15)	salbutamol	PO	4000	60	histamine	+1.1 (PC)
		IN	200	15	histamine	+3.6 (PC)
DeCotiis et al (16)	terbutaline	SC	5/kg	NS*	methacholine	+2.4 (PD)
Bandouvakis et al(17)	fenoterol	IN	800	45	histamine	+3 (PC)
					methacholine	+4 (PC)
Salome et al (18)	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(19)	salbutamol	IN	200	30	histamine	+2.5 (PD35)**
Salome et al (20)	fenoterol	IN	100	15	histamine	+1.8 (PD)
			200	15	histamine	+1.9 (PD)
			400	15	histamine	+3.1 (PD)
Chung et al(21)	salbutamol	IN	200	30	methacholine	+3 (PD35)
Ahrens et al (22)	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 (23)	salbutamol	IN	200	30	histamine	+2.8 (PD)
				240	histamine	0 (PD)
Britton et al (24)	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 (25)	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 (26)	salbutamol	IN	2500	40	histamine	+3.2 (PC)
				40	methacholine	+2.9 (PC)
Higgins et al (27)	salbutamol	IN	1855***	60	histamine	+2.3 (PD)

* NS = Not stated ** PD_{35} = PD_{35}sGaw *** Cumulative dose

dosimeter method or tidal breathing technique. Values are expressed as provocative dose (PD) or provocative concentration (PC) at which a certain (mostly 20%) decrease in baseline FEV_1 or specific airway conductance (sGaw) occurs. Changes after drug administration can be expressed as doubling doses (DD).
The effects of $ß_2$-agonists on airway responsiveness will be considered separately on airway sensitivity as well as on the maximal degree of airway narrowing. A distinction will be made between the effect after a single dose (acute protection) and the effect during long-term treatment. For acute protection the effect on allergen-induced airway hyper-responsiveness will also be mentioned.

2.3 Short-acting $ß_2$-agonists

2.3.1 Acute protection after a single dose

Numerous studies have examined the effect of a single dose of a short-acting $ß_2$-agonist on airway sensitivity (Table 2.1). A shift of the dose-response curve of histamine or methacholine to the right was found.[14-26] The maximum increase in PD_{20} or PC_{20} varied from 0.3 to 4.3 DD and was usually reached within 30 minutes after drug inhalation. The effect was more pronounced when the drug was inhaled than after oral administration.[15 18] After inhalation, a dose relationship has been found in several studies.[20,22,24] Few data exist on the duration of the protective effect. Salome et al. found that this was less than 3 hours in all subjects, whereas in most subjects the improvement in FEV_1 was well maintained 4 hours after drug administration.[20] Similar results were obtained by Ahrens et al.[22] and Joad et al.[23] This difference in the duration of the bronchodilating and protective effects suggests that the protection is not only a result of smooth muscle relaxation, but is also determined by other mechanisms. This is supported by in vitro studies in which relaxation of guinea pig trachea with ß-agonists did not affect the concentration-response relationship to carbachol.[28]
Data on the effect of short-acting $ß_2$-agonists on the maximal degree of airway narrowing are limited. Bel et al. did not find a protection against excessive airway narrowing after single doses of 200 and 400 μg salbutamol, despite an effect on the position of the dose-response curve.[29] On the contrary, a steepening of the slope of the dose-response curve was found.
Short-acting $ß_2$-agonists were generally held not to influence the late asthmatic reaction and the allergen-induced increase in airway responsiveness. However, two recent studies revealed an inhibition of the late asthmatic reaction after inhalation of single doses of salbutamol of 2.5[30] and 0.5 mg.[31] Two-and-a-half mg salbutamol also protected against the allergen-induced increase in airway sensitivity up to 7.5 hours after administration. Whether this is the result of functional antagonism or of other properties

Table 2.2 Long-term effect of short-acting inhaled β_2-agonists on airway sensitivity to histamine or methacholine.

Reference	Drug and frequency	Daily dose (μg)	Duration	Test	Interval	Mean change in PC_{20} or PD_{20} (DD)
Adults:						
Peel et al (32) n = 8	salbutamol q.i.d.	800	1 mo	histamine	>6 h 2 wk	-0.3(PC) +0.5(PC)
Kraan et al (33) 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(34) n = 15	salbutamol q.i.d.	1600	12 mo	histamine	>8 h	-0.3(PC)
Wiebicke et al (35) n = 12	salbutamol q.i.d.	800	3 wk	histamine methacholine	>6 h >6 h	+0.6(PC100)* 0(PC100)*
Haahtela et al (36) n = 43	terbutaline b.i.d.	750	22 mo	histamine	6 h	+0.3(PC15)**
Vathenen et al (37) n = 8	terbutaline t.i.d.	2150	2 wk	histamine	23 h	-1.5(PC)
Sears et al (38) n = 64	fenoterol q.i.d.	1600	24 wk	methacholine	>6 h	0.6(PC)***
Kerstjens et al (39) n = 91	terbutaline q.i.d.	2000	24 mo	histamine	>8 h	0(PC)
Children:						
Kerrebijn et al (40) 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 (41) 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 (42) 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 (43) n = 58	salbutamol t.i.d.	600	22 mo	histamine	> 8 h	0(PD)

* PC_{100} = provocative concentration which causes an increase in sRaw by 100%
** PC_{15} = provocative concentration which causes a decrease in FEV_1 by 15%
*** Difference between regular and on demand treatment

Table 2.3 Acute protection of long-acting β_2-agonists against airway sensitivity to histamine or methacholine.

Reference	Drug	Dose (μg)	Test	Interval (hours)	Mean change in PC_{20} or PD_{20} (DD)
Derom et al (46)	salmeterol	50	methacholine	1	+2.4 (PC)
				12	+1.6 (PC)
12 adults		100		1	+3.2 (PC)
				12	+2.4 (PC)
Campos Gongora et al(47)	salmeterol	50.*	histamine	1	+2.7 (PC)
				12	+1.8 (PC)
12 adults		50.**		1	+2.4 (PC)
				12	+1.5 (PC)
Cheung et al (48)	salmeterol	50	methacholine	1	+3.3 (PC)
12 adults					
Verberne et al(49)	salmeterol	50	methacholine	1	+3.8 (PD)
				12	+2.0 (PD)
20 children				24	+1.2 (PD)
Simons et al (50)	salmeterol	25	methacholine	0.5	+2.0(PC)
				12	+2.1(PC)
20 children	salmeterol	50		0.5	+2.8(PC)
				12	+2.5(PC)
Maconochie et al (51)	salmeterol	12.5	histamine	1	+1.5(PC)***
				12	+0.9(PC)***
8 adults		50		1	+1.7(PC)***
				12	+0.8(PC)***
		100		1	+2.6(PC)***
				12	+2.0(PC)***
Rabe et al (52)	salmeterol	50	methacholine	0.5	+3.6(PC)
				12	+2.5(PC)
12 adults				24	+1.9(PC)
Solèr et al (53) 13 adults	salmeterol	50	histamine	14	+1.1(PD)
Nix et al(56)	formoterol	12	methacholine	2	+1.1 (PC)
12 adults				5	+1.5 (PC)
Ramsdale et al (57)	formoterol	12	methacholine	0.5	+3.8 (PC)
				12	+2.6 (PC)
16 adults		24		0.5	+4.3 (PC)
				12	+3.0 (PC)
Becker et al (58)	formoterol	12	methacholine	0.5	+2.9 (PC)
				12	+1.7 (PC)
16 children		24		0.5	+3.6 (PC)
				12	+2.1 (PC)
Von Berg et al (59)	formoterol	12	histamine	2	+3.3 (PD_{100})****
				8	+2.7 (PD_{100})****
5 children					

Wong et al (54) 6 adults	formoterol	24	histamine	24	+1.2(PC)
Sovijärvi et al (55) 12 adults	formoterol	12	histamine	3	+1.8(PD)***
Rabe et al (52) 12 adults	formoterol	12	methacholine	0.5 12 24	+3.5(PC) +2.5(PC) +1.1(PC)

* metered dose inhaler
** dry powder inhaler
*** PC_{15} or PD_{15} histamine
**** PD_{100} = provocative dose which causes an increase in sGaw by 100%

of the drug is still the subject of debate.

2.3.2 Protection during long-term treatment

Table 2.2 summarizes the results of studies on airway sensitivity during long-term treatment with short-acting inhaled β_2-agonists in adults and children.[32-43] The duration of these studies varied from 2 weeks to 2 years. No significant changes in airway sensitivity were found after treatment periods from 2 weeks to 6 months by Peel et al.[32] and Wiebicke et al.[35] in adults and by Raes et al.[41] and Waalkens et al.[42] in children. The slight increases in airway sensitivity reported by Kraan et al.[33] and Kerrebijn et al.[40] are within the intra-subject reproducibility of the measurement and may be the result of selecting patients in a stable condition, so that the risk of deterioration is greater than the chance of improvement. Although statistically significant, the change in airway sensitivity in the 12 months study by Van Schayck et al. is small and unlikely to be of clinical importance.[34] Two long-term studies (22 and 24 months) in adult asthmatic patients comparing treatment with a β_2-agonist alone and in combination with an inhaled corticosteroid did not show an increase in airway sensitivity in the β_2-agonist group.[36,39] However, the results in both studies may have been influenced by the high drop-out rate (10/43 respectively 44/91) in the β_2-agonist only group, mainly because of symptoms. A 22-month study in children did not reveal an increase in airway sensitivity during treatment with 200 μg salbutamol 3 times daily, but in this study, too, there was a considerable drop-out rate because of symptoms.[43]

In two separate studies[37,44] a rebound increase in airway sensitivity of 1.5 and 1.65 DD was found 23 and 59 hours after the cessation of maintenance treatment with a β_2-agonist for periods of 2 and 3 weeks.

Vathenen et al.[37] as well as O'Connor et al.[45] reported a reduction of the protective effect of terbutaline against histamine- and methacholine-induced airway obstruction

Table 2.4 Long-term effect of inhaled long-acting β_2-agonists on airway sensitivity to histamine or methacholine

Reference	Drug (b.i.d)	Dose (μg)	Duration	Test	Interval (hours)	Mean change in PC_{20} or PD_{20} (DD)
Dahl et al (68) n=12	salmeterol	50	4 weeks	histamine	NS	+2.2(PC)
Roberts et al (69) n=12	salmeterol	50	6 weeks	methacholine	NS	+2.2(PC)
Cheung et al (48) n=12	salmeterol	50		methacholine	1	+3.3(PC)
			4 weeks		1	+1.0(PC)
			8 weeks		1	+1.0(PC)
Beach et al (70) n=10	salmeterol	50	6 weeks	methacholine	24	+0.0(PD)
					72	+0.2(PD)
Booth et al (71) n=10	salmeterol	50		methacholine	12	+0.9(PD)
			4 weeks		12	+1.2(PD)
			8 weeks		12	+0.7(PD)
Meijer et al (72) n=20 children	salmeterol	50		methacholine	8	+1.9(PC)
			1 week		8	+1.7(PC)
			8 weeks		8	+1.2(PC)
			16 weeks		12	+1.1(PC)
Bhagat et al (73) n=10	salmeterol	50		methacholine	1	+3.3(PC)
			2 days		1	+2.4(PC)
			3 days		1	+2.0(PC)
			4 days		1	+1.5(PC)
			5 days		24**	+1.9(PC)
Kalra et al (74) n=8	salmeterol	50		methacholine	1	+2.8(PC)
			2 days		1	+1.8(PC)
			3 days		1	+1.9(PC)
			4 days		1	+1.6(PC)
			5 days		24**	+1.7(PC)
Yates et al (75) n=12	salmeterol	50		methacholine	15***	+2.4(PC)
			1 week		15***	+1.0(PC)
	salmeterol+budesonide***	50		methacholine	15***	+3.4(PC)
			1 week		15***	+2.3(PC)
Booth et al (76) n=22	salmeterol	50		methacholine	1	+3.3(PD)
			4 weeks		1	+2.0(PD)
			8 weeks		1	+2.0(PD)
Verberne et al (77) n=15 children	salmeterol	50		methacholine	12	+1.7(PD)
			1 month		12	+0.6(PD)
			2 months		12	+1.0(PD)
			3 months		12	+0.6(PD)
			4 months		12	+0.8(PD)
Cheung et al (78) n=12	salmeterol	50		histamine	1	+3.4(PC)
			2 weeks		1	+1.5(PC)
			4 weeks		1	+1.5(PC)
	salmeterol+theophilline			histamine	1	+3.5(PC)

	*****		2 weeks		1	+1.8(PC)
			4 weeks		1	+1.7(PC)
Milot et al (79)	salmeterol	50		methacholine	1	+3.3(PC)
					12	+2.5(PC)
n=26			4 weeks		1	+1.7(PC)
					12	+1.1(PC)
	salmeterol+ics			methacholine	1	+2.4(PC)
	*****				12	+1.2(PC)
			4 weeks		1	+1.4(PC)
					12	+0.3(PC)
Woolcock et al (80)	salmeterol	50	8 weeks	histamine	NS*	+0.8(PC/PD)
			24 weeks		NS*	+0.8(PC/PD)
n=140	salmeterol	100	8 weeks	histamine	NS*	+0.6(PC/PD)
			24 weeks		NS*	+0.6(PC/PD)
Wong et al (81) n=14	salmeterol	50	4 days	methacholine	0.25	+1.6(PC)
Weersink et al (82) n=16	salmeterol	50	6 weeks	methacholine	7 (day)	+1.5(PC)
					7 (night)	+2.4(PC)
Yates et al (83) n=17	formoterol	24		methacholine	12	+1.9(PC)
			2 weeks		12	+0.5(PC)

* NS = Not stated
** 10 minutes after pretreatment with 200 μg salbutamol
*** 15 minutes after pretreatment with 200 μg salbutamol
**** salmeterol plus budesonide 800 μg b.i.d
***** salmeterol plus theophylline (mean serum level 9.9 $\pm$ 1.1 mg/l)
****** salmeterol plus inhaled corticosteroid treatment

after 14 and 7 days maintenance treatment compared with the first treatment day, despite a well-maintained bronchodilating effect. The latter study reported a greater reduction in the protection of bronchoconstriction induced by adenosine monophosphate than by methacholine, suggesting a more pronounced tolerance to the mast-cell stabilizing effects of a β_2-agonist than to the effects on bronchial smooth muscle. The clinical implications of the rebound phenomenon and the development of tolerance to non-bronchodilating effects of short-acting β_2-agonists are not yet clear.

2.4 Long-acting β_2-agonists

2.4.1 Acute protection after a single dose

Several studies have been published on the effects of single doses of salmeterol[46-52] and formoterol[52,54-59] on methacholine- and histamine-induced airway obstruction. The results are summarized in Table 2.3. Except in the study by Cheung et al. only the position of the dose-response curve (airway sensitivity) was studied. The peak increase in PD_{20} or PC_{20} varied between 1.1 and 3.8 DD for salmeterol and between 1.2 and 4.3 DD for

formoterol. Several studies suggest that the magnitude of the protection is dose-related for both salmeterol and formoterol.[46 50 51 57 58] The duration of protection at least lasts up to 12 hours after inhalation.[46 47 50 51 53 57 58] Three studies described a protective effect up to 24 hours after inhalation of either 50 μg salmeterol or 12 and 24 μg formoterol.[49 52 54] In the study in children, the maximum protective effect was reached one hour after inhalation (3.8 DD) and gradually decreased, being 2.0 DD at 12 hours and 1.2 DD at 24 hours. Nearly half of the children had PD_{20} measurements within the normal range up to 12 hours after inhalation.[49] In this study the effect on FEV_1 at 24 hours was no longer significantly different from that after placebo, although a residual bronchodilation seemed to persist. Comparable data were found in a study in adults with asthma for salmeterol as well as formoterol.[52 54]

After a single dose of 50 μg salmeterol a small decrease (3.2%) in the maximal response plateau was observed.[48]

The first study of salmeterol on allergen induced obstruction described an inhibition of the early and late phase reaction over 34 hours, together with an inhibition of the allergen induced rise in airway sensitivity to histamine.[60] Comparing the results after allergen challenge with those after saline, the authors conclude that the inhibition was not the result of prolonged bronchodilation or functional antagonism, but was due to other, anti-inflammatory mechanisms. Others have argued against this conclusion because, although airway sensitivity started at a higher baseline as a result of the bronchodilating effect of salmeterol, it showed a progressively lower increase during the late phase after allergen challenge.[61] Several studies now have been published regarding the effects of salmeterol[62 63 64 65] and formoterol[66 67] on the early and late responses after allergen challenge and the effects on blood and sputum eosinophils and activation markers. Except the study by Pedersen et al,[63] which showed minor effects on serum eosinophilic cationic protein and serum eosinophil protein X, none of these studies revealed anti-inflammatory effects of either salmeterol or formoterol. The overall conclusion from these studies is that salmeterol and formoterol are able to partially inhibit the late-asthmatic reaction due functional antagonism on airway smooth muscle, without affecting the anti-inflammatory response.

2.4.2 Protection during long-term treatment

Several studies, lasting from a few days to 24 weeks, have investigated the effects of salmeterol and formoterol on airway responsiveness during continuous treatment.[48 68-83] (Table 2.4) Depending on the interval between the last dose of the long-acting $ß_2$-agonist and the time of measurement these studies show protection against methacholine- and histamine induced bronchoconstriction varying between 0.6 and 2.4

DD up to 12 hours after the last dose. Cheung et al.[48] were the first to publish the development of tolerance to the bronchoprotective effect of salmeterol during treatment with twice daily 50 μg salmeterol for several weeks, despite the maintenance of the bronchodilating response. One hour after inhalation of salmeterol the protection dropped from 3.3 DD after the first dose to 1.0 DD after 4 and 8 weeks of continuous treatment. Except from the study by Booth et al.,[71] other studies have confirmed the development of tolerance for salmeterol as well as for formoterol.[73-79, 83] From the data now available, this seems to establish within the first days of treatment,[73] after which residual protection (between 1 and 2 DD) remains for at least four months.[77] Inhaled corticosteroids and theophylline do not prevent the development of tolerance.[74-76 78 83] Three studies also showed tolerance for the bronchoprotection by salbutamol after regular salmeterol treatment.[73-75] So far, the clinical relevance of this is not yet clear. Tolerance has also been described for the inhibition of salmeterol on indirect stimuli such as exercise,[84] cold air challenge[85] and allergen-induced bronchoconstriction[86] during continuous treatment for several weeks. After stopping maintenance treatment with either salmeterol or formoterol no rebound increase in airway responsiveness has been found.[48 59 69 70 83]

2.5 Conclusion

Airway hyper-responsiveness is an important characteristic of asthma, which may be related to the severity of asthma symptoms and the long-term outcome of asthma. It can be distinguished in airway sensitivity and the maximal response plateau. It is attractive to hypothesize that treatment regimens that aim at reducing airway responsiveness will contribute to a better long-term outcome of asthma. Short-acting β_2-agonists as well as two recently developed long-acting β_2-agonists, salmeterol and formoterol, have an acute protective effect on airway sensitivity, reflected in a right-ward shift of the dose-response curve, induced by non-specific stimuli such as histamine and methacholine. For short-acting β_2-agonists the duration of protection is only about 3 hours; for the long-acting β_2-agonists it may last 12-24 hours. No effect on the maximal degree of airway narrowing will occur, although after a single dose of salmeterol a small decrease in the maximal response plateau was noted.

Maintenance treatment with short-acting β_2-agonists will not decrease airway sensitivity; in fact, some studies have reported a small increase. The clinical relevance of this has been discussed over several years now, but so far continuous treatment with short-acting β_2-agonists has not been unequivocally related to deterioration of asthma. However, international guidelines do recommend to use these drugs on an as needed basis. Several studies with the long-acting β_2-agonists salmeterol and formoterol now have shown that

tolerance develops to the bronchoprotective effects after direct stimuli as histamine and methacholine as well as after indirect stimuli. This develops within several days after the start of continuous treatment, but residual protection remains. Tolerance can not be prevented by inhaled corticosteroids. Furthermore, continuous use of long-acting β_2-agonists also resulted in less bronchoprotection by inhaled salbutamol. Theoretically, this might have clinical implications when short-acting β_2-agonists are used intermittently together with a continuous long-acting β_2-agonist. As far as tolerance to the bronchodilator effect is concerned, this is less evident, although long-acting β_2-agonists may show a relatively small shift of the dose-response curve for short-acting β_2-agonists to the right without effecting the maximum bronchodilation.[87 88] There are no adverse clinical effects during prolonged treatment with long-acting β_2-agonists, such as an increase in number or severity of exacerbations.[89]

After treatment with a short-acting β_2-agonist for 1 or 2 weeks a rebound increase in airway sensitivity was found. This has not been found after discontinuation of treatment with long-acting β_2-agonists.

2.6 References

1. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; **136:** 225-44.
2. Hogg JC, James AL, Pare PD. Evidence for inflammation in asthma. *Am Rev Respir Dis* 1991; **143:** S39-42.
3. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. *Am Rev Respir Dis* 1989; **140:** 1745-53.
4. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985; **131:** 599-606.
5. Losewicz S, Gomez E, Wells C, Ferguson H, Richman P, Devalia J, Davies RJ. Airway inflammation changes in stable asthma. *Br Med J* 1988; **297:** 1515-6.
6. Losewicz S, Wells C, Gomez E, Ferguson H, Richman P, Davies RJ. Morphological integrity of the bronchial epithelium in mild asthma. *Thorax* 1990; **45:** 12-5.
7. Sterk PJ, Bel EH. Bronchial hyperresponsiveness: The need for a distinction between hypersensitivity and excessive airway narrowing. *Eur Respir J* 1989; **2:** 267-74.
8. Pee S de, Timmers MC, Hermans J, Duiverman EJ, Sterk PJ. Comparison of maximal airway narrowing to methacholine between children and adults. *Eur Respir J* 1991; **4:** 421-8.
9. Josephs LK, Gregg I, Holgate ST. Does non-specific responsiveness indicate the severity of asthma? *Eur Respir J* 1990; **3:** 220-7.
10. De Baets FM, Goeteyn M, Kerrebijn KF. The effect of two months of treatment with inhaled budesonide on bronchial responsiveness to histamine and house-dust mite antigen in asthmatic children. *Am Rev Respir Dis* 1990; **142:** 581-6.
11. Neijens HJ, Degenhart HJ, Raatgeep HC, Kerrebijn KF. Study on the significance of bronchial hyperreactivity in the bronchus obstruction after inhalation of cat dander allergen. *J Allergy Clin Immunol* 1979; **64:** 507-15.
12. Postma DS, de Vries K, Koëter GH, Sluiter HJ. Independent influence of reversibility of airflow obstruction and non-specific hyperreactivity on the long-term course of lung function in CAO. *Am Rev Respir Dis* 1986; **134:** 276-80.

13. Gerritsen J, Koëter GH, de Monchy JGR, van Lookeren Campagne JG, Knol K. Change in airway responsiveness to inhaled house dust from childhood to adulthood. *J Allergy Clin Immunol* 1990; **85:** 1083-9.
14. Casterline CL, Evans R, Ward GW. The effect of atropine and albuterol aerosols on the human bronchial response to histamine. *J Allergy Clin Immunol* 1976; **58:** 607-13.
15. Cockroft DW, Killian DN, Mellon JJA, Hargreave FE. Protective effect of drugs on histamine-induced asthma. *Thorax* 1977; **32:** 429-37.
16. De Cotiis BA, Braman SS, Corrao WM. The protective effect of theophylline and terbutaline on a methacholine inhalation challenge. *Am Rev Respir Dis* 1980; **121:** 125.
17. Bandouvakis J, Cartier A, Roberts R, Ryan G, Hargreave FE. The effect of ipratropium and fenoterol on methacholine- and histamine-induced bronchoconstriction. *Br J Dis Chest* 1981; **75:** 295-305.
18. Salome CM, Schoeffel RE, Woolcock AJ. Effect of aerosol and oral fenoterol on histamine and methacholine challenge in asthmatic subjects. *Thorax* 1981; **36:** 580-4.
19. Chung KF, Morgan B, Keyes SJ, Snashall PD. Histamine dose-response relationships in normal and asthmatic subjects. *Am Rev Respir Dis* 1982; **126:** 849-54.
20. Salome CM, Schoeffel RE, Yan K, Woolcock AJ. Effect of aerosol fenoterol on the severity of bronchial hyperreactivity in patients with asthma. *Thorax* 1983; **38:** 854-8.
21. Chung KF, Snashall PD. Methacholine dose-response curves in normal and asthmatic man: effect of starting conductance and pharmacological antagonism. *Clin Sci* 1984; **66:** 665-73.
22. Ahrens RC, Harris JB, Milavetz G, Annis L, Ries R. Use of bronchial provocation with histamine to compare the pharmacodynamics of inhaled albuterol and metaproterenol in patients with asthma. *J Allergy Clin Immunol* 1987; **79:** 876-82.
23. Joad JP, Ahrens RC, Lindgren S, Weinberger MM. Relative efficacy of maintenance therapy with theophylline, inhaled albuterol and the combination for chronic asthma. *J Allergy Clin Immunol* 1987; **79:** 78-85.
24. Britton J, Hanley SP, Garrett HV, Hadfield JW, Tattersfield AE. Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. *Thorax* 1988; **43:** 300-5.
25. Salome CM, Wright W, Sedgwick CJ, Woolcock AJ. Acute effects of fenoterol (Berotec) and ipratropium bromide (Atrovent) alone and in combination on bronchial hyperresponsiveness in asthmatic subjects. In: Black J, Armour C, eds. Mechanisms in asthma: *Pharmacology, physiology, and management.* Alan R. Liss Inc., New York, 1988: 405-19.
26. Phillips GD, Finnerty JP, Holgate ST. Comparative protective effect of the inhaled β_2-agonist salbutamol (albuterol) on bronchoconstriction provoked by histamine, methacholine, and adenosine 5'-monophosphate in asthma. *J Allergy Clin Immunol* 1990; **85:** 755-62.
27. Higgins BG, Powell RM, Cooper S, Tattersfield AE. Effect of salbutamol and ipratropium bromide on airway calibre and bronchial reactivity in asthma and chronic bronchitis. *Eur Respir J* 1991; **4:** 415-20.
28. Gustafsson B, Persson CGA. Effect of different bronchodilators on airway smooth muscle responsiveness to contractile agents. *Thorax* 1991; **46:** 360-5.
29. Bel EH, Zwinderman AH, Timmers MC, Dijkman JH, Sterk PJ. The protective effect of a β_2 agonist against excessive airway narrowing in response to bronchoconstrictor stimuli in asthma and chronic obstructive lung disease. *Thorax* 1991; **46:** 9-14.
30. Twentyman OP, Finnerty JP, Holgate ST. The inhibitory effect of nebulized albuterol on the early and late asthmatic reactions and increase in airway responsiveness provoked by inhaled allergen in asthma. *Am Rev Respir Dis* 1991; **144:** 782-7.
31. Palmqvist M, Balder B, Löwhagen O, Melander B, Svedmyr N, WÅhlander L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting β_2-agonist. *J Allergy Clin Immunol* 1992; **89:** 844-9.
32. Peel ET, Gibson GJ. Effects of long-term inhaled salbutamol therapy on the provocation of asthma

by histamine. *Am Rev Respir Dis* 1980; **121:** 973-8.
33. Kraan J, Koëter GH, van der Mark ThW, Sluiter HJ, de Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J Allergy Clin Immunol* 1985; **76:** 628-36.
34. Schayck CP van, Graafsma SJ, Visch MB, Dompeling E, van Weel C, van Herwaarden CLA. Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsentisization to salbutamol. *J Allergy Clin Immunol* 1990; **86:** 793-800.
35. Wiebicke W, Jörres R, Magnussen H. Comparison of the effects of inhaled corticosteroids on the airway response to histamine, methacholine, hyperventilation and sulfur dioxide in subjects with asthma. *J Allergy Clin Immunol* 1990; **86:** 915-23.
36. Haahtela T, Järvinen M, Kava T, Kiviranti K, Koskinen S, Lehtonen K, Nikander K, Persson T, Reinikainen K, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R, Laitinen L. Comparison of a $ß_2$-agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. *N Engl J Med* 1991; **325:** 388-92.
37. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988; **i:** 554-7.
38. Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery EM, Yates DM, Lucas MK, Herbison GP. Regular inhaled ß-agonist treatment in bronchial asthma. *Lancet* 1990; **336(ii):** 1391-6.
39. Kerstjens HAM, Brand PLP, Hughes MD, Robinson NJ, Postma DS, Sluiter HJ, Bleecker ER, Dekhuijzen PNR, de Jong PM, Mengelers HJJ, Overbeek SE, Schoonbrood DFME, and the Dutch Chronic Non-specific Lung Disease Study Group. A comparison of bronchodilator therapy with or without inhaled corticosteroid therapy for obstructive airways disease. *New Engl J Med* 1992; **327:** 1413-9.
40. Kerrebijn KF, van Essen-Zandvliet EEM, Neijens HJ. Effects of long-term treatment with inhaled corticosteroids and ß-agonists on the bronchial responsiveness in asthmatic children. *J Allergy Clin Immunol* 1987; **79:** 653-9.
41. Raes M, Mulder P, Kerrebijn KF. Long-term effect of ipratropium bromide and fenoterol on the bronchial hyperresponsiveness to histamine in children with asthma. *J Allergy Clin Immunol* 1989; **84:** 874-9.
42. Waalkens HJ, Gerritsen J, Koëter GH, Krouwels FH, van Aalderen WMC, Knol K. Budesonide and terbutaline or terbutaline alone in children with mild asthma: effects on bronchial hyperresponsiveness and diurnal variation in peak flow. *Thorax* 1991; **46:** 499-503.
43. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF, and the Dutch Chronic Non-specific Lung Disease Study Group. Effects of 22 months treatment with inhaled corticosteroids and/or $ß_2$-agonists on lung function, airway responsiveness, and symptoms in children with asthma. *Am Rev Respir Dis* 1992; **146:** 547-54.
44. Wahedna I, Wisniewski A, Wong C, Pavord I, Tattersfield AE. Airway effects of regular broxaterol and salbutamol treatment in asthmatic subjects. *Thorax* 1991; **46:** 770P.
45. O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled $ß_2$-agonists in asthma. *New Engl J Med* 1992; **327:** 1204-8.
46. Derom EY, Pauwels RA, Van Der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992; **89:** 811-5.
47. Campos Gongora H, Wisniewski AFZ, Tattersfield AE. A single-dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991; **144:** 626-9.
48. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting $ß_2$-adrenoceptor agonist, salmeterol, on airway responsiveness in patients with mild asthma. *New Engl J Med* 1992; **327:** 1198-203.
49. Verberne AAPH, Hop WCJ, Bos AB, Kerrebijn KF. Effect of a single dose of inhaled salmeterol

on baseline airway calibre and methacholine-induced airway obstruction in asthmatic children. *J Allergy Clin Immunol* 1993; **91**: 127-34.

50. Simons FER, Soni NR, Watson WTA, Becker AB. Bronchodilator and bronchoprotective effects of salmeterol in young patients with asthma. *J Allergy Clin Immunol* 1992;**90**:840-6
51. Maconochie JG, Denyer LH, Hassani H. Dose-related protection against histamine-induced bronchoconstriction by inhaled salmeterol. *Int J Clin Pharmacol Ther* 1994;**32**:329-34
52. Rabe KF, Jörres R, Nowak D, Behr N, Magnussen H. Comparison of the effects of salmeterol and formoterol on airway tone and responsiveness over 24 hours in bronchial asthma. *Am Rev Respir Dis* 1993;**147**:1436-41
53. Solèr M, Joos L, Bolliger CT, Elsasser S, Perruchoud AP. Bronchoprotection by salmeterol: cell stabilization or functional antagonism? Comparative effects on histamine- and AMP-induced bronchoconstriction. *Eur Respir J* 1994;7:1973-7
54. Wong BJ, Dolovich J, Ramsdale EH, O'Byrne P, Gontovnick L, Denburg JA, Hargreave FE. Formoterol compared with beclomethasone and placebo on allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992;**146**:1156-60
55. Sovijärvi ARA, Reinikainen K, Freudenthal Y, Andersson P, Riska H. Preventive effects of inhaled formoterol and salbutamol on histamine-induced bronchoconstriction - a placebo-controlled study. *Respiration* 1992;**59**:279-82
56. Nix A, Nichol GM, Robson A, Barnes PJ, Chung KF. Effect of formoterol, a long-lasting β_2-adrenoceptor agonist, against methacholine-induced bronchoconstriction. *Br J Clin Pharmacol* 1990; **29**: 321-4.
57. Ramsdale EH, Otis J, Kline PA, Gontovnick LS, Hargreave FE, O'Byrne PM. Prolonged protection against methacholine-induced bronchoconstriction by the inhaled β_2-agonist formoterol. *Am Rev Respir Dis* 1991; **143**: 998-1001.
58. Becker AB, Simons FER, McMillan JL, Faridy T. Formoterol, a new long-acting selective β_2-adrenergic receptor agonist: Double-blind comparison with salbutamol and placebo in children with asthma. *J Allergy Clin Immunol* 1989; **84**: 891-5.
59. Von Berg A, Berdel D. Efficacy of formoterol metered aerosol in children. *Lung* 1990; (suppl.): 90-8.
60. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990; **336**: 1338-42.
61. Britton J, Pavord I, Wong C, Tattersfield AE. β_2-agonists in asthma (Letter). *Lancet* 1991; **337**: 300-1.
62. Taylor IK, O'Shaughnessy KM, Choudry NB, Adachi M, Palmer JBD, Fuller RW. A comparative study in atopic subjects with asthma of the effects of salmeterol and salbutamol on allergen-induced bronchoconstriction, increase in airway reactivity, and increase in urinary leukotriene E_4 excretion. *J Allergy Clin Immunol* 1992; **89**: 575-83.
63. Pedersen B, Dahl R, Larsen BB, Venge P. The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X. *Allergy* 1993;**48**:377-82.
64. Weersink EJM, Aalbers R, Koëter GH, Kauffman HF, De Monchy JGR, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of salmeterol. *Am J Respir Crit Care Med* 1994;**150**:1262-7.
65. Pizzichini MMM, Kidney JC, Wong BJO, Morris MM, Efthimiadis A, Dolovich J, Hargreave FE. Effect of salmeterol compared with beclomethasone on allergen-induced asthmatic and inflammatory responses. *Eur Respir J* 1996;**9**:449-55.
66. Wong BJ, Dolovich J, Ramsdale EH, O'Byrne P, Gontovnick L, Denburg JA, Hargreave FE. Formoterol compared with beclomethasone and placebo on allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992;**146**:1156-60.
67. Palmqvist M, Balder B, Löwhagen O, Melander B, Svedmyr N, Wählander L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting β_2-agonist.

J Allergy Clin Immunol 1992;**89**:844-9.
68. Dahl R, Pedersen B, Venge P. Bronchoalveolar lavage studies. *Eur Respir Rev* 1991; **1**(4): 272-5.
69. Roberts JA, Bradding P, Holgate ST, Howarth PH. Effects of a six week course of salmeterol on bronchial reactivity. *Thorax* 1992; **47**: 230P.
70. Beach JR, Young CL, Harkawat R, Gardiner PV, Avery AJ, Coward GA, Walters EH, Hendrick DJ. Effect on airway responsiveness of six weeks treatment with salmeterol. *Pulm Pharmacol* 1993;**6**:155-7.
71. Booth H, Fishwick K, Harkawat R, Devereux G, Hendrick DJ, Walters EH. Changes in methacholine induced bronchoconstriction with the long acting $ß_2$-agonist salmeterol in mild to moderate asthmatic patients. *Thorax* 1993;**48**:1121-4.
72. Meijer GG, Postma DS, Mulder PGH, Van Aalderen WMC. Long-term circadian effects of salmeterol in asthmatic children treated with inhaled corticosteroids. *Am J Respir Crit Care Med* 1995;**152**:1887-92.
73. Bhagat R, Kalra S, Swystun VA, Cockcroft DW. Rapid onset of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1995;**108**:1235-9.
74. Kalra S, Swystun VA, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1996;**109**:953-6.
75. Yates DH, Kharitonov SA, Barnes PJ. An inhaled glucocorticosteroid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled $ß_2$-agonist. *Am J Respir Crit Care Med* 1996;**154**:1603-7.
76. Booth H, Bish R, Walters J, Whitehead F, Walters EH. Salmeterol tachyphylaxis in steroid treated asthmatic subjects. *Thorax* 1996;**51**:1100-4.
77. Verberne AAPH, Hop WCJ, Creyghton FBM, Van Rooij RWG, Van den Berg M, De Jongste JC, Kerrebijn KF. Airway responsiveness after a single dose of salmeterol and during four months of treatment in children with asthma. *J Allergy Clin Immunol* 1996;**97**:938-46.
78. Cheung D, Wever AMJ, De Goeij JA, De Graaff CS, Engelstätter R, Steen H, Sterk PJ. The effect of theophylline on tolerance to the bronchoprotective action of salmeterol in asthmatics in vivo. *Am J Respir Crit Care Med* 1996;**153**:A805.
79. Milot J, Boulet LP, Laviolette M, Côté J, Malo JL, Cartier A. Tolerance to the salmeterol protective effects on methacholine-induced bronchoconstriction and influence of inhaled corticosteroids. *Am J Respir Crit Care Med* 1996;**153**:A804.
80. Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996;**153**:1481-8.
81. Wong AG, O'Shaughnessy AD, Walker CM, Sears MR. Effects of long-acting and short-acting ß-agonists on methacholine dose-response curves in asthmatics. *Eur Respir J* 1997;**10**:330-6.
82. Weersink EJM, Douma WR, Postma DS, Koëter GH. Fluticasone propionate, salmeterol xinafoate, and their combination: treatment of nocturnal asthma. *Am J Respir Crit Care Med* 1997;**155**:1241-6
83. Yates DH, Sussman HS, Shaw MJ, Barnes PJ, Chung KF. Regular formoterol treatment in mild asthma. *Am J Respir Crit Care Med* 1995;**152**:1170-4.
84. Ramage L, Lipworth BJ, Ingram CG, Cree JA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Respir Med* 1994;**88**:363-8.
85. Gustafsson P, Ekström T, Forsström E, Rimás M. Bronchial hyperresponsiveness and protection by salmeterol against bronchial cold air challenge during regular salmeterol treatment in steroid treated asthmatics. *Eur Respir J* 1995;**8**:455s.
86. Giannini D, Carletti A, Dente FL, Bacci E, Bancalari L, Di Franco A, Vagaggini B, Paggiaro PL. Tolerance to salmeterol in allergen induced bronchoconstriction. *Am J Respir Crit Care Med* 1995;**151**:A39.
87. Newnham DM, Grove A, McDevitt DG, Lipworth BJ. Subsensitivity of bronchodilator and systemic $ß_2$-adrenoceptor responses after regular twice daily treatment with eformoterol dry powder in asthmatic patients. *Thorax* 1995;**50**:497-504.

88. Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. *Lancet* 1995;**346**:201-6.
89. Devoy MAB, Fuller RW, Palmer JBD. Are there any detrimental effects of the use of inhaled long-acting $ß_2$-agonists in the treatment of asthma? *Chest* 1995;**107**:1116-2450.

Chapter Three

The role of inhaled long-acting bronchodilator therapy

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3

The role of inhaled long-acting bronchodilator therapy

3.1 Summary

Inhalation of a single dose of salmeterol or formoterol results in prolonged bronchodilation for at least 12 h and also protects against various bronchoconstricting stimuli, such as methacholine, exercise and allergens. This suggests that twice daily salmeterol and formoterol may result in reduced asthma symptoms and improved lung function during chronic treatment.

Studies of twice daily treatment with salmeterol for up to 12 months have shown an improvement in symptoms and peak flow measurements, and no increase in exacerbation rate or worsening of asthma has been noted. Although some reduction of the protection against methacholine-induced bronchoconstriction was found in adults and children during long-term treatment with salmeterol alone, significant protection remained and showed no further reduction over time. Inhaled corticosteroids are the mainstay of asthma treatment in children with moderate-to-severe asthma, according to international consensus reports. Results from a recently published adult study found that the addition of salmeterol to low-dose inhaled corticosteroid compared favourably with increasing the corticosteroid dose.

Further studies are required to determine the place of long-acting bronchodilators in the management of childhood asthma.

3.2 Introduction

Childhood asthma leads to considerable morbidity, but in Western Europe, at least, mortality rates are very low. Hospital admissions for asthma have increased, especially in the young age group.[1] Most children with asthma suffer from exercise-induced and/or nocturnal symptoms and frequently miss school because of their asthma.[2] Attention should be paid not only to preventing the disease and provocating stimuli, but also to effective symptom control. Inflammatory changes in the airway wall mucosa are regarded as the pathophysiological basis of the disease[3] and, according to recent reports, inhaled corticosteroids are the mainstay of asthma treatment, particularly for children with moderate-to-severe asthma.[4 5] It might, therefore, be hypothesized that early intervention in the inflammatory process in young children with asthma prevents deterioration or, eventually, completely reverses the disease.

So far, the study with the longest follow-up has shown an improvement of symptoms, airway obstruction and bronchial responsiveness during 3 yrs of treatment with an inhaled corticosteroid (budesonide, 600 μg day) plus a short-acting β_2-agonist three times daily in children with mild-to-moderate asthma.[6 7] During the study period, 60% of the children achieved a period of symptomatic remission, which was defined as a symptom-free period during which no additional bronchodilator therapy was required

lasting for at least 8 months. Nearly 70% of children relapsed from their remission during the study, however, and less than 50% of the children with a symptomatic remission at the end of the study period had forced expiratory volume in one second (FEV_1) values and histamine responsiveness in the normal range. In a follow-up study in which the inhaled corticosteroid therapy was tapered off, there was a rapid reappearance of symptoms and deterioration in lung function.[8] Thus, with current medication regimens, it seems impossible to cure asthma. Moreover, most children are not symptom-free and show lung function abnormalities.

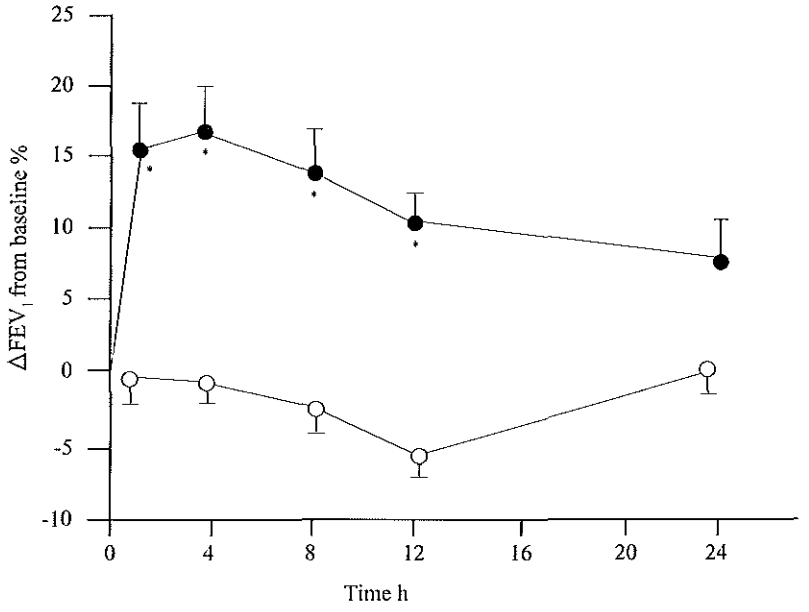

Figure 3.1
Bronchodilator action of a single dose of salmeterol (—●—), 50 μg, compared with placebo (—○—) in children. *p ≤ 0.001

Recently, two inhaled long-acting bronchodilators, salmeterol and formoterol, have become available. Their long duration of action makes twice daily dosing possible, which may reduce symptoms and improve lung function over the 24 h period.

3.3 Clinical studies

3.3.1 Single dose studies

The first study of salmeterol in adults with asthma showed that a single dose produced

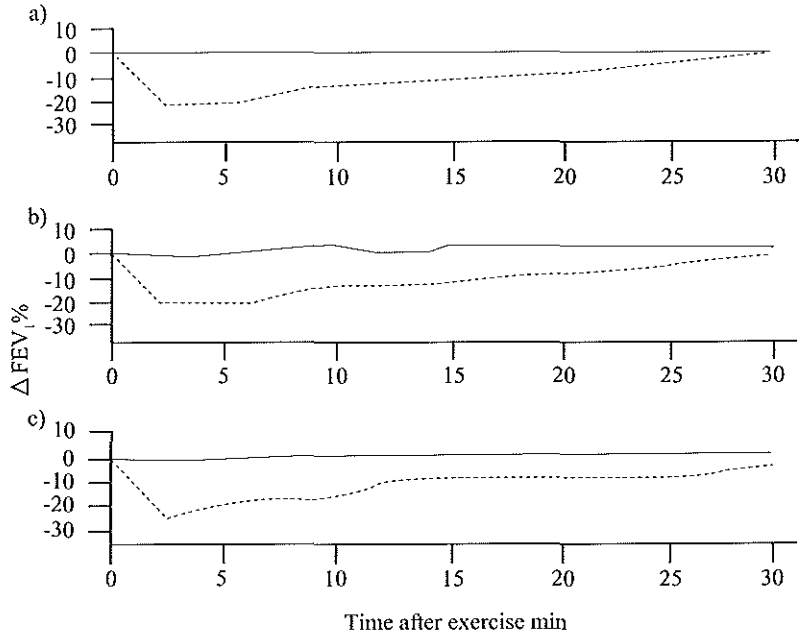

Figure 3.2
Effect of salmeterol (——), 50 μg, compared with placebo (·····), on exercise-induced bronchoconstriction in children with asthma. Exercise challenges were performed: a) 1 hour; b) 5 hours; c) 9 hours after administration of salmeterol or placebo.

bronchodilation, determined from measurements of peak expiratory flow rates lasting up to 12 h after dosing.[9] The duration of the bronchodilatory effect has also been investigated in children with asthma. Single doses of salmeterol, 25 and 50 μg, produced significant bronchodilation lasting for up to 12 h after inhalation, and the effect had not completely disappeared 24 h after dosing (Fig. 3.1).[10 11] The onset of the effect was slower for salmeterol than for salbutamol; however, 30 min after inhalation, the effect of both treatments was equal. From 3-12 h after inhalation, the effect of salmeterol was superior to salbutamol.[10] Single doses of formoterol, 12 μg and 24 μg, also produced long-lasting bronchodilation in children.[12]

The duration of protection against exercise-induced bronchoconstriction has been studied in 13 children with asthma for up to 12 hours after inhalation.[13 14] Salmeterol, 50 μg,

reduced the exercise-induced fall in FEV_1 in all children at all time points (Figure 3.2). Formoterol showed significant protection up to 12 h after dosing.[14] Protection against methacholine-induced bronchoconstriction has been investigated in two paediatric cross-over studies, with 20 children in each study.[10 11] Twelve hours after inhalation of salmeterol airway responsiveness to methacholine was reduced by about two DD, and 24 h after inhalation there was still significant protection of more than one DD (Figure 3.3). These results are comparable with the results from adult studies for protection against exercise[15 16] and methacholine.[17 18] In adults, long-lasting protection has also been found against bronchoconstriction caused by allergens,[19 20] histamine[21] and hyperventilation.[22] No data for these bronchoconstrictors are available in children.
In conclusion, single doses of salmeterol in children result in prolonged bronchodilation and protection against bronchoconstrictors, such as methacholine and exercise.

3.3.2 Multiple dose studies
Salmeterol and formoterol are recommended for use as regular preventive therapy. Results for continuous treatment are, therefore, more relevant clinically than single dose studies. Data from paediatric patients are beginning to appear.
A double-blind, parallel-group multicentre study of 847 children with asthma has compared the effect of salmeterol, 25 and 50 μg b.i.d., with salbutamol, 200 μg b.i.d., during 12 months of treatment.[23] Treatment was given either by a metered-dose inhaler (MDI) or by a dry powder inhaler (Diskhaler™). More than half of the study population was receiving inhaled corticosteroid treatment and another 18% were using sodium cromoglycate when they entered the study. These treatments were unchanged during the study period.
Mean morning and evening peak flows improved in the first week of treatment with salmeterol and were sustained during the 3 month observation period. Salmeterol, 50 μg b.i.d., was significantly better than salbutamol, 200 μg b.i.d., in terms both of morning and evening peak flow rates. Children receiving salmeterol, 50 μg b.i.d., had slightly more symptom-free nights and more days without rescue bronchodilator therapy compared with the salbutamol groups. There were no changes in the rate of asthma exacerbations during the 12 month period in either treatment group. The Diskhaler™ and MDI were equally effective for the same dose.
In another study, salmeterol, 100 μg b.i.d., was added to inhaled corticosteroid treatment in 11 children with severe asthma who attended a residential school for asthma. A significant improvement in morning and evening peak flow rates and FEV_1 values was seen in the salmeterol group during the 8 weeks of treatment.[24] There was a trend for improvement in all symptom parameters.

The effect of long-term treatment with salmeterol on airway responsiveness has been studied in a double-blind, parallel-group study comparing salmeterol, 50 μg b.i.d., with salbutamol, 200 μg b.i.d., in 30 children.[25] Throughout the 4 month treatment period, a constant significant protection against methacholine challenge was observed with salmeterol compared with baseline values and salbutamol. The protective effect during chronic treatment, however, was significantly less than the effect after the first dose of salmeterol (0.7 and 1.7 DD, respectively). This reduction of protection has also been observed by Cheung et al[26] in adults with asthma, but was not seen in another study by Booth et al[27]. It is of interest that both studies which showed a reduction of protection were performed in asthmatics not receiving concomitant inhaled corticosteroid therapy, whilst in the study in which the protective effect was maintained[27], most of the participants were receiving maintenance treatment with inhaled corticosteroids. The clinical relevance of this effect is not yet clear. Tolerance to the protective effect against exercise-induced bronchoconstriction has also been shown.[28] Recently, for salmeterol, sub-sensitivity was suggested after two weeks of treatment. However, this study has been extensively criticized for methodological reasons.[29]
In summary, salmeterol, 50 μg b.i.d., has been shown to reduce symptoms and improve lung function in children with asthma. The protection against methacholine challenge is less during prolonged treatment than after a single dose. Significant protection seems to remain and there are no signs of further deterioration over periods of several months.

3.4 Adverse events

The most commonly reported adverse events in children receiving long-term treatment with salmeterol, 50 μg b.i.d., were upper respiratory tract infections and related symptoms.[23] [25] Pharmacologically predictable adverse events, such as tremor, tachycardia and palpitations, were low, as was headache. No clinical significant effects on heart rate and rhythm were noted during 24 h electrocardiographic monitoring.[30]

3.5 Place in asthma treatment

3.5.1 In general

The data now available for children are similar to those for adults and show a reduction of asthma symptoms and improvement in lung function, as determined by measurement of peak flow or FEV_1. A reduction in protection against methacholine challenge has been observed in one paediatric and one adult study but its clinical relevance, if any, is unclear. Tolerance to nonpulmonary effects, such as tremor, increased QTc interval and elevated blood glucose, has been observed after 2 weeks of treatment with salmeterol in healthy individuals.[31] There are no data to suggest that an increase in

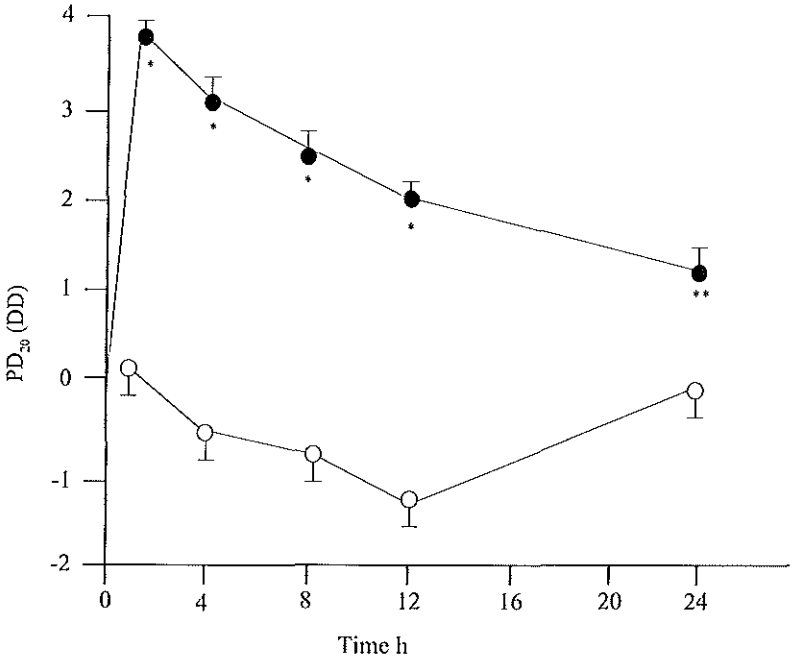

Figure 3.3
Protective effect of salmeterol (–●–), 50 µg, compared with placebo (–○–), against methacholine challenge in children with asthma. *p: ≤0.001; **p: ≤ 0.01

exacerbation rate or worsening of asthma control may occur during continuous treatment with salmeterol. There is still some debate about the continuous use of short-acting $ß_2$-agonists,[32] and a rebound increase in airway responsiveness has been described for these agents.[34 35] The data available for salmeterol show that a clinically important rebound phenomenon is unlikely.[25 26 35]

3.5.2 Exercise-induced asthma

Exercise-induced asthma is thought to occur in up to 90% of asthmatic patients and is particularly common in children because of their heightened and often unstructured levels of physical activity. Its psychosocial effects are also more pronounced in childhood because of the importance of physical activity, whether as organized sport or

play, to the development of the child and the importance society places on performing well. While short-acting bronchodilators have demonstrated effectiveness in relieving exercise-induced symptoms and can also be used before exercise to reduce its effects,[36] the extended duration of action of the long-acting bronchodilators suggests that they may be particularly well suited for the management of exercise-induced asthma.

The effects of long-acting bronchodilators in exercise-induced asthma have been clearly demonstrated for salmeterol in a large study involving 161 adults[37] and in two smaller studies in children. In one double-blind cross-over placebo-controlled study in 13 children who suffered from exercise-induced asthma, salmeterol was demonstrated to have a protective effect during exercise tests for up to 9 h after inhalation.[13] In a similar study involving 17 children, salmeterol was found to protect 65% of children for 6.5 h after inhalation.[38] In contrast, salbutamol provided protection against bronchospasm for 30 min but not at 2.5 h or longer.

Similar results have been shown for formoterol (12 μg) in a study of 15 asthmatic children (aged 6-12 years) with exercise-induced bronchoconstriction.[14] Formoterol was found to be significantly better in limiting exercise-induced bronchoconstriction than both salbutamol (200 μg) and placebo in exercise tests performed 3 and 12 hours after dosing.

3.5.3 Anti-inflammatory effects

Although anti-inflammatory effects of salmeterol have been well described *in vitro*,[39-42] no influence on chronic airway wall inflammation could be detected after 6 or 8 weeks of b.i.d. treatment with salmeterol in adults with asthma.[42,43] In the absence of studies comparing long-acting bronchodilators with prophylactic treatment using inhaled corticosteroids or sodium-cromoglycate, it would be best to use these drugs only in addition to inhaled corticosteroid treatment. One should also realize that most patients in the previously mentioned long-term studies were receiving prophylactic treatment.

The most urgent question at this time seems to be whether patients who are poorly controlled with a conventional dose of inhaled corticosteroids would gain more benefit from the addition of a long-acting bronchodilator to the conventional dose of inhaled corticosteroid than increasing the dose of inhaled corticosteroid. This is an important issue, particularly in children where the potential risks of inhaled corticosteroids may be more significant than in adults.

Recently, two adult studies have addressed this question. Greening et al[44] studied 429 adults with asthma who still had symptoms despite maintenance treatment with inhaled beclomethasone dipropionate (BDP), 200 μg b.i.d. Patients were randomized to receive either salmeterol, 50 μg b.i.d., in addition to BDP, 200 μg b.i.d., or a higher dose of

inhaled corticosteroid (BDP, 500 μg b.i.d.) for a 6 month period. Mean morning and evening peak flows were higher in the salmeterol treated group; there was also a significant reduction in diurnal variation. Use of rescue bronchodilator and daytime and nighttime symptoms were lower in the salmeterol group.
Another study by Woolcock[45] has compared the addition of salmeterol in two doses, 50 μg and 100 μg b.i.d., to BDP, 500 μg b.i.d., with BDP, 1000 μg b.i.d. Both salmeterol groups were superior regarding morning and evening peak flow measurements and certain symptom scores[34]. Airway responsiveness was measured in part of the study population; no changes of more than one DD from baseline were detected in either treatment group. Both studies lack a control group in which the inhaled corticosteroid dose was not increased.

3.6 Conclusion

Further studies in asthmatic children will be necessary to define the place of long-acting bronchodilators in the treatment of childhood asthma. It may well be that children, especially those who usually have good reversibility of bronchoconstriction, may benefit more from adding a long-acting bronchodilator to a conventional dose of inhaled corticosteroids than from increasing the dose of inhaled corticosteroid.

3.7 References

1. Anderson HR. Increase in hospital admissions for childhood asthma: trends in referral, severity, and readmissions from 1970 to 1985 in a health region of the United Kingdom. *Thorax* 1989;**44**:614-9.
2. Lenney W, Pedersen S, Boner AL, et al. Efficacy and safety of salmeterol in asthma. Submitted.
3. Djukanovic R, Roche WR, Wilson JW, et al. Mucosal inflammation in asthma (State of the art). *Am Rev Respir Dis* 1990;**142**:434-57.
4. Woodhead M (ed.). Guidelines on the management of asthma. *Thorax* 1993;**48**(2):S1-S24.
5. Warner JO. Asthma: a follow up statement from an international paediatric asthma consensus group. *Arch Dis Child* 1992;**67**:240-8.
6. Essen-Zandvliet EEM van, Hughes MD, Waalkens HJ, et al. Effects of 22 months treatment with inhaled corticosteroids and/or β_2-agonists on lung function, airway responsiveness and symptoms in children with asthma. *Am Rev Respir Dis* 1992;**146**:547-54.
7. Essen-Zandvliet EEM van, Hughes MD, Waalkens HJ, et al. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide). Can it be achieved? *Eur Respir J* 1994;**7**(1):63-8.
8. Waalkens HJ, Essen-Zandvliet EEM van, Hughes MD, et al. Cessation of long-term treatment with inhaled corticosteroid (budesonide) in children with asthma results in rapid deterioration. *Am Rev Respir Dis* 1993;**148**(5):1252-7.
9. Ullman A, Svedmyr N. Salmeterol, a new long acting inhaled beta 2 adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988;**43**(9):674-8.
10. Simons FER, Soni NR, Watson WTA, Becker AB. Bronchodilator and bronchoprotective effects of salmeterol in young patients with asthma. *J Allergy Clin Immunol* 1992;**90**:840-6.
11. Verberne AAPH, Hop WCJ, Bos AB, Kerrebijn KF. Effect of a single dose of inhaled salmeterol on baseline airway caliber and methacholine-induced airway obstruction in asthmatic children. *J*

Allergy Clin Immunol 1993;**91**:127-34.

12. Graff-Lonnevig V, Browaldh L. Twelve hours bronchodilating effect of inhaled formoterol in children with asthma: a double-blind cross-over *versus* salbutamol. *Clin Exp Allergy* 1990;**20**:429-32.
13. Green CP, Price JF. Prevention of exercise induced asthma by inhaled salmeterol xinafoate. *Arch Dis Child* 1992;**67**:1014-7.
14. Boner A, Spezia E, Piovesan P, Chiocca E, Maiocchi G. Inhaled formoterol in the prevention of exercise-induced bronchoconstriction in asthmatic children. *Am J Respir Crit Care Med* 1994;**149**:935-9.
15. Dhillon DP. Studies in exercise-induced asthma. *Eur Respir Rev* 1991;**1**(4):265-7.
16. Anderson SD, Rodwell LT, Du Toit J, Young IH. Duration of protection by inhaled salmeterol in exercise-induced asthma. *Chest* 1991;**100**(5):1254-60.
17. Derom EY, Pauwels RA, Vanderstraeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992;**89**:811-5.
18. Rabe KF, Jörres R, Nowak D, Behr N, Magnussen H. Comparison of the effects of salmeterol and formoterol on airway tone and responsiveness over 24 hours in bronchial asthma. *Am Rev Respir Dis* 1993;**147**:1436-41.
19. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;**336**(8727):1338-42.
20. Palmqvist M, Balder B, Lōwhagen O, Melander B, Svedmyr N, Wählender L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting $ß_2$-agonist. *J Allergy Clin Immunol* 1992;**89**:844-9.
21. Campos Gongora H, Wisniewski A, Britton J, Tattersfield AE. Single dose comparison of inhaled salmeterol and salbutamol on airway reactivity in asthmatic patients. *Eur Respir J* 1990;**3**:114s.
22. Malo JL, Ghezzo H, Trudeau C, L'Archévêque J, Cartier A. Salmeterol, a new inhaled $ß_2$-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *J Allergy Clin Immunol* 1992;**89**(2):567-74.
23. Boner A. Salmeterol: long-term studies in children. *Eur Resp J* 1992;**5**:318s.
24. Langton Hewer S, Hobbs J, French D, Lenney W. Pilgrim's progress: the effect of salmeterol in chronic severe asthma. Submitted.
25. Verberne AAPH, Hop WCJ, Creighton FBM, Van Rooij RWG, Van den Berg M, De Jongste JC, Kerrebijn KF. Airway responsiveness after a single dose of salmeterol and during four months of treatment children with asthma. *J Allergy Clin Immunol* 1996;**97**:938-46.
26. Cheung D, Timmers MC, Zwinderman AH, et al. Long-term effects of a long-acting $ß_2$-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *New Eng J Med* 1992;**327**:1198-203.
27. Booth H, Fishwick K, Harkawat R, et al. Changes in methacholine induced bronchoconstriction with the long acting $ß_2$ agonist salmeterol in mild to moderate asthmatic patients. *Thorax* 1993;48:1121-4.
28. Ramage L, Lipworth BJ, Ingram CG, Cree IA, Dhillon DP. Reduced protection against exercise-induced bronchoconstriction after chronic dosing with salmeterol. *Resp Med* 1994;**88**:363-8.
29. Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. *Lancet* 1995;**346**:201-6.
30. Pollard SJ, Bronsky EA, Chervinsky P, et al. The safety of salmeterol in pediatric patients as assessed by 24-hour electrocardiogram monitoring. *Am J Resp Crit Care Med* 1994;**149**:A350.
31. Maconochie JG, Minton NA, Chilton JE, Keene ON. Does tachyphylaxis occur to the non-pulmonary effects of salmeterol? *Br J Clin Pharmac* 1994;**37**:199-204.
32. Sears MR, Taylor DR, Print CG, et al. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;**336**:1391-6.
33. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988;(**3**):554-8.

34. Wahedna I, Wong CS, Wisniewski AFZ, Pavord ID, Tattersfield AE. Asthma control during and after cessation of regular beta$_2$-agonist treatment. *Am Rev Respir Dis* 1993;**148**:707-12.
35. Beach JR, Young CL, Harkawat R, Gardiner PV, Avery AJ, Coward GA, Walters EH, Hendrick DJ. Effect on airway responsiveness of six weeks treatment with salmeterol. *Pulmonary Pharmacology* 1993;**6**:155-7.
36. Sly RM. Beta-adrenergic drugs in the management of asthma in athletes. *J Allergy Clin Immunol* 1984;**73**:680-5.
37. Kemp J, Bukstein D, Busse W. Effect of salmeterol, salbutamol and placebo in the prevention of exercise-induced bronchospasm. *Chest* 1993;**104**(Suppl):10S.
38. Anderson SD, Rodwell LT, Du Toit JD, Young IH. Duration of protection by inhaled salmeterol in exercise-induced asthma. *Chest* 1991;**199**:1254-60.
39. Johnson M. The preclinical pharmacology of salmeterol: non-bronchodilator effects. *Eur Respir Rev* 1991;**1**(4):257-60.
40. Advenier C, Qian Y, Law Koune J-D, Molimard M, Candenas M-L, Naline E. Formoterol and salmeterol inhibit bradykinin- and histamine-induced airway microvascular leakage in guinea-pig. *Br J Pharmacol* 1992;**105**:792-8.
41. Eda R, Sugiyama H, Hopp RJ, Okada C, Bewtra AK, Townley RG. Inhibitory effects of formoterol on platelet-activating factor induced eosinophil chemotaxis and degranulation. *Int Arch Allergy Immunol* 1993;**102**:391-8.
42. Roberts JA, Bradding P, Holgate ST, Howarth PH. Effects of a six week course of salmeterol on bronchial reactivity. *Thorax* 1992;47:230p.
43. Gardiner PV, Ward C, Booth H, Allison A, Hendrick DJ, Walters EH. Effect of eight weeks of treatment with salmeterol on bronchoalveolar lavage inflammatory indices in asthmatics. *Respir Crit Care Med* 1994;**150**:1006-11.
44. Greening AP, Ind PW, Shaw G, Study Group Allen & Hanburys Limited. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1994;**344**:219-24.
45. Woolcock A. Comparison of the effect of addition of salmeterol with doubling the inhaled steroid dose in asthmatic patients. *Am J Resp Crit Care Med* 1994;**149**(4):A280.

Chapter Four

Effect of a single dose of inhaled salmeterol on baseline airway caliber and methacholine-induced airway obstruction in asthmatic children

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4

Effect of a single dose of inhaled salmeterol on baseline airway caliber and methacholine-induced airway obstruction in asthmatic children

4.1 Summary

Salmeterol is a new inhaled selective β_2-adrenergic receptor agonist with a long duration of action. We studied the duration of the bronchodilation and the protective effect against methacholine-induced airway obstruction of a single dose of salmeterol in a double-blind, randomized, placebo-controlled cross over design.

Seventeen boys and 3 girls with mild-to-moderate asthma participated in the study. On two separate days either 50 μg salmeterol or placebo was inhaled. FEV_1 and PD_{20} methacholine were determined before and 1, 4, 8, 12 and 24 hours after inhalation.

Salmeterol resulted in a significant bronchodilation compared with placebo up to 12 hours ($p=0.0001$). At 24 hours there was a residual effect that approached significance; mean FEV_1 being 8.3% ± 2.4 above baseline ($p=0.06$). Significant protection against airway sensitivity to methacholine after salmeterol inhalation was found at all time points ($p<0.005$). Twenty-four hours after administration mean PD_{20} was still 1.22 ± 0.29 DD above baseline. No important adverse effects were noted.

We conclude that a single dose of 50 μg salmeterol in children with asthma gives a long-lasting bronchodilation, exceeding 12 hours, which is comparable to the results in adult studies. The duration of the protection against airway sensitivity to methacholine exceeds 24 hours.

4.2 Introduction

Asthma is characterized by a variable degree of airway obstruction and airway hyperresponsiveness to several stimuli.[1] Persistent airway obstruction and airway hyperresponsiveness predispose to chronic symptoms and constitute a risk factor for the continuation of childhood asthma into adulthood.[2,3] Airway hyperresponsiveness can be characterized by an increase in airway sensitivity and by an increase in maximal response.[4]

Currently available short-acting inhaled β_2-agonists, like salbutamol, are strong bronchodilators. Their bronchodilating effect lasts for approximately 4 to 6 hours.[5] After inhalation of therapeutic doses, protection against histamine-induced airway responsiveness and other acute challenges, such as allergen, exercise, and cold air, lasts less than 4 hours.[5,6] They do not influence the maximal degree of airway narrowing; moreover, a steepening of the dose-response curve for methacholine and histamine has been reported.[7]

Salmeterol xinafoate (Glaxo, England) is a new selective β_2-agonist with a long duration of action on smooth muscle contractility in vitro.[8] Its long-lasting bronchodilating effect in vivo has been established in several studies in adult asthmatic patients.[9,10,11] More

than one half of the maximum bronchodilation remains 12 hours after administration.[9] In healthy volunteers, protection against histamine-induced airway obstruction lasted 12 hours after inhalation of 200 μg salmeterol.[12] In adult asthmatic patients protection against methacholine- and histamine-induced airway obstruction remained for at least 12 hours after salmeterol doses of 50 and 100 μg and appeared to be dose-related.[10,11] The aim of our study was to establish the duration of the bronchodilating effect and the degree and duration of the protection against airway sensitivity to methacholine of a single dose of 50 μg salmeterol in children with mild-to-moderate asthma.

4.3 Material and methods

4.3.1 Patients

Twenty children with mild-to-moderate asthma, 17 boys and 3 girls, were studied. The patient characteristics are summarized in Table 4.1. The children were selected from the outpatient department of Pediatric Respiratory Medicine, Sophia Children's Hospital, University of Rotterdam. The following inclusion criteria were fulfilled at a prestudy visit: (1) mild-to-moderate asthma according to American Thoracic Society criteria,[1] that is, reversible airway obstruction with an increase of at least 15% in forced expiratory volume in one second (FEV_1) after inhalation of a bronchodilator, (2) age between 7 and 16 years, (3) baseline FEV_1 greater than 60% predicted, (4) airway hyperresponsiveness to methacholine, that is, the dose of methacholine to produce a 20% fall in FEV_1 (PD_{20}) equal to or less than 150 μg,[13] (5) ability to produce reproducible lung function tests, that is, coefficient of variation in three consecutive measurements of FEV_1 less than 5%. All children were atopic to one or more inhaled allergens. Their asthma had been stable for at least 1 month, with no respiratory tract infections. Maintenance treatment, which had not been changed during the previous 6 months, consisted of inhaled corticosteroid (12 children), disodium cromoglycate (3 children), or both (3 children). All patients used a β_2-agonist on demand.

4.3.2 Study design

The study was double-blind, randomized, placebo-controlled, and crossover. The children visited the lung function laboratory on two separate occasions within 4 weeks; the interval between visits being no shorter than 3 days, preferably approximately 1 week. They arrived at 8 AM, having abstained from inhaled bronchodilators for at least 12 hours. After a short rest (15 minutes), baseline heart rate, blood pressure and FEV_1 were measured. Then a methacholine provocation test was performed. At least 1 hour after the last concentration of methacholine, when FEV_1 had returned to within 10% of baseline, the study medication was inhaled. This consisted of either placebo or 50 μg

Table 4.1 Patient characteristics at entry of the study.

Subject	Age (yr)	Sex	FEV_1 (%pred.)	FEV_1/FVC (%)	PD_{20} (μg)	Maintenance treatment
1	9	M	94	77	19	B C S
2	10	M	103	86	80	B C S
3	10	M	86	76	83	B S
4	14	M	65	64	25	B C S
5	13	M	76	90	109	B
6	9	M	82	78	94	B S
7	13	M	57	60	3	B S
8	16	M	88	79	24	B S
9	12	M	103	77	14	B S
10	16	M	61	52	26	B S
11	11	M	81	63	19	B S
12	12	M	90	86	35	B C
13	10	M	81	69	16	B S
14	11	M	88	83	47	B S
15	16	M	99	78	123	B
16	12	F	85	77	41	B C
17	8	M	90	74	42	B S
18	7	F	89	69	16	B S
19	10	F	71	70	7	B C
20	9	M	81	66	22	B S
Mean	11.8		84	74	30*	
SD	2.7		12.7	9.6		

M, male; *F*, female; *B*, inhaled β_2-agonist on demand; *C*, inhaled disodium cromoglycate; *S*, inhaled corticosteroid.
* Geometric mean

salmeterol, administered as two puffs from a metered dose inhaler in conjunction with a Volumatic (Glaxo) spacer device. The two puffs were given one by one.
After each puff the child had to take five breaths of sufficient magnitude to move the valve of the Volumatic.[14] During the study days no other bronchodilators were allowed except the study medication. Inhaled corticosteroid and/or disodium cromoglycate were continued as before entry into the study. On both study days they were inhaled at the same time of the day for all patients.
Repeated measurements of heart rate, blood pressure, and FEV_1 as well as repeated methacholine provocation tests were performed 1, 4, 8, 12 and 24 hours after administration of the study medication. During the day the children stayed in the laboratory of the outpatient department of pediatric respiratory medicine, during the night they slept in the hotel accommodation of the hospital together with one of their parents.

The study was approved by the medical ethics committee of the University Hospital/Sophia Children's Hospital Rotterdam. Informed consent was obtained from all patients and their parents.

4.3.3 Lung function measurements

All FEV_1 measurements were performed on a spirometer with a digital volume transducer (Vicatest-P2, Mijnhardt, Zeist, The Netherlands). Reference values used were those of the European Community for Coal and Steel.[15] Before each methacholine provocation, FEV_1 was measured in triplicate; the best value was taken. Methacholine provocation was performed according to standardization recommendations.[16,17] Aerosol-dispersed methacholine bromide in unbuffered saline was given in doubling concentrations (0.125 to 32 mg/ml). The aerosol was generated by a DeVilbiss 646 (De Vilbiss Co., Somerset, PA, USA) nebulizer, which was operated with 3 ml solution in the nebulizer cup. The nebulizer was attached to a Rosenthal-French dosimeter (Laboratory for Applied Immunology, Fairfax, Virginia, USA) driven by air at 137.8 kPa (20 psi). The aerosol was delivered directly into the mouth through a mouthpiece. The patient inspired slowly from functional residual capacity to total lung capacity. During inspiration the dosimeter was triggered for 0.6 seconds. After the inspiration had been completed, the child was asked to hold his or her breath for about 2 seconds. A total of 20 μl of aerosolized solution was delivered to the mouth in four consecutive breaths. Mouth doses were 2.5 to 640 μg methacholine. In order to exclude reactions to the diluent, saline solution was inhaled before methacholine in a similar way. FEV_1 was measured in triplicate 3 minutes after saline or methacholine inhalation. The interval between consecutive doses was 5 minutes. The next methacholine dose was not given if FEV_1 had fallen below 80% of baseline. PD_{20} was calculated from a log dose-response plot with linear interpolation of data points.

4.3.4 Statistical analysis

Wilcoxon's signed-rank test was used to compare active treatment with placebo after verifying that no significant treatment-order and period effects were present.[18] To allow for the multiplicity of testing at five time points, p values less than 0.01 (0.05/5) were considered to be statistically significant according to Bonferroni's procedure. The effect of study treatment on FEV_1 was expressed as percent change from baseline FEV_1 on the same day. Logarithmic transformation was performed in all analyses of PD_{20}. The magnitude of the protection against methacholine-induced airway sensitivity was expressed in DD change from baseline PD_{20} on the same day. In patients who did not

reach a 20% fall in FEV_1 after the maximum dose of 640 μg methacholine, PD_{20} was considered to be 640 μg for statistical calculations. As in these cases, PD_{20} is known to exceed 640 μg, and all but one of these occurred during active treatment; this will underestimate the treatment effect of salmeterol. Therefore an additional analysis was performed, with use of survival analysis techniques, allowing for such censored data. With these methods full use is made of the information that some PD_{20} values are known to exceed 640 μg, instead of assuming them to be equal to 640 μg. Because PD_{20} values appeared log normally distributed, it was possible to make adjusted estimates (i.e. which take account of the censored values) of the mean difference in DD at the different time points.[19]

4.4 Results

Twenty children entered the study. Mean baseline values of FEV_1 and baseline geometric means of PD_{20} at the two different treatment days did not differ significantly; 2.20 and 2.13 L (83% and 82% predicted), respectively, 34.7 and 30.9 μg,respectively, methacholine during placebo and active treatment. There were no effects of treatment order or period effects on FEV_1 and PD_{20} at all time points.

During placebo administration two children had an exacerbation of their asthma at 8 and 12 hours, respectively, after administration. Both children received salbutamol for relief of their symptoms. Measurements of FEV_1 and methacholine responsiveness within 12 hours after the salbutamol dose were not performed.

4.4.1 Effect on airway caliber

Salmeterol gave a long-lasting bronchodilatation (Figure 4.1). Compared with placebo the effect on FEV_1 was highly significant at all time points up to 12 hours ($p = 0.0001$). The maximum mean effect on FEV_1 was reached after 4 hours; the change from baseline being 18.6% $\pm$ 2.5% (mean $\pm$ SEM). However, after 1 hour bronchodilation was near its maximum (17.7% $\pm$ 2.3%). Twelve hours after inhalation of salmeterol mean FEV_1 was 10.8% $\pm$ 2.7% ($p = 0.0001$) above baseline, and after 24 hours it was still 8.3% $\pm$ 2.4% above baseline. Compared with placebo this value was approaching significance ($p = 0.06$). After placebo administration mean FEV_1 decreased with a maximum of 6.9% $\pm$ 2.0% after 12 hours. Results of FEV_1/forced vital capacity (FVC)% were comparable to those of FEV_1. Salmeterol inhalation resulted in a significant increase in FEV_1/FVC% at 1, 4, 8 and 12 hours compared with placebo ($p < 0.002$). After 24 hours the mean FEV_1/FVC% was respectively 0.77 and 0.75 after salmeterol and placebo ($p = 0.19$).

After salmeterol, individual results showed an increase in FEV_1 from baseline of at least

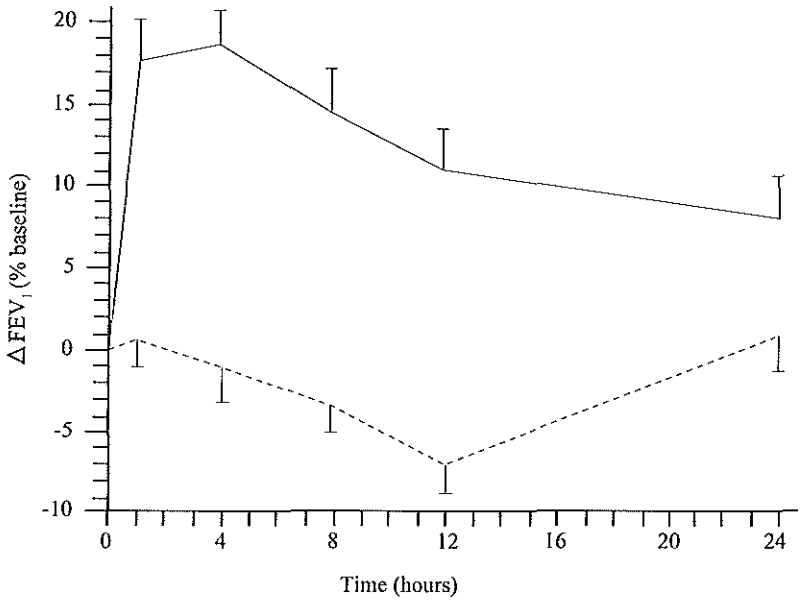

Figure 4.1
Change in FEV_1 in percent change from baseline (SEM).
Salmeterol, (——); placebo, (·····).

10% in 17 of 20 children. The maximum bronchodilating effect in these responders varied between 12% and 46%. Seven patients had their peak effect after 1 hour, seven after 4 hours and three after 8 hours. Also the time period for which FEV_1 was more than 10% above baseline varied between patients. Eight of the 17 responders had FEV_1 values of more than 10% above baseline during 24 hours. In the remainder of responders FEV_1 values of more than 10% above baseline were maintained for up to 12 hours in three patients, up to 8 hours in one, and up to 4 hours in five.

4.4.2 Effect on airway sensitivity to methacholine

The protection against methacholine-induced airway obstruction lasted for 24 hours. Figure 4.2 shows the geometric means of PD_{20} after salmeterol and placebo. The changes from baseline, expressed in DD are shown in Figure 4.3. Salmeterol resulted in a decrease in airway sensitivity to methacholine, with a maximum mean effect of nearly four DD 1 hour after inhalation, which gradually diminished to slightly more than one DD after 24 hours. Compared with placebo the protection of salmeterol was

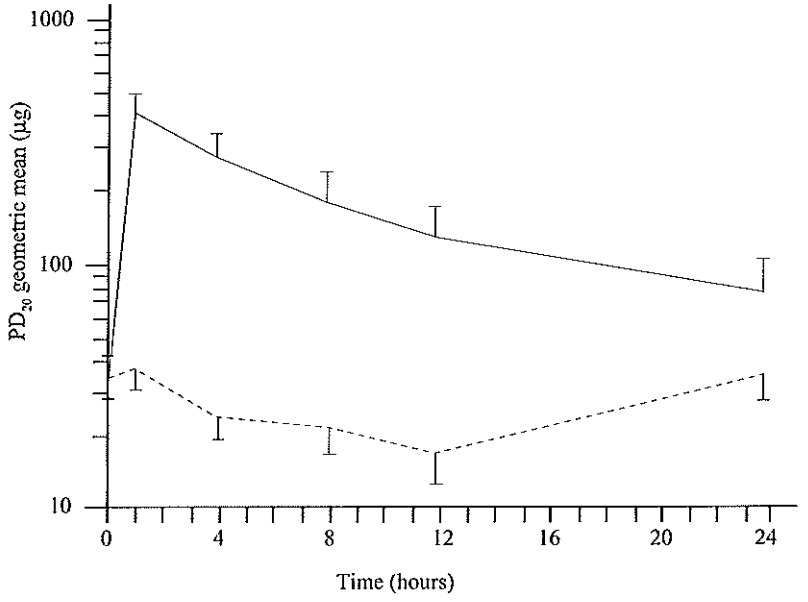

Figure 4.2
PD_{20} methacholine (geometric mean ± SEM). Salmeterol, (——); placebo, (·····).

Table 4.2 Number of patients in which PD_{20} exceeded 640 μg methacholine

Time	Number of patients in which PD_{20} exceeded 640 μg	Unadjusted mean differences in PD_{20} (DD)	Adjusted mean differences in PD_{20} (DD)
1 hour	10	3.7 (3.1-4.2)	4.5 (3.8-5.2)
4 hours	5	3.7 (3.0-4.4)	4.1 (3.3-4.9)
8 hours	3	3.2 (2.5-3.9)	3.5 (2.9-4.1)
12 hours	2	3.0 (2.3-3.7)	3.2 (2.5-3.9)
24 hours	1*	1.3 (0.5-2.1)	1.2 (0.4-2.0)

Except the one marked (*), this occurred after salmeterol treatment. Unadjusted and adjusted mean differences (95% confidence intervals) in PD_{20}, expressed as DD, at different time points between salmeterol and placebo treatment are given. Unadjusted values were calculated, assuming PD_{20} in these patients was 640 μg. Adjusted mean differences were calculated, using survival analysis techniques, taking account for the censored PD_{20} values, which actually exceed 640 μg.

highly significant at all time points ($p < 0.0002$ up to 12 hours, $p = 0.005$ at 24

hours). After placebo administration no changes of more than one DD occurred.
No PD_{20} was reached after the maximum methacholine dose, i.e. 640 μg, in a substantial number of the children after salmeterol administration and in one patient 24 hours after placebo. Because in these subjects PD_{20} was considered to be 640 μg, the above mentioned treatment results underestimate the treatment effect. Taking into account that PD_{20} actually exceeded 640 μg in these patients, adjusted mean differences between salmeterol and placebo treatment were calculated (Table 4.2).

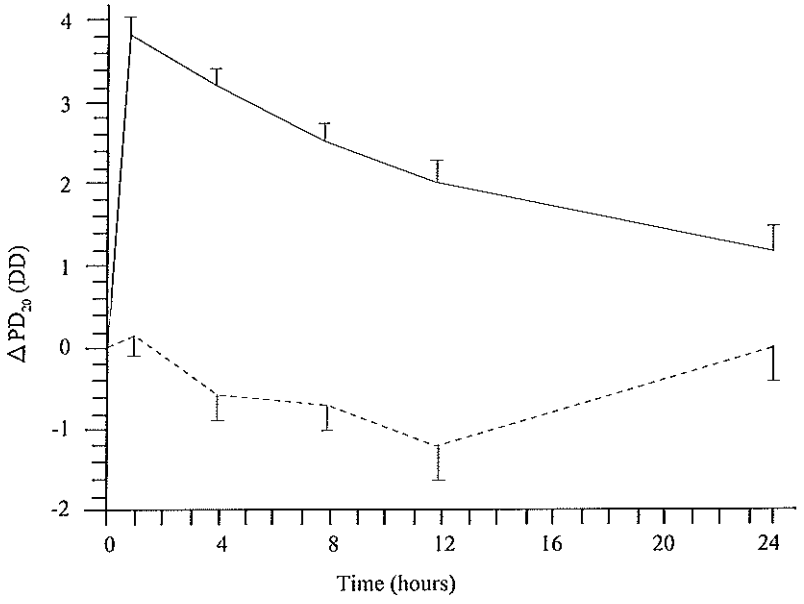

Figure 4.3
PD_{20} changes from baseline (doubling doses ± SEM). Salmeterol, (——); placebo,(------).

One hour after administration of salmeterol 18 patients had PD_{20} values in the normal range, that is PD_{20} equal to or greater than 150 μg methacholine.[13] For the other subsequent time points after 4, 8, 12 and 24 hours, respectively, 17, 11, 8 and 5 children had their PD_{20} in the normal range.
The peak response of the protective effect was measured at 1 hour after salmeterol administration in all subjects and varied between 2.2 and 6.1 DD. In only five patients the protective effect became less than one DD within 24 hours.
No correlation was found between the degree of the protective effect and the degree of

bronchodilation at different time points (r = -0.14, -0.15, -0.12, -0.14 and 0.11, respectively, $p > 0.5$).

4.4.3 Adverse events

No adverse effects, like tremor or palpitations, were noted during the study. Two children complained of headache; one during placebo and one during salmeterol treatment. Heart rate, systolic and diastolic blood pressure during active treatment did not significantly differ between salmeterol and placebo.

4.5 Discussion

In adult studies 50 μg salmeterol had been shown to be efficacious.[9-11] In a first study in children this dose was well tolerated.[20]

In this study the bronchodilating effect of 50 μg salmeterol exceeded in most patients 12 hours, although in a few patients it was less. Twenty-four hours after administration there was still a small but significant reduction of airway sensitivity to methacholine. Although at this time point the bronchodilating effect of salmeterol was not significantly different from that after placebo, a residual effect on FEV_1 seemed to exist in approximately one half of the patients. The changes of FEV_1 and PD_{20} after placebo administration were small and likely to be caused by diurnal variation.

In adult studies the peak level of FEV_1 after a single dose of 50 μg salmeterol was comparable with that after 200 μg salbutamol.[10,11,21] Mean peak increases were 15%, 12% and 18%, respectively, which is comparable with the results in our study (18.6 % $\pm$ 2.5%). The relatively small increase in FEV_1 may be due to the mild degree of airway obstruction at entry. Studies in adults report a significant bronchodilation after single doses of salmeterol 50 and 100 μg up to 12 hours after inhalation.[9-11] Our results are in keeping with those of Ullman and Svedmyr,[9] who found that one half of the bronchodilator effect remained after 12 hours. Another study showed almost identical mean FEV_1 values 1 and 12 hours after 50 μg salmeterol.[10] No data are available on the bronchodilating effect 24 hours after inhalation.

The protective effect against methacholine-induced airway obstruction was studied in adult asthmatic patients by Derom et al.[11] Salmeterol, 50 and 100 μg, gave a significant dose-related protection up to 12 hours after inhalation, whereas the effect of 200 μg salbutamol was no longer significantly different from that of placebo at 4 hours. Twelve hours after salmeterol mean PC_{20} was 1.5 DD after 50 μg and 2.4 DD after 100 μg salmeterol, which is comparable with 2.0 DD in our study after 50 μg. The peak effect was smaller, 2.4 and 3.3 DD, respectively, after 50 and 100 μg salmeterol. In a study on histamine-induced airway obstruction, the peak effect was 2.7 DD, which is also less

than we obtained, but the protection 12 hours after inhalation of 50 μg salmeterol was again similar (1.79 DD).[10] Whether these differences result from differences in patient characteristics or differences in pharmacokinetics between children and adults is unknown. Campos Gongora et al[10] found 50 μg salmeterol four times as potent as 200 μg salbutamol in regard to the protective effect against histamine-induced airway obstruction, whereas 50 μg salmeterol equaled the protective effect against methacholine of the same dose of salbutamol in another study.[11] No data are available on the effect of methacholine- and histamine-induced airway obstruction 24 hours after inhalation. In a recent saline challenge study 32 hours after 50 μg salmeterol, there was no significant effect on either FEV_1 or airway responsiveness to histamine.[22] Malo et al.[21] studied the effect of a single dose of 50 μg salmeterol on hyperventilation with cold dry air up to 24 hours. The mean duration of the protective effect of salmeterol was 15.9 hours, compared with 3.5 hours for salbutamol. However, 24 hours after inhalation only one patient showed a significant protection. In our study 24 hours after inhalation 15 of the 20 children showed a clinically relevant protection against methacholine-induced airway obstruction, that is, more than one DD above baseline. So the duration and degree of the protective effect of salmeterol may be different for different stimuli, which is also known for short-acting $ß_2$-agonists.[5]

Non-bronchodilating properties of salmeterol have been suggested from in vitro studies on human lung fragments[23] as well as from the study by Twentyman et al[22] who revealed a complete inhibition of the rise in nonspecific airway responsiveness over a 34-hour period after allergen challenge. Mechanisms other than bronchodilation and functional antagonism were held responsible for the protection of a short-acting $ß_2$-agonist against several constrictor stimuli in guinea pig trachea preparations.[24] The data in our study do not allow any conclusion on whether the long-lasting effect of salmeterol on airway sensitivity is the result of inhibition of smooth muscle contractility or of other non-bronchodilating properties. The fact that no correlation was found between the degree of the protective effect and the bronchodilating effect, which is also known for short-acting $ß_2$-agonists,[5] supports the view that mechanisms in addition to smooth muscle inhibition play a role.

It is unlikely that our results have been influenced by the concomitant use of inhaled corticosteroids or disodium cromoglycate or both. No significant direct protective effect on methacholine-induced airway responsiveness of these drugs exists.[6] During both study days these drugs were administered at the same time point for all patients, so they only could have influenced the placebo and the active treatment in the same way. Because all patients were using their maintenance treatment for at least 6 months, it is unlikely that the chronic dosing effect has changed within the study period. It is in

agreement with this that no period effect was found.

In this study 50 μg salmeterol was shown to have a long-lasting bronchodilating effect and a prolonged duration of action against airway sensitivity to methacholine in children with mild-to-moderate asthma. So, twice daily dosing may result in a 24-hour protection. This is of considerable clinical importance because in general the tolerance to various external stimuli increases together with a decrease in airway sensitivity.[25] A direct correlation has been found between the degree of airway sensitivity to a nonspecific stimulus and the amount of allergen that can be tolerated.[26,27] However, for short-acting β_2-agonists a steepening of the dose-response curve is known, and maximal airway narrowing is not influenced.[7] Because we studied only airway sensitivity and not the effect on maximal airway narrowing after methacholine, we can not speculate on the potential hazardous effects of higher doses of allergen or other strong stimuli reaching the airways because of long-lasting better patency. Further studies will be necessary to determine this effect as well as the degree and duration of the protective effect of salmeterol against other stimuli in asthmatic children and to determine the effect on the nonspecific airway responsiveness during long-term treatment.

4.6 References

1. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987; **136**: 225-44.
2. Gerritsen J, Koëter GH, Postma DS, Schouten JP, Knol K. Prognosis of asthma from childhood to adulthood. *Am Rev Respir Dis* 1989; **140**: 1325-30.
3. Weiss ST, Speizer FE. Increased levels of airway responsiveness as a risk factor for development of chronic lung disease. *Chest* 1984; **86**: 3-4.
4. Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. *Eur Respir J* 1989; **2**: 267-74.
5. Kerrebijn KF. Beta Agonists. Kaliner M, Barnes P, Persson C, eds. Asthma, its pathology and treatment. *Lung biology in health and disease*, volume 49, New York: Marcel Dekker, 1991: 523-59.
6. Van Essen-Zandvliet EEM, Kerrebijn KF. The effect of antiasthma drugs on bronchial hyperresponsiveness. *Immunol Allergy Clin North Am* 1990; **10**: 483-501.
7. Bel EH, Zwinderman AH, Timmers MC, Dijkman JH, Sterk PJ. The protective effect of a beta$_2$-agonist against excessive airway narrowing in response to bronchoconstrictor stimuli in asthma and chronic obstructive lung disease. *Thorax* 1991; **46**: 9-14.
8. Ball DI, Brittain RT, Coleman RA, Denyer LH, Jack D, Johnson M, Lunts LHC, Nials AT, Sheldrick KE, Skidmore IF. Salmeterol, a novel, long-acting beta$_2$-adrenoceptor agonist: characterization of pharmacological activity in vitro and in vivo. *Br J Pharmacol* 1991; **104**: 665-71.
9. Ullman A, Svedmyr N. Salmeterol, a new long-acting inhaled β_2-adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988; **43**: 674-8.
10. Campos Gongora H, Wisniewski AFZ, Tattersfield AE. A single dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991; **144**: 626-9.

11. Derom EY, Pauwels RA, Van der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992; **89**: 811-5.
12. Maconochie JG, Forster JK, Fowler P, Thomas M. An initial comparison of salmeterol and salbutamol against histamine-induced bronchoconstriction in healthy subjects. *Br J Clin Pharmacol* 1988; **25**: 115P.
13. Duiverman EJ, Neijens HJ, Van Strik R, Affourtit MJ, Kerrebijn KF. Lung function and bronchial responsiveness in children who had infantile bronchiolitis. *Pediatr Pulmonol* 1987; **3**: 38-44.
14. Gleeson JGA, Price JF. Nebuhaler technique. *Br J Dis Chest* 1988; **82**: 172-4.
15. Quanjer PhH, ed. Standardized lung function testing. Report of the Working Party "Standardization of Lung Function Tests", European Community for Coal and Steel. *Bull Eur Physiopathol Respir* 1983; **19**(suppl 5): 1-95.
16. Eiser NM, Kerrebijn KF, Quanjer PH. Guidelines for standardization of bronchial challenges with (non-specific) bronchoconstricting agents. SEPCR Working group "Bronchial Hyperreactivity". *Bull Eur Physiopathol Respir* 1983; **19**: 495-514.
17. Birnie D, Thoe Schwartzenberg GWS, Hop WCJ, Van Essen-Zandvliet EEM van, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? *Thorax* 1990; **45**: 199-202.
18. Koch GG. The use of non-parametric methods in the statistical analysis of the two-period changeover design. *Biometrics* 1972; **28**: 577-84.
19. Dixon WJ, ed. Lognormal accelerated failure time model. In: *BMDP Statistical Software Manual*, Volume 2. Berkeley: University of California Press, 1990; 770-1.
20. Verberne A, Lenney W, Kerrebijn K. A 3-way crossover study comparing twice daily dosing of salmeterol 25 mcg and 50 mcg with placebo in children with mild to moderate reversible airways disease. *Am Rev Respir Dis* 1991; **143**: A20.
21. Malo JL, Ghezzo H, Trudeau C, L'Archévêque J, Cartier A. Salmeterol, a new inhaled beta$_2$-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *J Allergy Clin Immunol* 1992; **89**: 567-74.
22. Twentyman OP, Finnerty JP, Harris AL, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet 1990*; **2**: 1338-42.
23. Butchers PR, Vardey DJ, Johnson M. Salmeterol; a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Br J Pharmacol* 1991; **104**: 672-6.
24. Gustafsson B, Persson CGA. Effect of different bronchodilators on airway smooth muscle responsiveness to contractile agents. *Thorax* 1991; **46**: 360-5.
25. Josephs LK, Gregg I, Holgate ST. Does non-specific bronchial responsiveness indicate the severity of asthma? *Eur Respir J* 1990; **3**: 220-7.
26. De Baets FM, Goeteyn M, Kerrebijn KF. The effect of two months of treatment with inhaled budesonide on bronchial responsiveness to histamine and house-dust mite antigen in asthmatic children. *Am Rev Respir Dis* 1990; **142**: 581-6.
27. Neijens HJ, Degenhart HJ, Raatgreep HC, Kerrebijn KF. Study on the significance of bronchial hyperreactivity in the bronchus obstruction after inhalation of cat dander allergen. *J Allergy Clin Immunol* 1979; **64**: 507-15.

Chapter Five

Airway responsiveness after a single dose of salmeterol and during four months treatment in children with asthma

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Airway responsiveness after a single dose of salmeterol and during four months treatment in children with asthma

5.1 Summary

Inhalation of a single dose of the long-acting β_2-adrenoceptor agonist salmeterol protects against methacholine-induced airway obstruction and other bronchoconstricting stimuli for at least 12 hours. Hypothetically, twice daily dosing of salmeterol may result in continuous protection.

This study was designed to investigate the protective effect of a single dose of salmeterol and of continuous twice daily treatment on airway responsiveness to methacholine.

In a double-blind, parallel study, salmeterol 50 μg b.i.d was compared with salbutamol 200 μg b.i.d. Thirty children with mild asthma, who had little or no bronchial obstruction and were hyperresponsive to methacholine ($PD_{20} \leq 150$ μg) were allocated to receive either salmeterol or salbutamol. Airway responsiveness was measured before study entry, 12 hours after a single dose and monthly during 4 months of daily treatment. Measurements were always performed at the same time of the day, 12 hours after the last dose of medication was administered.

No significant differences in FEV_1 were found between treatments at any time point. PD_{20} significantly increased after the first dose of salmeterol was given (geometric mean 100 μg). Geometric mean PD_{20} values were significantly better during salmeterol treatment than during salbutamol treatment, 52 and 25 μg respectively ($p=0.005$).

The protection provided by salmeterol during maintenance treatment was less than after the first dose ($p<0.001$). However, protection did not diminish during the 4 month treatment period and remained significant compared with baseline ($p=0.003$).

5.2 Introduction

Salmeterol xinafoate has a bronchodilating effect that lasts for at least 12 hours when administered as a single dose of 50 μg in adults and children with asthma.[1,2,3,4] Protection against methacholine-induced[2,3] and histamine-induced airway obstruction lasts for 12 up to 24 hours. Single-dose studies also show a prolonged protection against other bronchoconstricting stimuli, such as exercise[5], hyperventilation with dry cold air,[6] and allergen.[7] Theoretically, twice daily dosing of salmeterol can provide a 24-hour protection against various bronchoconstricting stimuli and therefore decrease symptoms in patients with asthma. Studies comparing salmeterol 50 μg twice daily with salbutamol 200 μg four times daily during 12 weeks have indeed shown better symptom control in the group treated with salmeterol.[8,9,10] In patients with mild asthma a reduction of the acute protective effect of salmeterol against methacholine-induced bronchoconstriction from a 3.3 DD after the first dose to 1.0 DD after stopping maintenance treatment at 4 and 8 weeks was found.[11] The bronchodilating effect did

not change during the study period. Another study in patients with mild-to-moderate asthma, of whom the majority were treated with inhaled corticosteroids, did not show this reduction in protection against methacholine-induced airway obstruction.[12] The two studies differ in the time point at which methacholine challenges were performed, respectively 1 and 12 hours after salmeterol; whereas in the study by Cheung et al[11] maintenance treatment was also stopped for 36 hours. A recent study in adult patients with symptomatic asthma, who were already being treated with a low dose of inhaled corticosteroids, showed a response more favourable in symptoms and peak flow values when salmeterol was added than when the inhaled corticosteroid dose was increased.[13] This study, however, does not include data on airway responsiveness. We investigated the protective effect of salmeterol against methacholine-induced bronchoconstriction after a single dose and during 4 months of maintenance treatment and compared this to the effect of salbutamol.

5.3 Material and methods

5.3.1 Patients

Between July 1992 and January 1993, thirty children, aged 7 to 16 years, with mild asthma according to the American Thoracic Society's criteria[14], were recruited from the outpatient department for Pediatric Respiratory Medicine, Sophia Children's Hospital, Rotterdam. The patients had to be capable of performing lung function tests reproducible, i.e., a coefficient of variation in three consecutive measurements of FEV_1 less than 5%. Because airway responsiveness is partly determined by the degree of bronchial smooth muscle constriction,[15] we selected children who had a consistent increase in airway responsiveness but little or no bronchoconstriction. They had to meet the following criteria: (1) a dose of methacholine that produced a 20% fall in FEV_1 (PD_{20} methacholine) equal to or less than 150 μg (this being more than two standard deviations below the mean value in healthy children)[16], (2) a baseline FEV_1 and FVC greater than 70% of predicted (reference values according to Zapletal et al[17]) and (3) a FEV_1/FVC greater than 70%. The inclusion criteria had to be fulfilled at a prestudy visit. All patients were atopic to one or more inhaled allergens, as determined by measurement of specific IgE in serum and/or positive skin test results. Their households were adapted to reduce house dust mite exposure, and keeping of domestic animals was discouraged. Asthma treatment before the study consisted of an inhaled β_2-agonist on demand only or in combination with maintenance treatment with disodium cromoglycate. Inhaled corticosteroids and maintenance treatment with oral corticosteroids were not allowed in the year preceding the study. Disodium cromoglycate was stopped 2 weeks before the start of the run-in period. If during this period the symptoms of ast-

hma increased significantly, the patient was excluded from the study. None of the children had acute episodes of asthma or respiratory tract infections for at least 1 month before entry into the study.

Thirty children, 20 boys and 10 girls, were allocated randomly to treatment groups (15 in each group). The baseline characteristics were the same for each treatment group (Table 5.1). Nine children in each treatment group had had disodium cromoglycate medication discontinued. The median duration of asthma was 5 and 6 years, respectively, for the salmeterol and salbutamol groups. The exacerbation rate was low in both groups, reflected by the mean number of prednisolone courses per patient in the previous year, respectively, 0.13 and 0.20. None of the children had been hospitalized for treatment of asthma in the year before entering the study.

5.3.2 Study design

The study had a double-blind, parallel group design and consisted of a 2-week run-in period, a 4-month treatment period and a 2-week follow-up period. The study was based on an intention to treat principle. At the first visit to the lung function laboratory, before the start of the run-in period, children were randomly allocated to receive either salmeterol 50 μg twice daily or salbutamol 200 μg twice daily. During the run-in period no medication was given, except for salbutamol in case of symptoms. The first dose of the study drug was taken at the end of the run-in period, 12 hours before the second visit. Thereafter the 4-month treatment period started, and children took their study medication two times a day with an interval of approximately 12 hours. For acute relief of acute asthma symptoms, salbutamol was allowed at a maximum dose of 200 μg six times daily. Exacerbations of asthma were treated with a standard short course of prednisolone (starting with 30 mg on the first day and tapering off to 0 in 1 week according to a scheme that depended on body weight). Salbutamol and the study medication were administered as Rotadisks in combination with a Diskhaler^{R} (Glaxo, Greenford, United Kingdom). All children were instructed in use of this inhalation device before entry into the study, and technique was checked at every visit. During the follow-up period after the study medication was stopped, salbutamol was used as needed. Children visited the lung function laboratory at the start and at the end of the run-in period, monthly during the treatment period, and at the end of the follow-up period. At each visit heart rate, blood pressure, FEV_1, PEF and airway responsiveness to methacholine were measured. All lung function measurements were performed between 8.30 and 9.30 am, 12 hours after the last dose of the study drug was given. To verify compliance with the last dose, patients were asked for the exact time of drug inhalation. If this was not 12 hours earlier, lung function measurements were

Table 5.1 Patient characteristics at entry of the study

Salmeterol

Subject no.*	Sex	Age (yr)	FEV_1 (% pred)	FVC (% pred)	FEV_1/ FVC(%)	PD_{20} (μg)	Medication	Atopy**
1	M	9	93	97	80	34	B	HD, C, D
2	F	13	90	112	68	6	B	HD, Gr, D
3	M	7	106	107	84	23	BC	HD
4	M	11	99	90	92	75	B	HD, Gr
5	M	15	96	115	68	17	BC	HD, Gr, C, D
6	F	12	104	107	82	4	BC	HD, Gr, C, D
7	M	6	87	94	78	48	B	HD, Gr, C
8	M	11	74	82	75	16	B	Gr
9	M	7	84	79	90	92	BC	HD, Gr
10	M	7	102	97	89	49	BC	HD, Gr, C, D
11	F	10	94	95	85	66	BC	HD, Gr, C, D
12	M	8	95	87	92	39	B	HD, C, D
13	F	12	99	99	85	136	BC	Gr
14	M	10	97	96	85	54	BC	HD, Gr, C, D
15	M	10	76	77	83	25	BC	C
Mean		10.3	93.0	95.5	82.5	32.4‡		
SD		2.5	9.5	11.6	7.6			

rescheduled. Rescue salbutamol was allowed up to 8 hours before the measurements were taken. No FEV_1 measurements or methacholine provocation tests were performed within the first 4 weeks after prednisolone was taken. During the run-in and follow-up periods and during the first 2 weeks of every month of treatment, a record card was completed daily. Separate daytime and nighttime scores from 0 to 3 were given for the presence and severity of cough, wheezing and dyspnea. PEF was recorded in triplicate twice daily before inhalation of the study drug, with the use of a mini-Wright peak flow meter (Clemente Clarke International Ltd., Harlow, Essex, U.K.). The use of rescue salbutamol was also recorded. At the start and the end of the treatment period, blood samples were taken and analyzed for hematologic and biochemical parameters, and urine was analyzed for protein, glucose and blood.

The study was approved by the Medical Ethics Committee of the University Hospital/Sophia Children's Hospital Rotterdam. Written informed consent was obtained from all patients and their parents.

Salbutamol

Subject no.*	Sex	Age (yr)	FEV_1 (% pred)	FVC (% pred)	FEV_1/ FVC(%)	PD_{20} (μg)	Medication†	Atopy**
16	F	11	103	106	82	101	B	HD
17	M	7	111	121	80	28	BC	HD,Gr,C
18	M	11	103	113	76	123	B	HD,Gr
19	M	11	81	103	66	27	BC	HD,Gr,C,D
20	F	7	96	111	75	14	BC	HD,Gr,C
21	F	11	84	89	81	24	BC	HD,Gr,C
22	M	12	91	84	90	83	BC	HD,D
23	F	11	92	94	83	107	BC	HD
24	F	7	103	103	86	25	B	HD,Gr,C
25	F	7	87	81	93	20	BC	HD,Gr,C,D
26	M	12	82	86	80	27	BC	HD
27	M	12	77	82	78	26	B	HD
28	M	10	73	73	83	45	B	HD
29	M	8	96	88	91	41	B	HD,C,D
30	M	10	89	97	78	21	BC	HD
Mean		10.3	91.2	95.3	81.5	37.2‡		
SD		2.0	10.9	13.6	7.0			

* Subject numbers do not indicate the sequence of entry into the study
† B: inhaled β_2-agonist on demand; C: inhaled disodium cromoglycate
‡ Geometric mean
** HD: house dust mite; Gr: grass pollen; C: cat; D: dog

5.3.3 Lung function measurements

FEV_1 was measured according to the European Community for Steel and Coal recommendations[18] with a water-sealed spirometer (Mijnhardt, Zeist, The Netherlands). The largest value from an envelope curve consisting of three to five attempts was recorded. Reference values of Zapletal et al[17] were used. PEF was measured in triplicate and the best value was recorded with the use of the patient's own mini-Wright peak flow meter. Methacholine provocation tests were performed with a modification of the dosimeter method of Chai, as described previously.[19] Nebulized methacholine bromide in unbuffered saline solution was given in doubling concentrations (0.125 to 32 mg/ml). The aerosol was generated by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA) attached to a Rosenthal-French dosimeter (Laboratory for Applied Immunology, Fairfax, VA) and driven by air at 137.8 kPa (20 psi) with a timing adjustment of 0.6 second. A total of 20 μl of aerosolized solution was

delivered to the mouth in four consecutive breaths. Mouth doses were 2.5 to 640 μg methacholine. Saline solution was inhaled before methacholine to exclude a non-specific response. The effect of each dose was determined by measuring FEV_1 in triplicate 3 minutes after each administration. The PD_{20} methacholine was calculated from a log dose-response plot by linear interpolation of data points.

5.3.4 Statistical analysis

FEV_1 results were expressed as percent predicted value according to reference values.[17] All PD_{20} values were logarithmically transformed before analysis. For patients in whom a 20% fall in FEV_1 was not reached after the maximum dose of 640 μg methacholine, PD_{20} was considered to be 640 μg. As this only occurred in two patients, both in the salmeterol group, this has resulted in a slight underestimation of the effect of salmeterol. PD_{20} values were analyzed as geometric mean, as well as changes from baseline, expressed in DD. Comparisons of PD_{20} and of FEV_1 between and within treatment groups were done by using repeated-measures analysis of variance.[20] Comparisons of PD_{20} and of FEV_1 at and between specific time points were done using the t-test and the paired t-test, respectively. The percentage of days with symptoms and mean morning and evening PEF for individual patients were calculated from the daily record card for each study period. If the number of days scored on the daily record card was less than 7 (out of the required 14 days), the percentage of days with symptoms or peak flow values were considered inestimable for that item in that period and were not included in the analysis. Comparisons of the percentages of days with symptoms in various study periods were done by using Mann-Whitney U test. Comparisons of mean morning and of mean evening PEF at and between specific study periods were done using the t-test and the paired t-test, respectively. For all analyses, a p-value of 0.05 (two-sided) was considered the limit of significance.

5.4 Results

During the study, six prednisolone courses were given: three during salmeterol treatment (subject 2, one course and subject 7, two courses), and three during salbutamol treatment (subjects 18, 19 and 29). Two children (subjects 7 and 18), one in each treatment group, withdrew during the treatment period because of an increase in symptoms; it was considered unethical to continue administration of blinded medication. After withdrawal, both children began receiving inhaled corticosteroids. Measurements obtained from these subjects were included up to the last visit before withdrawal. Compliance with treatment schedules were checked by counting the used blister packs at each visit. The compliance gradually improved during the treatment

period in both treatment groups: from 1.11 to 1.76 blister packs/day for the group treated with salmeterol and from 1.10 to 1.90 blister packs/day for the group treated with salbutamol.

5.4.1 Airway caliber

Results of FEV_1 expressed in liters as well as percent predicted value, are listed in Table 5.2. At the beginning of the run-in period, FEV_1 was similar in both groups. Twelve hours after the first dose of either of the study drugs was administered, we found no significant change in FEV_1 compared with baseline values. At no time, either after the first dose was given or during the treatment period, were there any significant changes in FEV_1 within or between the two treatment groups. Two weeks after the discontinuation of salmeterol treatment, a small but significant ($p=0.005$) decrease in FEV_1 occurred. However, the fall in FEV_1 after continuous treatment was stopped did not differ between the salmeterol and salbutamol groups ($p=0.10$).

5.4.2 Airway responsiveness

Baseline PD_{20} methacholine values were similar in the salmeterol and salbutamol group (geometric mean, 32 and 37 μg methacholine, respectively). There was a strong correlation between PD_{20} values at the different time points and baseline values of PD_{20} within treatment groups. Therefore to reduce the variation caused by interindividual differences in baseline PD_{20}, not only geometric mean PD_{20} values were analyzed but

Table 5.2 Results of FEV_1 expressed in liters (mean ± sem) and as % predicted (mean ± sem) for both treatment groups at different time points

	Salmeterol		Salbutamol	
	liters	% predicted	liters	% predicted
Baseline	1.98 ± 0.17	93.0 ± 2.5	2.00 ± 0.13	91.2 ± 2.8
Visit 2 (12 hours after first dose)	2.08 ± 0.22	95.5 ± 4.2	1.99 ± 0.12	90.3 ± 2.8
Treatment period				
1 month	2.04 ± 0.19	94.3 ± 3.1	2.03 ± 0.14	90.3 ± 3.4
2 months	1.97 ± 0.17	92.8 ± 2.9	2.06 ± 0.12	92.0 ± 2.4
3 months	2.07 ± 0.19	93.2 ± 3.0	1.98 ± 0.12	88.0 ± 2.1
4 months	2.02 ± 0.19	90.5 ± 4.1	1.96 ± 0.15	87.3 ± 3.1
Follow-up	1.95 ± 0.18	85.7 ± 3.1	1.97 ± 0.14	86.2 ± 2.1

also changes in PD_{20} from baseline. The results of these analyses were similar. Figure 5.1 shows both geometric mean PD_{20} values and PD_{20} changes in DD. Table 5.3 shows individual data at each time point.

At the end of the run-in period, 12 hours after the first 50 μg dose of salmeterol was given, PD_{20} methacholine increased by 1.66 DD compared with baseline. After administering 200 μg of salbutamol PD_{20} fell with a 0.54 DD ($p<0.001$ salmeterol vs

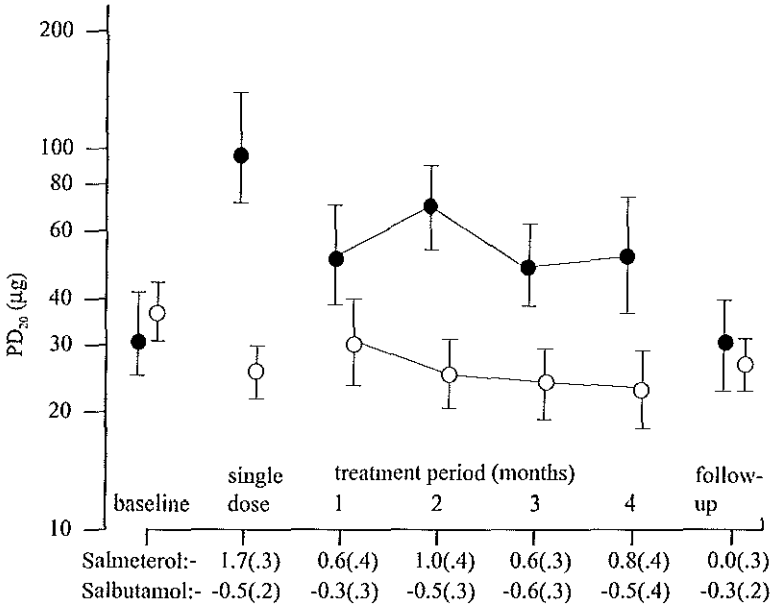

Figure 5.1
PD_{20} methacholine (geometric mean ± SEM) at all time points. Mean PD_{20}changes from baseline in DD (± SEM) for salmeterol and salbutamol treatment are listed below the time points.
Salmeterol, (-●-); salbutamol, (-○-).

salbutamol). Geometric mean PD_{20} values at this time point were 100 and 26 μg methacholine, respectively, for salmeterol and salbutamol ($p=0.001$). The individual results are plotted in Figure 5.2.

During the treatment period no significant changes in geometric mean PD_{20} were found within both groups from 1 to 4 months. The geometric mean PD_{20} during the treatment

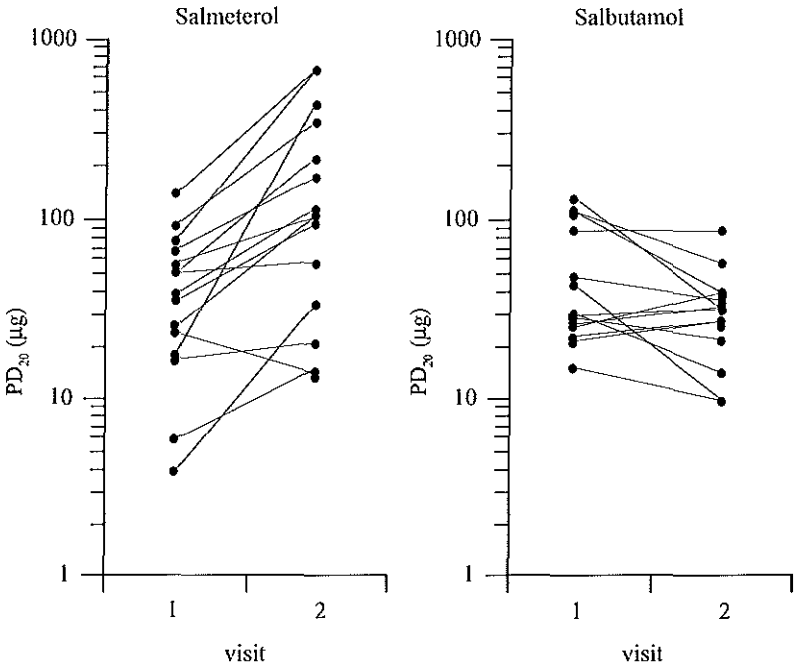

Figure 5.2
Individual results of PD_{20} methacholine for both treatment groups at baseline period (visit 1) and 12 hours after the first dose of the study drug was given (visit 2).

period was 52 µg methacholine for the salmeterol group, compared with 25 µg methacholine for the salbutamol group ($p=0.005$). The geometric mean PD_{20} was less during maintenance treatment with salmeterol than after the first dose was given ($p<0.001$), but still significant compared with baseline ($p=0.003$). Two weeks after maintenance treatment was stopped, geometric mean PD_{20} values were not significantly different between groups. For both treatment groups these values did not differ significantly from the values at the time of entry into the study.

5.4.3 Daily record cards

Symptom scores were generally low. Mean percentages of days with at least one symptom were 46% and 40%, respectively, for the salbutamol and salmeterol groups

Table 5.3 Individual data of PD_{20} (μg methacholine) at different time points

Salmeterol

Subject no.	Baseline	Single dose	Treatment period (months)				Follow up
			1	2	3	4	
1	34	89	127	73	24	21	11
2	6	14	*	*	13	12	11
3	23	13	20	26	41	35	13
4	75	>640	428	504	103	221	199
5	17	410	169	179	56	216	51
6	4	32	18	28	21	11	8
7	48	55	*	201	*	*	*
8	16	20	19	19	*	21	32
9	92	338	142	118	50	101	105
10	49	208	59	51	63	68	48
11	66	167	35	31	28	136	20
12	39	108	10	101	65	13	50
13	136	>640	101	117	>640	>640	69
14	54	99	51	50	54	44	20
15	25	97	23	46	44	*	*
Geometric mean	32	100	52	71	50	54	32

during the run-in period. For the group treated with salmeterol, these percentages were 32%, 25%, 22% and 23%, respectively during the four consecutive treatment periods. These percentages did not significantly differ from the percentages for the group treated with salbutamol, which were 35%, 34%, 30% and 25%, respectively (all $p > 0.59$). No significant differences were found when the various symptoms - cough, wheezing and shortness of breath - were analyzed separately. This applied also to the separate morning and evening symptom scores. Morning and evening PEF did not differ significantly within or between groups, although both tended to increase during salmeterol treatment.

5.4.4 Adverse events

During salmeterol treatment 17 adverse events were reported in 10 patients; during

Salbutamol

Subject no.	Baseline	Single dose	Treatment period (months)				Follow up
			1	2	3	4	
16	101	36	77	31	29	52	90
17	28	13	35	11	34	60	58
18	123	28	30	31	23	*	*
19	27	28	123	62	45	*	43
20	14	9	10	9	10	10	16
21	24	29	7	55	33	19	21
22	83	80	174	183	140	37	75
23	107	52	56	31	72	26	33
24	25	37	29	17	16	13	13
25	20	24	7	11	13	10	12
26	27	28	20	12	16	24	16
27	26	20	20	15	21	51	22
28	45	32	47	67	7	11	24
29	41	9	*	11	8	9	20
30	21	25	30	36	50	104	44
Geometric mean	37	26	31	26	25	24	28

* No measurements

salbutamol treatment 36 adverse events were reported in 12 patients. Most adverse events were upper respiratory tract symptoms. Headache occurred slightly more often during salbutamol treatment (eight periods of headache in four patients) than during salmeterol treatment (one headache). There were no significant changes in systolic and diastolic blood pressure or heart rate in any group during treatment.

5.5 Discussion

The results of this study show that twice daily treatment with salmeterol in children with mild asthma results in continuous, stable protection against methacholine-induced bronchoconstriction. This protection, however, is less than the protection provided after a first single dose. After maintenance treatment was stopped for 2 weeks, no residual protection remained, indicating that there was no sustained reduction of airway

responsiveness.

Salbutamol instead of placebo was used in the control group; otherwise, because of its bronchodilatory effect salmeterol could be recognized as the effective treatment. Twice daily treatment with salbutamol resulted in a slight but not significant fall ($p=0.06$) in PD_{20} during the 4 month period compared with baseline measurements; this was probably the result of withdrawal of disodium cromoglycate in more than half of the children before they entered the study. Some authors have suggested an increase in airway responsiveness and a deterioration of asthma as a result of regular $ß_2$-agonist treatment.[21,22] It is unlikely, however, that regular use of salbutamol is the explanation for the decrease of PD_{20} in the salbutamol group in our study, because the fall in PD_{20} was already present at the end of the run-in period in which children used salbutamol "as needed".

We selected children with mild asthma, who were hyperresponsive but with little or no bronchoconstriction to avoid interference of airway caliber and PD_{20}. We chose to measure airway responsiveness 12 hours after the last dose of the study drug was administered, which is the normal dose interval during maintenance treatment with salmeterol and therefore clinically relevant. Furthermore, a longer interval might introduce a rebound increase in airway responsiveness, as has been shown for up to 59 hours after stopping regular treatment with the short-acting $ß_2$-agonists terbutaline[23] and salbutamol.[24] Until now, a rebound increase in PD_{20} after stopping regular treatment with salmeterol has not been demonstrated.[11,12,25,26]

In our study the protective effect of salmeterol was probably caused by the prolonged effect of the drug on airway smooth muscle. This is functional antagonism, a well known phenomenon associated with other $ß_2$-adrenoceptor agonists.[27] In vitro experiments show evidence of an interaction of $ß_2$-agonists and methacholine at the level of intracellular signal transduction through phosphoinositide metabolism.[28]

After the first dose of salmeterol was given we found an improvement in PD_{20} of 1.7 DD which is comparable with the results of previous studies.[2,3,29] During the treatment period from 1 to 4 months, this protection was constant but reduced to 0.7 DD.

Two studies in adult patients with asthma investigated the immediate protective effect of salmeterol and the effect during regular twice daily treatment.[11,12] Booth et al[12] examined 26 patients with mild-to-moderate asthma, in a parallel-group, placebo controlled study. The majority of their patients were also receiving inhaled corticosteroid treatment. As in our study, the interval between salmeterol administration and measurement of airway responsiveness to methacholine was 12 hours. They found a small but significant protection during 8 weeks of salmeterol treatment, which did not differ from the single dose effect.[12] A reduction in protection during regular twice daily

treatment with salmeterol was found by Cheung et al.[11] They reported a reduction in protection from 3.3 DD after the single dose of salmeterol was given to 1.0 DD after 4 and 8 weeks of treatment. Airway responsiveness was measured 1 hour after salmeterol administration, and maintenance treatment was stopped for 36 hours. Although unlikely from the data at the end of their study, a possible rebound increase in airway responsiveness could not be excluded. As in our study, Cheung et al[11] selected subjects with mild asthma who were not treated with inhaled corticosteroids. The reduction in protection occurred within 4 weeks after the start of maintenance treatment and remained at the same level after 8 weeks of treatment. The explanation for this tolerance remains unclear but may be the result of receptor downregulation. Tachyphylaxis to nonpulmonary effects (e.g., tremor, increased QTc interval, and elevated blood glucose levels) has been found after 2 weeks of treatment with salmeterol in healthy subjects.[30] The use of inhaled corticosteroids may protect against the development of tachyphylaxis to pulmonary effects of β_2-agonists and may explain the different results obtained by Booth et al.[12] Reversal of tachyphylaxis by systemic corticosteroids has been shown in vitro and in vivo.[31]

In our study significant, stable protection remained throughout the 4 months of treatment with salmeterol. So, if any downregulation of the β_2-receptors occurs, this seems incomplete. Because we selected children with little or no bronchoconstriction, the effect could not be explained by an improvement in airway caliber.[14] This is supported by the fact that no significant changes occurred in FEV_1 and that no correlation was found between the changes in PD_{20} and in FEV_1. Although the protection after 4 weeks of treatment was less than after the first dose was given, our data do not indicate an ongoing increase in airway responsiveness. A significant degree of protection remained during treatment, and this may be of clinical relevance, because a decrease in airway responsiveness will improve the tolerance to other exogenous stimuli. A direct correlation has been found between the degree of airway responsiveness to a non-specific stimulus and the amount of allergen that can be tolerated.[32,33] In our study the changes in airway responsiveness were not reflected by changes in symptom scores. However, this may be the result of selecting patients with mild asthma who already have very low symptom scores before the start of the study.

We conclude that the protective effect of salmeterol against methacholine-induced airway obstruction during 4 months of treatment is lower than the protection offered by a single dose. However, twice daily administration of salmeterol provides significant, stable protection compared with baseline and salbutamol treatment.

According to international consensus reports, asthma therapy should be directed against airway inflammation, and inhaled corticosteroids are now the mainstay of asthma

treatment.[34] It is unlikely from the data now available that salmeterol in itself influences chronic airway wall inflammation.[35] Addition of salmeterol to inhaled corticosteroid treatment may have beneficial effects on symptom scores and airway responsiveness.[13] Studies are now being performed in children with asthma to evaluate the effect of addition of salmeterol to treatment with a conventional dose of an inhaled corticosteroid, as compared with increasing the dose of an inhaled corticosteroid.

5.6 References

1. Ullman A, Svedmyr N. Salmeterol, a new long acting inhaled β_2-adrenoceptor agonist; a comparison with salbutamol. *Thorax* 1988;**43**:674-8
2. Derom EY, Pauwels RA, Van Der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992;**89**:811-5
3. Verberne AAPH, Hop WCJ, Bos AB, Kerrebijn KF. Effect of a single dose of inhaled salmeterol on baseline airway caliber and methacholine-induced airway obstruction in asthmatic children. *J Allergy Clin Immunol* 1993;**91**:127-34
4. Campos Gongora H, Wisniewski AFZ, Tattersfield AE. A single-dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991;**144**:626-9
5. Anderson SD, Todwell LT, DuToit J. Duration of protection by inhaled salmeterol in exercise-induced asthma. *Chest* 1991;**100**:1254-60
6. Malo JL, Ghezzo H, Trudeau C, L'Archévêque J, Cartier A. Salmeterol, a new inhaled β_2-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *J Allergy Clin Immunol* 1992;**89**:567-74
7. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;**1**:1338-42
8. Britton MG, Earnshaw JS, Palmer JBD. A twelve month comparison of salmeterol with salbutamol in asthmatic patients. *Eur Respir J* 1992;**5**:1062-7
9. Pearlman DS, Chervinsky P, LaForce C, Seltzer JM, Southern DL, Kemp JP, Dockhorn RJ, Grossman J, Liddle RF, Yancey SW, Cocchetto DM, Alexander WJ, Van As A. A comparison of salmeterol with albuterol in the treatment of mild-to-moderate asthma. *N Engl J Med* 1992;**327**:1420-5
10. Lundback B, Rawlinson DW, Palmer JBD. Twelve months comparison of salmeterol and salbutamol as dry powder formulations in asthmatic patients. *Thorax* 1993;**48**:148-53.
11. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting β_2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;**327**:1198-203.
12. Booth H, Fishwick K, Harkawat R, Devereux G, Hendrick DF, Walters EH. Changes in methacholine induced bronchoconstriction with the long acting β_2-agonist salmeterol in mild to moderate asthmatic patients. *Thorax* 1993;**48**:1121-4.
13. Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1994;**344**:219-24.
14. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225-44.
15. Ryan G, Latimer KM, Dolovich S, Hargreave FE. Bronchial responsiveness to histamine in relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. *Thorax* 1982;**37**:423-9.
16. Duiverman EJ, Neijens HJ, Van Strik R, Affourtit MJ, Kerrebijn KF. Lung function and bronchial responsiveness in children who had infantile bronchiolitis. *Pediatr Pulmonol* 1987;**3**:38-44.

17. Zapletal A, Samanek M, Paul T. Lung function in children and adolescents. Methods, reference values. In: Zapletal A, ed. *Progress in respiration research.* Basel: Karger, 1987;**22**:114-218 .
18. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault YC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function test european community for steel and coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993;6(suppl 16):5-40.
19. Birnie D, Thoe Schwartzenberg GWS, Hop WCJ, Van Essen-Zandvliet EEM, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter method for the assessment of bronchial responsiveness in children with asthma depend on age? *Thorax* 1990;**45**:199-202.
20. Schluchter MD. Unbalanced repeated measure models with structured covariance matrices. In: *BMDP Statistical Software Manual.* Dixon WJ, ed. Volume 2. Berkeley: University of California Press, 1990:1207-44.
21. Sears MR, Taylor RD, Print CG, Lake DC, Li Q, Flannery EM, Yates DM, Lucas MK, Herbison GP. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;**336**:1391-96.
22. Schayck CP van, Dompeling E, Herwaarden CLA van, Folgering H, Verbeek ALM, Hoogen HJM van der, Weel C van. Bronchodilator treatment in moderate asthma or chronic bronchitis: continuous or on demand? A randomized controlled study. *BMJ* 1991;**303**:1426-31.
23. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988;**1**:.554-8
24. Wahedna I, Wong CS, Wisniewski AFZ, Pavord AD, Tattersfield AE. Asthma control during and after cessation of regular β_2-agonist treatment. *Am Rev Respir Dis* 1993;**148**:707-12.
25. Beach JR, Young CL, Harkawat R, Gardiner PV, Avery AJ, Coward GA, Walters EH, Hendrick DJ. Effect on airway responsiveness of six weeks treatment with salmeterol. *Pulm Pharmacol* 1993;6:155-57.
26. Roberts JA, Bradding P, Holgate ST, Howarth PH. Effects of a six week course of salmeterol on bronchial reactivity. *Thorax* 1992;**47**:230P.
27. Ariens EJ. Pharmacology of airway smooth muscle. In: Nadel JA, Pauwels R, Snashall PD, eds. Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Oxford, England: *Blackwell Scientific*, 1987;7-22.
28. Van Amsterdam RGM, Meurs H, Ten Berge REF, Veninga NCM, Brouwer F, Zaagsma J. Role of phosphoinositide metabolism in human bronchial smooth muscle contraction and in functional antagonism by beta-adrenoceptor agonists. *Am Rev Respir Dis* 1990;**142**:1124-8.
29. Verberne AAPH, Kerrebijn KF. Effects of ß2-receptor agonists on airway responsiveness. *Life Sci* 1993;**52**:2181-91.
30. Maconochie JG, Minton NA, Chilton JE, Keene ON. Does tachyphylaxis occur to the non-pulmonary effects of salmeterol? *Br J Clin Pharmac* 1994;**37**:199-204.
31. Svedmyr N. Action of corticosteroids on beta-adrenergic receptors. Clinical aspects. *Am Rev Respir Dis* 1990;**141**:S31-8.
32. De Baets FM, Goeteyn M, Kerrebijn KF. The effect of two months of treatment with inhaled budesonide on bronchial responsiveness to histamine and house-dust mite antigen in asthmatic children. *Am Rev Respir Dis* 1990;**142**:581-6.
33. Neijens HJ, Degenhart HJ, Raatgreep HC, Kerrebijn KF. Study on the significance of bronchial hyperreactivity in the bronchus obstruction after inhalation of cat dander allergen. *J Allergy Clin Immunol* 1979;**64**:507-15.
34. British Thoracic Society, Woodhead M (editor). Guidelines on the management of asthma. *Thorax* 1993;**48** Suppl:S1-S24.
35. Roberts JA, Bradding P, Walls AF, Britten KM, Wilson S, Holgate ST, Howarth PH. The influence of salmeterol xinafoate on mucosal inflammation in asthma. *Am Rev Respir Dis* 1992;**145**:A418.

Chapter Six

One year treatment with salmeterol compared to beclomethasone in children with asthma

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6

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One year treatment with salmeterol compared to beclomethasone in children with asthma

6.1 Summary

The aim of this study was to compare the effects of salmeterol and beclomethasone on lung function and symptoms in children with mild to moderate asthma. Sixty-seven children not treated with inhaled corticosteroids were randomized in a double-blind parallel study either to salmeterol 50 µg b.i.d. or beclomethasone 200 µg b.i.d. After one year, FEV_1 significantly increased in the beclomethasone group, whereas in the salmeterol group there was a small reduction. Differences between groups were 14.2 % predicted ($p<0.0001$) and 7.0 % predicted ($p=0.007$) for pre- and postbronchodilator FEV_1 values, respectively. PD_{20} methacholine decreased by 0.73 DD ($p=0.05$) in the salmeterol group and increased by 2.02 DD ($p<0.0001$) in the beclomethasone group. Morning and evening PEF and symptom scores improved in both groups, although more in the beclomethasone group. Asthma exacerbations, for which prednisolone was needed, were more frequent in the salmeterol group (17 versus 2), as were the number of withdrawals due to exacerbations (6 versus 1). However, growth was significantly slower in the beclomethasone group (-0.28 SDS) compared with that in the salmeterol group (-0.03 SDS) ($p=0.001$). We conclude that treatment with a moderate dose of beclomethasone is superior to salmeterol in children with mild to moderate asthma and recommend that salmeterol should not be used as monotherapy.

6.2 Introduction

Short-acting inhaled beta-agonists offer rapid and effective symptom relief in asthmatic children.[1] Their limited duration of action, however, makes them less suitable for controlling symptoms throughout the entire 24-hour period. Furthermore, asthma is now recognized as a chronic inflammatory disease of the airway wall; hence, recent guidelines have focused on anti-inflammatory treatment by either cromoglycate or inhaled corticosteroids.[2 3] Despite this daily prophylactic treatment many children still suffer from asthma symptoms.[4] In particular, exercise-induced and nocturnal symptoms result in substantial discomfort to children as well as to their parents.[5] Taken as a single dose salmeterol, a new long-acting inhaled $ß_2$-agonist, has a bronchodilating effect of at least 12 hours in adults as well as children.[6 7] Protection against methacholine- and histamine-induced airway obstruction lasts for 12 to 24 hours.[7 8] Single-dose studies show prolonged protection against other bronchoconstricting stimuli such as exercise,[9] hyperventilation with dry cold air[10] and allergen.[11] Twice daily dosing of salmeterol may result in a 24-hour protection against various bronchoconstricting stimuli and therefore lessen symptoms in asthmatic patients. Compared with salbutamol, twice daily salmeterol for several weeks or months in adults as well as children results

in fewer symptoms, less need for additional bronchodilator treatment and better improvement in peak flow rates.[12 13] However, in these studies some of the patients were already on treatment with either cromoglycate or inhaled corticosteroid, making it difficult to estimate the true therapeutic potential of salmeterol. The aim of our study was to compare the effect of one year treatment with salmeterol with the effect of treatment with an inhaled corticosteroid. The primary efficacy outcome parameters were airway caliber, measured as forced expiratory volume in 1 second (FEV_1) and airway responsiveness to methacholine. Symptom scores, exacerbations, additional use of short-acting β_2-agonists and peak flow rates were considered as secondary outcomes.

6.3 Material and methods

6.3.1 Patients

Sixty-seven children aged 6 to 16 years with mild to moderate asthma were selected from the outpatient pediatric clinics of 9 hospitals, 6 university hospitals and 3 general hospitals. Patients were recruited between September 1992 and October 1994. All children had mild to moderate asthma according to the American Thoracic Society criteria.[14] Patients included in the study had to have: (1) a FEV_1, that was 55-90% of predicted value and/or a ratio of FEV_1 to forced vital capacity (FVC) that was 50-75%, (2) an increase of at least 10% in FEV_1 after inhalation of 0.8 mg salbutamol, (3) airway hyperresponsiveness to methacholine, i.e. a 20% fall in FEV_1 after inhalation of 150 μg or less methacholine (PD_{20} methacholine); this being more than two standard deviations below the mean value in healthy children[15], (4) an ability to produce reproducible lung function tests, i.e. a variation in three consecutive measurements of FEV_1 of less than 5%, (5) a history of stable asthma for at least 1 month without exacerbations or respiratory tract infections, (6) not used inhaled corticosteroids in the previous 6 months or cromoglycate in the previous 2 weeks. The study was approved by the medical ethics committees of the participating centers. Written informed consent was obtained from all patients and their parents.

6.3.2 Study design

The study was a double-blind, randomized clinical trial. It consisted of a 6 week run-in period, a treatment period of 54 weeks and a follow-up period after treatment of 2 weeks. In the run-in period the only medication allowed was salbutamol 200 μg on demand, with a maximum of 6 inhalations per day. In the first and the last week of the run-in period measurements of FEV_1 and FVC before and after bronchodilatation and measurements of PD_{20} methacholine were performed. Lung function inclusion criteria had to be fulfilled at one of these visits. At the end of the run-in period, patients were

allocated to one of the two treatments by an independent randomization center. Randomization was stratified for sex, age, center, baseline FEV_1-value, baseline PD_{20} and prior use of inhaled corticosteroids more than 6 months before starting the study, using a computerized minimization method.[16] Using this method a patient is allocated to a treatment so as to minimize any imbalance between the treatment groups for each stratification factor. Study treatment consisted of either salmeterol xinafoate 50 μg b.i.d. or beclomethasone dipropionate 200 μg b.i.d. All drugs were administered as Rotadisks^R in combination with a Diskhaler^R (Glaxo Wellcome, Greenford, United Kingdom). All children were instructed in the use of this inhalation device prior to entry into the study and their inhalation technique was checked at every visit. For relief of symptoms during the treatment period the use of salbutamol 200 μg Rotadisk was allowed, with a maximum dose of 6 inhalations per day. Asthma symptoms, which did not sufficiently improve with the maximum dose of rescue salbutamol, were treated with a standard course of prednisolone. On the first day this started with a dose of 30 or 35 mg, depending on the weight of patients, and was tapered off to zero in 7 days. During the treatment period the response to a bronchodilator (at 12, 24, 36 and 48 weeks) and PD_{20} methacholine (at 6, 18, 30, 42 and 54 weeks) were measured alternately at intervals of 6 weeks. After 54 weeks all patients stopped taking randomized treatment for a period of 2 weeks. During this follow-up period the only medication allowed was salbutamol on demand. At the end of this period PD_{20} methacholine was measured.

At each clinic visit FEV_1, FVC, PEF, height, body weight, heart rate, systolic and diastolic blood pressure were measured. Height was measured using a stadiometer in centimeters, corrected to one decimal place. Furthermore, the patients were asked about adverse events and the number of used blisters of study medication and rescue salbutamol were counted.

Throughout the study period patients kept diary cards on which symptoms and additional use of rescue medication were recorded. They also measured PEF using a mini-Wright peak flow meter (Clemente Clarke International Ltd., Harlow, Essex, U.K.) at home. Symptoms and PEF measurements were recorded during the first 2 weeks of each 6-week period between clinic visits. Dyspnoea, wheeze and cough in the morning and the evening were scored separately, using a scale from 0 to 3. PEF was measured in triplicate morning and evening before taking study medication and all three values were recorded.

Patients were withdrawn from the study if they needed 3 or more prednisolone courses within 3 months, if according to the investigator it was not ethical to continue blinded treatment, or if patients or parents wanted to discontinue.

All data were collected and checked by the coordinating center in Rotterdam to ensure completeness. Interim analyses of the study data were made by an independent statistician every year and reviewed by a data monitoring committee. Investigators were kept blind to the results of the interim analyses. The data monitoring committee allowed the study to continue until all patients had completed.

6.3.3 Lung function measurements

All lung function measurements were performed between 12 and 18 hours after inhalation of the study drug. For each patient the time of measurement was constant throughout the study period. Patients were instructed to take their last dose of study drug before the clinic visit at a fixed time the previous evening. Rescue salbutamol was not allowed in the 8 hours before lung function measurement. The time of inhalation of the last dose of study drug, and any use of salbutamol was checked before taking measurements, and, if necessary, lung function measurements were postponed. No lung function measurements were performed less than 4 weeks after a course of prednisolone.

FEV_1 and FVC were measured according to the recommendations of the European Community for Steel and Coal, by using a water sealed or dry rolling seal spirometer or pneumotachometer.[17] At least 3 manoeuvres were performed with FEV_1 and FVC within 5%. Maximal 5 manoeuvres were allowed and the largest FEV_1 and FVC were taken for the analysis. Reference values of Zapletal and coworkers were used.[18] Post-bronchodilator FEV_1 was measured after inhalation of 0.8 mg salbutamol in order to obtain maximal bronchodilatation.[19] Salbutamol was administered by a Volumatic spacer as 4 puffs of 0.2 mg, one at a time, inspiring slowly from functional residual capacity to total lung capacity and holding each breath for about 10 seconds. FEV_1 was measured 20 minutes after inhalation of the last puff. PEF was measured in triplicate, using the patient's own Mini Wright peakflow meter. Methacholine provocation tests were performed using a modification of the dosimeter method by Chai, as described previously.[20] Preparation of methacholine solutions was standardized in all centers. Nebulized methacholine bromide in unbuffered saline solution was given in doubling concentrations (0.125 to 39.2 mg/ml). The aerosol was generated by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA) attached to a Rosenthal dosimeter (Laboratory for Applied Immunology, Fairfax, VA), driven by air at 137.8 kPa (20 psi) with a timing adjustment of 0.6 seconds. Output of the nebulizers was measured before the start of the study. All parts of each nebulizer were marked with waterproof paint to prevent interchanging. Nebulizers were cleaned after each measurement to prevent precipitations, and orifices were checked weekly according to recommendations.[21]

Aerosolized solution was delivered to the mouth in 4 consecutive breaths. Mouth doses were 2.5 to 784 μg of methacholine. Saline was inhaled before methacholine to exclude a non-specific response. The effect of each dose was determined by measuring FEV_1 in triplicate 3 minutes after administration. PD_{20} methacholine was calculated by a computer program from a log-dose-response plot by linear interpolation. Airway responsiveness was only measured if FEV_1 before methacholine provocation was 80% or more of the individual's baseline value at entry into the study.
All centers used written guidelines for lung function measurements. Technicians attended a training course before the start of the study. Site visits were made once a year by the primary investigator and a pulmonary physiologist to inspect the equipment and the methods used.

6.3.4 Statistical analysis
The study was designed to have 90% statistical power to detect a difference of 8% predicted FEV_1 using a statistical significance level of 5%.
Changes in FEV_1 and the logarithm of PD_{20} within each group over the study period were assessed using paired t-tests for matched data. Changes in PD_{20} were reported as numbers of DD. Comparisons of FEV_1 and the logarithm of PD_{20} between groups at each clinic visit were made using analysis of covariance to adjust for mean pre-intervention levels. Comparisons of both morning and evening PEF measurements recorded during the 2-week diary periods were made using analysis of covariance to adjust for mean pre-intervention levels. Morning and evening PEF variability for each patient was expressed as the standard deviation of the PEF measurements. Each measure of day-to-day variability was compared between treatment groups using the Mann-Whitney test. Distributions of symptoms during the 2-week diary periods were compared using the Mann-Whitney test, as were the numbers of blisters of rescue salbutamol used over this period. Where patients failed to complete their daily record cards for more than 7 days in any 14 day period such assessments were not included in the analysis. Otherwise, when there were missing days in the record, pro rata adjustment was made to give a 2-week assessment. Comparison of heights between groups were made using analysis of covariance to adjust for pre-intervention levels. Heights were also expressed as SDS using Dutch reference growth charts.[22] Changes in SDS over time within each group were assessed using paired t-tests for matched data, and comparisons between groups at each clinic visit were made using analysis of covariance to adjust for mean pre-intervention levels. The analysis of covariance model was extended to allow for the effect of puberty on SDS and to test for a possible interaction between puberty and treatment. All reported p-values are for two-sided tests and for simplicity of presenta-

Table 6.1 Baseline characteristics at the start of the run-in period and at randomization by treatment group.

At start of run-in period:	Salmeterol (n=32)	Beclomethasone (n=35)
sex	9F/23M	13F/22M
age (years):		
mean(sd)	10.6 (2.9)	10.5 (2.3)
duration of asthma		
(mean) (years)	6.6	6.3
atopy status:		
none	0/32	6/35
house dust mite	30/32	28/35
cat	18/32	14/35
dog	19/32	20/35
grasspollen	25/32	18/35
previous treatment:		
cromoglycate	15/32	15/35
inh.corticosteroids	5/32	6/35
FEV_1 % predicted:		
mean (sd)		
prebronchodilator	85.6 (15.0)	86.3 (13.6)
postbronchodilator	100.8 (10.9)	99.7 (14.0)
PD_{20} (microgram):		
median (quartiles)	13.5 (6-35.5)	18 (8-40)
height (cm)(sd)	144.9 (16.4)	144.8 (12.6)
At randomization:		
FEV_1 % predicted		
mean (sd)		
prebronchodilator	82.0 (13.9)	84.4 (16.7)
postbronchodilator	99.2 (13.8)	99.2 (15.3)
PD_{20} (microgram):		
median (quartiles)	18 (6.5-46)	20.5 (8-39)
PEF (l/min) mean(sd)		
morning	297 (96)	284 (69)
evening	301 (94)	299 (71)
Days in two weeks with symptoms:		
median (quartiles)	6 (3-11)	6 (2-12)
Nights in two weeks with symptoms:		
median (quartiles)	7 (4-10)	6 (2-13)
height (cm)(sd)	145.6 (16.5)	145.3(12.5)

tion are without formal adjustment for multiple comparisons over time. Confidence intervals for means were calculated parametrically assuming normality. Confidence intervals for medians were calculated to be consistent with the results of the Wilcoxon test.[23]

6.4 Results

Between October 1992 and October 1994, 67 patients (45 boys, 22 girls) were enrolled into the study. Patient characteristics at entry (beginning of the run-in period) and at randomization were similar in the two treatment groups (Table 6.1).

Ten patients withdrew during the study period. Seven patients withdrew because of exacerbations (6 in the salmeterol group), 2 because of non-compliance (both in the beclomethasone group) and 1 because of dizziness and nausea (salmeterol group).

Compliance with study treatment did not differ between the groups: the median number of blisters used per day were 1.82 and 1.84 in the salmeterol and beclomethasone groups respectively; ie. 91 respectively 92% of the prescribed study medication was used.

6.4.1 Airway caliber

At the end of the 54-week treatment period the mean difference in FEV_1 between treatment groups was 14.2 % predicted (95% confidence interval(CI) 8.3;20.0)($p<0.0001$) in favour of the beclomethasone treated group. On average FEV_1 levels declined in the salmeterol treated group over the course of the study. However, at no time point was the reduction from pretreatment values statistically significant. At the end of treatment the average change was -4.5 % predicted (95%CI -9.0;0.1). In the beclomethasone treated group average FEV_1 levels significantly increased ($p<0.0001$) by about 10% predicted at all visits (Figure 6.1). Two weeks after discontinuation of beclomethasone treatment, a significant reduction in FEV_1 was noted ($p=0.02$). Despite this reduction FEV_1 levels at the end of the follow-up period were still significantly higher ($p<0.0001$) in the beclomethasone group than in the salmeterol group.

Postbronchodilator results of FEV_1 were similar to the prebronchodilator results. After one year, the mean treatment difference was 7.0 % predicted (95%CI 2.0;11.9)($p=0.007$). On average FEV_1 levels declined in the salmeterol treated group and increased in the beclomethasone treated group. At the end of treatment the changes were -4.0 % predicted (95%CI -8.2;0.2) and 3.2 % predicted (95%CI 0.2;6.3) respectively.

Figure 6.2 shows the mean levels of FEV_1 before and after bronchodilatation.

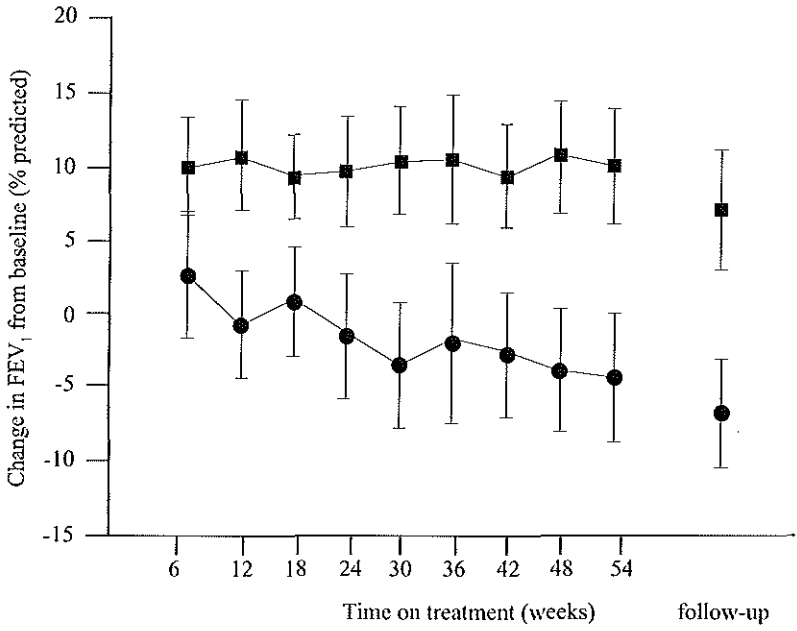

Figure 6.1
Changes in FEV_1% predicted (means, 95% CI) from baseline during treatment with salmeterol (●) or beclomethasone (■).

6.4.2 Airway responsiveness

At the end of the treatment period the difference between groups was 2.79 DD (95%CI 1.75;3.84)($p < 0.0001$). On average PD_{20} methacholine declined in the salmeterol treated group over the course of the study. At the end of treatment the average reduction was -0.73 DD (95%CI -1.46;0.00)($p=0.05$). Airway responsiveness gradually improved in the beclomethasone treated group. At the end of treatment the average increase in PD_{20} methacholine was 2.02 DD (95%CI 1.26;2.78)($p < 0.0001$) (Figure 6.3). After one year median PD_{20} values were 7 and 58 μg for the salmeterol and beclomethasone treated groups, respectively. Two weeks after discontinuation of beclomethasone PD_{20} methacholine dropped on average 0.76 DD ($p=0.004$) to a median of 47 μg, whereas after stopping salmeterol it dropped 0.4 DD ($p=0.09$).

The differences in improvement between treatment groups for FEV_1 and airway responsiveness are also reflected in the difference in the percentage of patients improving (Table 6.2).

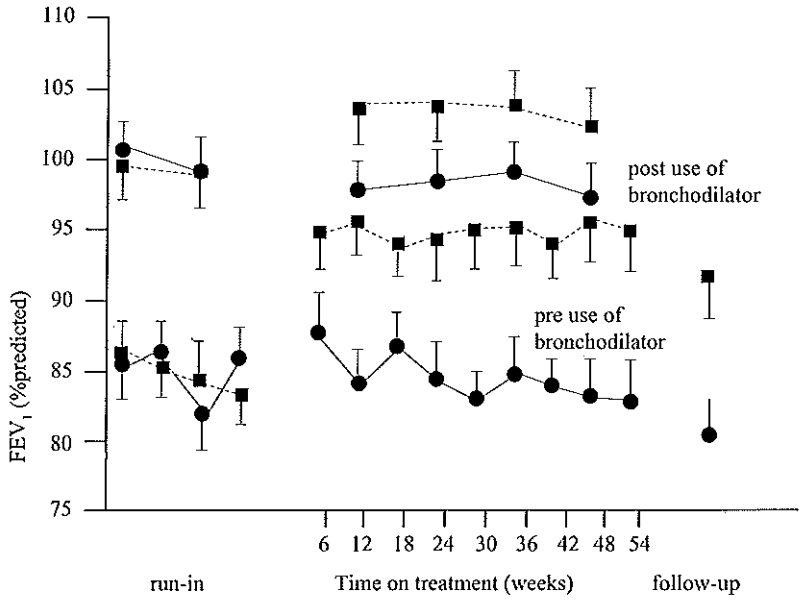

Figure 6.2
FEV_1% predicted (means, SEM) before and after bronchodilatation with 0.8 mg salbutamol during treatment with salmeterol (●) or beclomethasone (■).

Table 6.2 Percentage of patients showing an improvement in FEV_1 (pre- and postbronchodilator) and PD_{20} by treatment group

Treatment week	6	12	18	24	30	36	42	48	54	follow-up
Salmeterol										
FEV_1 pre	61	53	58	45	34	48	35	30	46	24
FEV_1 post		44		35		45		30		
PD_{20}	58		52		37		25		26	26
Beclomethasone										
FEV_1 pre	91	86	88	85	88	88	85	85	88	77
FEV_1 post		74		79		79		67		
PD_{20}	77		76		76		82		81	77

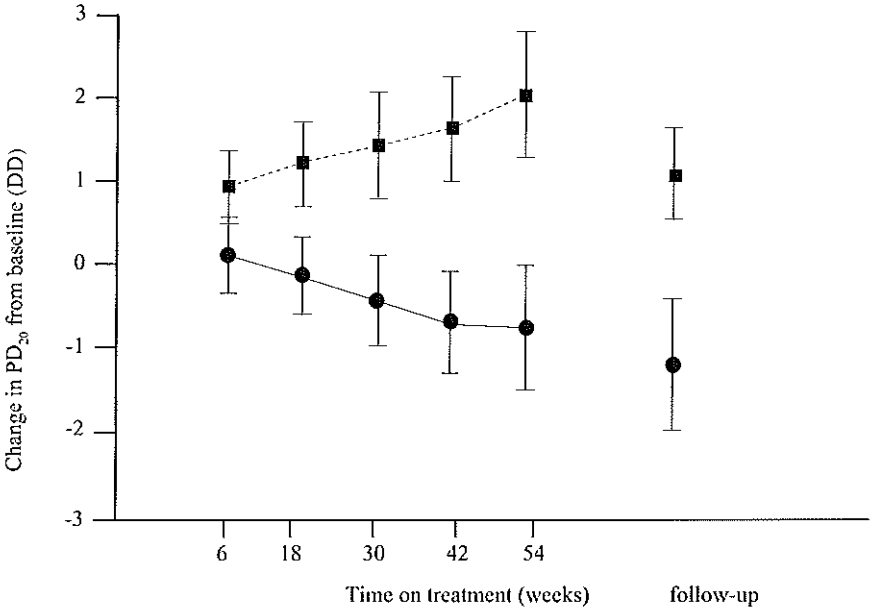

Figure 6.3
Changes is airway responsiveness in doubling doses (means, 95% CI) during treatment with salmeterol (●) or beclomethasone (■).

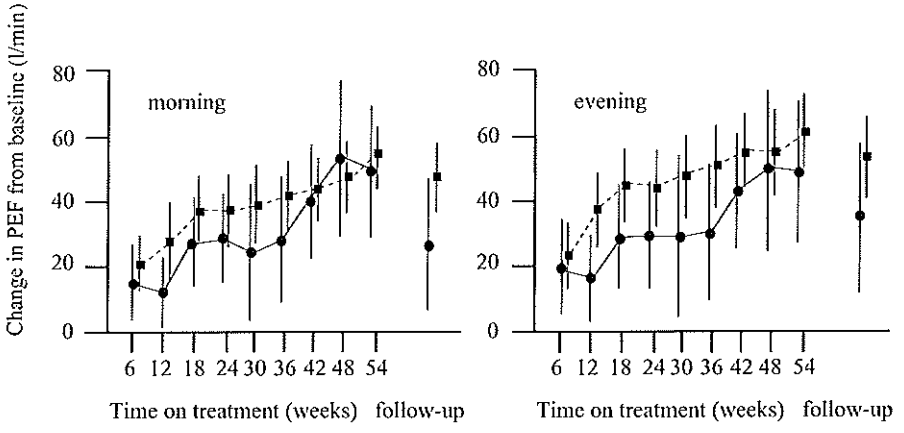

Figure 6.4
Mean changes in morning and evening PEF (l/min, 95% CI) during treatment with salmeterol (●) or beclomethasone (■).

6.4.3 Peak expiratory flow rates

Morning and evening PEF improved in both groups, with a tendency for more improvement in the group treated with beclomethasone (Figure 6.4). However, at the end of treatment there were no significant differences between groups. Mean increases in morning PEF were 48.8 l/min and 60.9 l/min for salmeterol and beclomethasone respectively, mean increases in evening PEF 48.9 l/min and 54.3 l/min. Day-to-day variability in both morning and evening PEF was also lower in the beclomethasone group (Figure 6.5).

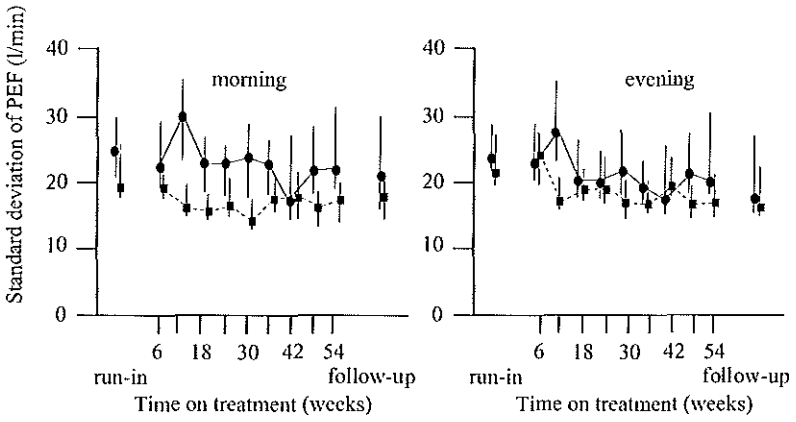

Figure 6.5
Standard deviation between days in morning and evening PEF (l/min, medians, 95% CI) during treatment with salmeterol (●) or beclomethasone (■).

6.4.4 Symptoms

Daytime and nighttime symptoms diminished in both treatment groups, with fewer symptoms in the patients treated with beclomethasone. However, the difference between salmeterol and beclomethasone was only significant at some time points. The percentage of children in the beclomethasone treated group reporting no symptoms during the 2-week diary card periods increased from 6% in the run-in period to 55% after one year of treatment (Figure 6.6). In comparison, 3% and 36% were asymptomatic during the corresponding periods in the salmeterol treated group. The need for additional salbutamol during daytime and nighttime, as noted on the diary cards, significantly diminished throughout the treatment period in the beclomethasone group (Figure 6.7). The median number of additional salbutamol inhalations per day, as counted from the

Table 6.3 Most common reported adverse events during the treatment period

	Salmeterol	Beclomethasone
Number of patients	32	35
Number of patients with any adverse event	30 (94%)	31 (89%)
Number of patients with:		
asthma	18 (56%)	3 (9%)
rhinitis	9 (28%)	5 (14%)
fever	8 (25%)	4 (11%)
nausea and vomiting	7 (22%)	4 (11%)
headache	6 (19%)	11 (31%)
malaise and fatigue	4 (13%)	10 (29%)
viral infections	4 (13%)	3 (9%)
breathing disorders	4 (13%)	3 (9%)
cough	3 (9%)	8 (23%)
upper resp. tract infection	3 (9%)	5 (14%)
viral respiratory infection	2 (6%)	10 (29%)
throat irritation	2 (6%)	3 (9%)
injuries	0	4 (11%)

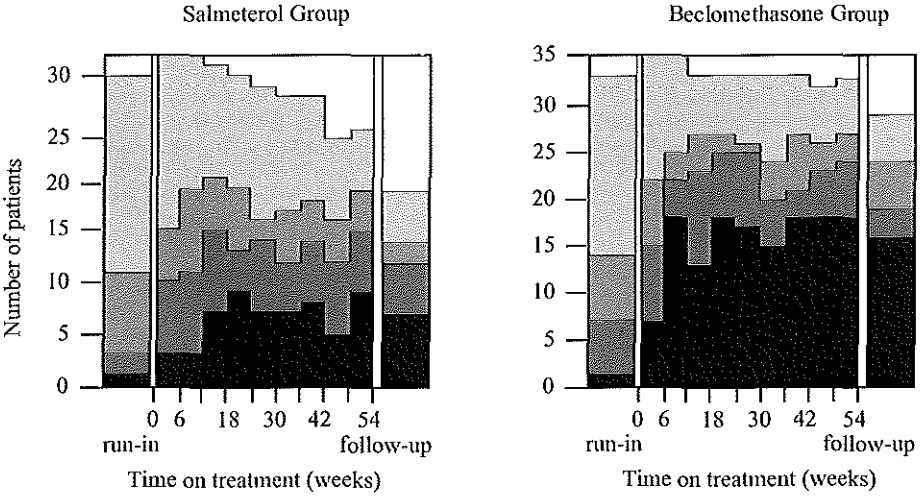

Figure 6.6
Number of days out of 14 with symptoms during salmeterol (left) and beclomethasone (right) treatment. Days with symptoms per two weeks: missing, □ ; 8-14, ▣ ; 4-7, ▩ ; 1-3, ▩ ; 0, ■.

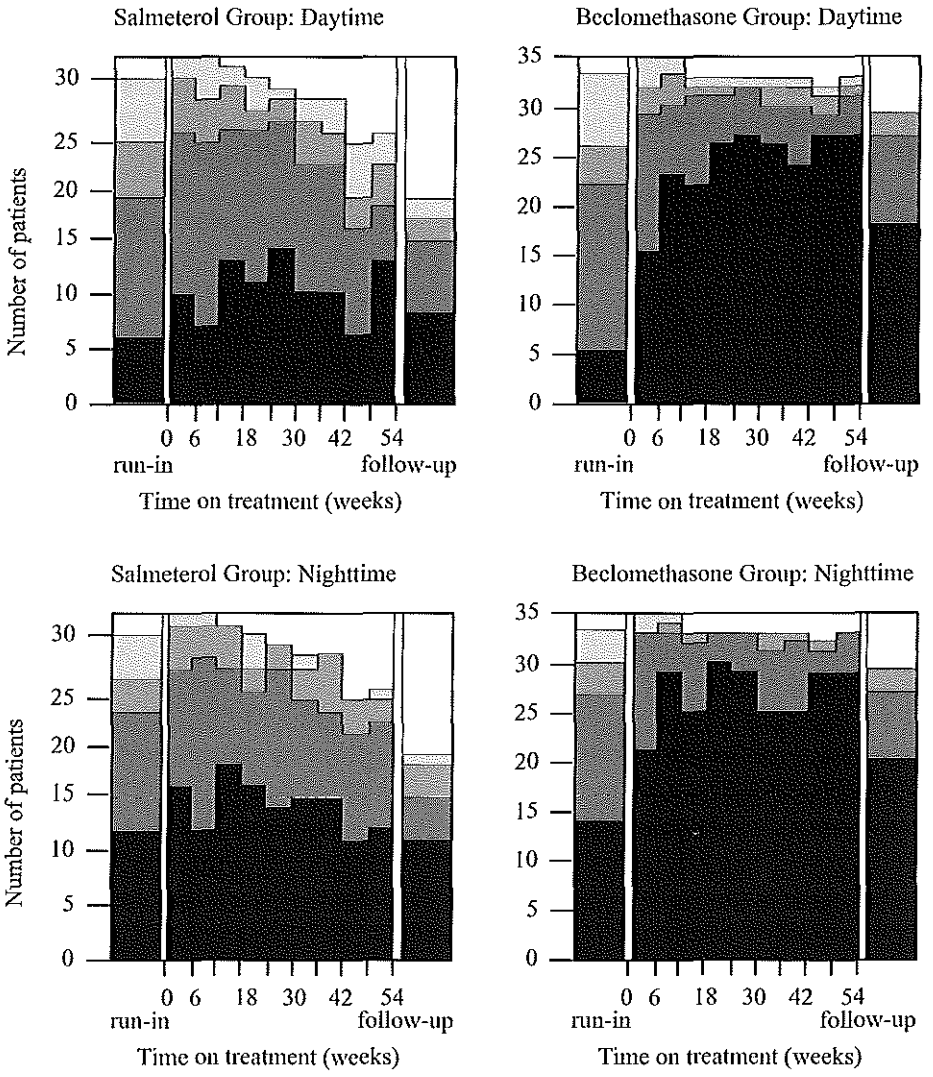

Figure 6.7
Number of blisters of salbutamol used in the 14 days period during daytime and nighttime for the salmeterol group and beclomethasone group:
missing, □; 15+,□; 8-14,□; 1-7,■; 0,■.

used blisters, during the treatment period was 0.44 in the salmeterol treated group and 0.07 in the beclomethasone treated group (p=0.0001).

During the treatment period 19 courses of prednisolone were given, 17 of these to 15

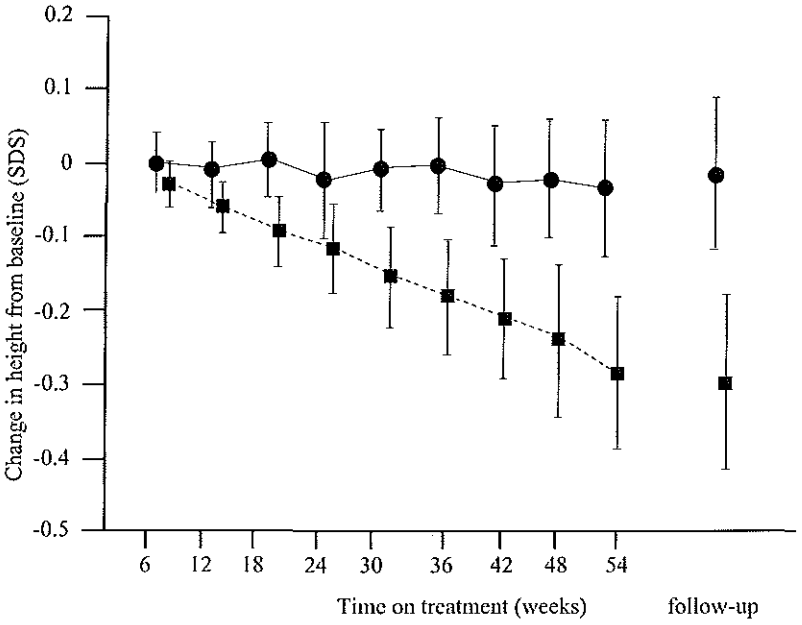

Figure 6.8
Change in height as SDS (means, 95% CI) during treatment with salmeterol (●) or beclomethasone (■).

patients in the salmeterol group (2 patients received 2 courses).

6.4.5 Adverse events

At no point during the treatment period were any significant changes in heart rate, systolic and diastolic blood pressure found in either treatment group.

Table 6.3 shows the most common reported adverse events.

The mean increase in height was 6.1 cm (95%CI 5.3;6.9) in the salmeterol treated group, compared with 4.7 cm (95%CI 4.0;5.3) in the beclomethasone treated group (p=0.007). SDS showed a change of -0.03 SDS in the patients treated with salmeterol compared to 0.28 SDS in the patients treated with beclomethasone (p=0.001) (Figure 6.8). No interaction was found with gender. A significant interaction (p=0.03) was found with puberty; the mean difference in SDS between groups was -0.10 (95%CI -0.29;0.10) for patients with puberty stages 2 and more and -0.37 (95%CI -0.58;-0.16) for prepubertal patients.

6.5 Discussion

This is the first long term study to compare treatment with a long-acting β_2-agonist with treatment with an inhaled corticosteroid. We selected children with mild to moderate asthma, who were not treated with an inhaled corticosteroid, as this is the category of patients for whom monotherapy with a long-acting β_2-agonist may be considered. For salmeterol twice daily 50 μg is recommended as the optimum dose in childhood asthma.[13] Inhaled corticosteroids are the most effective asthma treatment currently available and so they were chosen as the comparator treatment. A daily dose of 400 μg beclomethasone was chosen, as this is considered to be a moderate dose in the treatment of childhood asthma.[2]

The data from this study show that treatment with beclomethasone is superior to treatment with salmeterol in terms of airway caliber, airway responsiveness, symptoms and exacerbations. Two short-term studies have compared inhaled corticosteroid with salmeterol treatment. In an open uncontrolled study 23 children received either twice daily 100 μg budesonide or twice daily 50 μg salmeterol for 3 weeks.[24] FEV_1 values after budesonide were not significantly higher than after salmeterol. Both treatments improved symptoms and peak flow rates. A randomized study in 46 adults showed no significant difference in the effect on FEV_1 and PC_{20} after 6 weeks of treatment with salmeterol 50 μg twice daily, fluticasone 250 μg twice daily or the combination.[25] No differences may have been found in these studies because of the small number of patients included and/or the short duration of treatment. However, in our study significant differences between treatments in FEV_1 and airway responsiveness were apparent already after 6 weeks. The rapid improvement in FEV_1, which occurred within 6 weeks after the start of beclomethasone treatment compares well with the data found by Van Essen et al. in a three year follow-up study, in which budesonide 600 μg daily was given.[26] In that study, airway responsiveness also improved, but more gradually than in the current study. After 12 months inhaled corticosteroid treatment PD_{20} increased, on average, by less than 1.5 DD, whereas in this study it improved by 2 DD. It is likely that the patients in Van Essen's study had more severe asthma; their mean baseline FEV_1 was 76% of predicted, compared to 86% in the current study. Although baseline PD_{20} values were similar to the PD_{20} values in this study, more than half of their patients had recently been treated with inhaled corticosteroids and it is therefore possible that part of the potential improvement had already taken place. Another difference is that in Van Essen's study the inhaled corticosteroid was combined with an inhaled short-acting β_2-agonist and one might hypothesize that regular use of β_2-agonist reduces the improvement caused by the inhaled corticosteroid. This has been suggested in a study by Sears et al.[27], who found a negative effect of regular use of fenoterol on

several outcomes of asthma, compared to it's use on demand. However, a later publication indicated that the differences were small.[28]

With salmeterol we observed a tendency to gradual deterioration in FEV_1 and PD_{20}. This became more pronounced at the end of the one year treatment period. In a previous study of 4 months duration with salmeterol 50 μg b.i.d. we did not observe such a decline in FEV_1 or PD_{20}.[29] However, in that study lung function measurements were performed exactly 12 hours after inhalation of salmeterol and it could be argued that a negative effect was masked by the residual bronchodilator effect of salmeterol. This seems unlikely, because in the present study comparison of subgroups according to the measurement interval after salmeterol inhalation did not reveal any differences in the magnitude of the decrease in FEV_1 or PD_{20}. Tolerance to the bronchoprotective effects of salmeterol has been described and seems to occur within a period of a few days after starting daily treatment.[29 30] In the longest follow-up study so far,[29] there was no evidence for a progressive decline of protection after 4 months, and therefore it is unlikely that increasing tolerance explains our results. Salmeterol, on contrast to inhaled corticosteroid treatment, does not reduce airway wall inflammation.[31] We think it likely that ongoing inflammation might be the cause of the decrease in FEV_1 and PD_{20} in the salmeterol group. Thickening of the airway wall due to inflammatory changes will result in a lower airway diameter and might also explain the increase in airway responsiveness.[32] This is consistent with the finding that postbronchodilator FEV_1 during salmeterol treatment tends to decrease. Whether ongoing inflammation is the result of the underlying disease itself or whether it is negatively influenced by the use of a regular β_2-agonist could only have been shown by incorporating a control group with placebo treatment into the study design. This was considered not feasible. The drop in FEV_1 and PD_{20} after stopping salmeterol treatment suggests that during treatment there was a beneficial effect of salmeterol on airway caliber and airway responsiveness.

Despite a deterioration in FEV_1 and PD_{20} symptoms diminish and peak flow rates increase in the patients on salmeterol. This is in agreement with the clinical improvement found in previous studies in which salmeterol was compared to salbutamol.[12 13] However, in our study, salmeterol was compared to treatment with inhaled corticosteroid and improvements were less in the salmeterol group. Asthma exacerbations were rare in the children treated with beclomethasone. In contrast, they were the most frequent reason for withdrawal in the children treated with salmeterol. In most other studies with salmeterol a significant reduction of asthma exacerbations was not observed, despite reductions in symptoms.[12 13]

In this study treatment with 400 μg beclomethasone daily, administered as dry powder,

resulted in decreased growth compared to salmeterol treatment. So far, most studies with inhaled corticosteroids have not shown an effect on long- and intermediate-term growth in children.[33] Recently a study by Doull et al.[34] revealed growth impairment during 7 months of treatment with beclomethasone 400 μg/day as dry powder in children with mild asthma. Compared to placebo, a growth difference of 1.0 cm was found. This is consistent with our observed difference of 1.4 cm over a 12 months period. In both studies no differences were found between boys and girls. The study of Doull et al. only included prepubertal children. In our study the effect on growth was more marked in prepubertal children. Doull's study as well as this study used dry powder inhalators, compared to former studies in which usually metered dose inhalers were used.[33] Differences in delivery systems and thereby in lung and oropharyngeal deposition may account for the differences in effect on growth. From the scarce data available, children with asthma usually grow to their predicted height.[35] It is unlikely therefore, that a negative effect of inhaled corticosteroids on height will continue for years during treatment. One might speculate that it is a transient effect which will be followed by a catch-up growth later on. Further prospective long-term studies are necessary to address this issue.

From the results of this study we conclude that treatment with inhaled corticosteroid in a moderate dose provides better asthma control and lung function improvement compared with monotherapy with a long-acting β_2-agonist in children with mild to moderate asthma. This study shows that a reduction in symptoms and an increase in PEF may well occur during treatment with salmeterol without a corresponding improvement in airway caliber and airway responsiveness. Monotherapy with a long-acting β_2-agonist therefore carries a risk of masking the severity of the disease and probably allow ongoing inflammation. We suggest that salmeterol should not be used as a monotherapy in children with asthma. We further advocate the careful monitoring of individual growth during treatment with inhaled corticosteroids.

6.6 References

1. Kerrebijn KF. Beta agonists. In: *Asthma, its pathology and treatment*. Ed. Kaliner MA, Barnes PJ, Persson CGA. Marcel Dekker, Inc., New York 1991, 523-59.
2. Warner JO. Asthma: a follow-up statement from an international paediatric asthma consensus group. *Arch Dis Child* 1992;**67**:240-8.
3. Woodhead M (Ed.). Guidelines on the management of asthma. *Thorax* 1993;**48**:S1-24.
4. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Kerrebijn KF. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide). Can it be achieved? *Eur Respir J* 1994;**7**:63-8.
5. Lenney W, Wells NEJ, O'Neill BA. The burden of paediatric asthma. *Eur Respir Rev* 1994;**4**:49-62.

6. Ullman A, Svedmyr N. Salmeterol, a new long-acting inhaled beta-2-adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988;**43**:574-8.
7. Verberne AAPH, Hop WCJ, Bos AB, Kerrebijn KF. Effect of a single dose of salmeterol on baseline airway calibre and methacholine-induced airway obstruction in asthmatic children. *J Allergy Clin Immunol* 1993; **91**:127-34.
8. Campos Gongora H, Wisniewski AFZ, Tattersfield AE. A single dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991;**144**:626-9.
9. Green CP, Price JF. Prevention of exercise-induced asthma by inhaled salmeterol xinafoate. *Arch Dis Child* 1992;**67**:1014-7.
10. Malo JL, Ghezzo H, Trudeau C, L'Archévêque J, Cartier A. Salmeterol, a new inhaled beta-2-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *J Allergy Clin Immunol* 1992;**89**:567-74.
11. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;**336**:1338-42.
12. Lundback B, Rawlinson DW, Palmer JBD. Twelve month comparison of salmeterol and salbutamol as dry powder formulations in asthmatic patients. *Thorax* 1993;**48**:148-53.
13. Lenney W, Pedersen S, Boner AL, Ebbutt A, Jenkins MM. Efficacy and safety of salmeterol in childhood asthma. *Eur J Pediatr* 1995;**154**:983-90.
14. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225-44.
15. Duiverman EJ, Neijens HJ, Van Strik R, Affourtit MJ, Kerrebijn KF. Lung function and bronchial responsiveness in children who had infantile bronchiolitis. *Pediatr Pulmonol* 1987;**3**:38-44.
16. Pocock SJ. Clinical trials, a practical approach. John Wiley and sons, Ltd. Chichester, 1983.
17. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault YC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function test European Community for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J.* 1993;**6**(suppl 16):5-40
18. Zapletal A, Samanek M, Paul T. Lung function in children and adolescents. Methods, reference values. In: Zapletal A, ed. *Progress in respiration research.* Basel: Karger, 1987;**22**:114-218.
19. Merkus PJFM, Eelkman Roorda HM, van Essen-Zandvliet EEM, Duiverman EJ, Quanjer PhH, Kerrebijn KF. Assessment of bronchodilatation following spontaneous recovery from a histamine challenge in asthmatic children. *Thorax* 1992;**47**:355-9.
20. Birnie D, Thoe Schwartzenberg GWS, Hop WCJ, Van Essen-Zandvliet EEM, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter method for the assessment of bronchial responsiveness in children with asthma depend on age? *Thorax* 1990;**45**:199-202.
21. Merkus PJFM, Van Essen-Zandvliet EEM, Parlevliet E, Borsboom G, Sterk PJ, Kerrebijn KF, Quanjer PhH. Changes of nebulizer output over the years. Technical note. *Eur Respir J* 1992;**5**-:488-91.
22. Roede MJ, Van Wieringen JC. Growth diagrams 1980, the Netherlands. Third nation wide survey. *T Soc Gezondheidszorg* 1985;**63**:1-34.
23. Conover WJ. Practical non-parametric statistics. *Wiley.* New York, 1980.
24. Fuglsang G, Agertoft L, Vikre-Jorgensen J, Pedersen S. Influence of budesonide on the response to inhaled terbutaline in children with mild asthma. *Pediatr Allergy Immunol* 1995;**6**:103-8.
25. Weersink EJM, Douma WR, Koëter G, Postma DS. Is there a synergistic effect between salmeterol and fluticasone in asthmatics with nocturnal airway obstruction? *Am J Respir Crit Care Med* 1995;**151**:A268.
26. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF. Effects of 22 months treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness and symptoms in children with asthma. *Am Rev Respir Dis* 1992;**146**:547-54.

27. Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery EM, Yates DM, Lucas MK, Herbison GP. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;**336**:1391-6.
28. Taylor DR, Sears MR, Herbison GP, Flannery EM, Print CG, Lake DC, Yates DM, Lucas MK, Li Q. Regular inhaled beta-agonist in asthma; effects on exacerbations and lung function. *Thorax* 1993;**48**:134-8.
29. Verberne AAPH, Hop WCJ, Creyghton FBM, Van Rooij RWG, Van den Berg M, De Jongste JC, Kerrebijn KF. Airway responsiveness after a single dose of salmeterol and during 4 months' treatment in asthmatic children. *J Allergy Clin Immunol* 1996;**97**:938-46.
30. Verberne AAPH, McCormack EM, Fuller R, Devoy M. An overview of nine clinical trials of salmeterol in an asthmatic population. *Eur Respir J* 1995:**8**;A156s.
31. Gardiner PV, Ward C, Booth H, Allison A, Hendrick DJ, Walters EH. Effect of eight weeks of treatment with salmeterol on bronchoalveolar lavage inflammatory indices in asthmatics. *Am J Respir Crit Care Med* 1994;**150**:1006-11.
32. James AL, Paré PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989;**139**:242-6.
33. Wolthers OD. Long-, intermediate- and short-term growth studies in asthmatic children treated with inhaled glucocorticosteroids. *Eur Respir J* 1996;**9**:821-7.
34. Doull IJM, Freezer NJ, Holgate ST. Growth of prepubertal children with mild asthma treated with inhaled beclomethasone dipropionate. *Am J Respir Crit Care Med* 1995;**151**:1715-9.
35. Balfour-Lynn L. Growth and childhood asthma. *Arch Dis Child* 1986; **61**:1049-55.

Chapter Seven

Addition of salmeterol versus doubling the dose of beclomethasone in children with asthma

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Addition of salmeterol versus doubling the dose of beclomethasone in children with asthma

7.1 Summary

Studies in adults revealed that addition of salmeterol to a moderate dose of inhaled corticosteroid resulted in better symptom control and higher PEF compared with doubling the dose of inhaled corticosteroid. The aim of this 3-group study was to compare the effects of a moderate dose of beclomethasone, the same dose of beclomethasone with salmeterol and a doubling dose of beclomethasone on lung function and symptoms in children with moderate asthma. One hundred and seventy-seven children, already treated with inhaled corticosteroids, were randomized in a double-blind parallel study either to salmeterol 50 μg b.i.d., beclomethasone 200 μg b.i.d. or placebo. Beclomethasone 200 μg b.i.d. was continued in all groups. No significant differences between groups were found in FEV_1, PD_{20} methacholine, symptom scores and exacerbation rates after one year. Salmeterol resulted in slightly better PEF in the first months of treatment. FEV_1 and PD_{20} methacholine significantly improved in all groups. After one year mean changes in FEV_1 %predicted were 4.3% (95%CI 1.3;7.2), 5.8% (95%CI 2.9;8.7) and 4.3% (95%CI 2.1;6.5) for salmeterol, beclomethasone and placebo, respectively. Changes in airway responsiveness were 0.60 (95%CI 0.05;1.14), 1.30 (95%CI 0.73;1.87) and 0.80 (95%CI 0.33;1.27) doubling doses. Growth was significantly slower in the group with the doubling dose of beclomethasone. We conclude that no additional benefit was found of adding either salmeterol or more beclomethasone to a daily dose of 400 μg beclomethasone in this group of children with excellent compliance of medication.

7.2 Introduction

Salmeterol, taken as a single dose, has a bronchodilating effect of at least 12 hours in adults and children.[1 2] Protection against methacholine and histamine-induced airway obstruction lasts for 12 to 24 hours.[2 3 4] Single-dose studies show prolonged protection against other bronchoconstricting stimuli such as exercise,[5] hyperventilation with dry cold air[6] and allergen.[7 8] Compared with salbutamol, twice daily salmeterol for several weeks or months has been shown to result in fewer symptoms, less need for additional bronchodilator treatment and better improvement in peak flow rates in studies in adults and in children.[9 10 11] International guidelines recommended the use of long-acting $ß_2$-agonists, such as salmeterol, either as an addition to conventional doses of inhaled corticosteroids or as an additive treatment in patients on higher doses of inhaled corticosteroids.[12 13 14] Two studies in adults have focussed on this subject. The first study was carried out in patients treated by general practitioners who were still symptomatic on 400 μg budesonide or beclomethasone. Salmeterol 50 μg b.i.d. together

with beclomethasone 200 μg b.i.d. was compared with beclomethasone 500 μg b.i.d.[15] This study showed better peak flow rates, less diurnal variation of peak flows and fewer symptoms after 21 weeks treatment in the group in which salmeterol was added to the inhaled corticosteroid. Similar results were obtained in a 24-week study of patients treated in hospital, in whom addition of salmeterol 50 μg b.i.d. and salmeterol 100 μg b.i.d. to beclomethasone 500 μg b.i.d. was compared with beclomethasone 1000 μg b.i.d.[16] We set out to investigate whether beneficial effects also occur in children with asthma. The aim of our 3-group study was to compare the effects of one year treatment with beclomethasone 200 μg b.i.d., the same dose of beclomethasone together with salmeterol 50 μg b.i.d. and beclomethasone 400 μg b.i.d. The primary efficacy outcome parameters were airway calibre, measured as forced expiratory volume in 1 second (FEV_1) and airway responsiveness to methacholine. Symptom scores, exacerbations, additional use of short-acting $ß_2$-agonists and peak expiratory flows (PEF) were secondary outcome parameters.

7.3 Material and methods

7.3.1 Patients

One hundred and seventy-seven children aged 6 to 16 years with moderate asthma were selected from the outpatient paediatric clinics of 9 hospitals, 6 university hospitals and 3 general hospitals. Patients were recruited between September 1992 and May 1995. All children had mild to moderate asthma according to the American Thoracic Society criteria.[17] Patients included in the study had to have: (1) an FEV_1 between 55-90% of predicted value and/or a ratio of FEV_1 to forced vital capacity (FVC) of 50-75%, (2) an increase of at least 10% in FEV_1 after inhalation of 0.8 mg salbutamol, (3) airway hyperresponsiveness to methacholine, i.e. a 20% fall in FEV_1 after inhalation of 150 μg or less methacholine (PD_{20} methacholine), which is more than two standard deviations below the mean value in healthy children[18], (4) an ability to produce reproducible lung function tests, i.e. a variation in three consecutive measurements of FEV_1 of less than 5%, (5) a history of stable asthma for at least 1 month without exacerbations or respiratory tract infections, (6) used inhaled corticosteroids between 200 to 800 μg daily for at least 3 months before the start of the study. The study was approved by the medical ethics committees of the participating centers. Written informed consent was obtained from all patients and their parents.

7.3.2 Study design

The study was a double-blind, randomized clinical trial. It consisted of a 6 week run-in period, a treatment period of 54 weeks and a follow-up period of 2 weeks. In the run-in

period all patients received beclomethasone 200 μg b.i.d. Salbutamol 200 μg on demand was allowed as rescue medication, with a maximum of 6 inhalations per day. In the first and the last week of the run-in period FEV_1 and FVC before and after bronchodilatation and PD_{20} methacholine were assessed. Lung function inclusion criteria had to be fulfilled at at least one of these visits. At the end of the run-in period patients were allocated to one of the three treatments by an independent randomization center. Randomization was stratified by sex, age, center, baseline FEV_1, baseline PD_{20} and prior dose of inhaled corticosteroids, using a computerized minimization method.[19] Study treatment consisted of either salmeterol xinafoate 50 μg b.i.d., beclomethasone dipropionate 200 μg b.i.d. or placebo b.i.d., while beclomethasone 200 μg b.i.d. was continued in all treatment arms. All drugs were administered as Rotadisks^R in combination with a Diskhaler^R (Glaxo Wellcome, Greenford, United Kingdom). All children were instructed in the use of this inhalation device prior to entry into the study and their inhalation technique was checked at every visit. For relief of symptoms during the treatment period the use of salbutamol 200 μg Rotadisk was allowed, with a maximum of 6 inhalations per day. Asthma symptoms, which did not sufficiently improve with the maximum dose of rescue salbutamol, were treated with a standard course of prednisolone. On the first day this started with a dose of 30 or 35 mg, depending on the weight of patients, and this was tapered off to zero in 7 days. During the treatment period the reversibility of FEV_1 to salbutamol (at 12, 24, 36 and 48 weeks) and PD_{20} methacholine (at 6, 18, 30, 42 and 54 weeks) were measured alternately at intervals of six weeks. After 54 weeks all patients stopped taking randomized treatment for a period of 2 weeks. During this 2-week period patients continued with beclomethasone 200 μg b.i.d. and salbutamol on demand. At the end of this period PD_{20} methacholine was measured again.

At each clinic visit FEV_1, FVC, PEF, height, body weight, heart rate, systolic and diastolic blood pressure were measured. Height was measured using a stadiometer in centimeters, corrected to one decimal place. Furthermore, the patients were asked about adverse events and the number of used blisters of study medication and rescue salbutamol were counted.

Throughout the study period patients kept diary cards on which symptoms and additional use of rescue medication were recorded. They also measured PEF using a mini-Wright peak flow meter (Clemente Clarke International Ltd., Harlow, Essex, U.K.) at home. Symptoms and PEF measurements were recorded during the first two weeks of each six-week period between clinic visits. Dyspnea, wheeze and cough in the morning and the evening were scored separately, using a scale from 0 to 3. PEF was measured in triplicate in the morning and evening before taking study medication and all three

values were recorded. The highest value was used in the analysis. Patients were withdrawn from the study if they needed 3 or more prednisolone courses within 3 months, or if it was not ethical to continue blinded treatment according to the investigator or if patients or parents wanted to stop.
All data were collected and checked by the coordinating center in Rotterdam to ensure completeness. Interim analyses of the study data were made by an independent statistician every 6 months and reviewed by a data monitoring committee. Investigators were kept blind to the results of the interim analyses. The data monitoring committee allowed the study to continue until all patients had completed follow-up.

7.3.3 Lung function measurements
Lung function measurements were performed between 12 and 18 hours after inhalation of the study medication. For each patient the time of measurement was constant throughout the study period. Patients were instructed to take their last dose of study drug before the clinic visit at a fixed time the previous evening. Rescue salbutamol was not allowed in the 8 hours before lung function measurement. The time of inhalation of the last dose of study drug and any use of salbutamol were checked before performing measurements and, if necessary, these measurements were postponed. No lung function measurements were done within the 4-week period after a course of prednisolone.
FEV_1 and FVC were measured according to the recommendations of the European Community for Steel and Coal, by using a water sealed or dry rolling seal spirometer or pneumotachometer.[20] At least 3 manoeuvres were performed with FEV_1 and FVC within 5%. A maximum of 5 manoeuvres were allowed and the largest FEV_1 and FVC were taken for the analysis. Reference values of Zapletal and coworkers were used.[21] Postbronchodilator FEV_1 was measured 20 minutes after inhalation of 0.8 mg salbutamol.[22] Salbutamol was administered by a Volumatic spacer (Glaxo Wellcome, Greenford, UK) as 4 puffs of 0.2 mg, one at a time, inspiring slowly from functional residual capacity to total lung capacity and holding each breath for about 10 seconds. PEF was measured in triplicate, using the patient's own Mini Wright peakflow meter. Methacholine provocation tests were performed using a modification of the dosimeter method by Chai, as described previously.[23] Preparation of methacholine solutions was standardized in all centers. Nebulized methacholine bromide in unbuffered saline solution was given in doubling concentrations (0.125 to 39.2 mg/ml). The aerosol was generated by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA) attached to a Rosenthal dosimeter (Laboratory for Applied Immunology, Fairfax, VA), driven by air at 137.8 kPa (20 psi) with a timing adjustment of 0.6 seconds. Output of the nebulizers

was measured before the start of the study. All parts of each nebulizer were marked with waterproof paint to prevent interchanging. Nebulizers were cleaned after each measurement to prevent precipitations, and orifices were checked weekly according to recommendations.[24] Aerosolized solution was delivered to the mouth in four consecutive breaths. Mouth doses were 2.5 to 784 μg of methacholine. Saline was inhaled before methacholine to exclude a non-specific response. The effect of each dose was determined by measuring FEV_1 in triplicate 3 minutes after administration. PD_{20} methacholine was calculated by a computer program from a log-dose-response plot by linear interpolation. Airway responsiveness was only measured if FEV_1 before methacholine provocation was 80% or more of the individual's baseline value at entry into the study.

All centers used written guidelines for lung function measurements. Technicians attended a training course before the start of the study. Site visits were made once a year by the primary investigator and a pulmonary physiologist to inspect the equipment and the methods used.

7.3.4 Statistical analysis

The study was designed to have 90% statistical power to detect a difference of 6% predicted FEV_1 between any two (of three) treatment groups using a statistical significance level of 5%.

Changes in FEV_1 and the logarithm of PD_{20} within each group over the study period were assessed using paired t-tests for matched data. Changes in PD_{20} were reported as numbers of doubling doses (DD). Comparisons of FEV_1 and the logarithm of PD_{20} between groups at each clinic visit were made using analysis of covariance to adjust for mean pre-intervention levels. Comparisons of both morning and evening PEF measurements recorded during the 2-week diary periods were made using analysis of covariance to adjust for mean pre-intervention levels. Morning and evening PEF variability for each patient was expressed as the standard deviation of the PEF measurements. Each measure of day-to-day variability was compared between pairs of treatment groups using the Mann-Whitney test. Distributions of symptoms during the 2-week diary periods were compared using the Mann-Whitney test, as were the numbers of blisters of rescue salbutamol used over this period. Where patients failed to complete their daily record cards for more than 7 days in any 14 day period such assessments were not included in the analysis. Otherwise, when there were missing days in the record, pro rata adjustment was made to give a 2-week assessment. Comparison of heights between groups were made using analysis of covariance to adjust for pre-intervention levels. Heights were also expressed as standard deviation scores (SDS)

Table 7.1 Baseline characteristics at the start of the run-in period and at randomization by treatment group.

All values are means (sd) unless stated otherwise

At start of run-in period:	**Salmeterol** (n=60)	**Beclomethasone** (n=60)	**Placebo** (n=57)
sex, F/M	20/40	24/36	21/36
age, yr	10.8 (2.5)	11.4 (2.9)	11.1 (2.7)
height, cm	143.6 (15.4)	147.9 (17.0)	145.6 (15.4)
duration of asthma, yr	7.8 (3.5)	9.0 (3.1)	8.5 (3.1)
atopy status:			
none	7/60	5/60	7/57
house dust mite	46/60	45/60	44/57
cat	29/60	35/60	23/57
dog	34/60	31/60	30/57
grasspollen	30/60	28/60	26/57
inh.corticosteroid treatment:			
dose, μg	490 (154)	503 (201)	488 (149)
<400 μg	1/60	6/60	2/57
>400 μg	21/60	22/60	20/57
duration, yr	3.2 (2.2)	3.4 (2.1)	2.9 (2.0)
FEV_1, %predicted:			
prebronchodilator	87.2 (13.0)	85.3 (13.8)	86.5 (13.2)
postbronchodilator	103.2 (14.1)	100.9 (12.3)	102.2 (12.0)
PD_{20} μg*	24.5 (11-47.5)	22.5 (7.5-42.5)	26 (12-38)
At randomization:			
height, cm	144.1 (15.4)	148.5 (17.0)	146.1 (15.4)
FEV_1, %predicted:			
prebronchodilator	89.7 (11.8)	87.4 (12.3)	89.2 (13.4)
postbronchodilator	103.5 (13.1)	102.3 (11.4)	103.0 (13.6)
PD_{20}, μg*	29 (9-59)	20 (6-56)	27 (16.5-44)
PEF, l/min:			
morning	299 (79)	315 (90)	317 (79)
evening	306 (82)	323 (92)	325 (80)
Days in two weeks with symptoms*	6 (3-11)	5 (1.5-10)	4 (1-9)
Nights in two weeks with symptoms*	6 (3-10)	4.5 (1-11)	5 (1-9)

* median and quartiles

using Dutch reference growth charts.[25] Changes in SDS over time within each group were assessed using paired t-tests for matched data, and comparisons between groups at each clinic visit were made using analysis of covariance to adjust for mean pre-intervention levels. This model was extended to simultaneously investigate the effect of treatment group and puberty (pre- and postpubertal according to Tanner stages) on SDS. All reported p-values are for two-sided tests and for simplicity of presentation are without formal adjustment for multiple comparisons over time or for multiple comparisons between groups. Confidence intervals for means were calculated parametrically assuming normality.

7.4 Results

Between October 1992 and May 1995, 177 patients (112 boys, 65 girls) were enrolled into the study. Patient characteristics at entry (beginning of the run-in period) and at randomization were similar in the three treatment groups (Table 7.1).

Fifteen patients withdrew during the study period, 5 in the salmeterol treated group, 6 in the beclomethasone group and 4 in the placebo group. Eleven patients withdrew because of non-compliance or failure to return. Three patients withdrew as a result of an adverse event: 2 patients, both in the salmeterol group, because of alopecia or ingestion of corpus alienum, 1 patient in the beclomethasone group because of vomiting. Only 1 patient, treated with placebo, withdrew because of an exacerbation.

Compliance with study treatment was slightly better in the salmeterol group than in the beclomethasone group ($p=0.01$) and the placebo group ($p=0.01$). The median number of blisters of study medication used per day were 1.88, 1.77 and 1.75 in the salmeterol, beclomethasone and placebo groups respectively; i.e. 94%, 89% and 88% of the prescribed study medication. Compliance with maintenance beclomethasone treatment was comparable to that with study medication; the median number of blisters per day were 1.89, 1.81 and 1.75 respectively.

7.4.1 Airway calibre

At the end of the 54-week treatment period no significant differences in FEV_1 %predicted between treatment groups were found. Significant changes in FEV_1 occurred in all groups. Mean changes in prebronchodilator FEV_1 from baseline to the end of the treatment period were 4.3 %predicted (95% confidence interval(CI) 1.3;7.2)($p=0.005$), 5.8 %predicted (95%CI 2.9;8.7)($p=0.0002$), and 4.3 %predicted (95%CI 2.1;6.5)($p=0.0003$) for the salmeterol, beclomethasone and placebo groups respectively (Figure 7.1).

No differences between treatments were found after analyzing subgroups according to

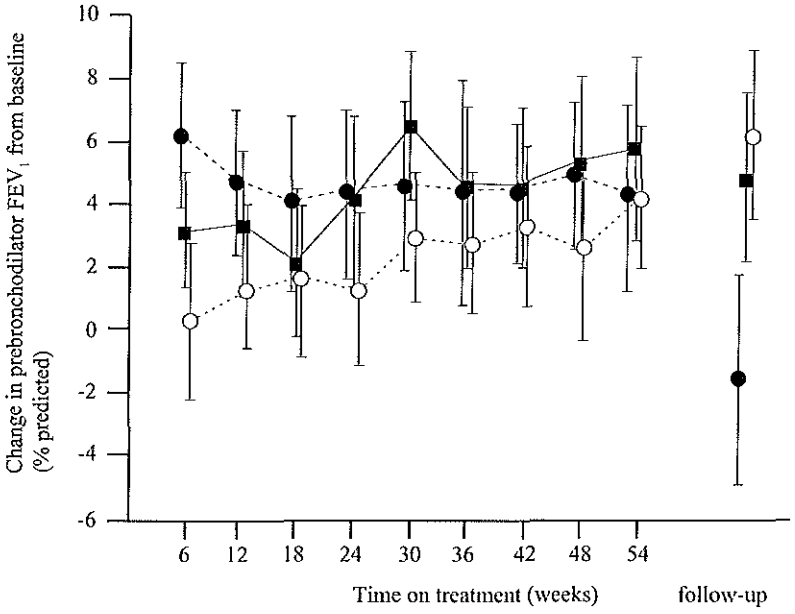

Figure 7.1
Changes in prebronchodilator FEV_1% predicted (means, 95% CI) from baseline during treatment with salmeterol (●), beclomethasone (■) or placebo (O).

prestudy dose and duration of inhaled corticosteroid, baseline levels of FEV_1 and PD_{20} and the numbers of daytime or nocturnal symptoms.

After stopping treatment with salmeterol, there was a significant fall in FEV_1 of 5.6 %predicted (95%CI 2.1;9.1)(p=0.003). Stopping of either beclomethasone or placebo did not result in significant changes.

Mean changes in postbronchodilator FEV_1 were -0.1 %predicted (95%CI -2.3;2.1-)(p=0.9), 3.5 %predicted (95%CI 1.6;5.4)(p=0.0005) and 2.0 %predicted (95%CI -0.6;4.5)(p=0.13) for the salmeterol, beclomethasone and placebo groups respectively (Figure 7.2). The difference between the levels in the salmeterol and beclomethasone groups was of borderline statistical significance at 48 weeks (p=0.04) but not at any other time point.

7.4.2 Airway responsiveness

At the end of the treatment period no significant differences between groups were

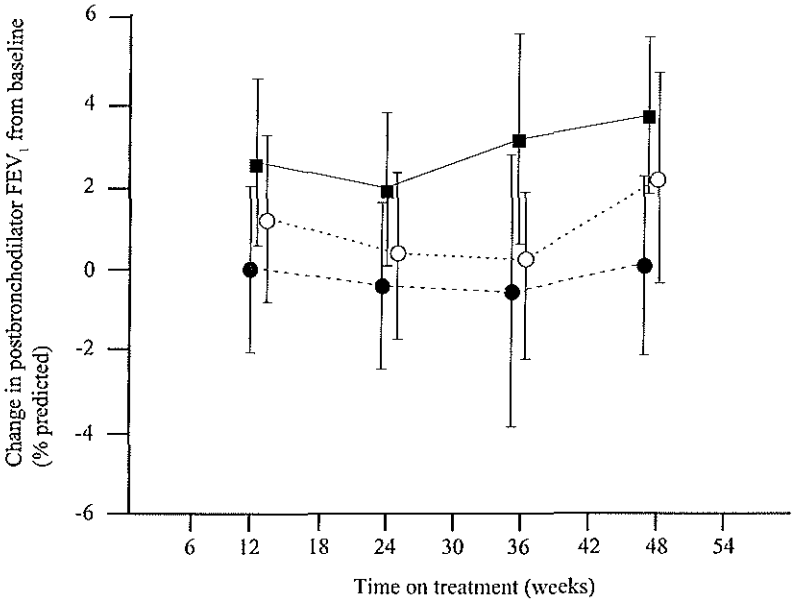

Figure 7.2
Changes in postbronchodilator FEV_1% predicted (means, 95% CI) from baseline during treatment with salmeterol (●), beclomethasone (■) or placebo (○).

found. Median PD_{20} values were 36, 39.5 and 35 μg for the salmeterol, beclomethasone and placebo groups, respectively. All three groups showed a significant improvement in PD_{20}. Changes in PD_{20} compared to baseline were 0.60 doubling doses (DD)(95%CI 0.05;1.14)(p=0.03), 1.30 DD (95%CI 0.73;1.87)(p=0.00003) and 0.80 DD (95%CI 0.33;1.27)(p=0.001) for the salmeterol, beclomethasone and placebo groups respectively (Figure 7.3).
After stopping salmeterol there was a small but not significant decrease in PD_{20} (-0.28 DD), whereas after stopping placebo treatment an increase in PD_{20} was found (0.6 DD)(p=0.003) and after stopping beclomethasone no change occurred. As for FEV_1, subgroup analysis revealed no trends in favor of any of the treatments.

7.4.3 Peak expiratory flow
Morning and evening PEF improved in all treatment groups. During the first months of treatment changes were larger in the salmeterol group than in the other two groups

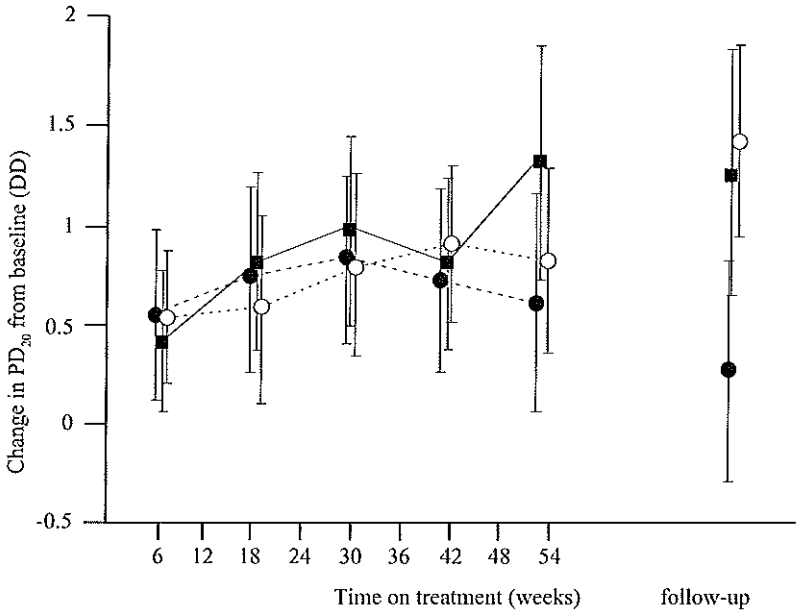

Figure 7.3
Changes in airway responsiveness in doubling doses (means, 95% CI) during treatment with salmeterol (●), beclomethasone (■) or placebo (O).

with the differences between the salmeterol and placebo groups being statistically significant at some time points (Figure 7.4). After one year mean increases in morning PEF were 41.8 l/min, 41.1 l/min and 27.3 l/min for the salmeterol, beclomethasone and placebo groups respectively. Mean increases in evening PEF were 38.6 l/min, 37.6 l/min and 24.9 l/min respectively. Differences between mean follow-up levels in the placebo group and each of the other two groups were of borderline statistical significance for both morning and evening PEF ($0.05 < p < 0.1$ for each comparison). There was some evidence that at the first follow-up visit, day to day variability was greater in the beclomethasone group than in the salmeterol group ($p=0.004$ am, $p=0.003$ pm) and the placebo group ($p=0.03$ am, $p=0.06$ pm), however, especially for the morning PEF day to day variability at baseline was already higher in the beclomethasone group. Day to day variability did not differ significantly between groups at any other follow-up visit.

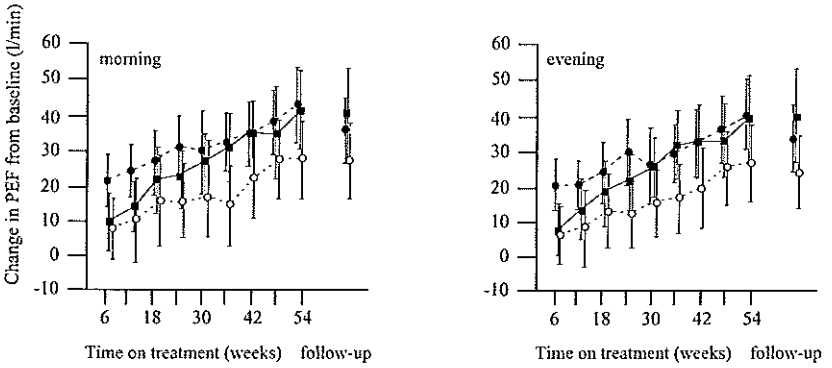

Figure 7.4
Mean changes in morning and evening PEF (l/min, 95% CI) during treatment with salmeterol (●), beclomethasone (■) or placebo (O).

7.4.4 Symptoms

Daytime and nighttime symptoms diminished in all treatment groups in a similar way. The percentage of children reporting no symptoms during the 2-week diary card periods increased from 3%, 13% and 11% for the salmeterol, beclomethasone and placebo

Table 7.2 Most common reported adverse events during the treatment period

	Salmeterol	**Beclomethasone**	**Placebo**
Number of patients	60	60	57
Number of patients with adverse event	59 (98%)	52 (87%)	52 (93%)
Number of patients with:			
headache	25 (42%)	16 (27%)	23 (41%)
rhinitis	21 (35%)	20 (33%)	14 (25%)
viral resp. infection	17 (28%)	18 (30%)	14 (25%)
asthma	16 (27%)	19 (32%)	16 (29%)
upper resp. tract infection	16 (27%)	15 (25%)	9 (16%)
cough	12 (20%)	16 (27%)	13 (23%)
fever	12 (20%)	7 (12%)	8 (14%)
nausea and vomiting	11 (18%)	5 (8%)	7 (13%)
diarrhea	8 (13%)	2 (3%)	4 (7%)

groups respectively in the run-in period to 34%, 39% and 35% after one year of treatment. At no time point were there statistically significant differences in symptom scores between the groups.

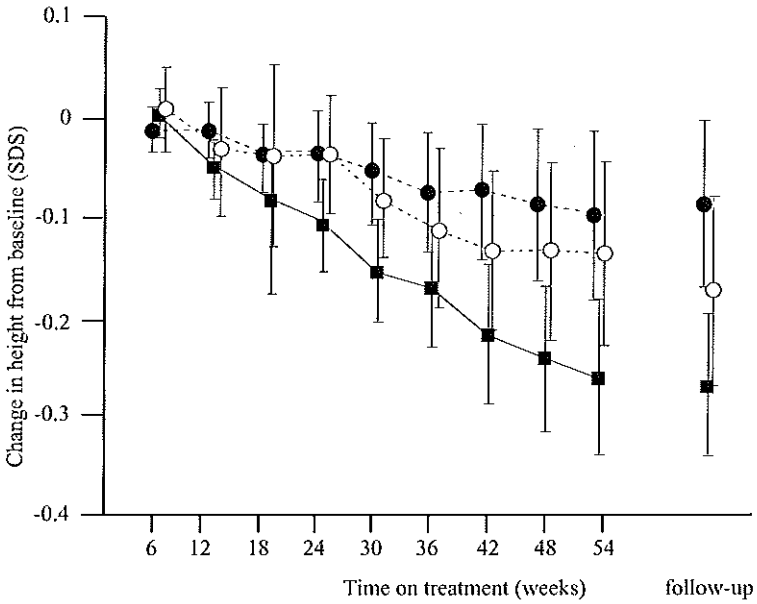

Figure 7.5
Change in height as SDS (means, 95% CI) during treatment with salmeterol (●), beclomethasone (■) or placebo (○).

There was some evidence that the use of additional salbutamol, as noted on the diary cards, differed between groups, particularly during the first two weeks of treatment this was higher in the beclomethasone group than in the other groups. The median number of additional salbutamol inhalations per day during the treatment period, as counted from the used blisters, was 0.19, 0.33 and 0.15 for the salmeterol, beclomethasone and placebo group respectively. The difference between the rates in beclomethasone and placebo was of borderline statistical significance ($p=0.06$).
During the treatment period 34 courses of prednisolone for exacerbations were given; 13 courses to 10 patients in the salmeterol treated group, 8 courses to 7 patients in the beclomethasone treated group and 13 courses to 10 patients in the placebo treated group.

7.4.5 Adverse events

During the treatment period no consistently, clinically significant differences in heart rate, systolic and diastolic blood pressure were found between treatment groups.

Table 7.2 shows the most commonly reported adverse events.

The mean increase in height was 5.1 cm (95%CI 4.5;5.7) in the salmeterol group, compared with 3.6 cm (95%CI 3.0;4.2) in the beclomethasone group and 4.5 cm (95%CI 3.8;5.2) in the placebo group. A slightly greater proportion of patients were pre-pubertal in the placebo group (47%), than in the salmeterol (43%) or beclomethasone (35%) groups. The reductions in SDS were greatest in the pre-pubertal patients. After adjustment for puberty, the reduction in SDS was significantly greater in the beclomethasone group than in the other two groups (p=0.006 compared with salmeterol, p=0.02 compared with placebo). Mean changes in SDS were -0.10 (95%CI -0.19;-0.02), -0.27 (95%CI -0.34;-0.19) and -0.16 SDS (95%CI -0.24;-0.07) for salmeterol, beclomethasone and placebo respectively (Figure 7.5).

7.5 Discussion

This is the first long term study comparing the addition of a long-acting β_2-agonist with a doubling dose of an inhaled corticosteroid in asthmatic children on maintenance treatment with inhaled corticosteroid. We selected children with moderate asthma, who had used 200 to 800 μg of inhaled corticosteroid for at least 3 months before the start of the study. During the six week run-in period they were treated with 200 μg beclomethasone b.i.d., which is considered a moderate dose in the treatment of childhood asthma.[14] Despite this treatment all children were symptomatic and had reversible airway obstruction and airway hyperresponsiveness. The dose chosen for salmeterol (50 μg b.i.d.) is recommended as the optimum dose in childhood asthma.[11] The data from this study show no statistically significant differences in airway calibre, airway responsiveness, symptom scores and exacerbation rates between 200 μg beclomethasone b.i.d., 200 μg beclomethasone plus salmeterol 50 μg b.i.d. or 400 μg beclomethasone b.i.d. Although FEV_1 and airway responsiveness tended to be slightly better in the group on the high dose of inhaled corticosteroid, only for postbronchodilator FEV_1 was the difference between this group and the salmeterol group of borderline statistical significance (p=0.04) at the end of the treatment period. During the first months patients in the salmeterol group tended to have higher peak flow values. Our results differ somewhat from those from the two adult studies which have compared the addition of salmeterol with increasing the inhaled corticosteroid dose.[15] [16] Both studies lasted about 6 months and showed a significantly better improvement in PEF with the addition of salmeterol. Symptoms were less in patients on salmeterol

treatment, especially in the study by Woolcock et al.[16] and at some time points in the study by Greening et al.[15] These studies as well as our study selected patients with reversible airway obstruction. In contrast with our study, in which inclusion criteria were based on airway caliber and airway responsiveness, inclusion criteria of both adult studies were based on symptom scores in the run-in period prior to randomization and PEF variability. This may have led to the selection of highly symptomatic patients. In the study by Woolcock et al.[16] beclomethasone was increased from 1000 to 2000 μg. This dose increase may already be on the "flat part" of the dose response curve for anti-asthma effects of inhaled corticosteroids[26], which could explain the better symptomatic improvement with salmeterol. In the study by Greening et al.[15] the beclomethasone dose increased from 400 to 1000 μg daily. This lower dose range was probably on the "steep part" of the dose-response curve, which may explain why differences in symptom scores between salmeterol and the high dose of inhaled corticosteroid were less obvious. Based on PEF and FEV_1 data, the degree of obstruction in the adult studies is more severe than in our study: the mean baseline PEF in the study by Greening[15] was 74% predicted, the mean baseline FEV_1 in the study by Woolcock[16] was about 72% predicted compared to 86% in our study. This suggests that there might have been more room for improvement by a bronchodilator in these studies and may explain why in our study, no differences were found between salmeterol and beclomethasone in terms of FEV_1 improvement. However, subgroup analysis by FEV_1 at baseline did not reveal a larger effect of salmeterol in those asthmatic children who had more severe airway obstruction. For PEF, also in our study salmeterol was slightly superior to beclomethasone during the first 24 weeks of treatment, although the differences between salmeterol and beclomethasone in our study were less than in the adult studies and not consistently statistically significant.

All treatment groups in our study showed a significant improvement in airway responsiveness compared with baseline. For the beclomethasone group this was 1.3 DD, comparable with 1.5 DD found after one year of treatment with 600 μg budesonide daily in an earlier study on asthmatic children.[27] However, the latter study selected children not on inhaled corticosteroids before inclusion into the study. Further data of that study showed a plateau of PD_{20} improvement after 22 months of treatment.[28] It is remarkable that a comparable improvement in PD_{20} was found in our study despite the fact that the mean duration of inhaled corticosteroid use before study entry was about 3 years. No differences between subgroups who had used inhaled corticosteroids for less than 2 years and those who had been on corticosteroid treatment for 2 years or more were found. In the study by Woolcock et al.[16], the improvement in airway responsiveness was far less: 0.6 DD after salmeterol 50 μg b.i.d. and 0.4 DD after

beclomethasone 1000 μg b.i.d. However, their study population consisted of patients with longstanding asthma, who had been taking inhaled corticosteroids for several years and so their response might have reached a plateau. Furthermore, for the patients who doubled their inhaled corticosteroid dose, the plateau phase of the dose-response curve for PD_{20} may have been reached. An explanation for the relatively favorable response in our study as well as that in the study by Van Essen et al.[28] may be that in children the inflammatory changes in the airways are more reversible by inhaled corticosteroid treatment than they are in adults, who typically have longstanding asthma. There is some evidence that a delay in the introduction of inhaled corticosteroid leads to a smaller improvement in lung function in children as well as adults.[29 30] In our study even the placebo group, who continued with 400 μg beclomethasone, showed a significant improvement in FEV_1 as well as in PD_{20}; an effect, which is most likely attributable to the excellent compliance of nearly 90% of prescribed medication, which was probably better than before due to the strict study protocol and patient control.

All treatment groups showed a decrease in height growth over the one year treatment period. Several explanations are possible: growth of asthmatic children may be impaired as a result of their chronic disease or due to delayed puberty[31] or may be due to the adverse effects of the inhaled corticosteroid. The unusually high compliance with medication in this study may have resulted in effects on growth with relatively low doses of inhaled corticosteroid. Furthermore, the use of a dry powder inhaler with a high lung deposition may play a role. The effect on growth in this study appeared to be dose-dependent. The changes in SDS in the two groups treated with 400 μg beclomethasone daily (resp. -0.10 and -0.16 SDS) were less than those found in another study in which the same dose of beclomethasone was given for the first year (mean change in SDS was -0.28).[32] Since children had used inhaled corticosteroids for a number of years before entry into the current study, this may suggest that the effect of inhaled corticosteroids on growth diminishes during prolonged treatment. Further long-term studies are necessary to address this issue.

We conclude that adding salmeterol or doubling the dose of beclomethasone gave no additional benefit over that from 200 μg beclomethasone b.i.d. in this selected group of children with moderate asthma, in which the compliance with medication was excellent. This study also showed that with strict monitoring of the children and frequent control visits the compliance with medication was high, which resulted in considerable improvements in lung function and symptoms. Further studies are needed in order to evaluate which of the approaches is best in children with severe asthma. The possible advantage of a higher dose of inhaled corticosteroid in suppressing airway inflammation should than be balanced against the adverse effects, especially on growth.

We advocate careful monitoring of children for medication compliance and growth during treatment with inhaled corticosteroids.

7.6 References

1. Ullman A, Svedmyr N. Salmeterol, a new long-acting inhaled beta-2-adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988;**43**:574-8..
2. Verberne AAPH, Hop WCJ, Bos AB, Kerrebijn KF. Effect of a single dose of salmeterol on baseline airway caliber and methacholine-induced airway obstruction in asthmatic children. *J Allergy Clin Immunol* 1993;**91**:127-34.
3. Derom EY, Pauwels RA, Van der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992;**89**:811-5.
4. Campos Gongora H, Wisniewski AFZ, Tattersfield AE. A single dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991;**144**:626-9.
5. Green CP, Price JF. Prevention of exercise-induced asthma by inhaled salmeterol xinafoate. *Arch Dis Child* 1992;**67**:1014-7.
6. Malo JL, Ghezzo H, Trudeau C, L'Archévêque J, Cartier A. Salmeterol, a new inhaled beta-2-adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *J Allergy Clin Immunol* 1992;**89**:567-74.
7. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;**336**:1338-42.
8. Weersink EJM, Aalbers R, Koëter GH, Kauffman HF, De Monchy JGR, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of salmeterol. *Am J Respir Crit Care Med* 1994;**150**:1262-7.
9. Britton MG, Earnshaw JS, Palmer JBD. A twelve month comparison of salmeterol with salbutamol in asthmatic patients. *Eur Respir J* 1992;**5**:1062-7.
10. Lundback B, Rawlinson DW, Palmer JBD. Twelve month comparison of salmeterol and salbutamol as dry powder formulations in asthmatic patients. *Thorax* 1993;**48**:148-53.
11. Lenney W, Pedersen S, Boner AL, Ebbutt A, Jenkins MM. Efficacy and safety of salmeterol in childhood asthma. *Eur J Pediatr* 1995;**154**:983-90.
12. International consensus report on diagnosis and treatment of asthma. National Heart, Lung and Blood Institute, National Institutes of Health Bethesda. *Eur Resp J* 1992;**5**:601-41.
13. Guidelines on the management of asthma. Woodhead M, Editor. *Thorax* 1993;**48**:S1-24.
14. Warner JO. Asthma; a follow up statement from an international paediatric asthma consensus group. *Arch Dis Child* 1992;**67**:240-8.
15. Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1994;**344**:219-24.
16. Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996;**153**:1481-8.
17. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225-44.
18. Duiverman EJ, Neijens HJ, Van Strik R, Affourtit MJ, Kerrebijn KF. Lung function and bronchial responsiveness in children who had infantile bronchiolitis. *Pediatr Pulmonol* 1987;**3**:38.-44
19. Pocock SJ. Clinical trials, a practical approach. *John Wiley and sons, Ltd.* Chichester, 1983.
20. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault YC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function test European Community for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993;6(suppl 16):5-40.

21. Zapletal A, Samanek M, Paul T. Lung function in children and adolescents. Methods, reference values. In: Zapletal A, ed. *Progress in respiration research*. Basel: Karger, 1987;**22**:114-218.
22. Merkus PJFM, Eelkman Roorda HM, Van Essen-Zandvliet EEM, Duiverman EJ, Quanjer PhH, Kerrebijn KF. Assessment of bronchodilatation following spontaneous recovery from a histamine challenge in asthmatic children. *Thorax* 1992;**47**:355-9.
23. Birnie D, Thoe Schwartzenberg GWS, Hop WCJ, Van Essen-Zandvliet EEM, Kerrebijn KF. Does the outcome of the tidal breathing and dosimeter method for the assessment of bronchial responsiveness in children with asthma depend on age? *Thorax* 1990;**45**:199-202.
24. Merkus PJFM, Van Essen-Zandvliet EEM, Parlevliet E, Borsboom G, Sterk PF, Kerrebijn KF, Quanjer PhH. Changes of nebulizer output over the years. Technical note. *Eur Respir J* 1992;**5**-:488-91.
25. Roede MJ, Van Wieringen JC. Growth diagrams 1980, the Netherlands. Third nation wide survey. *T Soc Gezondheidszorg* 1985;**63**:1-34.
26. Lipworth BJ. Airway and systemic effects of inhaled corticosteroids in asthma: dose response relationship. *Pulm Pharmacol* 1996;**9**:19-27.
27. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF. Effects of 22 months treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness and symptoms in children with asthma. *Am Rev Respir Dis* 1992;**146**:547-54.
28. Van Essen-Zandvliet EEM, Hughes MD, Waalkens HJ, Duiverman EJ, Kerrebijn KF. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide). Can it be achieved? *Eur Resp J* 1994;**7**:63-8.
29. Agertoft L, Pedersen S. Effects of long-term treatment with an inhaled corticosteroid on growth and pulmonary function in asthmatic children. *Respir Med* 1994;**88**:373-81.
30. Overbeek SE, Kerstjens HAM, Bogaard JM, Mulder PGH, Postma DS. Is delayed introduction of inhaled corticosteroids harmful in patients with obstructive airways disease (asthma and COPD)? *Chest* 1996;**110**:35-41.
31. Wolthers OD. Long-, intermediate- and short-term growth studies in asthmatic children treated with inhaled glucocorticosteroids. *Eur Respir J* 1996;**9**:821-7.
32. Verberne AAPH, Frost C, Roorda RJ, Van der Laag H, Kerrebijn KF and the Dutch Paediatric Asthma Study Group. One year treatment with salmeterol compared to beclomethasone in children with asthma. *Am J Respir Crit Care Med*, in press.

Chapter Eight

Summary and conclusions

8

Summary and conclusions

8.1 Summary

The long-acting β_2-agonist, salmeterol, was first introduced for the treatment of asthma in adults at the end of the eighties.[1] The subject of this thesis was to evaluate the effect of salmeterol after a single dose and during long-term treatment on several outcome parameters of asthma in children, and to establish the place for salmeterol in the treatment of childhood asthma. Primary outcome parameters were airway calibre, as measured by FEV_1, and airway responsiveness, as measured by PD_{20} methacholine. In the long-term studies secondary outcome parameters were symptom scores, PEF, use of additional short-acting β_2-agonists and exacerbations of asthma.

Chapter 1 gives a general introduction and describes the pathophysiology of asthma as an inflammatory disease of the airways. Current guidelines for the treatment of asthma are discussed. The place of long-acting β_2-agonists in the stepwise treatment of asthma is not unequivocally defined in international consensus reports.[2 3 4 5] They are positioned either as additional treatment to higher doses of an inhaled corticosteroid, or one step earlier, when a moderate dose of an inhaled corticosteroid is not sufficient to reduce symptoms. The pharmacology and clinical aspects of inhaled corticosteroids, short-acting β_2-agonists and long-acting β_2-agonists are described in more detail. These drugs now form the cornerstones of asthma treatment for children and adults.

Chapter 2 gives a review of the literature on the effects of short-acting and long-acting β_2-agonists on airway responsiveness to histamine and methacholine. Short-acting β_2-agonists have a short-lasting acute protective effect, shifting the dose-response curve to a bronchoconstrictor to the right. Long-term treatment with short-acting β_2-agonists has no beneficial effect on airway responsiveness; some studies have even suggested worsening of airway responsiveness from the continuous use of short-acting β_2-agonists. Single doses of the long-acting β_2-agonists salmeterol and formoterol give prolonged protection for up to at least 12 hours against methacholine- and histamine-induced bronchoconstriction. After prolonged use for weeks or months, long-acting β_2-agonists still protect against bronchoconstricting stimuli. Some tolerance seems to develop for this protective effect, while the bronchodilating effect is maintained.

Chapter 3 reviews the literature on clinical studies with salmeterol and formoterol, focusing on paediatric data. After single doses both drugs produce bronchodilation for up to at least 12 hours. Also, there is prolonged protection against various bronchoconstricting stimuli, such as exercise, methacholine, histamine and allergens. Twice daily use for weeks or months results in better symptom control and increased

peak flow values compared with treatment with a short-acting β_2-agonist. Although tolerance seems to develop for the protective effects, residual protection remains over several months of treatment. The clinical relevance of this is not yet clear. Adverse events did not differ from those found in studies with short-acting β_2-agonists.

Chapter 4 presents a single dose study with salmeterol. In a double-blind, placebo-controlled, cross-over design the effect of 50 μg salmeterol on airway caliber and airway responsiveness to methacholine was investigated. Twenty children with mild to moderate asthma participated; the majority of children was on treatment with inhaled corticosteroids or disodium cromoglycate. Salmeterol in a dose of 50 μg resulted in significantly better FEV_1 values for up to 12 hours after inhalation. The maximum response was reached 4 hours after administration with a mean FEV_1 of 18.6% $\pm$ 2.5% (mean$\pm$sem) above baseline. Significant protection against airway responsiveness to methacholine was found for up to 24 hours after salmeterol inhalation. At this time point the mean PD_{20} was still 1.22 $\pm$ 0.29 DD above baseline. The maximum protection was nearly 4 DD at 1 hour. We concluded that a single dose of salmeterol resulted in prolonged bronchodilation and protection against methacholine-induced airway obstruction.

In **chapter 5** the effect of a single dose of salmeterol on airway responsiveness was compared with the effect of treatment for 4 months. In a double-blind, parallel study, 30 children were either randomized to salmeterol 50 μg b.i.d daily or salbutamol 200 μg b.i.d. Children with mild asthma, not on treatment with inhaled corticosteroids or disodium cromoglycate, who had little or no bronchial obstruction and were hyperresponsive to methacholine were selected. Airway responsiveness was measured before study entry, 12 hours after a single dose, and monthly during 4 months of daily treatment. Measurements were always performed at the same time of the day, 12 hours after the last dose of medication was administered. No significant differences in FEV_1 were found between treatments at any time point. PD_{20} methacholine increased by 1.7 $\pm$ 0.3 DD after the first dose of salmeterol. This protection was reduced to 0.6 $\pm$ 0.3 DD after 1 month, which was still significantly different from baseline and from salbutamol treatment, and remained at the same level during the rest of the treatment period. We concluded from these data that tolerance to the protective effect of salmeterol to methacholine-induced bronchoconstriction develops within 1 month after starting treatment, and that no further tolerance occurred during several months of treatment.

Chapter 6 describes the results of a multicenter study which compares salmeterol 50 μg b.i.d with beclomethasone 200 μg b.i.d during one year. Sixty-seven children with mild to moderate asthma, not on treatment with inhaled corticosteroids during the previous 6 months and not on treatment with disodium cromoglycate during 2 weeks before entering the study, were randomized in a double-blind parallel study. After one year of treatment there was a highly significant difference in favor of the beclomethasone treatment of 14.2% predicted (95%CI 8.3;20.0) for prebronchodilator FEV_1, 7% predicted (95%CI 2.0;11.9) for postbronchodilator FEV_1 and 2.79 DD (95%CI 1.75;3.84) for PD_{20} methacholine. In the salmeterol group FEV_1 as well as PD_{20} tended to decrease during the 1 year treatment period, despite improvement in symptoms and PEF. Mean decreases were 4.5% predicted (95%CI -9.0;0.1) for FEV_1 and 0.73 DD (95%CI -1.46;0.00) for PD_{20}. Between groups there were no significant differences in symptoms (daytime and nighttime), use of additional short-acting bronchodilators and peak flow values (morning and evening), although results in the beclomethasone group tended to be better. Asthma exacerbations for which prednisolone was needed, were far more frequent in the salmeterol group, as were the numbers of withdrawals due to exacerbations in this group. However, growth in the beclomethasone group was significantly retarded compared with that in the salmeterol group (-0.28 SDS versus -0.03 SDS). We concluded that treatment with a moderate dose of beclomethasone is superior to treatment with salmeterol in terms of lung function parameters, symptom scores and exacerbations of asthma in children with mild to moderate asthma. We strongly recommend that salmeterol should not be used as monotherapy and advocate careful monitoring of individual growth during treatment with inhaled corticosteroids.

Chapter 7 describes the results of a multicenter study in which the addition of salmeterol (50 μg b.i.d.) to a moderate dose of an inhaled corticosteroid (beclomethasone 200 μg b.i.d.)(SI200 group) was compared to doubling the dose of inhaled corticosteroid (beclomethasone 400 μg b.i.d.)(I400 group) and to the initial inhaled corticosteroid dose (beclomethasone 200 μg b.i.d.)(I200 group). In a double-blind, placebo-controlled, parallel study, 177 children participated. After one year of treatment no significant differences between the three treatment groups were found for pre- and post-bronchodilator FEV_1 values and PD_{20} methacholine. However, significant improvements from baseline values in FEV_1 and PD_{20} were found in all groups. For FEV_1 mean changes were 4.3% (95%CI 1.3;7.2), 5.8% (95%CI 2.9;8.7) and 4.3% predicted (95%CI 2.1;6.5), for the SI200, I400 and I200 groups, respectively. For PD_{20} mean changes were 0.6 DD (95%CI 0.05;1.14), 1.3 DD (95%CI 0.73;1.87) and 0.8 DD (95%CI 0.33;1.27) for the SI200, I400 and I200 groups, respectively. For morning

and evening peak flow values there was a slight advantage of the SI200 group compared to the I200 group, especially during the first months of the treatment period. No significant differences were found between groups for symptom scores, use of additional salbutamol and exacerbation rates. Growth was significantly more retarded in the I400 group. Compliance was high in all treatment groups, where nearly 90% of prescribed medication was used. We concluded that adding salmeterol or doubling the dose of beclomethasone had no additional benefit to 200 μg beclomethasone b.i.d. in this selected group of children with moderate asthma, in which the compliance with medication was excellent.

8.2 Conclusions and general discussion

Comparable with the results in studies in adult asthmatics, we confirmed in asthmatic children that salmeterol had a prolonged bronchodilating and protecting effect against methacholine induced bronchoconstriction.[6] In agreement with the results of the study by Cheung et al,[7] we found a reduction of the protective effect during 4 months treatment with salmeterol. However, our study revealed that significant residual protection remains, and no tendency to further reduction during treatment is apparent. As the patients in the study by Cheung and in our study were not on treatment with inhaled corticosteroids, one could hypothesize that inhaled corticosteroids prevent the development of tolerance. However, recent data reject this hypothesis.[8 9 10] The clinical relevance of the development of tolerance is not yet clear, and so far there is no convincing evidence of tolerance for the bronchodilating effect of the drug.[7 11] However, the reason that tolerance for the bronchodilating effect has not been found may also be that improvements in airway caliber are limited due to a ceiling effect when maximum dilatation has been reached, while for dose response curves there is a more open-end scale.[11]

The two long-term multicenter studies described in chapter 6 and 7 were performed to position salmeterol in the treatment of childhood asthma. The first study clearly showed the superiority of a moderate dose of beclomethasone to salmeterol monotherapy in terms of lung function improvements (airway caliber as well as airway responsiveness), symptom scores and exacerbation rates. Remarkably, even in the salmeterol group symptoms, as scored on the diary cards, and PEF improved, although airway caliber and airway responsiveness tended to decrease and frequent exacerbations occurred. During treatment with 400 μg beclomethasone daily we found a decrease in the standard deviation score of height which may be clinically relevant. A recent Dutch paediatric endocrinology consensus considered a change of 0.25 SDS per year clinically relevant. The use of a powder inhaler, with relatively high lung deposition and possible

oropharyngeal deposition, and a high compliance rate in the study may have influenced our results regarding growth. So far data from the literature do not support a clinically relevant growth effect of inhaled corticosteroid treatment.[12]

The second long-term study in which the addition of salmeterol to 200 μg beclomethasone b.i.d. was compared to doubling the dose of beclomethasone and to the initial beclomethasone dose, did not reveal significant differences between treatment groups on lung function, airway responsiveness, symptoms, use of additional β_2-agonists or exacerbations. Only for salmeterol there was a slight advantage on peak flow values, especially during the first months of treatment. All treatment groups, inclusive the placebo group in which the beclomethasone 200 μg b.i.d. was continued, showed considerable improvements of all parameters. Therefore, our results were not due to a ceiling effect. We could not identify special subgroups, which could benefit more from one of the treatments. The compliance with study medication as well as with maintenance beclomethasone was high, nearly 90% in all groups. The substantial improvement in the placebo group, which continued beclomethasone 200 μg b.i.d., can only be explained by this high compliance, which is likely to be better than before entry into the study. This study also suggested a dose-related effect of beclomethasone on growth.

Translating our findings to current asthma treatment guidelines, we conclude that, in agreement with these guidelines, salmeterol should not be used as a monotherapy, but only in addition to inhaled corticosteroid treatment. On the basis of our data, we could not define a dose of inhaled corticosteroid above which introduction of a long-acting β_2-agonist, such as salmeterol, should be the next step. Regarding the aims of the guidelines to reduce symptoms and exacerbations all three treatment regimens studied in our second multicenter study seem to fulfill these criteria, with no clear advantage of one therapeutic regimen above others. However, our studies clearly showed a dose-dependent effect of beclomethasone on growth. Therefore, careful monitoring of growth is necessary in all children during treatment with inhaled corticosteroids. It seemed that addition of salmeterol may have advantages on peak flow values and use of rescue medication during the first few weeks of treatment, without resulting in better symptom control, better airway caliber and less airway responsiveness after a year of treatment. On the basis of these data in combination with the knowledge of developing tolerance during maintenance treatment with salmeterol, at least to non-bronchodilating effects, one could argue whether salmeterol could better be used for short periods of time (a number of weeks) during symptomatic episodes, instead of as maintenance therapy for months or years. Our study also showed that with frequent control of patients and check of their medication use, it was possible to achieve excellent compliance rates with

subsequent improvements in asthma control. As we studied children with moderate asthma, we can not extrapolate our results to more severe asthma. It might well be possible that both increasing the inhaled corticosteroid dose and/or adding salmeterol has benefits in this group.

8.3 Directions for future research

Our data raise some questions about the reliance on symptom scores and peak flow values, especially during treatment with long-acting bronchodilators. All current asthma guidelines use these parameters as an indicator of asthma severity. The underlying hypothesis is that asthma is a chronic inflammatory process of the airways, which should be treated. Recent data on treatment with inhaled corticosteroids in adult asthmatics showed that airway responsiveness to methacholine correlates best with the inflammatory changes found in bronchial biopsies, whereas symptom scores, use of $ß_2$-agonists, PEF and FEV_1 were not.[13] One could therefore argue whether measurements of airway responsiveness are useful in determining the severity of asthma and subsequent treatment. Further studies are necessary to address the question whether measurements of airway responsiveness will prove superior as a guide to improve treatment adjustments. Therefore, it will be necessary to develop methods to assess airway inflammation in a non-invasive way. So far, parameters as serum eosinophilic cationic protein and eosinophil derived neurotoxin (eosinophil protein X) in serum and urine, have been related to disease activity.[14] [15] Some evidence exists that urinary eosinophil derived neurotoxin may be a useful marker of airway inflammation during treatment with inhaled corticosteroids.[16] Recently, the amount of hydrogen peroxide in exhaled air of asthmatic children was found to be increased compared to controls.[17] All these parameters however, show considerable overlap between patients and controls and it remains to be proven that this variability is actually due to differences in airway inflammation.

Our studies showed a dose-dependent effect of beclomethasone on growth in children. Although data from the literature so far,[12] do not support an effect of inhaled corticosteroids on long-term growth or adult height, new long-term studies are necessary to test the hypothesis, that the negative effect on growth will diminish during further treatment. Besides beclomethasone, other inhaled corticosteroids, such as budesonide and fluticasone, need to be studied for their effects on growth during long-term treatment.

Studies in children with more severe asthma are necessary to address whether in this group of patients higher doses of inhaled corticosteroids and/or addition of salmeterol are beneficial.

8.4 References

1. Ullman A, Svedmyr N. Salmeterol, a new long-acting beta-2-adrenoceptor agonist; comparison with salbutamol in adult asthmatic patients. *Thorax* 1988;**43**:574-8.
2. Warner JO. Asthma: a follow up statement from an international paediatric asthma consensus group. *Arch Dis Child* 1992;**67**:240-8.
3. Guidelines for the diagnosis and management of asthma. National Heart, Lung and Blood Institute, National Asthma Education Program Expert Panel Report. *J Allergy Clin Immunol* 1991;**88**:425-534.
4. International consensus report on diagnosis and treatment of asthma. National Heart, Lung and Blood Institute, National Institutes of Health Bethesda. *Eur Respir J* 1992;**5**:601-41.
5. Guidelines on the management of asthma. Woodhead M, editor. *Thorax* 1993;**48**:S1-S24.
6. Derom EY, Pauwels RA, Van der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992;**89**:811-5.
7. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting beta-2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;**327**:1198-203.
8. Kalra S, Swystun VA, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1996;**109**:953-6.
9. Yates DH, Kharitonov SE, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled beta-2-agonist. *Am J Respir Crit Care Med* 1996;**154**:1603-7.
10. Booth H, Bish R, Whitehead F, Walters EH. Salmeterol tachyphylaxis in steroid treated asthmatic subjects. *Thorax* 1996;**51**:1100-4.
11. Cockcroft DW, Swystun VA. Functional antagonism: tolerance produced by inhaled beta-2-agonists. *Thorax* 1996;**51**:1051-6.
12. Wolthers OD. Long-, intermediate- and short-term growth studies in asthmatic children treated with inhaled glucocorticosteroids. *Eur Respir J* 1996;**9**:821-7.
13. Sont JK, Van Krieken JHJM, Evertse CE, Hooijer R, Willems LNA, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996;**51**:496-502.
14. Zimmerman B, Lanner A, Enander I, Zimmerman RS, Peterson CG, Ahlstedt ST. Total blood eosinophils, serum eosinophil cationic protein and eosinophil protein X in childhood asthma. *Clin Exp Allergy* 1993;**23**:564-70.
15. Koller DY, Herouy Y, Gotz M, Hagel E, Urbanek R, Eichler I. Clinical value of monitoring eosinophil activation in asthma. *Arch Dis Child* 1995;**73**:413-7.
16. Kristjansson S, Strannegard IL, Strannegard O, Peterson C, Enander I, Wennergren G. Urinary eosinophil protein X in children with atopic asthma: a useful marker of antiinflammatory treatment. *J Allergy Clin Immunol* 1996;**97**:1179-87.
17. Jöbsis Q, Raatgreep HC, Hermans PWM, De Jongste JC. Hydrogen peroxide in exhaled air is increased in stable asthmatic children. *Eur Respir J* 1997;**10**:519-21

Samenvatting

Salmeterol, een lang-werkende $ß_2$-agonist werd voor het eerst geïntroduceerd voor de behandeling van astma bij volwassenen aan het eind van de jaren tachtig.[1] Doel van dit proefschrift was het effect van salmeterol na een eenmalige dosering en tijdens onderhoudsbehandeling te onderzoeken en de plaats van salmeterol in de behandeling van astma bij kinderen te bepalen. Primaire uitkomstvariabelen van het onderzoek waren de luchtwegdiameter, gemeten als FEV_1 en de luchtweggevoeligheid, gemeten als PD_{20} voor metacholine. In de lange-termijn onderzoeken waren secundaire uitkomstvariabelen: symptoom scores, piekstroom, gebruik van extra kort-werkende $ß_2$-agonisten en aantal astma-exacerbaties.

Hoofdstuk 1 omvat een algemene introductie en beschrijft de pathofysiologie van astma als een inflammatoire ziekte van de luchtwegen. De huidige richtlijnen voor de behandeling van astma worden besproken. De plaats van de lang-werkende $ß_2$-agonisten in het stapsgewijze behandelschema voor astma ligt niet onomstotelijk vast in de diverse internationale consensusrapporten.[2 3 4 5] Ofwel worden zij als additionele middelen geplaatst wanneer reeds hoge doseringen inhalatiecorticosteroïden worden gebruikt, ofwel een stap eerder, wanneer ondanks een lagere dosering inhalatiesteroïden nog steeds astma symptomen persisteren. De farmacologische en klinische aspecten van inhalatiesteroïden, kort-werkende en lang-werkende $ß_2$-agonisten worden uitvoeriger besproken. Deze medicamenten zijn nu de hoekstenen van de behandeling van astma bij kinderen en volwassenen.

Hoofdstuk 2 geeft een literatuuroverzicht van de effecten van kort-werkende en lang-werkende $ß_2$-agonisten op de luchtweggevoeligheid voor histamine en metacholine. Kort-werkende $ß_2$-agonisten hebben een kortdurend beschermend effect, waarbij de dosis-effect curve voor de luchtwegvernauwende prikkel naar rechts verschuift. Tijdens lange-termijn behandeling met kortwerkende $ß_2$-agonisten treedt geen vermindering van de luchtweggevoeligheid op; sommige onderzoeken hebben zelfs een verslechtering van de luchtweggevoeligheid gesuggereerd tijdens chronisch gebruik van kort-werkende $ß_2$-agonisten. Eenmalige dosering van de lang-werkende $ß_2$-agonisten salmeterol en formoterol geeft langdurige bescherming tot ten minste 12 uur, tegen luchtwegvernauwing geïnduceerd door metacholine of histamine. Bij gebruik van weken tot maanden treedt wel tolerantie op voor dit beschermend effect tegen luchtwegvernauwende stimuli, echter er blijft bescherming bestaan. Tolerantie treedt niet op voor het bronchusverwijdende effect.

Hoofdstuk 3 beschrijft de literatuur betreffende klinische onderzoeken met salmeterol

en formoterol, met name de resultaten van onderzoeken bij kinderen. Na een eenmalige dosering hebben beide middelen een luchtwegverwijdend effect van tenminste 12 uur. Ook is er een langdurig beschermend effect tegen luchtwegvernauwende stimuli, zoals inspanning, metacholine, histamine en allergeen. Bij tweemaal daags gebruik gedurende weken tot maanden zijn er minder astma symptomen en zijn de piekstroom waarden hoger ten opzichte van behandeling met kort-werkende $ß_2$-agonisten. Hoewel er tolerantie optreedt voor het beschermende effect, blijft er bescherming bestaan gedurende behandeling van enkele maanden. De klinische relevantie hiervan is nog niet geheel duidelijk. Bijwerkingen van de lang-werkende $ß_2$-agonisten zijn niet verschillend van die van kort-werkende $ß_2$-agonisten.

In **hoofdstuk 4** worden de resultaten gepresenteerd van het onderzoek van een eenmalige dosis salmeterol. In een dubbel-blind, placebo-gecontroleerd, cross-over onderzoek wordt het effect van 50 μg salmeterol op luchtwegdiameter en luchtweggevoeligheid bestudeerd. Twintig kinderen met matig tot ernstig astma participeerden in het onderzoek; de meerderheid van de kinderen werd behandeld met inhalatiesteroïden of cromoglicaat. Vijftig μg salmeterol resulteerde in betere FEV_1-waarden gedurende de eerste 12 uur na inhalatie. De maximale respons werd 4 uur na inname bereikt: de gemiddelde FEV_1 waarde lag 18.6 % boven de uitgangswaarde. Significante bescherming tegen metacholine geïnduceerde luchtwegvernauwing trad op tot 24 uur na inhalatie van salmeterol (gemiddelde PD_{20} 1.22 verdubbelingsdosis boven de uitgangswaarde). De maximale bescherming bedroeg bijna 4 verdubbelingsdoses 1 uur na inhalatie. Wij concludeerden dat een eenmalige dosis van 50 μg salmeterol resulteerde in langdurige luchtwegverwijding en bescherming tegen metacholine.

In **hoofdstuk 5** wordt het effect van een eenmalige dosering salmeterol op luchtweggevoeligheid vergeleken met het effect tijdens chronische behandeling van 4 maanden. In een dubbel-blind, parallel onderzoek werden 30 kinderen gerandomiseerd tussen ofwel salmeterol 50 μg twee maal daags ofwel salbutamol 200 μg twee maal daags. Voor dit onderzoek werden kinderen gerecruteerd met mild astma, die geen onderhoudsbehandeling hadden met inhalatiesteroïden of cromoglicaat en nauwelijks of geen luchtwegvernauwing hadden, maar wel een toegenomen luchtweggevoeligheid voor metacholine. De luchtweggevoeligheid werd gemeten voor de start van het onderzoek, 12 uur na de eerste dosis en daarna maandelijks gedurende de 4 maanden durende behandelinsperiode. De metingen werden altijd op hetzelfde tijdstip van de dag verricht, 12 uur na de laatste dosis van de medicatie. Voor FEV_1 werden geen significante verschillen tussen de beide behandelingen gevonden. PD_{20} voor metacholine steeg met

1.7 ± 0.3 verdubbelingsdosis na de eerste gift salmeterol. Deze bescherming verminderde tot 0.6 ± 0.3 verdubbelingsdosis na 1 maand, hetgeen over de rest van de behandelingsperiode niet veranderde en significant beter was dan de uitgangswaarde en de behandeling met salbutamol. Wij concludeerden dat tolerantie ten aanzien van het beschermende effect tegen metacholine geïnduceerde luchtwegvernauwing optreedt binnen 1 maand na start van salmeterol onderhoudsbehandeling, echter dat geen toenemende tolerantie optreedt in het verloop van enkele maanden.

Hoofdstuk 6 beschrijft de resultaten van een multi-center onderzoek waarin behandeling met salmeterol 50 μg twee maal daags vergeleken wordt met beclometason 200 μg twee maal daags. Zevenenzestig kinderen met mild tot matig-ernstig astma, die tenminste 6 maanden voor de aanvang geen inhalatiesteroïden hadden gebruikt en tenminste 2 weken geen cromoglicaat, werden gerandomiseerd in een dubbel-blind parallel onderzoek. Na een jaar behandeling was er een significant voordeel voor de beclometason groep: in deze groep was de FEV_1 waarde gemeten voor bronchusverwijding gemiddeld 14.2 % van voorspeld hoger, de FEV_1 waarde na bronchusverwijding gemiddeld 7 % van voorspeld hoger en de PD_{20} voor metacholine 2.79 verdubbelingsdoses hoger. In de salmeterol was er een tendens tot verslechtering voor zowel de FEV_1 als PD_{20}, ondanks het feit dat symptomen en piekstroom waarden verbeterden. De gemiddelde daling was 4.3 % voorspeld voor FEV_1 en 0.73 verdubbelingsdosis voor PD_{20}. Voor wat betreft symptomen (dag- en nacht-), gebruik van extra kort-werkende $ß_2$-agonisten en piekstroom waarden (ochtend- en avond-) waren er geen significante verschillen tussen de behandelingsgroepen, hoewel beclometason behandeling iets betere resultaten gaf. Astma exacerbaties waarvoor prednisolon nodig was, kwamen aanzienlijk vaker voor in de salmeterol groep, evenzo was het aantal kinderen dat om deze reden uit de studie viel aanzienlijk hoger in de salmeterol groep. In de beclometason groep was echter de groei vertraagd ten opzichte van de salmeterol groep (-0.28 SDS versus -0.03 SDS). Wij concludeerden dat behandeling met beclometason in deze gemiddelde dosering superieur is ten opzichte van behandeling met salmeterol ten aanzien van longfunctie, symptoom scores en astma exacerbaties. We adviseerden salmeterol niet als monotherapie te gebruiken en bepleitten controle van de groei tijdens behandeling met inhalatiesteroïden.

Hoofdstuk 7 beschrijft de resultaten van een multi-center onderzoek waarin toevoeging van salmeterol (50 μg twee maal daags) aan beclometason (200 μg twee maal daags)(SI200 groep) vergeleken wordt met beclometason 400 μg twee maal daags (I400 groep) en met beclometason 200 μg twee maal daags (I200 groep). In een dubbel-blind,

placebo-gecontroleerd, parallel onderzoek participeerden 177 kinderen. Na 1 jaar behandeling waren er geen significante verschillen tussen de 3 behandelingsgroepen voor FEV_1 en PD_{20} metacholine. Echter in alle 3 de groepen waren er significante verbeteringen ten opzichte van de uitgangswaarden. Voor FEV_1 waren de gemiddelde veranderingen respectievelijk 4.3 % (95%CI 1.3;7.2), 5.8 % (95%CI 2.9;8.7) en 4.3 % voorspelde waarde (95%CI 2.1;6.5) voor de SI200, I400 en I200 groep. Voor PD_{20} metacholine waren de gemiddelde veranderingen respectievelijk 0.6 (95%CI 0.05;1.14), 1.3 (95%CI 0.73;1.87) and 0.8 verdubbelingsdoses (95%CI 0.33;1.27) voor de SI200, I400 en I200 groep. Voor de ochtend- en avond piekstroom waarden was er een gering voordeel voor de SI200 groep ten opzichte van de I200 groep, met name in de eerste maanden van de behandelingsperiode. Er waren geen significante verschillen tussen de groepen wat betreft symptoom scores, gebruik van extra salbutamol en astma exacerbaties. De lengtegroei was significant verminderd in de I400 groep. Het gebruik van de voorgeschreven medicatie was zeer goed in alle groepen, waarbij bijna 90% van de voorgeschreven medicatie werd gebruikt. Wij concludeerden dat toevoeging van salmeterol of verdubbeling van de inhalatiesteroïd dosis geen extra voordeel heeft boven 200 μg beclometason twee maal daags in deze selecte groep kinderen met astma, waarin het gebruik van de voorgeschreven medicatie uitmuntend was.

Conclusies en algemene discussie

In overeenstemming met de resultaten van onderzoeken bij volwassenen met astma, vonden wij dat salmeterol ook bij kinderen een langdurig bronchusverwijdend en beschermend effect tegen metacholine geïnduceerde luchtwegvernauwing heeft.[6] Evenals de resultaten uit onderzoek van Cheung et al,[7] vonden wij een vermindering van het beschermende effect tijdens 4 maanden behandeling met salmeterol. Echter, ons onderzoek liet zien dat significante bescherming blijft bestaan zonder dat er een tendens is tot verdere vermindering in de tijd. Aangezien de patiënten in zowel het onderzoek van Cheung als in ons onderzoek geen inhalatiesteroïden gebruikten, zou men kunnen veronderstellen dat gebruik van deze het ontstaan van tolerantie zouden kunnen voorkomen. Recente onderzoekingen verwerpen echter deze hypothese.[8 9 10] De klinische relevantie van het ontstaan van deze tolerantie is niet geheel duidelijk, en tot dusverre is er geen overtuigend bewijs dat tolerantie ook ontstaat voor het bronchusverwijdend effect.[7 11] Anderzijds, de reden dat tolerantie niet is aangetoond voor het bronchusverwijdend effect zou ook gelegen kunnen zijn in het feit dat verbeteringen van de luchtwegdiameter gelimiteerd zijn bij het bereiken van maximale verwijding, terwijl voor de dosis-effect curve bij metacholine er een open-eind is.[11]

De twee lange termijn onderzoeken zoals beschreven in hoofdstuk 6 en 7 waren bedoeld

om de plaats van salmeterol in de behandeling van astma bij kinderen aan te geven. Het eerste onderzoek toonde de superioriteit van beclometason in een gebruikelijke dosering ten opzichte van salmeterol monotherapie zowel wat betreft longfunctie verbetering (luchtwegdiameter en luchtweggevoeligheid), symptoom scores als aantal exacerbaties. Opvallend was dat in de salmeterol groep symptomen en piekstroom waarden verbeterden, hoewel de luchtweg diameter en luchtweggevoeligheid enigszins verslechterden en er frequent exacerbaties optraden. Behandeling met 400 μg beclometason per dag resulteerde wel in een mogelijk klinisch relevante afname van de standaard deviatie score voor lengtegroei. Een recente Nederlandse kinderendocrinologie consensus bijeenkomst beoordeelde een afname van 0.25 standaard deviatie score per jaar als klinisch relevant. Het is mogelijk dat in ons onderzoek het gebruik van een poederinhalator met relatief hoge longdepositie en mogelijk ook in de mond-keelholte, en de hoge compliance hebben bijgedragen aan deze groeiremming. Tot dusverre worden in de literatuur geen aanwijzingen gevonden voor een klinisch relevant effect van inhalatiesteroïden op de groei.[12]

Het tweede lange-termijn onderzoek vergeleek het effect van toevoeging van salmeterol aan twee maal daags 200 μg beclometason met verdubbeling van de beclometason dosering en met de initiële beclometason dosering. Dit onderzoek leverde geen significante verschillen op tussen de drie behandelingsgroepen voor wat betreft longfunctie, luchtweggevoeligheid, symptomen, gebruik van extra kort-werkende $ß_2$-agonisten en aantal exacerbaties. Toevoeging van salmeterol gaf wel iets hogere piekstroom waarden, met name in de eerste maanden van de behandeling. Alle behandelingsgroepen lieten evenwel significante verbeteringen zien in de gemeten parameters, zodat de resultaten niet verklaard kunnen worden uit een gebrek aan ruimte voor verbetering. Wij konden geen specifieke subgroepen identificeren, die betere resultaten hadden met een van de behandelingsmethoden. Het gebruik van studie medicatie en onderhoud beclometason was uitmuntend; bijna 90% van de voorgeschreven medicatie werd gebruikt. De verbetering in de placebogroep die beclometason, twee maal daags 200 μg, continueerde, is toe te schrijven aan dit hoge medicatiegebruik, hetgeen waarschijnlijk beter was dan voor het onderzoek. Dit onderzoek toonde een dosis-afhankelijk effect van beclometason op de lengte-groei.

Wanneer we de gegevens uit bovenstaande onderzoeken vertalen naar de huidige richtlijnen voor de behandeling van astma, kunnen we concluderen dat, in overeenstemming met deze richtlijnen, salmeterol niet als monotherapie gebruikt dient te worden, maar alleen in combinatie met inhalatiesteroïden. Op basis van onze resultaten, konden we geen dosering inhalatiesteroïd aangeven, waarboven toevoeging van een lang-werkende $ß_2$-agonist, zoals salmeterol, de volgende stap in het

behandelplan dient te zijn. De behandeldoelen zoals gesteld in de richtlijnen om symptomen en exacerbaties te verminderen worden in alle 3 de behandelingsgroepen gehaald, zonder dat een van de behandelingen hier een evident voordeel biedt. Ons onderzoek liet wel een duidelijk dosis-afhankelijk effect van beclometason op de lengtegroei zien. Dit maakt een nauwkeurig vervolgen van de lengtegroei bij kinderen behandeld met inhalatiesteroïden noodzakelijk. Toevoeging van salmeterol lijkt in de eerste weken een positief effect te hebben op piekstroom waarden en gebruik van extra kort-werkende $ß_2$-agonisten, zonder een effect op symptoom scores, luchtweg diameter en luchtweggevoeligheid. Dit gegeven gecombineerd met het feit dat tolerantie, tenminste voor de niet-luchtwegverwijdende effecten, ontstaat tijdens langdurige continue behandeling, zou een argument kunnen zijn om salmeterol in te zetten in kortere periodes (enkele weken), wanneer er meer symptomen zijn, in plaats van als continue behandeling voor maanden tot jaren. In ons onderzoek bleek dat frequente controle van de patiënten, waarbij ook het medicatiegebruik werd gecontroleerd, leidde tot een goede compliance en daarmee verbetering van het astma. We kunnen onze resultaten niet extrapoleren naar ernstig astma, daar we in deze onderzoeken alleen kinderen met matig-ernstig astma onderzocht hebben. Het is zeer wel mogelijk dat bij ernstig astma zowel verhoging van de dosering inhalatiesteroïd als toevoeging van salmeterol wel voordeel bieden.

Aanbevelingen voor toekomstig onderzoek

Onze resultaten werpen de vraag op of symptoom scores en piekstroom waarden geschikte parameters zijn met name tijdens gebruik van lang-werkende luchtwegverwijders. De huidige richtlijnen gebruiken symptomen en piekstroom waarden om de ernst van astma te classificeren. De hypothese is dat astma een chronische ontstekingsziekte van de luchtwegen is, die als dusdanig behandeld moet worden. Recente data bij volwassen astmapatiënten die behandeld werden met inhalatiesteroïden lieten zien dat de mate van luchtweggevoeligheid voor metacholine het best correleerde met de mate van ontsteking in de luchtwegbiopten, terwijl symptomen, gebruik van $ß_2$-agonisten, piekstroom en FEV_1 hier niet mee correleerden.[13] Mogelijk is het bepalen van de luchtweggevoeligheid van nut ter bepaling van de ernst van het astma. Verder onderzoek is nodig om de vraag te beantwoorden of bepaling van de luchtweggevoeligheid een geschiktere parameter is als leidraad voor de behandeling. Het is tevens nodig om niet-invasieve methoden te ontwikkelen, die de mate van luchtwegwandontsteking weergeven. Verschillende eiwitten, zoals "eosinophilic cationic protein" en "eosinophil derived neurotoxin (eosinophil protein X)" in bloed en urine correleren met de ziekte-activiteit.[14] [15] Er zijn

enkele aanwijzingen dat "eosinophil derived neurotoxin" uitgescheiden in de urine een waardevolle graadmeter is van het ontstekingsproces tijdens behandeling met inhalatiesteroïden.[16] Uit recent onderzoek blijkt dat de hoeveelheid waterstofperoxide in de uitademingslucht bij kinderen met astma verhoogd is ten opzichte van controles.[17] Alle bovengenoemde bepalingen vertonen een grote overlap tussen patiënten en controles en verder onderzoek zal nodig zijn om aan te tonen in hoeverre dit relateert aan de ontsteking in de luchtwegwand.

Ons onderzoek toonde een dosis-afhankelijk effect van beclometason op de lengtegroei bij kinderen. Hoewel de huidige literatuurgegevens een effect van inhalatiesteroïden op de lange-termijn groei en op de uiteindelijke volwassen lengte niet ondersteunen,[12] dienen nieuwe lange-termijn studies te worden opgezet om dit verder te onderzoeken. Onze hypothese dat de negatieve effecten op de lengtegroei mogelijk verminderen tijdens langere behandeling moet verder onderzocht worden. Naast beclometason dienen ook de effecten van de andere inhalatiesteroïden, zoals budesonide en fluticason, op de lengtegroei onderzocht te worden.

Onderzoek bij kinderen met ernstig astma is nodig om aan te geven of in deze groep hogere doseringen inhalatiesteroïd en/of salmeterol een gunstiger effect hebben.

Referenties

1. Ullman A, Svedmyr N. Salmeterol, a new long-acting beta-2-adrenoceptor agonist; comparison with salbutamol in adult asthmatic patients. Thorax 1988;43:574-8
2. Warner JO. Asthma: a follow up statement from an international paediatric asthma consensus group. Arch Dis Child 1992;67:240-8
3. Guidelines for the diagnosis and management of asthma. National Heart, Lung and Blood Institute, National Asthma Education Program Expert Panel Report. J Allergy Clin Immunol 1991;88:425-534
4. International consensus report on diagnosis and treatment of asthma. National Heart, Lung and Blood Institute, National Institutes of Health Bethesda. Eur Respir J 1992;5:601-41
5. Guidelines on the management of asthma. Woodhead M, editor. Thorax 1993;48:S1-S24
6. Derom EY, Pauwels RA, Van der Straeten MEF. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. J Allergy Clin Immunol 1992;89:811-5
7. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting beta-2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. N Engl J Med 1992;327:1198-203
8. Kalra S, Swystun VA, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. Chest 1996;109:953-6
9. Yates DH, Kharitonov SE, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled beta-2-agonist. Am J Respir Crit Care Med 1996;154:1603-7
10. Booth H, Bish R, Whitehead F, Walters EH. Salmeterol tachyphylaxis in steroid treated asthmatic subjects. Thorax 1996;51:1100-4
11. Cockcroft DW, Swystun VA. Functional antagonism: tolerance produced by inhaled beta-2-agonists. Thorax 1996;51:1051-6

12. Wolthers OD. Long-, intermediate- and short-term growth studies in asthmatic children treated with inhaled glucocorticosteroids. Eur Respir J 1996;9:821-7
13. Sont JK, Van Krieken JHJM, Evertse CE, Hooijer R, Willems LNA, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax 1996;51:496-502
14. Zimmerman B, Lanner A, Enander I, Zimmerman RS, Peterson CG, Ahlstedt St. Total blood eosinophils, serum eosinophil cationic protein and eosinophil protein X in childhood asthma. Clin Exp Allergy 1993;23:564-70
15. Koller DY, Herouy Y, Gotz M, Hagel E, Urbanek R, Eichler I. Clinical value of monitoring eosinophil activation in asthma. Arch Dis Child 1995;73:413-7
16. Kristjansson S, Strannegard IL, Strannegard O, Peterson C, Enander I, Wennergren G. Urinary eosinophil protein X in children with atopic asthma: a useful marker of antiinflammatory treatment. J Allergy Clin Immunol 1996;97:1179-87
17. Jöbsis Q, Raatgreep HC, Hermans PWM, De Jongste JC. Hydrogen peroxide in exhaled air is increased in stable asthmatic children. Eur Respir J 1997;10:519-21

Curriculum vitae

Anja Verberne werd geboren op 23 januari 1960 te Eindhoven. Het Atheneum-B diploma behaalde zij in 1978 aan het Augustinianum te Eindhoven. Hierna werd de geneeskunde studie aan de Rijksuniversiteit Leiden aangevangen, waar zij in 1983 haar doctoraalexamen en in 1985 haar artsexamen behaalde. In augustus 1985 werd zij aangesteld als assistent geneeskundige niet in opleiding in het Juliana Kinderziekenhuis te Den Haag, alwaar zij haar opleiding tot kinderarts startte op 1 januari 1986 (opleider Dr. H.E. Zoethout). Haar academische stage werd gevolgd op de kinderafdeling van het Academisch Ziekenhuis Leiden (opleider Prof.Dr. L.J. Dooren). Het laatste half jaar van de opleiding werd als fellow doorgebracht; de eerste 4 maanden op de afdeling neonatologie in Leiden (hoofd: Prof.Dr. J.H. Ruys), vanaf november 1989 op de afdeling kinderlongziekten in het Sophia Kinderziekenhuis te Rotterdam (hoofd: Prof.Dr. K.F. Kerrebijn). Na haar registratie als kinderarts in januari 1990 bleef zij hier werkzaam als staflid. Naast een verdere subspecialisatie in de kinderlongziekten werd wetenschappelijk werk verricht in de vorm van klinisch onderzoek naar salmeterol. De werkzaamheden werden deels verricht op de kinderlongziekten afdeling in Rotterdam, deels als coördinator van een groot multi-center onderzoek door de Dutch Paediatric Asthma Study Group. Vanaf november 1993 combineerde zij dit als deeltijdbaan met een baan als algemeen kinderarts in het Ignatius Ziekenhuis Breda. Vanaf juni 1996 is zij alleen nog werkzaam als kinderarts in Breda. Zij is getrouwd met Bart Vaessen en is moeder van 3 kinderen: Maud (6 jaar), Floor (4 jaar) en Kai (2 jaar).

Dutch Paediatric Asthma Study Group

The Dutch Paediatric Asthma Study Group consists of a steering committee (K.F. Kerrebijn, J.A.M. Raaymakers, S.J. Pocock, J.M. Bogaard) and members from the departments of paediatric respiratory medicine of the Emma Children's Hospital/Children's AMC, Amsterdam (J.C. van Nierop), the University Hospital of Amsterdam (A.F. Nagelkerke, B. Thio), the St Antonius Hospital, Nieuwegein (T.J. Schouten), the Asthma Center Heideheuvel, Hilversum (E.E.M. van Essen-Zandvliet, C.E. van Marle, A. Denteneer), the Beatrix Children Clinic/University Hospital Groningen (J. Gerritsen, M.H. Grol), Hospital De Weezenlanden, Zwolle (R.J. Roorda), the University Hospital Maastricht (J.J.E. Hendriks), the Juliana Children's Hospital, The Hague (E.J. Duiverman, J.M. Kouwenberg), the Wilhelmina Children's Hospital, Utrecht (J. van der Laag, H.J.L. Brackel), and the Sophia Children's Hospital, Rotterdam (A.A.P.H. Verberne).

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List of abbreviations

AC	adenylyl cyclase
AMP	adenosine monophosphate
AP-1	activating protein-1
BDP	beclomethasone dipropionate
b.i.d.	twice daily
cAMP	cyclic 3'-5'-adenosine monophosphate
CI	confidence interval
CREB	cAMP responsive element binding protein
DD	doubling dose(s)
DNA	deoxyribonucleic acid
DPI	dry powder inhaler
ECP	eosinophilic cationic protein
EDN	eosinophil-derived neurotoxin
EPO	eosinophilic peroxidase
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GM-CSF	granulocyte macrophage-colony stimulating factor
IFN	interferon
IL	interleukin
MBP	major basic protein
MDI	metered dose inhaler
NF-κB	nuclear factor-κB
PC_{20}	provocative concentration which causes a decrease in FEV_1 by 20%
PD_{20}	provocative dose which causes a decrease in FEV_1 by 20%
PEF	peak expiratory flow
PKA	protein kinase A
SDS	standard deviation scores
SEM	standard error of the mean
sGaw	specific airway conductance
Th	T helper lymphocyte