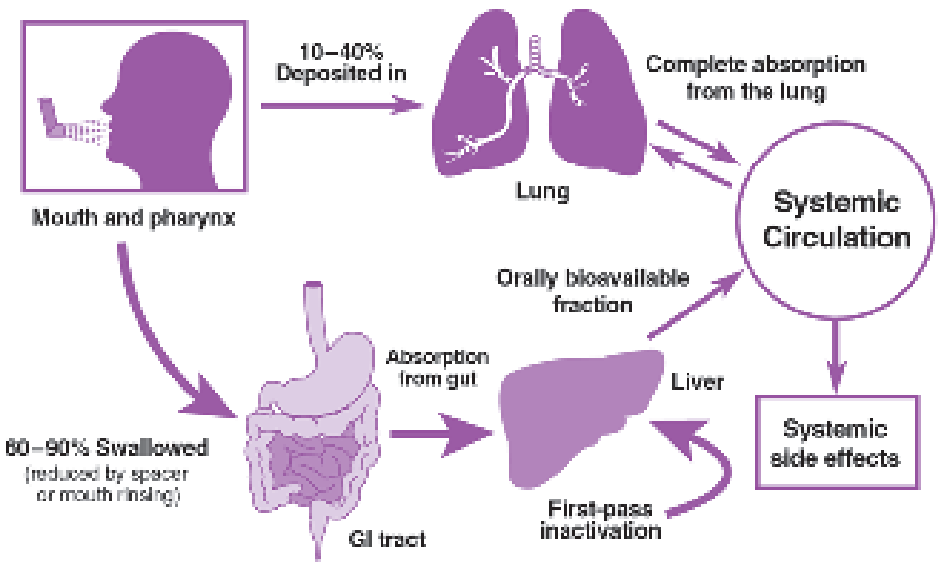
Lung Biology in Health and Disease Volume 163
Executive Editor: Claude Lenfant

# Inhaled Steroids in Asthma

## Optimizing Effects in the Airways




edited by

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# INTRODUCTION

Because of its subject, this monograph is unique among the more than 150 volumes that have been published in the Lung Biology in Health and Disease series. The reason is that it focuses on a single class of medications, i.e., the steroids in asthma.

Of course, appreciation of the role that glucocorticosteroids can play in the treatment of asthma is not new, but their introduction into the practice of medicine and the reception they have received from physicians has been relatively slow and cautious.

Just think! In January 1971, the Ciba Foundation convened a "Study Group on Identification of Asthma." Its aim was to "define asthma"; yet, in one of the chapters of the report on the pharmacology of asthma, one finds mention of "the need to prevent or minimize damage to cells, and this can be done by stabilizing cell membranes and reducing the activity of destructive enzymes. Such protection results from the use of glucocorticoids and some acidic anti-inflammatory drugs" (1).

Two years later, in 1973, the report of an international conference on asthma held in late 1972 was published. One of the contributors reported that "one function of steroids in phagocytic cells may be stabilization of the mem-

branes, both the plasma membrane and lysozomes themselves." He later concluded: "If inflammation in acute or chronic disease (i.e., asthma) is due to the release of mediators of inflammation from intercellular organelles or granules, then cortisone may be an effective anti-inflammatory agent" (2).

We all know that since then the field of asthma research has progressed in a remarkable manner. Scientists have shown definitively that inflammation in response to the injurious event is the culprit, and among the various therapeutic agents, steroids have emerged as a powerful and effective regimen—perhaps not in all cases, but certainly in many. Today, it is an accepted belief that asthma, albeit a serious disease, can be controlled and that very few patients, if any at all, will die of it if they are carefully monitored and treated.

Research in academia has played a great role in all these advances. However, as the editors of this volume point out, the role that the pharmaceutical industry agreed to take on has been pivotal in bringing to patients the full benefit of all the research done in academia and in its own laboratories.

This volume is a major contribution to this series. It assembles what we know today about steroids and the most common mode of administration in asthma, i.e., inhalation. Undoubtedly, it is one more step toward improving the treatment of asthma patients. The reader need only take note of the editors and authors of this monograph to be sure of the excellence and utility of this publication. As the executive editor of the Lung Biology in Health and Disease series, I am grateful to them for giving me the opportunity to present it to our readership.

**Claude Lenfant, M.D.**
Bethesda, Maryland

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# PREFACE

In the era that has witnessed the sequencing of the human genome, asthma remains a debilitating and sometimes fatal disease for those unfortunate enough to suffer from the inability to breathe inflicted by this condition. Now recognized as a disease that flourishes in association with the increases in standard of living in the twentieth century, asthma continues to defy all efforts of modern science and medicine to develop a cure. Although a number of exciting new strategies to treat the disorder have emerged over the last two decades, none has yet succeeded in managing the symptoms or diminishing the profound exacerbations of asthma as effectively as glucocorticoids. While we harbor great hope that new strategies for treating asthma will succeed, we must accept for the time being that glucocorticoids remain the most effective asthma medications and acknowledge that this may be true for decades to come.

Probably the biggest advances in inhaled glucocorticoid medications have resulted from efforts to improve targeting of these drugs to the lungs. This evolved from concerted and inspired scientific investigations in both industry and academia. It is extraordinarily important for the field of asthma—as well as for any other field of medicine that endeavors to utilize pulmonary drug delivery—that the accomplishments of the numerous individuals involved in these efforts be doc-

umented, chronicled, and evaluated. The intent of this volume is to gather the available information on inhaled glucocorticoid targeting in the lungs, based on the expertise and experience of leading asthma researchers and clinician–investigators. We hope that readers of this volume find it useful as a source of ideas, as a wellspring for future studies, and as an essential reference for the scientific literature on inhaled glucocorticoids.

The production of this volume would not have occurred without the important efforts of a number of individuals. We would like to express our gratitude to Dean Phizacklea of AstraZeneca for his generous support of this project. We also acknowledge the invaluable assistance of Mrs. Nicola Heller and Dr. Syed Shahabuddin, who were instrumental in documenting the discussion sessions of the volume. We also thank Christina Max for her help with the subject index. Our thanks also go to the staff of Marcel Dekker, Inc., and to Dr. Claude Lenfant, who provided the medium for this communication. Special thanks go to Ms. Bonnie Hebden for tackling the monumental task of assembling this volume with cheerfulness and great competence.

Finally, we are pleased to note that another important goal both of the meeting from which this volume evolved and of this book has been the desire of three of us (Robert P. Schleimer, Paul M. O'Byrne, and Stanley J. Szefler) to honor our beloved coeditor and colleague, Dr. Ralph Brattsand, and to recognize his inspired and outstanding contributions to the field of inhaled glucocorticoid treatment for asthma.

**Robert P. Schleimer**
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# CONTENTS

# Part One

## INTRODUCTION

# 1

## Drug Development of Inhaled Steroids

### A Pharmacologist's View Based on Experiences from the Budesonide Project

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### I. Introduction

The introduction of inhaled steroids (IS) has been successful for asthma therapy from the viewpoints of the patient and the medical community, as well as from the health care cost of society (see Chap. 2). The special steroids required for that development were selected and documented more by combining rational thought and functional in vivo testing than upon solid mechanistic knowledge about steroid kinetics and dynamics in airways/lung tissue. The IS budesonide (BUD) has played an important role in that development, and during the 1980s and 1990s BUD became the major tool for extending the clinical documentation of IS therapy. This introductory chapter gives me the chance to sum up some pharmacological considerations and personal thoughts after three decades of work with topical steroids. Even if these experiences are based mainly upon the BUD project, these considerations may exemplify motivations behind IS development and point out common problems for the preclinical and clinical documentation of IS. The present chapter comprises a short background of topical steroids, the structure-activity work behind budesonide, a discussion of the resulting clinical profile, mechanisms behind its airways selectivity, a list of still open pharmacological

issues concerning IS therapy and, finally, comments on the animal models used during the selection and documentation of BUD.

The success of the early phases of the BUD project (on which I concentrate here) seems in retrospect to have depended on the dedication and competence of a small internal research organization, which then was cross-fertilized with complementary knowledge and resources by a company deal happening at the best time for the project. The initial qualifications of the internal organization were some knowledge about steroid synthesis (not discussed here) and animal testing, clear aims for a topical steroid project, and importantly a desire to test new medicinal chemistry, pharmacological and medical approaches forward that goal. The only mode to drive the project at that time was via empericism, based on chemistry and determination of the resulting in vivo profile. How completely that strategy contradicts the sacred drug development strategy of today! The present strategy starts from new, validated molecular mechanisms, proceeds with establishment of high through screening methods for those mechanisms, followed by the testing of huge chemical libraries, and then ideally results in a preproject with structural optimization based on in vivo animal work. For the BUD project it can be concluded in retrospect that the major pharmacological preferences of BUD would never have been detected by the present strategy, as its rapid hepatic inactivation and its effect-prolonging esterification were unraveled first nearly one and more than two decades, respectively, after the project start. However, the impact of these mechanisms directed us during the in vivo selection procedures so that the functional preferences of BUD appeared well before the kinetic background could be clarified!

## II. Skin Steroids: The Forerunners of IS

Local/topical drug therapy of a delimited target area offers two theoretical advantages over systemic treatment. By such administration a much higher local drug concentration can be achieved, better exploiting upper parts of the dose-response curve of efficacy. Second, local administration reduces the total dose, diminishing the risk of adverse systemic reactions when this lower dose is distributed into the general circulation. Together these advantages increase the therapeutic ratio of a drug. However, such therapy may also shorten rather than extend the duration of local efficacy (1), as the higher local concentration enhances the diffusion gradient away from the site of action, and plasma does not reconstitute the local site with new drug over prolonged periods as is it does in systemic therapy. Topical skin therapy is a special case of local treatment, since the stratum corneum barrier strongly impairs and retards topical uptake of drugs. In this therapy a very small percentage of the applied steroid reaches the epidermal/dermal drug targets (2), and this delivery is spread over a number of hours from the reservoir formed by

drug dissolution into the hornified layer. When the absorbed steroid leaves its targets via the dermal vessels and enters the systemic circulation, there is a strong dilution diminishing the plasma steroid level below the threshold level for systemic efficacy. Therefore, during normal topical therapy steroids attain selectivity for their epidermal and dermal skin targets due to this combination of low and retarded uptake followed by strong dilution in blood.

IS originate directly or indirectly from topical skin steroids; their development has been lengthy and laborious (3). Due to the above kinetic properties, a topically selective therapy for skin was available in the early 1950s, while it took 20 years more until steroids attaining a topical selectivity for airways tissue were reported. Because airways/lung mucosa lacks a barrier like the stratum corneum, lipophilic steroids are nearly fully absorbed into airway tissue and proceed then rapidly into systemic circulation. This impairs selectivity in two ways: by shortening the local anti-inflammatory efficacy and by reinforcing the unwanted systemic actions. While the steroid reservoir in the horny layer extends dermal efficacy markedly, it is not possible to achieve a similar retardation at the airway surface, as the mucociliary clearance sweeps away undissolved drug particles from central airways within a few hours. Furthermore, a large part of the inhaled dose is orally deposited and swallowed and will, if anything, just add to unwanted systemic activity.

Thus, IS need special properties to overcome these obstacles. First, they need some form of binding to airways/lung tissue to extend the local efficacy and with that reduce the number of daily inhalations. This is especially true considering that the compliance of prophylactic asthma therapy is poor (see Chap. 20). Second, IS must rapidly undergo an effective first-pass inactivation in the systemic compartment.

## III. Early Failures and Successes of IS Therapy

Cortisone, hydrocortisone, and prednisolone were tested early by the inhalation route, but with a poor clinical outcome (for overview see Ref. 4) (Fig. 1). With today's knowledge we understand that topical therapy with cortisone and prednisone lacks efficacy, as they are prodrugs that gain affinity for the glucocorticoid receptor only after the liver-mediated reduction to hydrocortisone and prednisolone, respectively. When inhaled at daily doses of 7.5–18 mg, hydrocortisone and prednisolone achieved antiasthmatic efficacy only in some patients, but could still reduce urinary secretion of 17-hydroxysteroids (4). Their poor topical efficacy depends on a combination of the low receptor affinity (5) and the action of the enzyme 11β-hydroxy-steroid dehydrogenase (11β-HSD), which in lung favors their oxidation to cortisone and prednisone, respectively (6). The importance of that enzyme was confirmed by Schleimer and Kato, who in in vitro studies found that

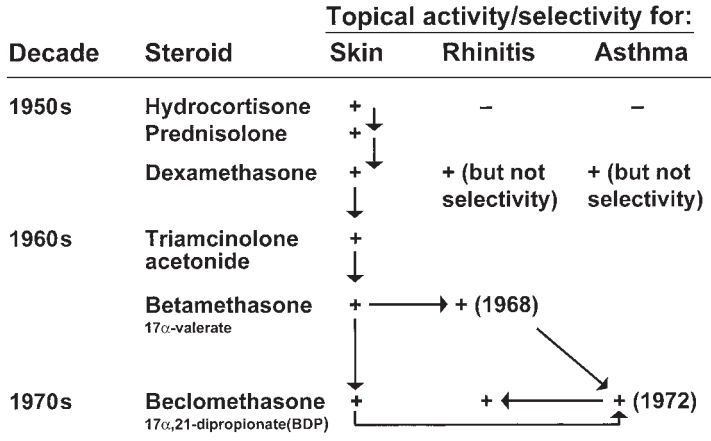



**Figure 1** Scheme for the early development of topical glucocorticosteroid therapy.

the conversion of hydrocortisone to cortisone in human lung and tracheal tissue was effectively blocked by 11β-HSD inhibitors (7). The orally deposited part of inhaled hydrocortisone and prednisolone has more than 50% bioavailability (5), mediating systemic activity.

Dexamethasone was the first steroid to show a clear antiasthmatic and antirhinitic efficacy by inhalation (4). It was clinically introduced in the 1960s in the United States as aerosol formulations of the water-soluble dexamethasone 21-phosphate ester. However, controlled clinical trials revealed soon that inhaled dexamethasone has a very poor topical selectivity, as a similar efficacy was achieved by the oral as by the inhaled route (8–10). Its low topical selectivity depends on a poor uptake and binding to airway tissue (11), probably due to its rather low lipophilicity (12), while on the other hand the orally deposited fraction has a bioavailability of 65% or more (5). During the 1960s new skin glucocorticosteroids were gradually developed, gaining topical efficacy also on more resistant dermatoses like psoriasis and stubborn eczema. The chemical approach of that development was the introduction of lipophilic substituents at the 17α- or 16α,17α-positions of the steroidal D-ring (Fig. 2), with the idea that this enhanced their intrinsic activity as well as their topical uptake (2). At the end of that decade the topical efficacy of a couple of these lipophilic steroids was tested on respiratory disorders. The first positive results were published in 1968 by Czech studies with betamethasone 17α-valerate (Fig. 2) on rhinitis, reporting both topical efficacy and selectivity (13,14), but this finding was not commercially developed. The asthma breakthrough came from work within the Allen & Hanbury Company with the skin steroid beclomethasone-17α,21-dipropionate (BDP) (Fig. 2) (for overview, see Ref. 4). The novel medical outcome was that steroidal antiasthmatic

efficacy was attained without clear concomitant indices of systemic steroidal activity. The pioneering clinical trial with inhaled BDP was performed by Morrow-Brown using asthmatic subjects with eosinophilic sputa. The trials started in June 1970, and the first full publication appeared in April 1972 (15), soon followed by confirmatory reports from other groups (3). The treatment regimen was standardized to four daily inhalations of 100 μg BDP or betamethasone 17α-valerate. Only patients with moderate to severe asthma were treated with steroids, as the symptoms of mild asthmatics were not at that time considered to be related to ongoing airway inflammation.

Very little effort had earlier been devoted to clarify the metabolic routes and rates of elimination of skin steroids (including BDP) due to the lack of sensitive analytical techniques and the perception that the very low steroid uptake made a clarification of their systemic kinetics less important. However, the positive clinical findings with inhaled BDP called for metabolic evaluation, and the first mechanistic explanation of its airway selectivity came within a couple of years when kineticists at the Allen & Hanbury Company reported an unexpected, high hepatic biotransformation rate of BDP into beclomethasone and polar metabolites with reduced glucocorticoid (GC) activity (16). They showed that this metabolism reduced the systemic impact of the large orally deposited and swallowed fraction (17,18). With that finding, one key mechanism behind airways/lung selectivity of inhaled steroids was elucidated.

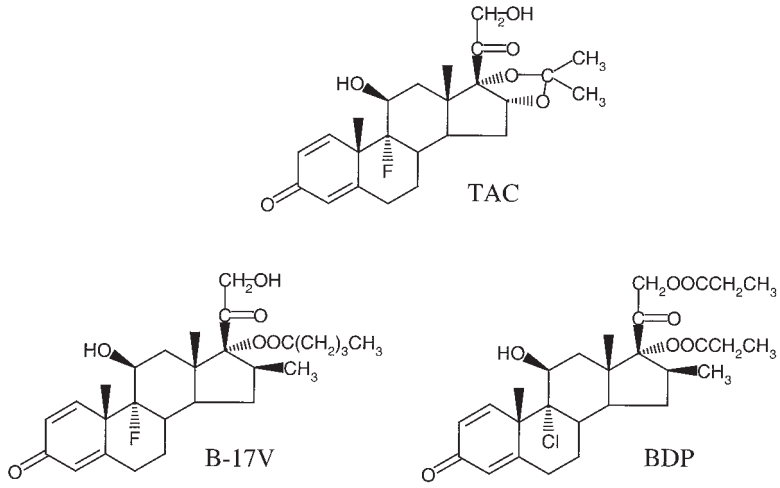



**Figure 2** Structure of the 16α,17α-acetal glucocorticosteroid triamcinolone acetonide (TAC) and the two 17α-ester glucocorticosteroids beclomethasone 17α,21-dipropionate (BDP) and betamethasone 17α-valerate (B-17V).

## IV. The Budesonide Project

### A. Medical and Biological Aims

Within the small Swedish pharmaceutical company Bofors Nobel-Pharma, a topical steroid project was started in the mid-1960s with the aim of enhancing the topical potency of the 16α,17α-acetal glucocorticosteroids. The pioneering steroid of that class was triamcinolone acetonide (TAC), the first steroid having efficacy on psoriasis and stubborn eczemas (Fig. 2). After initial chemical failures the project was reorientated in 1967, chemically by the medicinal chemist Arne Thalén and pharmacologically by myself. In addition to the original medical goal—enhanced topical efficacy for skin—we decided that the project should also aim for a generally improved topical selectivity (defined as an elevated ratio between the topical and systemic potencies compared to that of current topical steroids). Due to our lack of proper bioanalytical techniques, it was decided to perform the selectivity testing in vivo and include it as part of the primary screening. For that purpose a new variant of the cotton pellet test was developed, allowing estimation of the local anti-inflammatory as well as the resulting systemic GC actions. Two small cotton pellets (weighing 6 mg each) were implanted subcutaneously (s.c.) into adrenalectomized rats, and after one week a marked granuloma formation was formed within and around the pellets. At that stage the granuloma was composed of proliferating macrophages and fibroblasts, infiltrating and walling off the pellets from surrounding tissue. The extent of this cellular inflammation was determined as the increase of dry weight of the dissected granulomas. Before implantation the pellets were loaded with steroid microcrystals, formed by injecting a small volume of an acetone solution of the steroid. By dose-response studies in comparison with negative and positive controls, the local antigranuloma potency of a steroid was estimated as its $ED_{50}$, ranging from one to a few μg/pellet for potent steroids. The resulting systemic potency was judged as the extent of thymus involution and reduced body weight gain over the one-week study period. Based on the $ED_{50}$s for granuloma inhibition and thymus involution and the $ED_{25}$ for reduced body weight gain, the local/systemic potency ratio was calculated as a measure of the local selectivity of the compound. These results were complemented with determination of the topical potency in rodent ear edema models and in the human skin blanching test.

### B. Structure-Activity Work for Optimization of 16α,17α-Acetal Glucocorticosteroids

By structure-activity (SA) studies it was found that the nonsymmetrical 16α,17α-acetal of triamcinolone with acetaldehyde (Fig. 3, lower panel) gave a better local potency and selectivity than for the corresponding symmetrical acetal with acetone (TAC) (Fig. 2, upper panel). From this a stepwise optimization process was

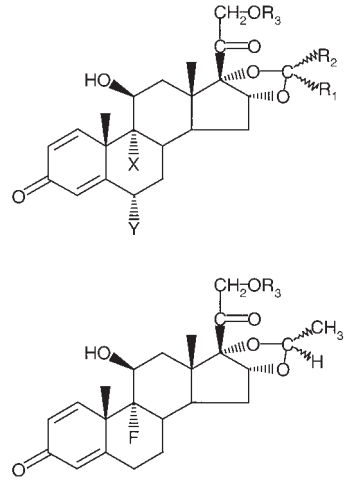



**Figure 3** Overview of structural variations of 16α,17α-acetal glucocorticosteroids tested in the budesonide project (upper panel). $R_1$ = H, or alkyl chain; $R_2$ = straight or branched alkyl chain; X and Y = H or F. The first lead on nonsymmetrical acetal is shown in the lower panel.

initiated in which the next aim was to determine the optimal size of the alkyl substituent for that improvement. Nonsymmetrical acetals substituted with straight or branched alkyl chains from 1 up to 9 carbons were synthesized on 16α-hydroxyprednisolone, triamcinolone, and fluocinolone (19). The uniform conclusion (Fig. 4) was that an *n*-propyl group (3 carbons) mediated a 10-fold higher local antigranuloma potency than was reached by TAAC, while the systemic actions were less potentiated so that the local/systemic activity ratio of the new acetals was markedly improved (19). The optimal glucocorticoid potency of *n*-propyl substitution was later confirmed in molecular biological models. In reporter gene constructs for GC-induced upregulation (via GRE-triggering) and for GC-induced downregulation (inhibition of the AP-1/TRE mediated pathway), the *n*-propyl substituted acetal (Fig. 5) had the highest potency, with much lower activity observed with shorter or longer acetals (12).

A practical drawback of nonsymmetrical acetals is that two diastereoisomers are formed (Fig. 4). The R-epimers had a somewhat higher local anti-inflammatory potency than the S-epimers, but in the rodent models that was also the case for their systemic activity (19–21). Accordingly, a difficult and expensive stereoselective synthesis or isomer separation was not justified. The next optimization step was to see how esterification of the 21-hydroxy group affected the improved profile of the nonsymmetrical acetals. A number of ester types were

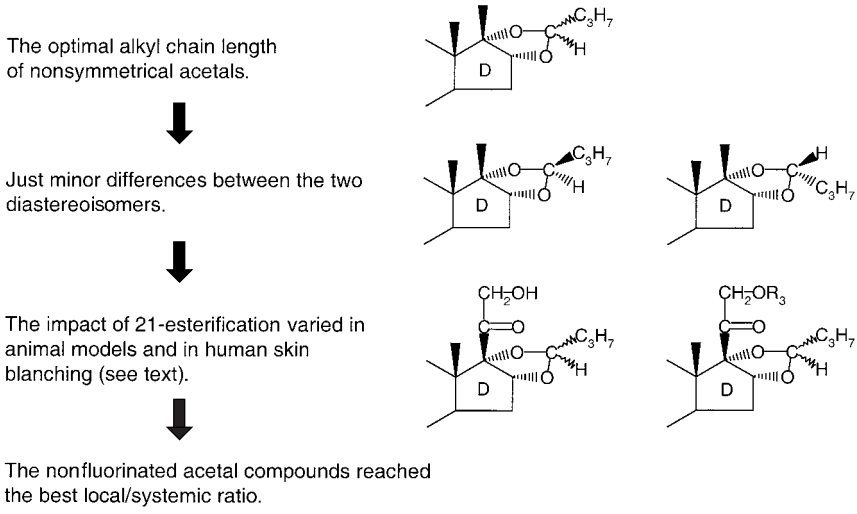



**Figure 4** Steps in the optimization of nonsymmetrical 16α,17α-acetals.

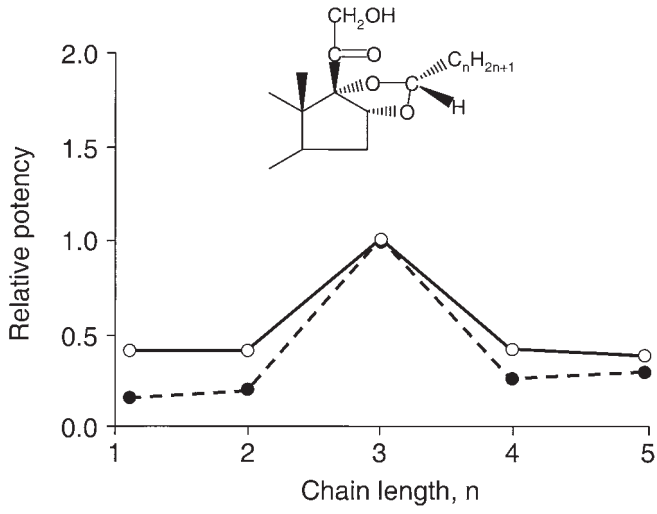
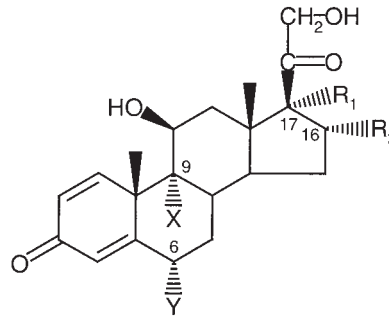
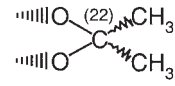
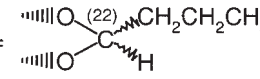



**Figure 5** Influence of acetal chain length on glucocorticosteroid potency in a homologous series of nonsymmetrical 16α,17α-acetals. The potency was determined on reporter genes transfected into a rat fibroblast line. Filled circles, GRE-mediated upregulation of CAT (chloramphenicol acyl transferase). Open circles, inhibition of AP-1–controlled β-galactosidase. (From Ref. 12.)

**Table 1** Topical/Systemic Ratios in Rat, Calculated Between the Potencies[a] to Inhibit Ear Edema (Topical Effect) and to Involute the Thymus (Systemic Effect)




| Fluoro substitution | | Acetal substituent in 16α,17α- positions | |
|---|---|---|---|
| | | Type A | Type B |
| | | $R_1R_2$ =  | $R_1R_2$ =  |
| X | Y | | |
| H | H | 0.11 | 1 (= budesonide) |
| H | F | 0.05 | — |
| F | H | 0.05 | 0.88 |
| F | F | 0.08 | 0.46 |

[a]All potencies are calculated in relation to BUD. The table shows the importance of the 16α,17α-acetal type and the extent of B-ring fluorination. The type A acetals are 6α-F = flunisolide; 9α-F = TAC; 6α-F,9α-F = fluocinolone acetonide.
*Source*: Adapted from Ref. 20.

synthesized on the new 16α,17α-acetal compounds. In the rat models used, esterification enhanced local as well as systemic potencies, while no potentiation of the topical activity was seen in the human skin blanching tests (19,21). These equivocal results, together with potential ester stability problems in formulations, led us to concentrate on the 21-hydroxy nonesterified acetals. The final structural evaluation of the new acetals was on the impact of fluorination in the steroid skeleton, preferably in the B-ring. In our rat models, fluoro substituents at the 9α- or the 6α,9α-positions enhanced the systemic more than the local activity (19,20), so that the nonfluorinated compounds achieved the best local/systemic activity ratio (Table 1). Based on this systematic structure-activity work performed over the period 1969–1972 and comprising a large number of structures, a patent application for novel, topical 16α,17α-acetals was filed in Sweden in May 1972, followed one year later by international applications (22).

### C. The Budesonide Inhalation Project

The budesonide project was strongly affected by two external events over 1972 and 1973. As described above, the first asthma publication on the successful use of inhaled BDP appeared in the spring of 1972 (we never noted the preceding Czech betametason 17α-valerate work on rhinitis), claiming that inhaled BDP differentiated between local and systemic actions. We thought that this "skin" steroid had neither been designed for, nor been specifically selected for, such differentiation, whereas our clear objective over the preceding years had been to synthesize and screen new steroids for an improved relation between their local and systemic actions. By this directed work we reasoned that the new nonsymmetrical acetals might reach an even better differentiation. Because systemic adverse actions are not a major problem in topical skin therapy, it was thought that the medical preferences of the new acetals would best be exploited in topical therapy of mucous membrances, e.g., on airway or bowel mucosa. The second external event affecting the project happened in spring 1973, when it was announced that the topical steroid project was going to be taken over scientifically as well as commercially by Astra, due to a strategic deal between the Astra and Bofors Groups. The key part of the Bofors preclinical steroid group agreed to go along, and these eight people moved in 1974 with the project to Lund, and merged there with the respiratory, pharmaceutical, and medical expertise of Astra Draco.

During autumn 1974, BUD (Fig. 6) was selected as the single drug candidate from the preclinical project. Its high topical potency had at that time been confirmed in further models (the rat and mouse ear edema models, and the human skin blanching test), and its profile had been compared also to the 17α-esters BDP (Fig. 2) and betamethasone 17α-valerate (Fig. 2). BUD had double the topical potency of these esters (23,24, data in files of Astra Draco). When selectivity was calculated as the topical-systemic potency ratio gained in the rodent models, BUD achieved a 10-fold higher ratio than for the esters (23, data in files of Astra Draco), as well as for the acetonides (Table 1).

The IND-work started in 1975 with documentary work on the kinetic, meta-

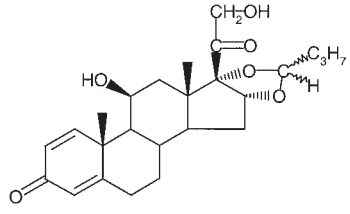



**Figure 6** Structure of the nonsymmetrical 16α,17α-acetal glucocorticosteroid budesonide (BUD), selected for topical treatment of inflammatory and allergic disorders.

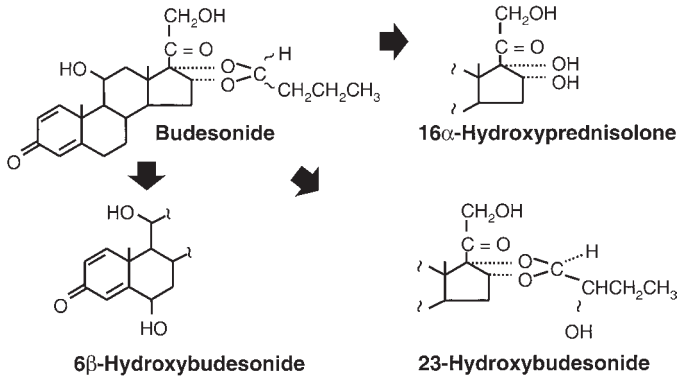



**Figure 7** Main metabolic pathways of BUD, mediated by hepatic CYP3A.

bolic, toxicological, and pharmaceutical properties of BUD. In vitro work on human tissues (liver, blood, and lung) was included as soon as we had tritiated BUD and bioanalytical methods for that work. Acute Phase I studies on the systemic tolerance were performed in collaboration with Professor Karl-Erik Andersson and Associate Professor Pavo Hedner at Lund University Hospital. These volunteer trials revealed a 3–4 times lower systemic potency of oral BUD compared to oral BDP, measured as reduction of morning plasma cortisol (24). When compared by inhalation (in CFC-based pMDI-formulations) BUD had half the cortisol-depressing potency of BDP (24).

The improved systemic tolerance of BUD was shown to depend on a rapid and extensive hepatic metabolism (25,26). The BUD biotransformation appeared to be rather stereospecific (Fig. 7). The R-diastereoisomer undergoes a splitting of the 16α,17α-acetal (27), and this is a unique route compared to TAC and flunisolide, which have metabolically stable acetonides. Following a CYP3A-catalyzed oxygenation of the 22-carbon of the BUD-R epimer, the formed intermediary hemiortho-ester is immediately hydrolyzed to 16α-OH-prednisolone and butyric acid (Fig. 8). The BUD-S diastereoisomer is hydroxylated in 6β-position (Fig. 7), which is a well-known metabolic pathway for steroids, and it can also undergo hydroxylation of its acetal to form 23-OH-BUD (Fig. 7). These metabolites have a 50–100 times lower receptor affinity than does BUD (28), and their potency is further reduced by glucuronidation and sulfation. These transformations explain the finding that BUD is several times more rapidly metabolized than BMP (the rate-limiting step in BDP inactivation) and TAC (25,26). The bioavailability of BUD was determined in volunteers (25), and its low oral bioavailability (approximately 10%) was at that time a novel finding for IS (see Chaps. 9 and 10). With the analytical techniques used in these early studies, there were no signs of metabolic transformations of BUD within lung tissue or in blood (26).

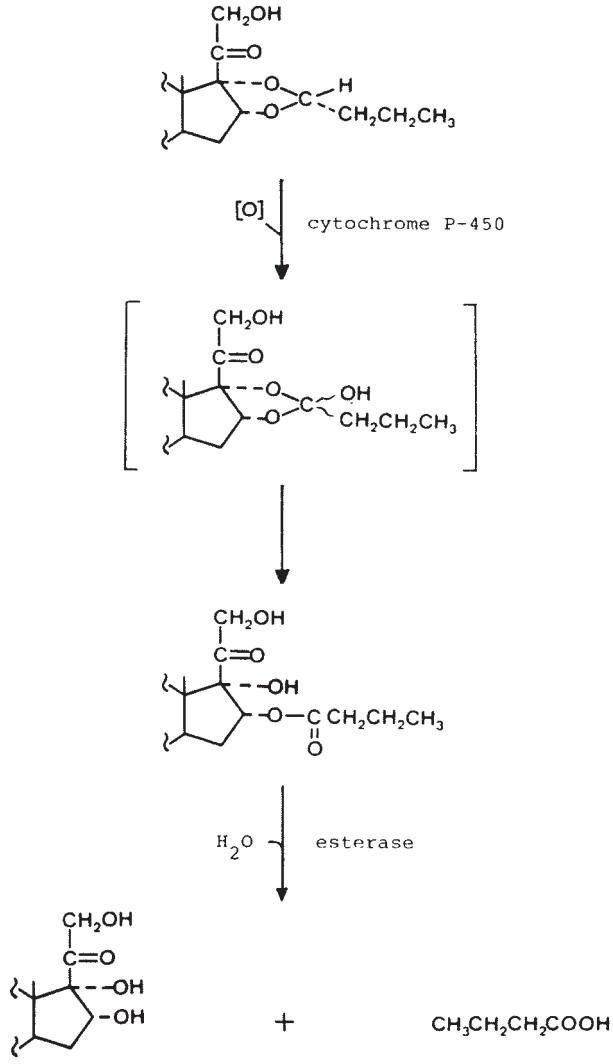



**Figure 8** Biochemical pathway for the 16α,17α-acetal splitting of (22R)-budesonide. (Adapted from Ref. 27.)

Thus, the human pharmacological testing confirmed a profile preference for BUD over BDP, as BUD had twice the topical potency in the skin blanching test, while its cortisol-depressing potency after inhalation was just half that of inhaled BDP (24). Based on these data, asthma trials with BUD were started around 1977, using a conventional CFC pMDI aerosol.

### D. Some Highlights from the Clinical Documentation of Budesonide/Pulmicort

The early clinical trials comprised dose- and time-response studies on the antiasthmatic efficacy of Pulmicort. The trials were run in collaboration with, among others, Roger Ellul-Micallef (acute effects) (29), Ronald Dahl (subacute trials on clinical asthma) (30), and studies with allergic provocation (31) and John Togood (subacute tests with various efficacy and adverse effects readouts, with or without addition of spacer) (32–34). A dose-response relationship was found in the range of 100–1600 μg/day (29,30), and the efficacy was exerted locally as oral treatment giving a similar plasma level of BUD mediated just marginal efficacy (29,34). Depressed 8 A.M. cortisol was seen first at or above 1600 μg/day. A surprisingly rapid onset (improved PEF within a few hours) was detected in the acute testing of chronic mild asthmatics (29). Other novel findings were the full blunting of the immediate allergic reaction, when Pulmicort was inhaled for one month (31), and that Pulmicort reached the same antiasthmatic efficacy as high dose oral steroids (33). In the subacute studies the inhalation frequency was found to be important for unstable but not for stable asthma (32), creating the possibility to reduce the dosing frequency in proper prophylactic therapy. Based on these and other experiences, Pulmicort got its first European marketing license in the early 1980s.

Efficacy comparisons among IS require special designs for showing meaningful differences, and the designs and results of such drug comparisons are overviewed elsewhere in this volume (see Chaps. 14 and 16).

During the 1980s and 1990s, Pulmicort became the major tool for documenting extended achievements of IS therapy, and some of these findings are listed below:

The good safety profile of Pulmicort was exploited by giving higher doses, resulting in better disease control in patients with severe asthma and reducing the need for oral steroids (35,36). In addition to improved respiratory function and QoL, such treatment was shown to cut the total health care costs (37–39), especially for acute visits and hospitalization.

The safety profile allowed a more variable dosing (level and frequency) depending on current asthma severity, improving the patient's involvement and compliance (40).

Another use of the good, long-term safety data of Pulmicort was to introduce steroid inhalation therapy earlier in mild asthma. This led to a pioneering study where patients with newly detected asthma were treated for two years either with Pulmicort or Bricanyl (41). The marked improvement in patients using Pulmicort during the first months could not be reproduced by a later switch from bronchodilating to steroid therapy (42), showing that anti-inflammatory treatment has the best outcome when it is

introduced before airways remodelling has started. The results of these (41,42) and other BUD studies (43–45) have had a great impact on current guidelines for early asthma treatment.

Pulmicort has been the pioneer of efficacious once-daily inhalation therapy of mild asthma (46–48), with the potential to enhance the poor compliance of prophylactic asthma therapy (see. Chap. 20). The reversible esterification of BUD within airways/lung tissue (see below) may contribute to this property.

Improved inhalation devices have markedly contributed to these advances. The first steps were taken 20 years ago with extensible plastic tubes and spacers attached to the CFC-arerosols, which reduced the oral and improved the airways deposition (32). A real advance was reached in the late 1980s, when Pulmicort was introduced in the first multidose dry powder inhaler, the Turbuhaler, further improving Pulmicort's topical efficacy and selectivity by enhancing the airways/lung deposition (49) and by the ease of use of this device.

Due to its moderate water solubility, BUD is the first IS that can be effectively delivered by nebulization to severely ill adults or infants (50,51). This is not in the same way possible with less water soluble IS (e.g., the solubility of BDP and FP is 100 times lower than that of BUD).

Pulmicort today has a very extensive safety record, based on its estimated use over 25 million patient years. The rate of recorded severe adverse events is approximately one case per nearly 100,000 patient years (data on file at AstraZeneca), and the few cases recorded comprise primarily occasional bronchoconstriction and skin allergic reactions rather than systemic steroid actions. The safety data are excellent also in long-term trials (52). In a unique study on the final height of asthmatic children treated long-term (53) (see Chap. 15), Pedersen et al. showed minimal effects of inhaled Pulmicort. In another unique study on use during pregnancy, Pulmicort did not affect the malformation rate in a large cohort of Swedish infants (54).

## V. The Difficulty of Forecasting a Market Potential

Astra's goal in the 1970s for the takeover of the BUD-project was to complement its sprouting respiratory business, based on terbutaline and a theophylline formulation, with a steroid for the more severe asthmatics. The motivation was more to get a full line for asthma therapy, than for economic revenue of the steroid project itself. Bronchodilators were at that time considered to be the cornerstone of acute as well as prophylactic asthma treatment, and based on this view and early figures from the BDP and betamethasone 17α-valerate marketing, the worldwide turn-

over of a BUD product for asthma was in the mid-1970s roughly estimated to be $15 million per year. Due to the elucidation in the 1980s of airway inflammation as a central pathophysiological factor for all forms of asthma, the novel effects of IS on asthmatic hyperresponsiveness and the emerging excellent safety records, IS became the cornerstone of prophylactic asthma therapy. This therapeutic shift enhanced the market potential of IS drastically, so that the worldwide turnover of Pulmicort today is nearly 50 times higher than the first market estimation. However, it should be pointed out that the clinical success of Pulmicort and its devices has strongly contributed to that therapy shift.

## VI. Budesonide as a Tool for Elucidating Mechanisms Behind Airways/Lung Selectivity of IS: Personal Views

Current IS are either steroid 16α,17α-acetals (BUD, TAC, flunisolide) or derivatives of steroid 17α-esters (BDP,FP) (Fig. 2), and these extra substituents make IS more lipophilic than the parent steroids (12,55,56). Their enhanced lipophilicity affects several pharmacological properties (Fig. 9), and it seems to be the combination of these properties that mediates the topical efficacy and selectivity of IS. While the high receptor affinity and the extended binding to airways tissue are crucial for efficacy, their selectivity depends mainly on the airways/lung binding combined with the hepatic first-pass inactivation of systemically absorbed substance. The optimum extent of lipophilicity for each of these properties is not yet known. (For broader pharmacological overviews of IS activity and selectivity, see Chapters 9, 10, and 12.)

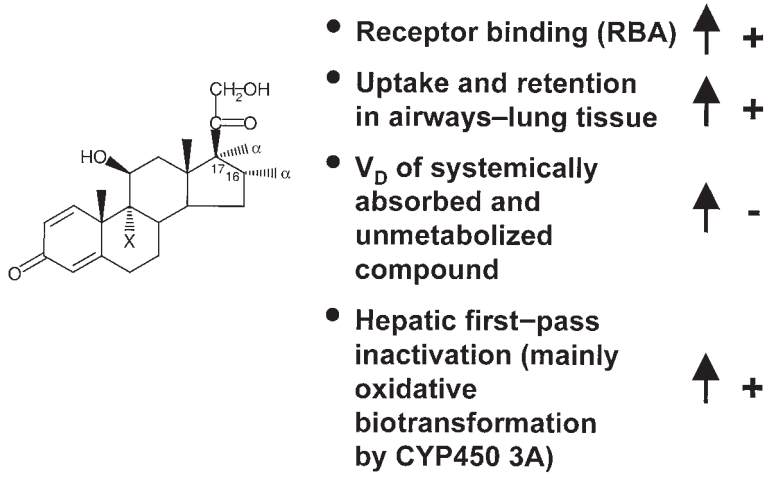



**Figure 9** Properties conferred by introduction of lipophilic substituents in the 17α or 16α,17α positions. +, Positive impact on the IS profile; −, negative impact.

### A. Basis of High Prolonged Efficacy at Airways/Lung Target

Up to a certain level, lipophilicity correlates positively with an increased affinity for the GC-receptor (GC-R) (12,28). The high affinity of IS compensates for the dilution occurring over the vast airways/lung surface and mediates steroid efficacy to the mucosal and submucosal targets. However, due to the uniformity of the GC-R, a raised receptor affinity also potentiates the systemic activity, which means that selectivity is not improved by high affinity in the same way as for efficacy (see Chap. 12).

It seems reasonable that glucocorticoid receptors within the airways/lung compartment can be triggered by just a minute part of the inhaled dose, due to the high-affinity–low-capacity character of that binding. Based on the $K_D$ of BUD (0.5 nmol/L) (28) and the volume of human airways/lung, just 1 μg would theoretically be sufficient for initially liganding these receptors. Animal and human kinetic data suggest that topical application of relevant doses gives initial BUD concentrations within airways/lung tissue 10- to 100-fold higher than for its $K_D$ (11,57–59). This indicates that the main obstacle for achieving anti-inflammatory activity at airways/lung level is not the initial triggering, but rather the difficulty to maintain the trigger long enough, since most of the deposited steroid is rapidly absorbed into the systemic circulation. Figure 10 shows the turnover cycle of triggered GC-R. Liganded receptors have been shown in vivo to have a half-life of several hours (see Chap. 5). After the ligand dissociation, the receptor has to be phosphorylated and associated with heat-shock proteins (HSP) in order to be able to bind new GC molecules. Thus, some hours after the initial triggering there is a need for GC to remain in local tissue in order to maintain the triggering of recycled and de novo synthesized receptors and achieve anti-inflammatory efficacy.

When the mucosal uptake and retention was studied in a tracheal perfusion model, IS were found to be both better absorbed and retained than was the case for

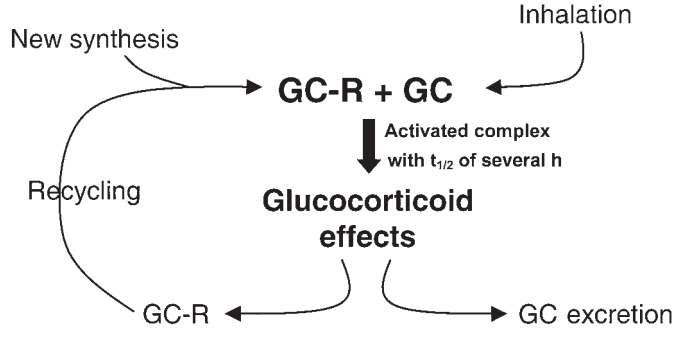



**Figure 10** Scheme of the turnover of triggered glucocorticoid receptor (GC-R).

**Table 2** Lipophilicity (log P), Receptor Binding Affinity (RBA), and Oral Bioavailability (F%) of IS

| Steroids | log P | RBA | F%[a] |
|---|---|---|---|
| *Systemic steroids* | | | |
| Hydrocortisone | 1.56 | 0.01 | 55 |
| Prednisolone | 1.65 | 0.06 | 80 |
| Dexamethasone | 1.95 | 0.13 | 65 |
| *Inhaled steroids (IS)* | | | |
| Beclomethasone dipropionate | 4.40 | 0.3 | 25 (?) |
| Beclomethasone monopropionate | 3.63 | 2.0 | |
| Triamcinolone acetonide | 2.53 | 0.5 | 23 |
| Flunisolide | 2.28 | 0.2 | 21 |
| Budesonide | 3.24 | 1 = Reference | 6–13 |
| Fluticasone propionate | 4.20 | 2.3 | <1 |
| Momethasone furoate | | 2.8 | <1 |

Log p and RBA values are data on file at AstraZeneca. The RBA determinations were performed in vitro in rat thymus cytosol.
[a]From human studies (5,59).

the less lipophilic systemic steroids (11). This enhanced and prolonged binding of IS to airways tissue is another reflection of their high lipophilicity (Fig. 9) and depends probably on enrichment of IS in lipophilic cellular compartments. Table 2 lists the lipophilicity and receptor affinitity of current IS. While they all differ from systemic steroids, there is a variation among IS contributing to their individual dose requirements, differing severalfold from, e.g., flunisolide to FP. None of these IS is inactivated within the target tissue, which means that the retained steroid pool has the propensity to extend receptor triggering as long as the tissue concentration of free IS is above a certain threshold. The neccessity of having a pool of tissue-bound, stable steroid is supported by the poor clinical outcome of soft steroids sensitive to tissue or plasma esterases (see Chap. 21). Even if such soft drugs would give the same initial receptor trigger (due to equipotent receptor affinity), they seem to be broken down within the target tissue over time, limiting their possibility to extend local receptor triggering.

During the 1990s a special kind of tissue binding was discovered for budesonide, which seems to contribute to its extended topical efficacy and high selectivity. During investigations in the tracheal superfusion model, it was revealed that budesonide stayed longer in large airways tissue than was anticipated from its own lipophilicity (11). Kinetic investigations clarified that BUD within cells can undergo a reversible esterification with fatty acids, forming very lipophilic BUD-esters lacking receptor affinity, but which are gradually hydrolyzed back to active

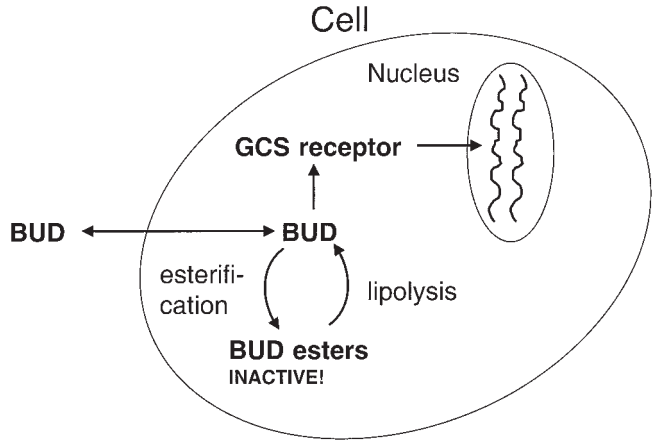



**Figure 11** Scheme of the reversible, intracellular esterification of BUD.

BUD (Fig. 11) (60). These BUD-esters have been documented in rat (11) and human airways/lung (58), as well as in the human nasal mucosa (61). These findings clarify early, poorly understood BUD results from an isolated perfused lung model, where a fraction of BUD bound so strongly to airways/lung tissue that tissue digestion was necessary to release that fraction (62). During that digestion with the multienzyme pronase, the BUD-esters were probably hydrolyzed and appeared as intact BUD, leading to the conclusion that BUD was not metabolized by airways/lung tissue.

BUD seems to be unique in undergoing this high esterification rate, as only a small ester formation was detected for TAC and none at all for BDP and FP. The reversible BUD ester formation was correlated to a long functional duration, both after topical application to the rat trachea (63) and after pulse exposure of cells in culture (64). In the latter model it could be clearly shown that when ester formation was blocked by an esterification inhibitor, BUD had a shorter duration of action (65). This, together with release experiments, support the concept that the BUD-ester pool is bioavailable both within the cell as well as for neighboring cells (11,64). This mechanism may explain why BUD—not having the top lipophilicity and receptor affinity among IS (Table 2)—can have the superior clinical documentation for once-daily efficacy in asthma.

### B. Basis of Low Systemic Activity

IS are bioavailable via the airways/lung and oral routes (Fig. 12). However, due to its effective hepatic first-pass inactivation, there is just a minute oral contribution when BUD is inhaled from the Turbuhaler followed by a mouth rinsing. CYP450

3A (66) is a ubiquitous hepatic enzyme, and this together with the low inhaled dose explains why BUD very seldom provokes drug interactions.

The airways/lung deposited fraction of the BUD dose is taken up into the systemic circulation via the bronchial and pulmonary vessels and has nearly full bioavailability (25; see also Chap. 10). Of the cardiac output, one quarter has the first pass to liver (leading to steroid inactivation), while the majority of airways/lung absorbed steroid is widely distributed before finally undergoing hepatic metabolism (Fig. 12). During this systemic disposition BUD has a moderate volume of distribution ($V_D$ = 183 L) and terminal plasma half-life ($t_{1/2}$ = 2.8 h), less than for the more lipophilic FP (for comparative figures, see Chap. 10). While the high lipophilicity favors local efficacy as discussed above, the same property is a drawback (Fig. 9) when the airways/lung absorbed fraction is distributed in the body, as this elevates the body burden at steady state. Thus, the ideal profile of an IS is to have a high lipophilicity in airways/lung compartment but low during systemic distribution, as that speeds up the subsequent disposition to liver for inactivation (67).

While the BUD-ester formation favors its local efficacy, the same mechanism would be a drawback if this reaction occurs to the same extent for systemically disposed BUD. However, this does not seem to be the case. First, the human $t_{1/2}$ of BUD (2.8 h) is not compatible with a profound peripheral disposition of very lipophilic BUD-esters. Second, analytical results (68) as well as kinetic modeling (see Chap. 13) show that after BUD inhalation the highest ester concentrations are found within airways/lung tissue, while the ester concentrations of

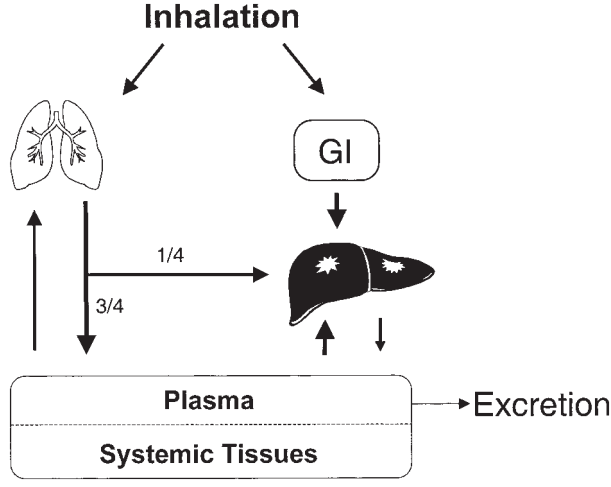



**Figure 12** Disposition of IS. GI = gastrointestinal tract. (Adapted from Ref. 12.)

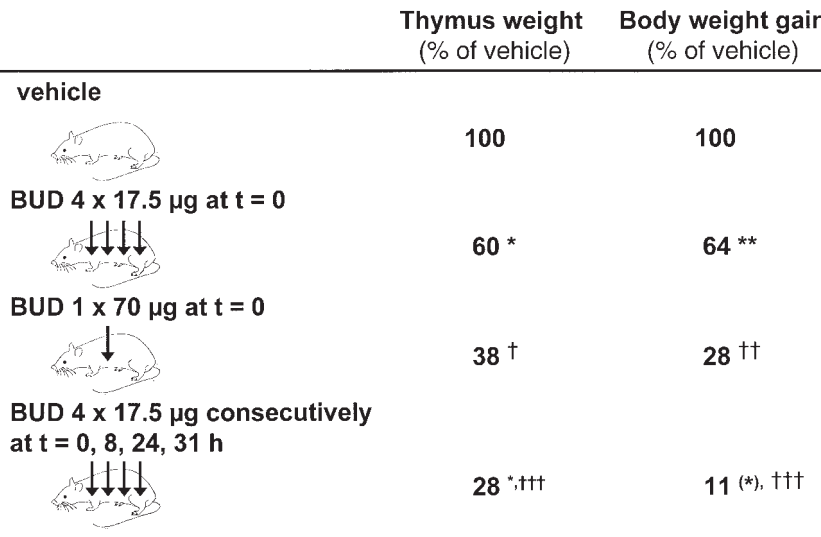



**Figure 13** Systemic efficacy of BUD as influenced by its uptake rate into systemic circulation. Rats (6 per group) were subcutaneously injected with suspensions of 70 µg BUD, leading to three different uptake rates. All groups had their own vehicle control, which did not differ among the groups. The systemic effects of all BUD groups differed significantly from the vehicle controls (significance levels not shown). The significances given are between the BUD groups. $*p = 0.1$; $*p < 0.05$; $**p < 0.01$ in comparison to BUD 1 × 70 µg; $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$ in comparison to BUD 4 × 17.5 µg at t = 0.

plasma and peripheral striated muscle are quite low. Interestingly, BUD esters are formed in airways/lung tissue after intravenous injection, and they stay longer in large airway tissue than elsewhere (see Chap. 13). This suggests that the high ester concentration recorded in airway tissue after inhalation depends both on the high local BUD concentration and on a high capacity of that tissue to esterify BUD. Thus, in this model BUD can to some extent exist in a more lipophilic form in airways/lung tissue than in the systemic compartment.

There are varying opinions on the issue of whether a slow or a rapid systemic uptake is most advantageous for reducing the systemic activity of IS. Figure 13 shows a simple test of that issue, with thymus involution and reduced body weight gain as steroid-sensitive systemic measures. The study was performed in a nonlung model, but the outcome is principally valid also for systemic uptake from airways/lung. A single subcutaneous injection of a suspension with 70 µg BUD/rat gave strong reductions of these measures, with maximal reductions after 3 days (thymus weight) and 2 days (body weight gain), respectively. This group

was compared to other groups where the rate of systemic uptake was changed. One group received simultaneous s.c. injections at four separate sites with 17.5 μg/each, as this speeds up the total uptake rate into the systemic circulation. Another group was s.c. injected with 17.5 μg/site, but with temporal delays for each of three remaining injections (all four given within 31 h). The clear-cut result indicates that the most rapid systemic uptake gave the least, while the slowest uptake mediated the worst systemic activity (Fig. 13). This result coincides with the common view for adverse potential of systemic steroids (69). Thus, while a prolonged deposition within airways/lung compartment potentiates the desired local activity of IS, this may at the same time worsen the systemic activity. Instead it would be advantageous if the bulk of airways/lung deposited steroid fraction, not utilized in the local binding to receptors or tissue, could be rapidly taken up into systemic circulation. This conclusion is based upon the high-affinity, low-capacity character of IS binding to the peripheral GC receptors. During a protracted systemic circulation relatively more of the steroid can be used for a prolonged receptor binding, while during a high plasma peak the binding capacity of peripheral receptors is overloaded, delivering more steroid to rapid metabolism and excretion. For reducing systemic activity by a retarded uptake, the systemic absorption rate must be so low that the resulting plasma steroid level is below the threshold for triggering GC-activity. This is further discussed in Chapter 23.

## VII. Remaining Pharmacological Questions About IS Efficacy and Selectivity

As stated in the introduction, the development of current IS has been governed more by empericism than by detailed knowledge about GC kinetics and dynamics in airways/lung tissue. In fact, we still lack answers to some crucial questions about the pharmacological basis of IS therapy. One important aim of this volume is to discuss these issues. If we can find clear answers to the questions listed in Table 3, a continued improvement in IS or IS-like drugs would be more likely.

## VIII. Comments on the Animal Models Used in the Preclinical Selection and Documentation of BUD

The preference of the initially used cotton pellet test was that this model, within a rather close dose interval, determined both local and systemic potencies, so that a local/systemic potency could be estimated. With this model it was possible to detect and optimize the local preferences of the nonsymmetrical 16α,17α-acetals (22,70). However, the cotton pellet model was laborious with regard to surgical techniques and preparation of the steroid-loaded pellets. More rapid evaluation models were therefore added: the ear edema tests in rats and mice, and the human

**Table 3** Remaining Pharmacological Questions

| Question | Impact of answer |
|---|---|
| *The overall profile questions* | |
| Is there a need for a slight contributory systemic activity for gaining the full IS efficacy? | Determines the prospects of soft steroids |
| What are the levels of non–protein-bound steroid in plasma, target, and non-target tissues? | Central for understanding the steroid exchange between tissues, and by that the building of proper kinetic models |
| *For achieving high/prolonged topical efficacy* | |
| What is the minimum daily period of receptor triggering for achieving anti-inflammatory efficacy in airways/lung? | Will aid in design of steroid and its formulation |
| What is the impact of mucociliary clearance on the local uptake of steroid in airways of mild, moderate, and severe asthmatics, respectively? | The maximal time for local uptake of intraluminally deposited steroid at varying asthma severity |
| What are the mechanisms behind the prolonged tissue binding at airways/lung target? | Will allow exploitation of potential, novel binding mechanisms |
| Why do not current IS provoke connective tissue atrophy in airways/lung tissue, as they do in dermal tissue? | May reveal whether atrophic risks will appear by a further enhancement of local steroid potency and/or duration |
| What are the main target cells for the anti-asthmatic action? | Will open prospects for a steroid targeting to these cells |
| Does the liganded steroid need to be metabolically stable over its whole liganded period? | Will aid in design of soft steroids |
| *For low systemic activity* | |
| What are the critical determinants of steady-state kinetics? | Will reveal the best mode to minimize body burden |
| *Nonkinetic modes for differentiation* | |
| Can the antiasthmatic and adverse actions be differentiated at the nuclear level? | Will open prospects for a safer oral steroid therapy |
| *Antiasthmatic shortcomings of IS* | |
| What important antiflammatory/immunosuppressive actions do IS lack? | Will determine the best complementary therapy |

skin blanching test (for judging topical efficacy over an epithelial barrier) and tests for thymus involution, growth inhibition, and HPA-axis function (for judging the systemic potential after various routes of administration). BUD showed the same preferences in all these models, showing that its promising profile was not coupled to the special administration mode in the cotton pellet model. The com-

parisons with the 17α-ester derivatives BDP and betamethasone 17α-valerate had to be done in mice, as these esters behave aberrantly in the rat (being more antagonists than agonists in that species) (71,72). Both rats and mice were acceptable species for picking up the kinetic preferences of BUD over earlier steroids, but today we know that the rat liver biotransforms BUD more slowly than the human liver does (the oral bioavailability in the rat is double that in humans). Another difference between rat and human liver metabolism—also known in retrospect—regards the impact of steroid nucleus fluorination on the nonsymmetrical acetals. In the rat model (Table 1), fluorination impaired selectivity due to a potentiated systemic activity, while we now know that this fluorination speeds up the inactivation rate in human liver. Therefore, in later projects based on nonsymmetrical acetals, we have concentrated upon fluorinated structures (73; see also Chap. 23).

No appropriate rodent models of inflamed airways were available when BUD was selected. The allergic provocation models used in the 1970s were based on high-dose sensitization (giving mainly IgG antibodies), where glucocorticoids exert a poor antianaphylactic activity. However, under using an antihistamine it could be shown that BUD reduced the release of SRS-A (today known as leukotrienes) (74). In the Astra Draco laboratories, novel, low-dose sensitization methods (giving mainly IgE antibodies) were developed for guinea pigs and rats, where BUD protected strongly against antigen-induced anaphylaxis and mediator release without concomitant anti-allergic drugs (75–77) (Table 3). Furthermore, in the early 1980s we developed two GC-sensitive models mimicking the late allergic reaction by giving particulate immune triggers, Sepharose-coupled ovalbumin to sensitized guinea pigs and Sephadex particles to rats which have innate dextran hypersensitivity. Intratracheal instillation of BUD protected against the late bronchoconstriction in guinea pigs (78,79) and against pulmonary edema and eosinophil recruitment in the rats (80,81). However, in these models the lung protection was coupled to concomitant systemic activity (measured as plasma cortisol depression in guinea pigs and thymus involution in rats), indicating a poor topical selectivity of BUD. This poor selectivity for rodent lungs (verified also for other IS, including FP) may depend on the lower number of airway generations in their small lungs, resulting in more peripheral deposition and therefore rapid systemic uptake. Subsequent drug developmental work (see Chaps. 21 and 23) indicates that a topical selectivity for rodent lungs is achievable with new steroids, with a much slower absorption rate or with a soft drug design.

BUD achieves a topical selectivity when applied to restricted parts of rodent airways (Table 4). When the application and the anti-inflammatory measure were restricted to rat large airways, a topical BUD dose of 11 μg/kg inhibited local TNFα release for more than 12 hours with just minor effects on plasma corticosterone (63). In a rat model of allergic rhinitis, topical BUD of 0.3 μg/kg blunted the late-phase plasma leakage from the allergen-provoked nose, while systemic activity required more than a 100-fold higher topical dose (82). The topical selectivity of inhaled BUD in allergic dog and pig has been shown by another approach,

**Table 4** Topical Efficacy/Selectivity of BUD in Animal Airways: Lung Models In Vivo

| Model | Anti-inflammatory efficacy | Selectivity | Ref. |
|---|---|---|---|
| IAR and LAR in guinea pig | + | (+) | 75,76,78,79 |
| IAR in rat | + | − | 77 |
| Sephadex-induced pulmonary edema in rat | + | − | 80,81 |
| IAR and LAR in sheep | + | n.i. | 87 |
| AHR in dog | + | + | 84–86 |
| LAR in pig | + | + | 83 |
| LAR in rat nose | +[a] | + | 82 |
| LPS-induced "late TNFα production" in rat large airways | +[a] | + | 63 |

IAR = Immediate allergic reaction; LAR = late allergic reaction.
n.i. = Not investigated.
[a]Used also for testing the duration of topical efficacy.

namely comparison of the lung protection after inhalation and intravenous infusion at doses giving the same area under the plasma level curve of BUD. In the pig, inhaled BUD (airways/lung deposited dose 10 μg/kg), but not infused BUD, protected against the allergen-induced late airways resistance, the deterioration of blood gases and pH, and the rise in urinary late $LTE_4$ excretion (83). In dog trials (83,84) BUD inhalation (airways/lung deposited dose 13 μgkg), but not infusion, ameliorated the allergen-provoked airways hyperresponsiveness (85).

Thus, contrary to the situation during early BUD development, it is now possible to judge the topical airways/lung efficacy and selectivity in animal models. However, for being optimal tools for selecting new steroids, these models need still better characterization of dose-response relationships of the local and the systemic actions, more information on the airways/lung deposition pattern, and better analysis of similarities and differences in the rates and routes of glucocorticoid biotransformation, compared to humans.

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## Discussion

**Dr. Jeffery:** You mentioned that, initially, there was mild surprise at how efficacious steroids were in the treatment of all asthma. However, there are subgroups of asthmatics who do not respond well to inhaled corticosteroid treatment—they are resistant or dependent. Is there any pharmacological explanation for the apparent failure of steroids in these subgroups?

**Dr. Brattsand:** The poor responsiveness is seen among some moderate-severe asthmatics, and such steroid insensitivity is known also in other inflammatory diseases. In most cases the poor responsiveness is not coupled to reduced receptor number or affinity of the active α-form of the receptor (but possibly by an enhanced expression of the blocking β-form of the receptor—see discussion below). The insensitivity does not comprise all glucocorticoid actions, suggesting that only some downstream actions (e.g., cytokine inhibition) are affected. An important line of future work is to see how severely inflammation (e.g., proinflammatory kinases) affects the receptor number and function, and whether these problems occur only in individuals having reduced receptor numbers and activity before the start of disease.

**Dr. Derendorf:** In one of your slides you suggested that 25% of the amount of drug absorbed in the lung undergoes hepatic first-pass metabolism. Wouldn't you consider pulmonary absorption equivalent to an intravenous bolus where the total amount absorbed is available for systemic availability?

**Dr. Brattsand:** As CYP4503A has a very high capacity for budesonide inactivation, the fraction undergoing hepatic first pass will be the same for both these modes of administration. After inhalation a fraction will be retained in airways-lung compartment, but as this fraction is minor it does not seem to have a major impact on the systemic activity of budesonide.

**Dr. Okret:** Does budesonide induce Cyp3A in the liver? Can this affect side effects?

**Dr. Brattsand:** We have no data to suggest that budesonide induces CYP3A either in vitro or in vivo.

**Dr. Persson:** The role of inhaled GC in identifying inflammation as a cardinal feature of asthma, even the mildest forms of the disease, must not be underestimated. We have known for a century that severe asthma is characterized by advanced eosinophilic inflammation in the airway mucosa. However, it is primarily through the discovery (during the 1970s–1980s) of exceptional efficacy of GC in mild asthma, in adults and children, that we have obtained the "facts" about the basic inflammatory nature of bronchial asthma.

# Part Two

## USE OF INHALED STEROIDS

# 2

# How Inhaled Corticosteroids Changed Asthma Therapy

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## I. Introduction

In the late 1960s and early 1970s, inhaled corticosteroids became available for clinical evaluation. The experience in these trials eventually led to their use in the treatment of asthma. This represented a major advance and has subsequently revolutionized, in my mind, the treatment of asthma. Prior to the availability of inhaled corticosteroids, asthma therapy primarily involved oral and inhaled bronchodilators. Treatment was, in large part, directed toward rescue therapy. The regular administration of asthma medications, although common, was not the usual practice, and side effects with oral bronchodilators were common.

In patients with more severe or persistent asthma, oral corticosteroid use was necessary, but these were prescribed with reluctance. The side effects associated with these medications were known, and long-term use led to significant adverse effects including weight gain, hyperglycemia, osteoporosis, and cataracts, to name but a few. Consequently, the prescribing of systemic corticosteroids was done with reluctance and limited to patients either with severe disease or at the time of an acute asthma exacerbation.

With the advent of effective, potent, inhaled corticosteroids, the need for systemic corticosteroids diminished. Inhaled corticosteroids for the treatment of

**Table 1** The Legacy of Inhaled Corticosteroids

| |
|---|
| Replaced oral corticosteroids |
| Provided effective, safe treatment of asthma |
| Provided insight into mechanisms of asthma: |
| modulate inflammation |
| prevent "remodeling" |
| restore lung function |

asthma emerged as the primary treatment for patients with persistent disease (1).

The legacy of inhaled corticosteroids is a milestone for long-term treatment of asthma in the annals of asthma treatment (Table 1). These medications have largely replaced the need and use of oral corticosteroids for patients with persistent asthma. In addition, inhaled corticosteroids have provided an effective, safe treatment for asthma. Along with their place in the treatment of asthma, inhaled corticosteroids have also been important in clinical research efforts to understand mechanisms of asthma, particularly those related to control of inflammation. As will be discussed below, studies with inhaled corticosteroids have provided insight into mechanisms of asthma, particularly the relationship of disease severity and markers of airway inflammation. Moreover, there is emerging evidence that early intervention with inhaled corticosteroids can prevent the loss in lung function found in some patients with asthma (2, 3). Such studies raise the possibility that airway remodeling may be modulated by early intervention with inhaled corticosteroid treatment.

To appreciate the use of inhaled corticosteroids and their eminent role in the treatment of asthma, it is important to briefly review early studies into their use in the treatment of asthma. This chapter will focus on the early use of these inhaled corticosteroids in the treatment of asthma, the inference that they have been effective in the prevention of asthma morbidity, and examples that they have provided a targeted therapeutic agent whose use has given insight into mechanisms of asthma.

## II. Early Trials with Inhaled Corticosteroids

In the late 1970s, the results of a number of clinical trials were published which suggested that inhaled corticosteroids were effective in the treatment of asthma. In one of the early studies, patients with long-standing oral corticosteroid–dependent asthma were identified and admitted to hospital for the evaluation of the effectiveness of inhaled corticosteroid treatment in replacing systemic

steroids (4). All of these patients had long-standing asthma and had an average daily prednisone requirement of at least 20 mg/day or 40 mg every other day. The subjects entered the hospital and were observed over a 3-day baseline period. Their dose of oral prednisone was then reduced to 5 mg/day; the subjects were begun either on inhaled triamcinolone (300 μg q.i.d.) or placebo. Over the following 2 weeks, they were monitored closely for deterioration or improvement in lung function as well as symptoms. In patients who received the active form of therapy, there was a gradual improvement in lung function over a 4-week period (Fig. 1). In contrast, subjects who received the placebo had a gradual deterioration in lung function (mean value = 9.9%).

This study was one of the first to show the efficacy of inhaled corticosteroids in replacing systemic steroids. In those treated with active inhaled steroids, there was an average improvement in $FEV_1$ of 13.5% despite the discontinuation of large doses of prednisone. Furthermore, the "switch" from systemic corticosteroids to the inhaled form was safe. Of the 13 patients treated with inhaled corticosteroids, only 2 had deterioration in their lung function and had to terminate participation in the study. Although the number of patients involved was small, these results were encouraging and indicated that inhaled corticosteroids could be substituted for the systemic form.

Earlier studies had had limited success in treating asthma patients with inhaled corticosteroids. With the advent of inhaled beclomethasone, the results of such clinical trials began to show more promise. The British Thoracic and Tuberculosis Association (5) undertook a multicenter trial to determine whether beclomethasone diproprionate or inhaled betamethasone valerate was superior to placebo in patients who were taking daily doses of prednisone to control their

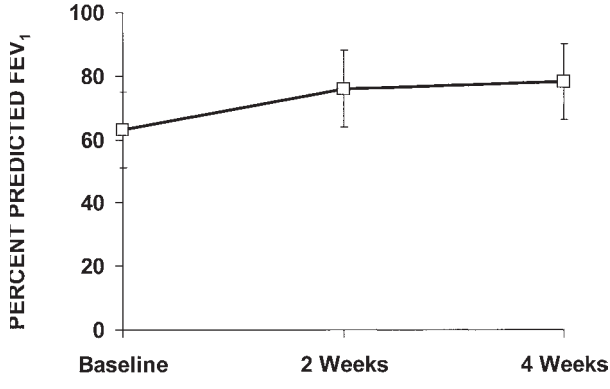


**Figure 1** Effect of triamcinolone treatment on $FEV_1$. (From Ref. 4.)

asthma. In this two-phase study, patients were identified who required approximately 10 mg/day of prednisone to control their asthma. They were then begun either on placebo or one of two doses of beclomethasone, 100 and 200 μg q.i.d., or betamethasone valerate, 200 μg q.i.d., and then monitored. During the first phase of this trial, a scheduled reduction in prednisone was followed. The enrolled patients were monitored carefully as prednisone was cautiously withdrawn. Five times as many patients were withdrawn from the study while receiving placebo when compared to the inhaled corticosteroid group ($p < 0.01$; Table 2).

Patients then entered phase 2 of the study, either when they came off prednisone or when the study physician had halted the reduction of prednisone. Over the next 24-week study period, a significantly greater number of patients were able to remain off prednisone if they were treated with inhaled corticosteroids versus placebo (Table 3).

A number of important observations were made in these two representative studies. First, it was possible to reduce or eliminate the need for prednisone by the use of inhaled corticosteroids. This was a major advance in the treatment of asthma, because it was possible to treat more severe asthma with a safe, inhaled dose of corticosteroids and avoid adverse effects associated with systemic corticosteroid use. Second, maintenance therapy with inhaled corticosteroids was effective in preventing asthma deterioration and the need to reinstitute systemic corticosteroid use. Third, in some patients improvement in lung function occurred despite the reduction in systemic corticosteroid use. This observation raised the possibility that the mode of action of inhaled corticosteroids may have some distinct therapeutic features.

Since these early studies, large numbers of clinical trials have shown the effectiveness of inhaled corticosteroids in asthma (6–10). Their use was associated with improvement in lung function, a reduction in symptoms, a replacement for the need of systemic corticosteroids, and even an improvement in one of the features of asthma, bronchial hyperresponsiveness. As was pointed out in an editorial in *The New England Journal of Medicine* in 1993 (11), "inhaled glucocorticoid therapy is effective in patients with asthma because a drug with high topical potency is deposited directly in the airways." The editorial went on to say, that "the chemical modifications that have given inhaled glucocorticoids their favorable

**Table 2** Patients Withdrawn Because of Treatment Failure

| Reasons | *p*-value | BDP100 | BDP200 | BV |
|---|---|---|---|---|
| Poor control | 16* | 3 | 3 | 1 |

*Compared to active treatment; $p < 0.01$.
*Source*: Ref. 5.

**Table 3** Patients Remaining Off Prednisone During Phase 2[a]

| | *p*-value | BDP100 | BDP200 | BV |
|---|---|---|---|---|
| No. completing phase 2 | 26 | 31 | 33 | 41 |
| No. remaining off prednisone | 4 | 15 | 25 | 23 |
| Percentage | 15 | 48 | 76 | 56 |

BDP, beclomethasone dipropionate.
[a]Active vs. placebo, $p < 0.001$.
*Source*: Ref. 5.

ratio of topical bronchial activity to systemic activity have led to compounds that are very effective in many children and adults with asthma when given in doses that cause no systemic effects . . .” (11).

Thus, the introduction of inhaled corticosteroids dramatically and effectively changed the conventional approach to asthma therapy. First, a focus and emphasis was placed upon inhaled medication with properties that modified the inflammatory component of asthma. Second, with the institution of inhaled corticosteroids, it was possible to replace, for most patients, the need for chronic use of systemic glucocorticosteroids. The obvious benefit of this approach was a reduction in side effects, which, for some individuals, were severe and debilitating. Finally, dosing of inhaled corticosteroids was adjusted to the severity of an individual patient’s asthma and their response to this form of treatment.

## III. The Benefits of Inhaled Corticosteroids for Asthma Complications

Asthma exacerbations are associated with increased morbidity, the possibility of death, and increased costs of health care. A study published in 1996 evaluated the impact of inhaled corticosteroids on acute asthma hospitalizations in Sweden from 1978 to 1991 (12). To accomplish this goal, the investigators evaluated the sales of inhaled corticosteroids versus hospitalizations in Sweden. To accomplish this goal, data on regional sales of inhaled corticosteroids and use of bed-days for asthma in 14 Swedish county councils for the period 1978–1991 were used. Drug sales were measured as defined daily doses (DDD) and related to the total population within each council.

Beginning in the late 1970s and early 1980s, there was an increase in the frequency with which these medications were prescribed. By 1991, there had been nearly a 14-fold increase in sales of inhaled steroids in the Sweden areas (Fig. 2). Parallel evaluations of hospitalizations in these districts revealed a drop in the need for hospitalization (Fig. 3). Although this study did not directly evaluate the influence of inhaled corticosteroids on need for asthma hospitalizations, the

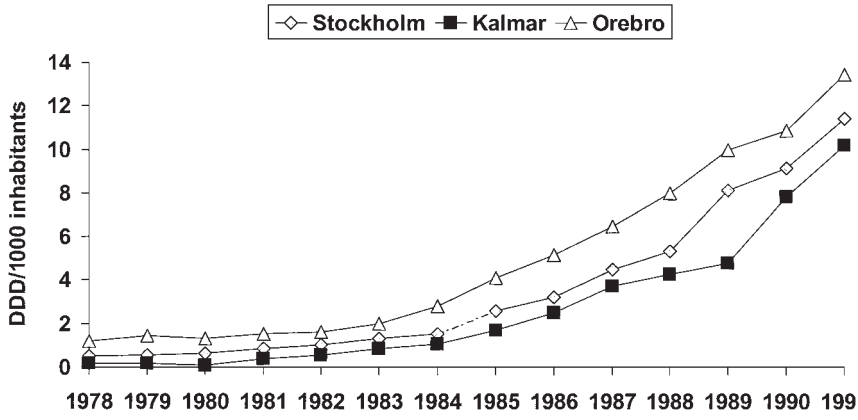


**Figure 2** Sales of inhaled corticosteroids in defined daily doses per 1000 inhabitants. (From Ref. 12.)

correlative events, i.e., the rise in inhaled corticosteroid prescriptions versus the fall in hospitalizations, indicate a possible "cause-and-effect" relationship.

The authors indicate that the results of their study support their hypothesis—there is a correlation between the sales of inhaled corticosteroids and improved asthma, as measured by the number of bed days in acute inpatient facilities. As the authors also indicate, the causal relationship between these two events needs to be validated by appropriate modeling of their interactions. Nonetheless, these novel observations strongly suggested a correlation between the change in prescribing habits and one marker of asthma morbidity—need for hospitalization.

In another study, Blais et al. (13) compared first treatment choices of inhaled corticosteroid with theophylline. In a large case-controlled study, a cohort of 13,563 newly diagnosed patients were first identified. The investigators compared the first-time users of inhaled corticosteroids with first-time users of theophylline as to their ability to prevent hospitalizations for asthma. The authors were able to demonstrate that there was a 40% reduction in the need for hospitalizations in those patients who received regular use of inhaled corticosteroids versus theophylline over the first 12 months of treatment. If, in contrast, the use of inhaled steroids was irregular, there was no reduction in the need for hospitalization. Hospitalization for asthma is a well-recognized indicator of more severe asthma. As Blais et al. (13) were able to demonstrate, treatment with inhaled corticosteroids within the year of asthma recognition not only reduced a major cost of asthma hospitalization, but, as suggested by the authors, may control factors that could lead to more severe or persistent disease.

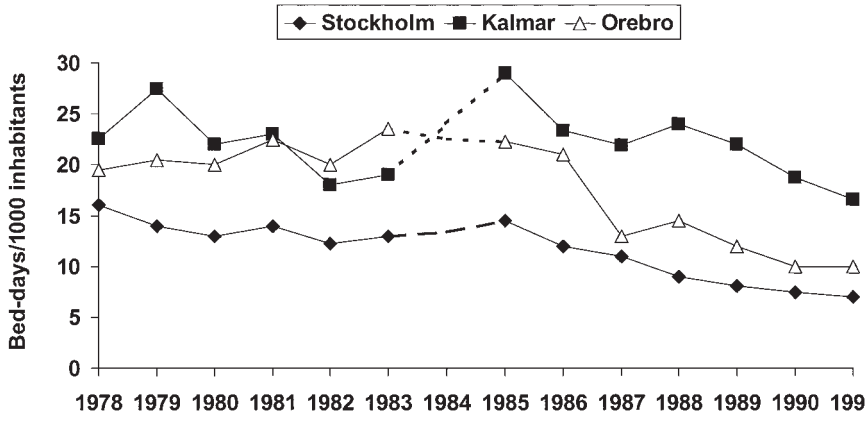


**Figure 3** Number of bed-days per 1000 inhabitants in acute somatic care due to asthma. (From Ref. 12.)

## IV. Benefit of the Addition of Inhaled Corticosteroids

A recent publication by Rowe and colleagues (14) evaluated the effect of the addition of inhaled budesonide, 1600 μg/day, to systemic corticosteroids for patients who had been treated for severe asthma in the emergency room. In this study, patients who were discharged from the emergency room following acute care were given 50 mg/day of prednisone for 7 days plus either placebo or budesonide (1600 μg/day). Three weeks after discharge from the emergency room, 12.8% of those who had received budesonide had a relapse of asthma versus 24.5% on placebo ($p = 0.649$). The evaluation of these patients indicated that following 7 days of prednisone and either placebo or budesonide, pulmonary functions were similar. However, the need for inhaled beta agonists, asthma symptoms, and quality of life (i.e., activities) were improved in those given inhaled corticosteroids. These results indicate the added benefit of inhaled corticosteroids to a short course of systemic corticosteroids for the treatment of acute asthma. Although not a component of this study, the results of Rowe et al. (14) raise the possibility that the addition of inhaled corticosteroids can improve those factors that contribute to the instability of asthma.

## V. Inhaled Corticosteroids as Research Tools for Mechanisms of Asthma

Inhaled corticosteroids can modify many aspects of the inflammatory process. Using this information, a number of investigators have evaluated effects of inhaled

corticosteroids on parameters of asthma. These studies have provided insight not only into how inhaled corticosteroids may be effective in the treatment of asthma, but also into the mechanisms by which inflammation occurs and is regulated. For example, Fahy and Boushey (15) evaluated the effect of low-dose beclomethasone (336 μg/day) on asthma control and sputum markers of inflammation. In this study, 24 subjects with mild asthma were identified and treated either with placebo or budesonide. During these treatment periods, lung function was measured and sputum was collected for analysis of inflammatory markers. As expected, inhaled corticosteroids (beclomethasone 336 μg/day) led to an improvement in the $FEV_1$ (Fig. 4). The investigators also collected sputum supplies. When treated with inhaled beclomethasone, there was a decrease in sputum eosinophils (Fig. 5). These data raise the possibility that improvement in lung function with inhaled corticosteroids is associated with the suppression of the eosinophilic inflammatory response in the airway. One interpretation is that the action of inhaled corticosteroids on inflammation in asthma is the reduction of eosinophils in the airway, which then is associated with a resolution of airway inflammation and improvement in airflow obstruction.

In classical studies by Haahtela and colleagues (3,16) in Finland, the investigators evaluated the effectiveness of early treatment with budesonide (1200 μg/day) for newly diagnosed asthma versus inhaled beta agonist alone for over a 2-year period. The results of their initial study were published in 1991 and indicated that inhaled budesonide was more effective than beta agonist alone and led to significant improvement in lung function (16). Laitinen and colleagues (17) obtained

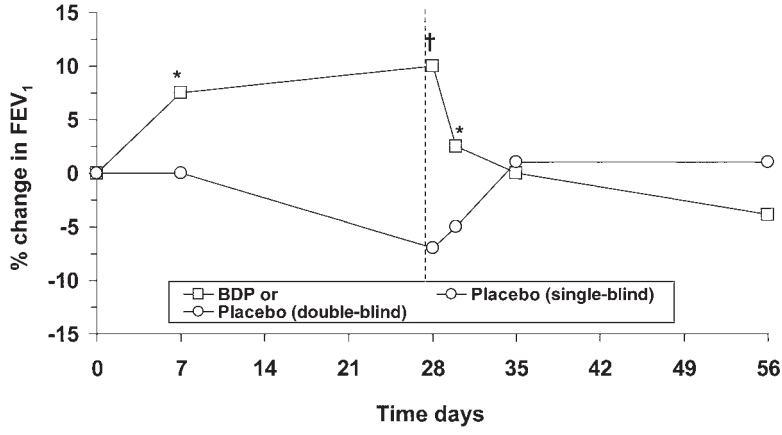


**Figure 4** The effect of beclomethasone (336 μg/d) vs. placebo on $FEV_1$. *$p < 0.05$ from baseline, but not significantly different from corresponding change in placebo. †$p < 0.05$ from baseline and from the corresponding change in placebo group. (From Ref. 15.)

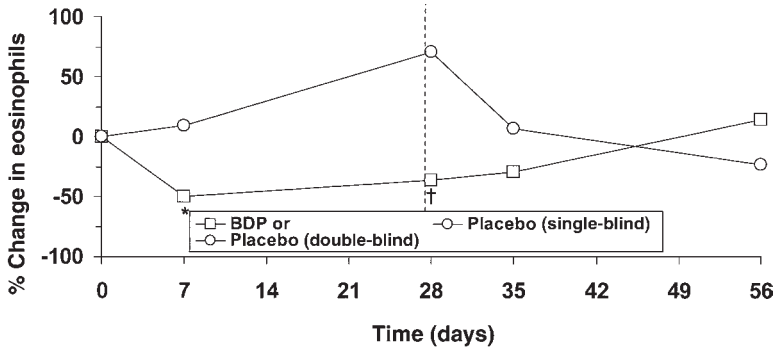


**Figure 5** The effect of beclomethasone (336 μg/d) vs. placebo on sputum eosinophils. $^{*}p < 0.05$, significantly different from baseline but not significantly different from corresponding change in placebo group. $^{\dagger}p < 0.05$, significantly different from the corresponding change in placebo group but not significantly different from baseline. (From Ref. 15.)

biopsies prior to and following inhaled corticosteroid or beta agonist treatment. They found that terbutaline had no significant effects on mast cells and eosinophils in tissues. In contrast, patients who received 1200 μg/day of budesonide had a significant reduction in mucosal mast cells and eosinophils. These studies are further evidence that not only do inhaled corticosteroids improve lung function, but their use is also associated with an inhibition of cellular markers of inflammation, i.e., mast cells and eosinophils.

In an extension of the original study, Haahtela and colleagues (3) extended the initial study with the following design. First, the patients who received 1200 μg/day of budesonide were given either a reduced dose, 400 μg/day, or placebo. Second, those individuals who had been treated with an inhaled beta agonist alone for 2 years were begun on inhaled budesonide (1200 μg/day).

A number of key findings emerged from this study. First, although there was a reduction in peak flow values on the lower dose of budesonide, measurements of lung function were generally stable. In those previously treated budesonide patients who were given an inhaled placebo, there was a deterioration in measurement of peak flow. Finally, the institution of budesonide at 1200 μg/day improved lung function in those patients previously treated with terbutaline alone. However, the improvement in lung function never achieved values noted when inhaled corticosteroid had been given initially with the diagnosis of asthma.

A number of conclusions are apparent from this study. First, it is possible to reduce the inhaled corticosteroid dose in individuals whose lung functions are stable and symptom control is good; the reduction does not necessarily lead to deterioration in asthma control. In contrast, stopping inhaled corticosteroids, even after 2 years of treatment, can be associated with a fall in lung function. These

findings indicate that inhaled corticosteroids control features of asthma during use, but inhaled corticosteroids do not "cure" the patient's asthma. Finally, if there is a delay in the initiation of effective anti-inflammatory therapy, "permanent changes," or remodeling, may occur such that a loss of lung function may be a consequence. This latter finding raises the possibility that there is a "window of opportunity" during which the initiation of inhaled corticosteroids is critical to achieve optimal control.

## VI. Summary

Inhaled corticosteroids have revolutionized the treatment of asthma. It is now appreciated that therapy directed toward airway inflammation is a critical component of treatment for persistent disease (1). Studies with inhaled corticosteroids have found them to be effective, safe in usual doses, and capable of modifying components of airway inflammation. We have learned a considerable amount about not only effective asthma treatment with inhaled corticosteroids but also, from a knowledge of their immunopharmacological action, how mechanisms of inflammation in asthma can be controlled. In the 25–30 years that inhaled corticosteroids have been under evaluation, the concepts of asthma have changed considerably. Our insight into these "new" concepts, e.g., inflammation, persistent disease, and airway remodeling, has been aided by observations with inhaled corticosteroids. We are still learning, and as the immunopharmacological mechanisms of corticosteroids are further understood, new knowledge about asthma will emerge. In the meantime, with the inhaled corticosteroids we have available now, an effective treatment for asthma is possible.

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## Discussion

**Dr. Szefler:** You presented an array of effects of asthma and the corresponding effect of inhaled corticosteroids. If you have one "marker" to pick in measuring the course of disease and target which one would it be?

**Dr. Busse:** That is a difficult question. Clearly, a measure of lung function is important. However, that is not the entire story. You would like an index of airway inflammation and a measure to assess exacerbation presentation. The assessment must be multifactorial.

**Dr. Pedersen:** One thing the use of inhaled steroids has taught us is that there is a substantial hidden morbidity in mild asthmatics. This group of patients shows marked improvements once they are treated with inhaled steroids. Their quality of life improves, and they have fewer exacerbations. So mild asthma should be taken seriously.

**Dr. Hamid:** Do you think that the introduction of inhaled steroids has focused our research? Do you agree that other mechanisms could be important, like smooth muscle function?

**Dr. Busse:** We have focused on inflammatory cells because they can be measured. We need to look at the other tissues, like airway smooth muscle, epithelium, and connective tissue.

**Dr. Schleimer:** When does bronchial hyperreactivity evolve? Does it precede antigen-dependent events early in life, or is it driven by antigen exposure? In some countries, mortality has increased during the era of inhaled steroid availability. Are the patients who are dying not using ICS?

**Dr. Busse:** Data indicate that ICS decrease asthma mortality. In the United States, ICS use is still low. Airway hyperresponsiveness is caused by many factors. It precedes antigen challenge but is increased by airway inflammation.

# 3

# Side Effects of Inhaled Corticosteroids

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## I. Introduction

Since the initial identification of corticosteroids as effective treatment for asthma (1–3), inhaled corticosteroids have evolved into the most important and useful drugs currently available to treat asthma (4–7). In addition, inhaled corticosteroids have been used to treat a variety of other pulmonary disorders including chronic obstructive pulmonary disease, sarcoidosis, allergic bronchopulmonary aspergillosis, and croup. Inhaled corticosteroids were initially developed in the 1950s, and their clinical benefits in asthma were first demonstrated by Gelfand (2). The development of topically potent inhaled corticosteroids along with their markedly superior side effect profile has led to these agents being the preferred route for the treatment of patients with asthma as well as a minority of patients with chronic obstructive pulmonary disease who demonstrate a reversible component to their airway obstruction. Indeed, inhaled corticosteroids are now recommended as first-line therapy for persistent asthma in national and international guidelines (4–7).

## II. Inhaled Corticosteroid Preparations

Beclomethasone dipropionate (BDP) was introduced as a topically active, lipophilic, inhaled cortcosteroid in the early 1970s (8). Newer lipophilic glucocorticosteroids followed including budesonide (BUD), fluticasone propionate (FP), and, most recently, mometasone. Each of these had increased glucocorticoid receptor specificity and more efficient first-pass hepatic metabolism, resulting in very low oral bioavailability.

There are currently six topically active glucocorticosteroids available by the inhaled route for the treatment of asthma: BDP, triamcinalone acetonide, flunisolide, BUD, FP, and mometasone. These drugs have used chlorofluorocarbons (CFC) as propellants in pressurized metered dose inhalers (pMDIs) or dry power inhalers (DPIs) to deliver the inhaled corticosteroid to the lungs. More recently, inhaled corticosteroids have been developed that use hydrofluoroalkanes (HFA) as the propellant in pMDIs to replace the ozone-depleting CFC pMDIs that have been in use until now. Their properties are different from the previous formulations, most notably because of their more peripheral lung deposition and possibly increased systemic absorption.

## III. Side Effects of Corticosteroids

Despite the wealth of evidence demonstrating the marked efficacy of inhaled corticosteroids in the treatment of asthma, which is unmatched by any other treatment, concerns about the side effects of inhaled corticosteroids have limited their prescription by many physicians and their use by many patients. This is because of concerns that the well-documented serious side effects of the regular use of even low doses of systemic corticosteroids to treat many disease, including severe asthma, may also occur with inhaled corticosteroids.

There is no doubt that inhaled corticosteroids are absorbed across the lung. Corticosteroids are not metabolized by the lungs, and, therefore, every molecule that is deposited in the lungs moves across into the systemic circulation and can exert effects beyond the lungs. In addition, a proportion of an inhaled corticosteroid dose is deposited in the oropharynx, is swallowed, and enters the portal circulation. The magnitude of the orophyrangeal deposition mainly depends on the inhaler device used, and the effects of the absorbed fraction depends on the efficiency of the first-pass hapatic metabolism of the corticosteroid, which is different for the various inhaled corticosteroids (9). This is low for BDP (approximately 40%), which means that a substantial amount of the swallowed (and clinically useless) BDP will also enter the systemic circulation. The hepatic first-pass metabolism is much better for budesonide (10%), FP ($<1\%$), and mometasone ($<0.1$). The side effects of inhaled glucocorticosteroids are dose-related, with little or no evidence of clinically relevant systemic unwanted effects at doses of

<400 μg/day of beclomethasone or budesonide in children and of <1000 μg/day in adults (10). These doses of inhaled corticosteroids are at the top of the efficacy dose-response curve for inhaled corticosteroids, which means that all the available inhaled corticosteroids have an excellent therapeutic ratio (the ratio between efficacy and side effects) (Fig. 1). This chapter will consider the side effects of inhaled cortcosteroids as those that are troublesome (mainly topical in the oropharynx), but which are not dangerous to patients, those that are systemic and potentially dangerous, but have been shown to have no clinical import, and finally those for which some clinical concerns remain.

### A. Topical Side Effects

The most common side effects that do occur with inhaled corticosteroids are local side effects in the oropharynx.

#### *Oral Candidiasis*

Clinically obvious oral candidiasis occurs in 5–10% of adult asthmatics treated with inhaled corticosteroids (11) but is much less common in children, where it occurs in only 1% (12). However, positive oropharyngeal cultures for candida, not associated with clinical symptoms, have been demonstrated in up to 45% of

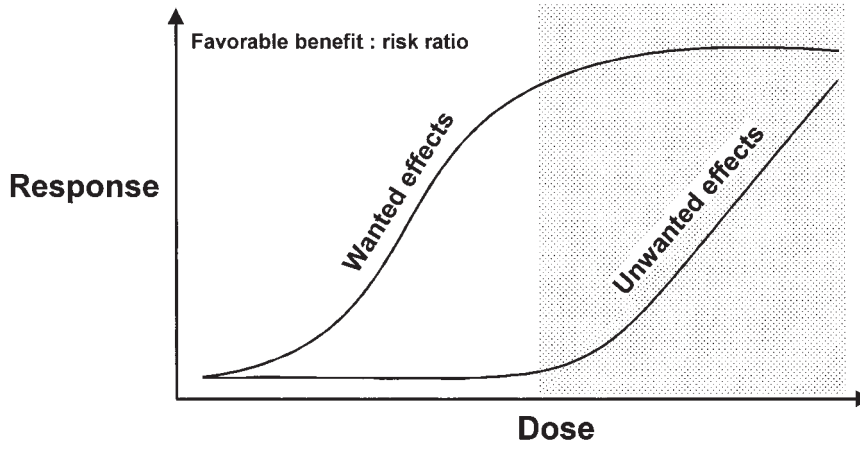


**Figure 1** Schematic dose-response curves for the wanted and unwanted effects of inhaled corticosteroids. The range in which the benefit:risk ratio is favorable is that at which the wanted effects in the lungs increase steeply with dose while the unwanted systemic effects increase gradually. At higher doses, the increase in risk greatly outweighs the slight remaining increase in benefit. This relationship appears to vary for different inhaled corticosteroids. (From Ref. 9.)

children and 70% of adults using corticosteroids (11). The most usual symptoms are pain and discomfort in the mouth, which can be associated with dysphagia. The risk of clinically obvious oral candidiasis is increased by the concomitant use of antibiotics with inhaled corticosteroids and is greatly reduced by the use of a large-volume spacer or Aerochamber® to deliver the inhaled corticosteroid (13) and by mouth rinsing after use. Oral candidasis is easily treated with 3–4 days of treatment with nystatin swished around the mouth and swallowed twice daily.

#### *Dysphonia*

Dysphonia, a more common topical side effect of inhaled corticosteroids, has been reported to occur in up to 30% of patients (11). It is more common in patients who use their voices a lot, is usually reversible with discontinuation, and is much more troublesome in patients who are using their voice to earn their income. Dysphonia is reported to be a much less frequent topical side effect if the inhaled corticosteroid is delivered by the DPI Turbuhaler® (14).

### B. Systemic Side Effects

There is a vast literature on the measurement of systemic side effects of inhaled corticosteroids in asthmatic patients. While several different indices indicating systemic activity can be measured, their clinical consequences are much less clear. Clinically relevant systemic unwanted effects should ideally be studied within the context of controlled, long-term clinical trials, which use clinically relevant doses in patients whose disease severities and ages are similar to the groups in which the drugs would normally be prescribed. Such studies require large numbers of patients and are difficult to conduct. As a substitute, the systemic effects of the various inhaled corticosteroids are often studied in short-term, cross-over studies on healthy volunteers or patients with mild disease who will tolerate treatment with placebo for a certain period. A recent study suggested that systemic unwanted effects are higher in healthy volunteers than in asthmatics (15). Therefore, the clinical relevance of findings from such studies for patients with moderate and severe asthma is not known. Thus, while all physicians who treat asthmatics should be conscious of the potential for the development of the types of adverse effects that occur in patients who use corticosteroids to treat asthma or other diseases, the reality is that none of these have been documented, as yet, to be clinically important. For these reasons the systemic side effects of inhaled corticosteroids include those that can be measured and for which there is no evidence of clinical import and those about which some clinical concerns remain.

#### *Effects on the Hypothalamic-Pituitary-Adrenal Axis*

Evaluation of the systemic effects of inhaled corticosteroids and studies of comparisons between inhaled corticosteroids have mainly focused on the effects on the

hypothalamic-pituitary-adrenal (HPA) axis. Measures of HPA axis function provide the most sensitive and easily measured markers of systemic effects of inhaled corticosteroid therapy. The clinical significance of small alterations in HPA axis function measured under controlled, artificial laboratory conditions is, however, doubtful. Some reduction in cortisol secretion merely reflects the normal functioning (feedback) of the HPA axis control mechanisms in response to exogenous steroid rather than a clinically significant abnormality, and the total corticosteroid exposure of the body may remain within the physiological range. Significant laboratory findings are, therefore, not predictive of important clinical effects.

The effects of corticosteroids on the HPA axis can be measured in a number of different ways, each assessing either basal levels or dynamic stimulation tests using corticotropin, corticotropin-releasing hormone, or a synthetic analog to assess adrenal cortical reserve in times of physiological stress (16). The most commonly used, easiest to obtain, but least sensitive, method has been the measurement of early morning serum cortisol levels. Much more sensitive measurements of the effects of excess glucocorticosteroids on the HPA axis are 24-hour urinary cortisol for basal levels (17) or the short tetracosactrin (ACTH) stimulation test (18) for dynamic stimulation.

All currently available inhaled corticosteroids can produce suppressive effects on the HPA axis, and the effect is dose-dependent; however, the different inhaled glucocorticosteroids are not equal with regard to their effects on the HPA axis (Fig. 2). For example, in children, a dose-dependent effect of urinary cortisols has been demonstrated with doses of BDP of 200–800 μg/day (19). By contrast, doses of BUD of 400 μg/day do not cause any effect on urinary cortisols, even when used for up to 1 year (20). In adults, many studies have examined the effects of ICS on HPA axis function, and there is no convincing evidence that doses of BDP of $<$1500 μg/day and BUD of $<$1600 μg/day have any measurable effect on the HPA axis (21). In addition, the effects of budesonide and fluticasone on HPA axis function depend on the inhaler devices compared and on whether the assessment is made after single or repeated dosing. The systemic potency ratio between fluticasone pMDI and budesonide pMDI on a μg for μg basis has usually been around 3:1 (i.e., three times as much budesonide is required to produce the same degree of systemic effect as fluticasone) (22,23). For the DPIs this ratio seems to be around 1.5:1 in adults (24) and around 1:1 in children (25). Also, higher doses of fluticisone demonstrated a twofold greater effect on the HPA axis when compared to higher doses of triamcinolone acetonide in adult asthmatics (26).

The measurable effects seen at higher doses clearly indicate systemic activity of the ICS but are of questionable clinical significance. There are only two case reports of clinically evident adrenal insufficiency in patients, treated with only inhaled glucocorticosteroids on withdrawal of the ICS. These were an adult who was treated with a very high dose of inhaled BUD (6400 μg/day(27) and a child who was using much lower doses (250 μg/day)(28).

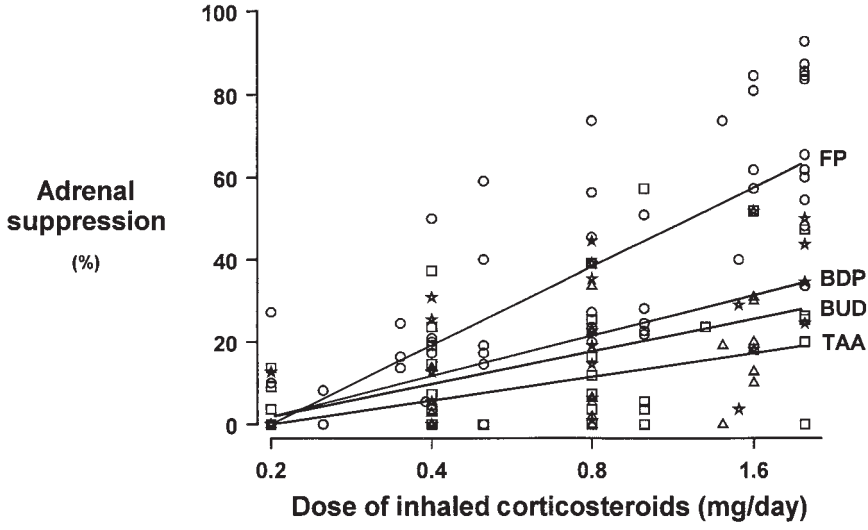


**Figure 2** Effects of increasing doses of four different inhaled corticosteroid preparations of adrenal cortisol output expressed as % supression from baseline measurements. FP: Fluticasone propionate; BDP: beclomethasone dipropionate; BUD: budesonide; TAA: triamcinalone acetonide. (From Ref. 16.)

### *Bone Demineralization and Osteoporosis*

Osteoporosis is an important complication of the use of ingested glucocorticosteroids, particularly in high-risk patients, such as postmenopausal women (29). This occurs through an increase in bone resorption and a decrease in bone formation and results in increased risk of fractures, especially hip and spine. Inhaled corticosteroids have been demonstrated to have effects on bone metabolism, although there is little evidence that, at the conventionally used doses, they cause osteoporosis and no evidence that they cause increased risk of fractures (30).

The effects of ICS on bone metabolism have been demonstrated by measuring serum osteocalcin, which indicates changes in bone formation, and urinary hydroxyproline, measured after a 12-hour fast, which increases with increased bone resorption. Pyridium cross-links in urine are another measure of bone resorption, which has the advantage over urinary hydroxyproline of not being diet dependent; however, to date, the effects of ICS on this latter measure of bone resorption has not been reported.

The effects of BDP and BUD on serum osteocalcin and urinary hydroxyproline have been studied in adults. Both have been shown to influence serum osteocalcin levels in a dose-dependent manner (31), but only BDP increases urinary hydroxyproline excretion at doses up to 2000 μg/day. More important than resorption or formation markers may be the interplay of the combination. In chil-

dren, doses of BUD of < 800 μg/day (32) and of FP of 200 μg/day (32) have no effect on any biochemical marker of bone turnover.

Bone densitometry has been measured in several studies in adults during periods of treatment with inhaled corticosteroids for up to 3 years. In one study adult asthmatics were taking varying doses of inhaled BDP (mean dose 630 μg/day)(33), while in another they were taking a mean dose of BDP or budesonide of 980 μg for 3 years. In addition in the EUROSCOP trial (34), evaluating the efficacy of inhaled budesonide in chronic obstructive lung disease (COPD), older patients (mean age 52 years) were treated with inhaled budesonide 800 μg/day for 3 years. In none of these studies was there any evidence that these patients had increases in bone loss. Finally, bone density has been followed longitudinally in a study of children using inhaled budesonide (mean dose 500 μg/day) over a mean of 4.5 years of treatment (35). No evidence was demonstrated for loss of bone density (Fig. 3). Also, to date there are no studies that have demonstrated that these biochemical markers of bone turnover are associated with increased risk of bone fracture.

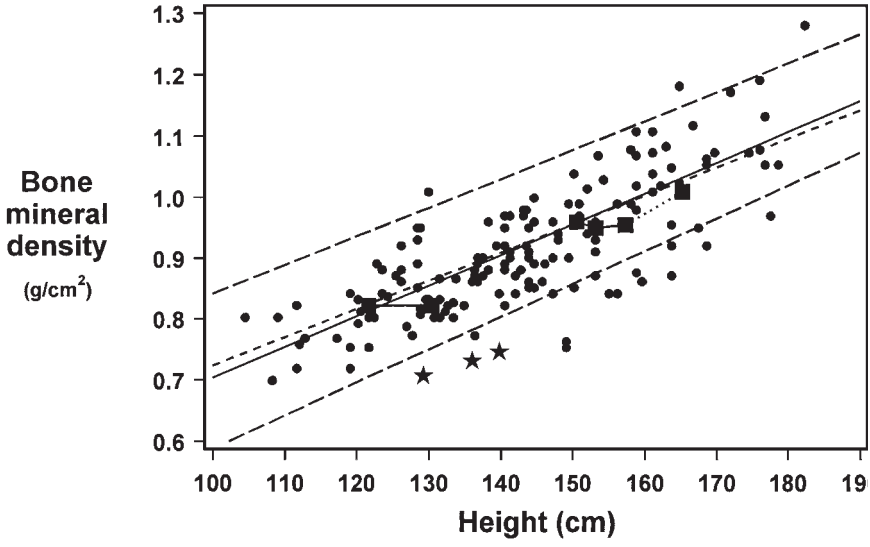


**Figure 3** Individual bone mineral density measured using dual energy x-ray absorptiometry (DEXA scan) in 157 asthmatic shildren treated continuously for 3–6 years with inhaled budesonide at a mean daily dose of 504 μg/day. For comparison, the 95% prediction interval and mean regression lines from measurements in 111 children with asthma who had never received treatment with inhaled corticosteroids are shown. Stars indicate 3 children who had received a diet with insufficient amounts of calcium. (From Ref. 35.)

### Glaucoma

An increased risk of open angle glaucoma has been reported in one case-control study in patients on high doses of BDP or BUD (36). The increased odds ratio (OR 1.44) was only seen in patients who were currently taking high doses of inhaled corticosteroids of >1600 μg/day. Further prospective studies are needed to confirm this finding.

### Posterior Subcapsular Cataracts

The fact that posterior subcapsular cataracts occur more frequently in patients taking ingested corticoseroids greatly complicates the issue of whether they occur with increased frequency in patients using inhaled glucocorticosteroids. All prospective studies in adults (37) and children (38) suggest that, once the confounding effect of ingested glucocorticosteroids is removed, there is no evidence that inhaled glucocorticosteroids increase the risk of developing posterior subcapsular cataracts. Two recent case-controlled studies have, however, indicated that high inhaled doses of BDP is associated with a slightly greater risk of posterior subcapsular cataracts in older patients (39,40). These studies did not, however, stratify for the known confounding risk of allergy for cataract development in these populations (41).

### Growth Retardation in Children

The concern that the use of inhaled corticosteroids will cause growth retardation in children has greatly limited their use in children. The studies that have examined this issue are reviewed in another chapter of this book.

### Skin Thinning and Easy Bruising

Corticosteroids applied topically to the skin cause skin thinning and atrophy. This is because the corticosteroid remains on the skin for several hours. By contrast, inhaled corticosteroids are rapidly absorbed across the airway mucosa and are unlikely to have this effect on the airway mucosa. Indeed, studies of airway biopsies of asthmatics who have been using inhaled corticosteroids for months or years have not demonstrated any evidence of airway mucosal atrophy (42), but rather repair of the epithelial damage that is a characteristic feature of asthma (43). However, skin thinning and bruising do occur as a dose-dependent systemic side effect of inhaled corticosteroid use, the latter related to increased capillary fragility. It is rare at daily doses of <800 μg/day of BDP or its equivalent, and its incidence increases with age and duration of treatment. In one study of older patients on high doses of BDP, the prevalence of easy bruising was 47% for those on inhaled glucocorticosteroids and 22% for those who were not (44). Also, in the recently published EUROSCOPE trial, a higher incidence of skin bruising was reported in older patients using budesonide 800 μg/day for 3 years (34).

### *Effects on Diabetes Mellitus Control*

Systemic corticosteroids can cause loss of diabetes mellitus control and consequent diabetic coma. One case report has demonstrated mild loss of diabetic control when a patient was treated with high doses of FP (45). The loss of control did not happen when doses were reduced to 500 μg/day. There have not been any reports of diabetic coma associated with the use of inhaled corticosteroids.

### *Risks of Lung Infection*

Lung infections are not increased in patients using inhaled glucocorticosteroids. Also, inhaled glucocorticosteroids do not increase the risks of reactivation of pulmonary tuberculosis, and therefore prophylactic isoniazid treatment is not needed when inhaled glucocorticosteroids are used in patients with inactive pulmonary tuberculosis. However, it is prudent to be more observant for reactivation of pulmonary tuberculosis particularly in endemic areas of the world. There is one case report of laryngeal aspergillosis developing during use of inhaled corticosteroids (46). This suggests that careful evaluation of persistent hoarseness involve direct observation of the vocal cords.

### *Steroid Psychosis*

Psychosis may occur in as high as 2% of patients treated with systemic corticosteroids and has been reported to occur very occasionally in patients taking inhaled corticosteroids. A total of eight patients have been reported thus far who developed symptoms within days of being treated with either inhaled BDP or budesonide (47,48). The psychosis resolved promptly after stopping the inhaled glucocorticosteroid.

## IV. Conclusions

Inhaled corticosteroids are the mainstay of the treatment of persistent asthma. Their systemic unwanted effects have been the focus of extensive research since their introduction in 1972. The availability of topically potent corticosteroids, with effective first-pass metabolism in the liver, has ensured that the efficacy obtained with the doses usually needed for the optimal management of asthma is not associated with clinically relevant unwanted effects in almost all patients. The advent of CFC-free formulations in the late 1990s brings a new group of inhaled corticosteroids among us, with vastly different properties from their predecessors. Further studies of these compounds relating to their degree of systemic absorption and consequent adverse effects are awaited.

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## Discussion

**Dr. Pedersen:** It is true that often a marked reduction in hospitalizations or mortality is not seen with increasing use of inhaled steroids. However, substantial reductions in hospitalization and exacerbations are consistently seen in countries that also include the use of inhaled steroids for patients with mild persistent asthma. These countries include Finland, Sweden, Denmark, and the Netherlands. The reason for this is that in the society 60–80% of hospitalizations with asthma exacerbation are seen in patients with mild asthma.

**Dr. Denburg:** Do we know about the long-term effects of IS given in childhood on bone mineral density in adulthood? Regarding "biochemical" adrenal suppression, what are the clinical effects of infection or surgery on induction of adrenal crises in IS-treated subjects?

**Dr. O'Byrne:** There are no studies which have specifically followed bone mineral density in adults who have been treated with IS in childhood. The studies in childhood preformed by Agertoft and Pedersen have, however, been very reassuring. There are also no reports of adrenal crisis in asthmatics only treated with IS alone during emergency surgery. However, most clinicians still treat such patients with supplemental hydrocortisone prior to surgery, just to be sure such an event does not occur.

**Dr. Pedersen:** Many children with asthma have a different growth pattern with delayed puberty and prolonged growth. This is independent of inhaled steroids. Some healthy children have a similar growth pattern. These subjects don't achieve the same peak bone mineral density as people who do not have this growth pattern. Therefore, bone mineral density in patients with asthma should be related to the bone mineral density of healthy volunteers with great caution since possible differences might be due to differences in growth patterns. The best control group would be healthy volunteers with delayed puberty or asthmatics who never received inhaled steroids.

**Dr. Hamid:** Is there any evidence that inhaled steroids cause thinning of bronchial epithelium? If not, what do you think are the reasons for the discrepancy between the effect on skin epithelium versus lung epithelium?

**Dr. O'Byrne:** One study has evaluated this in patients with a history of long term IS usage. There was no evidence of thinning of the epithelium (Broder I, Tarlo SM, Davies GM, Thomas P, Leznoff A, Sturgess J, Baumal R, Mintz S, Corey PN. Safety and efficacy of long-term treatment with inhaled beclomethasone dipropionate in steroid-dependent asthma. Can Med Assoc J 1987; 136:129–35). By contrast, the airway epithelial abnormalities associated with asthma are corrected by IS (Laitinen L, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and of an

inhaled $\beta_2$-agonist, terbutaline, on airway inflammation in newly diagnosed asthma. J Allergy Clin Immunol 1992; 90:32–42).

**Dr. Busse:** I have been impressed by the increase in early bruisability of the skin in some older patients receiving inhaled corticosteroids. There appears to be a dose-dependency to bruisability. Do we have observations as to whether the individuals with those changes in the skin may be more susceptible to changes in other potential manifestations such as osteoporosis?

**Dr. O'Byrne:** Boulet et al. (Boulet LP, Milot J, Gagnon L, Poubelle PE, Brown J. Long-term influence of inhaled corticosteroids on bone metabolism and density. Are biological markers predictors of bone loss? Am J Respir Crit Care Med 1999; 159;838–44) have found that there was a poor correlation between skin bruising and markers of bone metabolism.

**Dr. Pedersen:** I think the local mucosal effects of steroids have not been sufficiently examined in vivo. Hence, we cannot say much about effects of GC at the organizational level (e.g., in epithelial damage repair processes and other gross physiological processes occurring in asthmatic bronchi). Although the balance of effects is beneficial, GC may have some less desirable local effects; different GC may also differ in this respect, speculatively. On this note, what is your view regarding the possibility that GC increase neutrophilia in asthmatic bronchi?

**Dr. O'Byrne:** This does not appear to have any clinically important effect in most asthmatics, who get such a great clinical benefit from IS. It does raise the interesting question, however, whether in those patients with severe asthma not responding to IS, many of whom have an airway neutrophilia, this effect may be of importance.

**Dr. Szefler:** As we move to more aggressive therapy, there are two consequences that we must address: (1) the use of higher doses and (2) the use in younger patients. Those are two areas of weaknesses—lack of data establishing safety with high doses and use in younger children.

**Dr. O'Byrne:** The data to date with extensive exposure to conventional dosing strategies do not point to a significant concern for lingering effects.

**Dr. Busse:** Neutrophils in the airway have been found in a number of inflammatory conditions. In acute asthma from viral infections there is an increase in neutrophils, and these cells are likely to be important to the contribution of asthma severity. However, an increase in the numbers of neutrophils in the airway of patients with severe disease is difficult to relate to disease severity or as a consequence of treatment. In severe asthma, it will be necessary to assess cell function in addition to cell number. The importance of neutrophils to airway dysfunction and response to therapy is not resolved.

**Dr. Seale:** An additional aspect of the Cumming et al. study in the *New England Journal of Medicine* (Ref. 39) was the higher odds ratio when the total cumulative dose of inhaled steroids exceeded 2000 mg. However, this study may not have taken due account of the association between atopy and lens cataracts.

**Dr. Pedersen:** The risk of cataract is greater in patients with atopy. The risk of having severe asthma requiring high doses of inhaled steroids is increased in patients with atopy. Therefore, conclusions about an association between long-term use of high-dose inhaled steroids and an increased occurrence of cataracts cannot be made unless adjustments are made for possible differences in occurrence of atopy between the groups that are compared.

**Dr. Jeffery:** My comment follows those made by Drs. Busse and Persson concerning the possibility that inhaled steroids may increase the number of neutrophils. The complementary information is that severe, corticosteroid-dependent intractable asthmatics (Wenzel et al, 1997, AJRCCM 156:737–743) had relatively very few eosinophils and yet the severity of their asthma continued. I believe this highlights the dis-association between the reductive effects of steroids on eosinophilic inflammation and their lack of effect on remodeling, at least once the remodeling of the airway wall has occurred. Clearly it is important to treat early (in childhood) to prevent both inflammation and subsequent remodeling. However, once remodeling has occurred, we do not understand or know if corticosteroids have any beneficial effect on the subsequent disease process. We urgently need studies to address the role of corticosteroids in inhibiting the myofibroblast and smooth muscle response to allergen challenge and how this may be reversed.

**Dr. Boulet:** In one of our recent studies, we found that although they were very effect to reduce airway inflammation, ICS could not significantly change some markers of remodeling in mild recently diagnosed asthma suggesting that they could possibly be used even earlier than at the symptomatic stage of asthma, at least in some instances.

I also have a comment about the clinical relevance of the mentioned side effects of ICS. One of the problems we face is that often there is a generalization of side effects of ICS, whatever the dose, while as Dr. O'Byrne showed, they may occur mostly at high doses of ICS in susceptible individuals. Changes in markers of systemic action of ICS show a dose-response effect that becomes only significant over 1000–1500 μg in most adults. In the severe patient requiring high doses of ICS, more studies should be done to determine how to identify susceptible individuals and possibly offer preventive treatment, for example, to prevent bone loss. In such studies, however, the influence of disease severity should be taken into account, as well as other counfounding factors.

**Dr. O'Byrne:** Another issue to consider is that we are now using inhaled GCS differently, using lower doses in association with long-acting $B_2$-agonists in

asthma and using inhaled GCS to treat patients with COPD much less than previously.

**Dr. Derendorf:** I agree that we have focused too much on safety and not enough on efficacy. However, I wouldn't go so far as to say that there is no safety issue with ICS. We should try to minimize systemic exposure with ICS. Don't you believe that serum cortisol is a suitable parameter to quantify systemic exposure?

**Dr. O'Byrne:** It has been possible to measure biochemical markers of effects of higher doses of inhaled GCS on adrenals or bone; however, we also have a responsibility to understand the clinical consequences of these. To date, there is no evidence of a clinically important detrimental effect.

**Dr. Denburg:** What is known about the effects of IS on brain and behavior (e.g., cognitive or mood disorders)?

**Dr. O'Byrne:** There are studies that show that oral steroids can be associated with behavioral changes, including steroid psychosis. There are, in addition, anecdotal reports of steroid psychosis in patients using IS, which resolved when the IS were discontinued.

**Dr. Persson:** Further to the comment by Dr. Busse that infections are the cause of airway neutrophilia in asthma: Jonas Erjefalt has carried out in vivo experiments on processes that follow upon asthma-like, nonsanguineous shedding of airway epithelial cells. The instantaneous and speedy repair was associated with marked local neutrophilia remaining at the patchy repair sites until repair epithelium had migrated to fully cover the denuded basement membrane. Thus, I submit the possibility that increased neutrophilia in severe asthma in part reflects the increased occurrence of epithelial injury-repair processes. Interestingly, topical budesonide treatment did not reduce local neutrophilia, nor did it reduce the acute, speedy epithelial restitution.

**Dr. Busse:** There appears to be a subject susceptibility in the development of neuropsychiatric side effects from corticosteroids. First, the development of the psychiatric side effects appears to be largely limited to systemic steroids. Second, there appears to be patient susceptibility; that is, only some people experience these side effects. Which mechanisms mediate such side effects are not clear. Finally, the development of neuropsychiatric effects with steroids is very rare, at least from clinical experience.

**Dr. Denburg:** It may be worth studying the effects of IS on cognitive function in children (who appear to be compromised at school by their disease, but also maybe by its treatment)?

# Part Three

## MOLECULAR ASPECTS OF STEROID ACTION

# 4

# Mechanisms of Gene Regulation by the Glucocorticoid Receptor

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## I. Introduction

Glucocorticoids (GCs) are steroid hormones whose actions influence a diverse range of functions in the mammalian system. Many effects of GCs can be observed at the level of intermediary metabolism, including increased glucose production through the promotion of gluconeogenesis, enhanced delivery of amino acids from peripheral tissues, increased deposition of glycogen through activation of glycogen synthetase, enhanced lipolysis in extremities, and promotion of protein and RNA metabolism. These hormones act to suppress the immune and inflammatory responses, and under conditions of stress the secretion rate of GCs is relatively high. Hypersecretion of cortisol, the major human glucocorticoid hormone, results in a depressed immune state. GCs are also important in fetal development, with a crucial role in fetal lung maturation. Finally, GCs are important effectors of homeostasis, necessary for the maintenance of normal blood pressure and cardiac output as well as the maintenance of normal water and electrolyte balance.

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GCs mediate their effects through the ubiquitously expressed, intracellular glucocorticoid receptor (GR) (1). GCs pass through the plasma membrane and bind to GR in the cytoplasm. The cellular uptake of glucocorticoids has long been regarded as primarily a passive diffusion of these lipophilic molecules across the lipid bilayer, but evidence is slowly accumulating to suggest that in at least some instances this uptake may be a more regulated event than previously believed (2,3) and that the availability of hormone for receptor binding may be influenced by the activity of steroid transporters (4,5). Furthermore, the activity of the hormone may be modified in a cell-specific manner by modifying enzymes. Ligand binding promotes a conformational change, heat-shock protein (hsp) dissociation, homodimerization, and activation of the receptor to a DNA-binding form. GR translocates into the nucleus upon GC binding, where it stimulates or represses transcriptional activity of target genes, either by binding to specific DNA sequences known as glucocorticoid response elements (GREs) or by interacting with other proteins. The mechanisms of action of GR are the focus of this chapter and will be discussed in greater detail below.

## II. The Nuclear Receptor Superfamily

GR is a member of the nuclear receptor superfamily, the largest family of metazoan transcription factors. Nuclear receptors share several common properties based on structural features, but they can be classified broadly into four subclasses (6). Class I receptors comprise receptors for GR and other steroids, such as estrogens (ERα and ERβ), mineralocorticoids (MR), progestins (PR), and androgens (AR). This subclass of receptors functions through forming homodimers and binding to palindromic DNA motifs. Class II receptors heterodimerize with the retinoic acid X receptor (RXR) and characteristically bind to response elements consisting of direct repeats with different spacing. This class encompasses receptors for nonsteroidal ligands such as thyroid hormone (TR), retinoic acid (RAR), and vitamin D (VDR), together with a large number of receptors whose specific ligands have yet to be discovered; these receptors have fallen under the collective label of orphan receptors, despite a diversity of physiological functions. Class III receptors bind primarily to direct repeats as homodimers, while Class IV receptors typically bind to extended core sites as monomers.

The steroid receptors have a modular structure, with distinct domains performing different functions within the molecule (Fig. 1). Three major domains have been identified. The N-terminal domain is the least conserved and may vary greatly in length. It contains sequences responsible for activation of target genes, including the major transcriptional domain, activator function-1 (AF-1). The DNA-binding domain (DBD) is a highly conserved cysteine-rich region that forms two zinc-finger structures, which provide the receptor with the ability to interact with DNA. This domain also participates in receptor dimerization and is

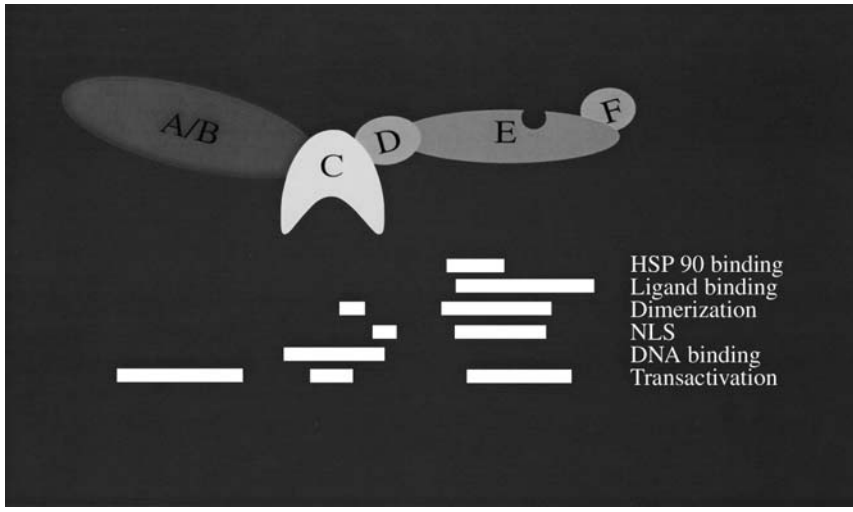


**Figure 1** Domain structure of GR. The receptor has a typical nuclear receptor structure, with distinct domains responsible for functions such as DNA binding and binding to the ligand. Note that the unactivated receptor is predominantly cytoplasmic, since prior to binding hormone the nuclear localization signal is masked by hsp90.

involved in translocation into the nucleus. The C-terminal domain, a less well-conserved hydrophobic region, is found in all receptors and is called the ligand-binding domain (LBD). This domain possesses the sequences important for ligand recognition (for the nuclear receptors with known ligands) and ensures specificity of the physiological response. This domain also contains regions involved in hsp binding, nuclear translocation, receptor dimerization, and ligand-dependent transactivation. Importantly, a second activator domain, activator function-2 (AF-2), is located within the LBD and appears to be the principal region involved in protein-protein interactions with cofactors, although interactions between the N-terminal domain and these proteins have also been demonstrated (7,8). The N-terminal domain, DBD, and LBD are each capable of acting independently when fused to heterologous proteins, indicating that they contain the information necessary for their individual functions (9). As expected, given their shared structural motifs, steroid receptors share a common mechanism in their functions as transcriptional activators.

## III. The Glucocorticoid Receptor

The fundamental role of GR in glucocorticoid physiology and during development has been investigated through the generation of GR-deficient mice by gene

targeting (10). The majority of $GR^{-/-}$ mice died within a few hours after birth as a result of respiratory failure. Further analysis revealed reduced expression of key gluconeogenic enzymes. The $GR^{-/-}$ mice also had elevated adrenocorticotropic hormone (ACTH) and corticosterone levels, indicative of impaired negative feedback regulation of the hypothalamus-pituitary-adrenal axis (HPA).

The human GR (hGR) gene is located on chromosome 5 and contains 10 exons. Two isoforms of hGR have been identified, hGRα and hGRβ (777 and 742 amino acids, respectively), derived from the same gene by alternative splicing (reviewed in Ref. 11). GRα is a ~94 kDa intracellular protein predominantly present in the cytosolic fraction of the cell in the absence of hormone. In this state the receptor forms part of a multiprotein complex, which includes one molecule of the receptor, two molecules of hsp90, and one molecule each of hsp70, hsp56, and hsp26 (12). While the roles of these hsps have been well documented, another molecular chaperone associated with GR, p23, has recently been shown to affect ligand efficacies (13). Upon hormone binding GRα undergoes a conformational change, which enables it to dissociate from the hsp complex. The receptor is subsequently hyperphosphorylated and the nuclear localization signals within the LBD unmasked, allowing GRα to translocate to the nucleus. As GRβ does not bind ligand, its functional role in the cell is a matter of debate (14–16) and it will not be discussed further here.

### A. Binding of Hormone

The conformation GR adopts on binding ligand is dependent on whether the ligand is an agonist or antagonist (17). Upon activation GR forms a homodimer that can bind to specific DNA sequences termed glucocorticoid response elements (GREs) present in the target genes. GR can also bind to so-called negative GREs (nGREs) or to other transcription factors (see below and Ref. 18). GREs and nGREs are usually located in the promoter region of target genes, but they have also been found within the structural gene, as is the case for the human growth hormone gene (19). GR homodimers bind to GREs with much higher affinity than monomers and dimerization is required for transactivation of target genes. Induction of dimerization is thus one of the main functions of ligand binding. Crystal structures of the LBDs of other nuclear receptors in the absence of ligand and in the presence of agonist or antagonist demonstrated that nuclear receptors appear to have similar folding patterns and a common ligand-binding pocket architecture (20). These studies, in conjunction with functional investigations, have revealed that positioning of the most carboxy-terminal α-helix (H12) of the LBD plays a major role in nuclear receptor transactivation activity. The positioning of H12 depends on whether the receptor is bound by agonist or antagonist (21). In the presence of an agonist bound to the receptor, H12 adopts a conformation that generates a surface for coactivator interaction, while receptor interaction with an

antagonist alters the H12 position, and receptor interactions with coregulators are thought to be modified as a consequence.

### B. DNA Binding

In the nuclear receptor superfamily, the highly conserved DBD is the region essential for interaction with DNA. Comparison of a large number of naturally occurring and synthetic DNA response elements identified a conserved half-site sequence AGAACA, which is preferentially recognized by GR, MR, PR, and AR, whereas the sequences AGGTCA or AGTTCA are favored by ER, TR, RAR, VDR, and many of the orphan receptors (32). The consensus GRE is composed of two 6-base-pair half-sites arranged as an imperfect palindrome with a 3-base-pair interval, to which GR binds as a homodimer (32). One receptor of the dimer contacts DNA specifically, while the other is regarded as essential for high-affinity binding. Sequences differing from the consensus GGTACANNNTGTTCT are generally bound with lower affinity by the receptor and may serve to attenuate the magnitude of the transcriptional response to GCs. For natural genes hormone-dependent activation from nonconsensus binding sites relies on cooperative interaction with adjacent or nearby transcription factors (32–36). Such interactions may serve to restrict a hormonal response to a specific cell type that expresses the appropriate set of cooperating factors (37). No consensus sequence has been described for an nGRE, and the reason why GR binding to such sites does not result in transactivation remains unclear. It is possible that these response elements either induce a slightly altered conformation of the DNA-bound receptor or may not allow efficient interaction with the GR (18). Supporting the idea that DNA binding per se is not enough to allow activation, mutation of some residues in the GR DBD not directly involved in the protein-DNA interaction inactivate or severely diminish transcriptional activation by GR. These mutations might result in an altered conformation of the DNA-bound receptor or its interaction with other proteins, affecting the transactivation function (38,39).

### C. Phosphorylation of GR

Posttranslational modifications are important in regulating transcription factor function, and phosphorylation of transcription factors may be important in regulation of nuclear translocation, regulation of DNA binding, and regulation of transactivation (40). The unliganded GR is a phosphoprotein that becomes hyperphosphorylated following ligand binding, mainly on serine residues in the N-terminal domain (41). In some studies, mutation of single or multiple phosphorylation sites in the mouse or human GR had little effect on the ability of these mutants to activate transcription (42), whereas others studies suggested that hypophosphorylation of the mouse receptor decreases transactivation (43). The role of phosphorylation in GR function thus awaits further clarification.

### D. Integration of the Hormone Signal on Chromatin

Induction of transcription from genomic DNA involves rearrangement of chromatin around the promoter and/or upstream segments, creating more open structures which facilitate access for the basal transcriptional complex. Examples of this are provided by studies on the mouse mammary tumor virus (MMTV) promoter and the rat tyrosine aminotransferase gene (44) Prior to GC induction, these genes are in a repressed state, and it has been demonstrated that the nucleosomes are structurally altered upon GC treatment (45).

In addition, the exciting progress in the identification and characterization of chromatin modifying proteins, particularly acetyltransferases and deacetylases, that associate directly or indirectly with nuclear receptors and other transcription factors has revealed roles for these cofactors/enzymes in transcriptional control. Acetylation neutralizes the positively charged lysine residues of the histone N-termini, decreasing their affinity for DNA and thus allowing the termini to be displaced from the nucleosome, thereby causing the nucleosome to unfold and facilitate access for the basal transcriptional machinery (46). GR may function at an early stage by recruiting histone acetylating factors to target gene promoters.

Steroid hormone receptors are thought to interact with several components of the basal transcription machinery. For example, the AF-1 domain of the GR was shown to interact with the TATA box binding protein, TBP. TAFs (TBP-associated factors) are required for GR activation in Hela nuclear extracts, suggesting an interaction between the receptor and these proteins (47,48). The interaction between steroid receptors and the basal transcription machinery is necessary for efficient hormone-dependent transcriptional control, but an additional set of proteins that do not bind DNA yet may modify transcriptional activity have been identified and designated as cofactors. These proteins can be further classified on a functional basis into coactivators and corepressors (49,50). The recent identification of an array of these proteins as coregulators for steroid hormone receptors and other sequence specific transcription factors, providing the means by which these factors modulate the activity of the basal transcription machinery (51,52).

## IV. Gene Regulation by Glucocorticoid Receptor

No single mechanism can explain all the observed GR-mediated transcriptional events, and it appears that GR utilizes a variety of means to promote or repress target genes. Receptor activation, chromatin acetylation, and nucleosome disruption are involved in gene regulation by GR, but additional parameters are necessary for the precise transcriptional control rendered by the receptor. For example, GR-mediated gene regulation involves complex interactions of the receptor with other regulatory factors. In addition, the promoter also plays a critical role in determin-

ing receptor action through the nature and arrangement of the GREs it contains, the nonreceptor factors that bind to it, and the arrangement of binding sites for these proteins in relation to the GRE (36).

### A. Activation of Transcription by GR

The classical mechanism for GR-dependent positive gene regulation entails binding of the hormonally activated receptor to one or more palindromic GRE sequences, usually located in the promoter region of glucocorticoid-responsive genes. It has been clearly established that positive gene regulation, in addition to GR binding to GREs and direct contact with the basal transcription machinery, involves complex interactions with both DNA-binding and non–DNA-binding proteins. Positive regulation also occurs in some instances in the absence of a GRE, probably through protein-protein interactions between the receptor and DNA-bound transcription factors (see below). Moreover, the cofactors that associate with DNA-bound receptor and/or other proteins act as a scaffold between sequence-selective receptors and the basal machinery, stabilizing the preinitiation complex on the promoter and thus enhancing transcription by RNA polymerase II (53,54).

In addition to GCs, other factors can modulate GR transcription and expression, for instance, the orphan estrogen-related receptor 2 (22) and the neurotransmitter dopamine (23). Another factor is cyclic AMP (cAMP), which has been shown to act through the protein kinase A pathway to modulate the GC sensitivity of a target cell by enhancing the DNA-binding activity of GR (24). Functional interactions between GR and other signal transduction molecules such as the growth regulators Rb and p53 (25, 26), AP-1 (27), NF-κB (28), and the STAT proteins (29,30) modulate GR activity and may partly account for differences in tissue sensitivity to glucocorticoids, reflecting another level of regulating receptor activity. Coregulators can also modulate glucocorticoid responsiveness, usually through modifying transcriptional activation (see below and Ref. 31).

#### *GR Interactions with Other DNA-Bound Transcription Factors*

Although GR is capable of controlling reporter gene expression from a simple GRE in vitro, transcriptional regulation in vivo and from natural promoters is more complex. The mouse mammary tumor virus long terminal repeat (MMTV-LTR) is a well-characterized DNA sequence containing GREs as well as binding sites for other transcription factors. Studies on the MMTV promoter have shown that CTF1/NF-1 and Oct-1 are required for optimal induction by either glucocorticoids or progestins (55). When activated GR binds to the LTR, the nucleosome undergoes a structural change, resulting in loss of chromatin repression and recruitment of a series of transcription factors (56,57). This is illustrated by interactions between GR and HNF3 on the tyrosine aminotransferase promoter or be-

tween GR and HNF1 on the rat insulin-like growth factor binding protein-1 gene. These interactions lead to functional synergism, suggesting that maximal promoter activity in these instances requires GR association with other sequence-specific transcription factors (58,59).

A more complex example is provided by GC-induced upregulation of transcription of the phosphoenolpyruvate carboxykinase (PEPCK) gene (60). The glucocorticoid response unit (GRU) in this gene promoter contains two GREs and three binding sites (AF1, AF2, and AF3) for accessory factors. The maximal glucocorticoid response is observed only when all these elements are occupied by their cognate proteins (61). Hepatic nuclear factor 4 (HNF4) and chicken ovalbumin upstream promoter factor (COUP-TF) bind to AF-1 and act as accessory factors for the glucocorticoid response (62). HNF3 and members of the CCAAT-enhancer binding protein (C/EBP) family bind to AF-2 (63), while the AF3 element of this gene is also bound by HNF4 and COUP-TF, all these factors acting as modulators of the GC response (62).

The mouse proliferin gene (pIfG) contains a composite element in which a GRE overlaps with an AP-1–binding site. On this element, the crucial determinant of GR function is the ratio of the two AP-1 family subunits, c-Jun and c-Fos, in the composition of the AP-1 complex, which binds DNA. GR enhances transcription from pIfG when AP-1 is composed of Jun-Jun homodimers, whereas it represses transcription when AP-1 is composed of Fos-Jun heterodimers (64), illustrating how a single DNA element can be differentially regulated by the composition of the factors binding to it.

A DNA-bound GR may also be influenced by additional factors. For example, the DNA-binding retinoblastoma protein Rb is capable of potentiating GR-mediated transcriptional activation (25), but this potentiation requires a cofactor, hBrm (a human homologue of the yeast protein SW12/SNF2).

### *GR-Mediated Activation of Transcription Independent of DNA Binding*

Not all effects of GR on transcriptional activation result from direct binding of receptor homodimers to canonical GREs. GR can mediate transactivation from some gene promoters without binding to DNA through protein-protein interactions between the receptor and DNA-bound transcriptional activators and/or components of the basal transcription machinery (54). This is the case for AP-1–controlled target gene promoters lacking GR binding sites (e.g., collagenase A) when the AP-1 complex is composed of a Jun-Jun homodimer (64).

Other examples of GR-mediated DNA-independent activation include an interaction between the receptor and STAT5 to amplify prolactin-stimulated transcription of the β-casein gene (29) and GR potentiation of transcriptional activation by STAT3 from an IL6 response element (30). GR and NF-IL6 (C/EBPβ) also appear to interact in a manner that allows each to enhance transcriptional ac-

tivation by the other from either a GRE or an NF-IL6 response element (66). It is important to note that in some of these examples (STAT3, NF-IL6) this transactivating effect is mutual. Bourk et al. reported that GR specifically potentiated the induction of transcription by C/EBPβ from the herpes simplex virus thymidine kinase (HSV tk) gene promoter proximal regulatory region in the absence of a GRE (67). Interestingly, these experiments suggest there is no direct contact between C/EBPβ and GR and that the interaction is instead mediated by cofactors.

### B. GR-Mediated Repression of Transcription

The mechanisms of transrepression of genes by GR are not as well characterized as those of the receptor's transactivating functions, and elucidation of these mechanisms has emerged as a topic for intense study (68). Repression of GC-controlled genes helps to control many aspects of differentiation, development, growth control, and homeostasis (69) and may be more important for normal development than transactivation, a notion supported by data from Reichardt and colleagues (70), who replaced the wild-type GR in mice with a mutated GR ($GR^{dim/dim}$), which does not bind to GREs. These mice are thus capable of transrepression but not transactivation of GC-controlled target genes (70). In contrast to $GR^{-/-}$ mice, $GR^{dim/dim}$ mice develop normally and are generally healthy, revealing that gene activation through GR binding to classical GREs is not necessary for development and survival in this model. $GR^{dim/dim}$ mice are also defective in repression of at least some genes that are regulated through nGREs as they are unable to repress the proopiomelanocortin (POMC) gene; as a consequence these mice exhibit elevated expression of this hormone.

Two principal mechanisms for repression by GR have been suggested, based on whether or not it is necessary for the receptor to bind DNA to transrepress target genes (Fig. 2). Another layer of complexity affecting the repressive effects mediated by the steroid receptors, including GR, has recently been proposed, where repression arises as a result of competition for limiting amounts of common mediators such as CBP/p300 (71,72).

#### *DNA-Dependent GR Repression of Transcription*

Competition between GR and positive transcription factors for binding to an overlapping site on the promoter region of genes is one mechanism that results in transcriptional repression (reviewed in Ref. 27). Several genes repressed by GR contain nGREs, to which, at least in vitro, GR binds. Examples include the genes encoding bovine prolactin (18), human osteocalcin (73), rat POMC (74), mouse proliferin (64), human corticotropin-releasing hormone (CRH) (75,76), human insulin (77), human interleukin-1β (78), and rat type 1 vasoactive intestinal polypeptide (79). The sequences of these nGREs usually differ substantially from the se-

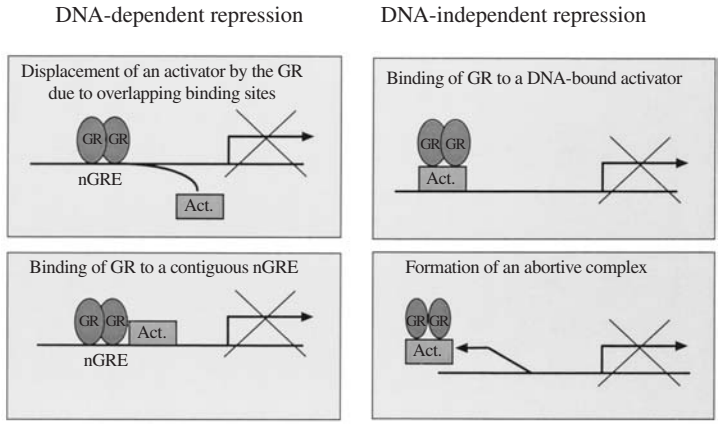


**Figure 2** Mechanisms of transcriptional repression by GR. In some cases receptor binding is required for repression of target genes by the activated receptor (upper and lower left), while in other situations direct binding of DNA is not necessary (upper and lower right).

quences of positive GREs (80). In most cases these elements overlap with other transcription factor–binding sites, suggesting a model involving competitive DNA binding.

It is unclear whether the GR bound to nGREs is functioning as a repressor molecule per se or whether it is acting as a transactivating competitor. While such competition may be considered a form of repression, low transcriptional rates are nearly always observed from these sequences, and the possibility that GR is functioning as a weak activator and simply displacing the stronger transactivators in these cases has not yet been conclusively ruled out.

The bovine prolactin promoter contains several nGREs, and one of these sites, termed PRL3nGRE, confers increased basal activity in the absence of GR via the binding of constitutive positive transcription factors. The activated GR represses this activity by displacement of these factors (81). In the case of PRL3nGRE only one GR moiety seems to contact the DNA (81,82).

In the case of the human osteocalcin gene, an nGRE overlaps the TATA box of the gene and the GR and TATA-binding protein (TBP) bind to their cognate sites in a mutually exclusive manner. Transient overexpression of TBP or a mutation in the promoter region that abrogates receptor binding prevented repression of the gene in the presence of glucocorticoids (73). The POMC nGRE may be unusual in that three GR monomers are thought to bind to it (74). However, this sequence only functions in the context of the POMC promoter and does not behave as an nGRE when fused to a heterologous promoter, implicating additional pro-

moter-bound factors in the repression of the POMC gene (see below). Glucocorticoid-mediated repression of human corticotropin-releasing hormone gene transcription also appears to involve monomeric GR binding, in contrast to the readily formed dimeric receptor complex observed with a positively regulated GRE (75).

These results suggest that repression through some nGREs may involve GR monomer interaction with the DNA, leading to a distinct conformational change, which precludes positive regulatory activity. On the other hand, results from the study with $GR^{dim/dim}$ mice argue against monomers being active in repression through nGREs, since with the loss of GR dimerization, negative regulation of prolactin mRNA expression was lost (70). However, in addition to losing their dimerization properties the receptors in these mice have also lost the ability to bind to DNA. It cannot, therefore, be determined whether a reported increase in PRL mRNA is due to an inability to bind DNA or a failure to dimerize.

As mentioned above, in the mouse proliferin gene GR and AP-1 bind to a composite site. On this element, the hormone-bound GR is inactive in the absence of c-Jun and inhibitory in the presence of Fos-Jun heterodimers (64). These researchers demonstrated an in vitro interaction between GR and c-Jun and, based on these observations, proposed that the GC response through this element was dependent on both protein-protein and protein-DNA interactions (64).

### *DNA-Independent Repression of Transcription by GR*

Nuclear receptor–mediated repression of gene transcription not requiring DNA binding by the receptor is thought to principally occur through transcriptional interference mediated via protein-protein interactions. Such interactions may repress transcription in two ways. In the first case, GR interaction with a positive acting transcription factor hampers DNA binding of that protein, while in the second model, GR interaction with a DNA-bound factor inhibits its transactivating properties. The best-studied examples of the latter mode of repression are the interactions between GR and the transcription factors AP-1 and NF-κB (see below). Repression of collagenase transcription was attributed to GR-mediated interference with AP-1 binding to or activity on its cognate site (83,84). The interference was mutual in that elevated expression of AP-1 or its activation by tumor promoters, pro-inflammatory cytokines, or growth factors inhibited GRE-controlled promoters. This provided further evidence for a protein-protein interaction, which was subsequently demonstrated by immunoprecipitation experiments. In vivo footprinting analysis carried out on the endogenous collagenase promoter (85) or on the tyrosine aminotransferase gene (86) revealed no major change in the level of occupancy of the respective AP-1– and GR-binding sites under repressed conditions. This showed that DNA-binding activity of AP-1 to the collagenase promoter is not altered under repression, suggesting that GR and the AP-1 complex interact without affecting each other's DNA-binding ability.

In contrast to ligand-dependent repression, Liu et al. have provided evidence that ligand-induced conformational changes of GR are not required in transrepression of the collagenase promoter (87). Their conclusion was based on studies with a GR mutant, GRL753F, which required a 200- to 300-fold higher concentration of GC than the wild-type receptor to transactivate a MMTV-CAT reporter gene, while in a reporter gene carrying the AP-1–inducible collagenase gene promoter, the concentration of GC required for 50% transrepression was the same for mutant and wild-type receptors. Significantly, this indicates that the activation and repression functions of GR are separable.

There are several other cases of repression mediated by GR independent of DNA binding by the receptor. The serum/glucocorticoid-inducible protein kinase (*sgk*) gene promoter, for example, contains several functional p53-binding sites and is repressed by GR through interference with the transactivation activity of p53 (26). The orphan nuclear receptor Nur77 is a mediator of the CRH induction of POMC transcription, and GCs inhibit POMC induction by antagonizing the Nur77-dependent transcription (88). GR interferes with Oct-1 activity mediated via an octamer element from the immunoglobulin heavy-chain intron enhancer through protein-protein interaction involving the homeo subdomain of Oct-1 (89). Others, by contrast, found that this GR-mediated repression does not apply to Oct-1 but to the lymphocyte-specific factor Oct-2A (55,90). The reason for the discrepancy between these observations is unclear.

### C. Cofactors Involved in Positive and Negative Transcriptional Regulation by GR

GR transcriptional activity is positively regulated by a number of cofactors, including SRC-1 (91), CBP/p300 (71), GRIP1/TIF2 (92), TIF1β (93), hBrm (94), and the η member of the 14-3-3 protein family (95). In addition, it was recently shown that homologs of the Ada adapter and Gen5 proteins from mammalian cells also enhanced the gene activation potential of the AF-1 domain from human GR (96). In contrast RAP46 (97) and RIP140 (98) have been shown to inhibit transcriptional activation by GR. In the latter study, RIP140 acted as an dominant negative inhibitor of all GR-mediated activities, including positive regulation through a GRE, synergy through cross-talk with AP-1, and negative regulation through an nGRE and cross-talk with NF-κB. This was mediated by a novel mechanism in the control of GR activity, with RIP140 blocking interactions between GR and coactivators to inhibit receptor-mediated transactivation (98). RIP140 appears to have a dual function within the cell, since this molecule was capable, albeit weakly, of enhancing transactivation by other nuclear receptors such as AR and, at some concentrations, ER (99). This type of dual role for cofactors has been described for NSD1, TIF1β, and recently for Zac1 (100–102). Hence, the primary role for these proteins may be to act as coregulators, fine-tuning a complex network of genes.

Importantly, the repressive activity of RIP140 did not occur when the GR was bound to the antagonist RU486. This is mirrored by data from work with other cofactors, where functional interactions with nuclear receptors occur in the presence of agonistic ligands but not antagonists (99). The majority of cofactors, including RIP140, have been shown to interact directly in a hormone-dependent manner with the AF-2 domain of steroid hormone receptors and are therefore called AF-2 cofactors (20,103). This interaction often involves one or several short conserved peptides with the amino acid sequence LXXLL (L = leucine, X = any amino acid) in the cofactor, which serves as an NR box or signature motif (104,105). Structural studies have revealed that the AF-2 domain undergoes an agonist-dependent conformational change, which facilitates coactivator interactions, whereas the binding of antagonists induces an alternative conformation in this domain, which appears to hinder coactivator binding (reviewed in Ref. 105). However, GR is also capable of functional interactions with coactivators that do not have the LXXLL motif (97).

Some coactivators (CBP/p300, SRC-1) have been shown to contain intrinsic histone acetyltransferase (HAT) activity, thus linking gene activation by receptors to histone modification and chromatin alterations (47,106). Recently, it has been shown that the largest member of the TBP-associated factors (TAFs), $TAF_{II}230/250$, also contains HAT activity, and furthermore, factors interacting with p300, such as P/CAF, are involved in localized chromatin remodeling and loss of chromatin repression. Transcriptional activation is thus a process that requires a number of factors, which have to be assembled in a regulated manner on the promoter to achieve an efficient response to any given signal (47,107).

### *Competition for Coactivators/Cofactors*

The conflicting results from in vivo DNA footprinting data, showing no change in the occupancy of the DNA sequences required for binding by AP-1 and GR during transrepression (85,86), and in vitro protein-DNA interaction studies, which demonstrated a requirement for both protein-protein and protein-DNA interactions (64), have led to an alternative concept of GR-mediated transrepression. This postulates that rather than GR interfering directly with the binding of the transcription factor to DNA, the two proteins may interact through transcriptional intermediary factors (TIFs), thus allowing both positive and negative effects on transcription (108). In line with this, CBP/p300 has been implicated in mediating the transcriptional effects both of nuclear receptors and the AP-1 and NF-κB transcription factor families (see below and Ref. 71). It was proposed that the inhibition of AP-1 activity by GR or RAR is the result of competition for limiting amounts of CBP/p300, since overexpression of CBP could relieve GR- or RAR-dependent AP-1 repression in transfection studies. Furthermore, data from our own laboratory demonstrate that overexpression of TIF-2 can partially rescue the

RIP140 inhibition of GR-mediated activation, supporting a mode of action based on competition between corepressors and coactivators (80). In line with our observations, CBP has recently been shown to rescue GR-mediated repression of RelA-dependent reporter gene transactivation.

However, competition by GR and AP-1 for limiting amounts of cofactor does not adequately account for all situations, since Pearce et al. demonstrated that when GR- and AP-1–binding sites were placed in a *cis* position (called a paired site) separated by ≥26 base pairs, synergy between GR and AP-1 was observed, irrespective of the composition of the AP-1 dimer (109). This may be due to the factors involved cooperating in the recruitment of CBP. Furthermore, when a site for only one of the factors was present, the other factor had an inhibitory effect (mutual inhibition) regardless of whether AP-1 was composed of Jun-Jun or Jun-Fos, suggesting that in this situation there may be competition for coactivators. However, this hypothesis does not explain the results obtained from paired sites with separations of 14–18 base pairs, since in this instance GR synergized with Jun-Jun and repressed Jun-Fos (109).

## V. Glucocorticoid Receptor and Inflammation

The use of synthetic glucocorticoids in the treatment of inflammation and autoimmune disease has been widespread, often with very successful outcomes. The evidence suggests this is due to the potent anti-inflammatory and immunosuppressive effects mediated by GR, including the downregulation of a number of pro-inflammatory cytokines and adhesion molecules (110). The majority of promoters for these genes do not contain recognizable GREs, and the mechanisms through which GR exerts its effects have been the source of intense study over recent years (reviewed in Ref. 111). The transcription factor NF-κB functions in a diametrically opposed manner to GR in inflammation and immunity, serving to upregulate pro-inflammatory cytokines, and it has been demonstrated that a key target for the immunosuppressive and anti-inflammatory activities of GCs is the repression of this family of transcription factors (28,112). Understanding the physiological antagonism between these molecules may thus be vital for the development off better and more sophisticated anti-inflammatory and immunosuppressive glucocorticoids.

Several lines of evidence point to an analogous mode of action between AP-1 and NF-κB repression by GR. Analagous to the situation with AP-1, GR interferes with NF-κB activity and negatively regulates the target gene promoters of this complex without binding to DNA (Fig. 3) (113). The DBD of GR is implicated in the transrepression of both molecules, and this region is thought to directly contact c-Jun (108) and the RelA subunit of NF-κB (114). Again as with AP-1, in vivo footprinting of the endogenous ICAM-1 promoter containing an NF-κB–binding site also showed that the receptor did not displace the NF-κB

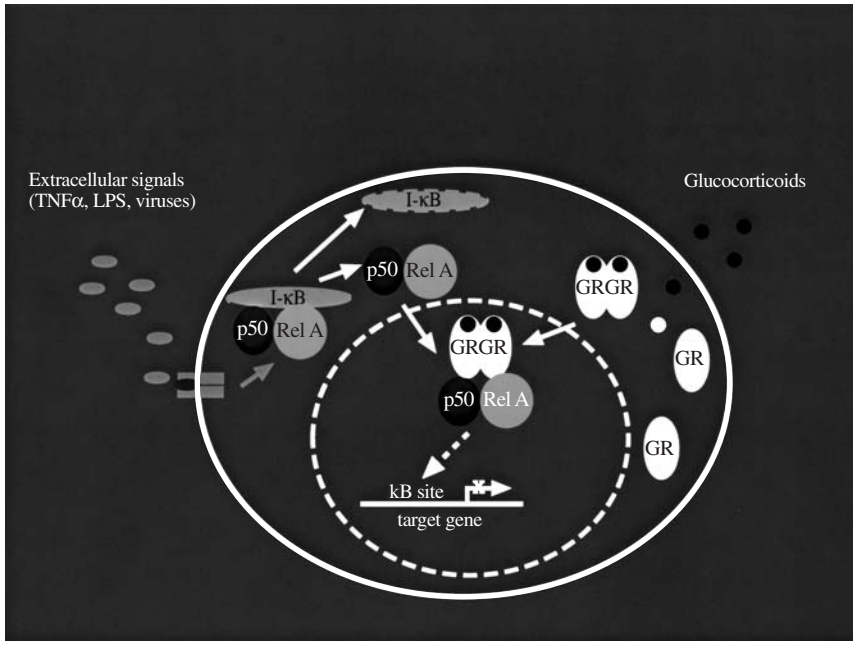


**Figure 3** Cross-talk between GR and NF-κB transcription factors. Activated GR is capable of repressing genes activated by NF-κB. This is mutual antagonism, as NF-κB is also capable of repressing GR-mediated transcriptional activation. GR may also modify the expression pattern of the NF-κB inhibitor molecule I-κB (see text).

complex during GR repression (J. Lidén, I. Rafter, M. Truss, and S. Okret, Biochem Biophys Res Commun 2000; 14:1008–1014.). In this regard, it is important to note that several dimerization-deficient GRs that do not bind to GREs as homodimers can still repress AP-1– and NF-κB–regulated genes (39,115). Synthetic ligands have been described for GR that are unable to induce transactivation by the receptor also retain the ability to transrepress AP-1 and NF-κB (20). These have been termed dissociating ligands.

The Rel homology domain in the p65 subunit of NF-κB (Rel A) was required for the physical interaction with GR in vitro. In addition, the transactivation domain was needed for functional interaction between these transcription factors (113). The DBD of GR is a further prerequisite for association between the two partners in vitro (114). Using chimeric receptors, exchanging the different functional domains of GR with domains from a nonactivating nuclear receptor, the DBD was shown to be the major domain involved in repression. This was further narrowed down to the C-terminal zinc finger, and finally it was demonstrated that two individual amino acid exchanges within this region were sufficient to abolish the majority of the repressive effect (39). However, other researchers have claimed

that additional domains may contribute to the maximal repression of NF-κB (reviewed in Ref. 116).

The interaction between GR and NF-κB leads to mutual antagonism, i.e., each factor represses the other, and their physical interaction may require an additional factor or factors. One potential candidate is CBP, which is known to interact with both proteins and could conceivably mediate their functional antagonism, possibly by altered assembly of the preinitiation complex. On the IL-6 gene promoter, for example, a strong synergism between p65 and CBP/p300 was demonstrated, and it was further shown that NF-κB–induced activation of this gene was repressed by GC treatment (117,118). Recently, Sheppard et al. showed that, as with AP-1, increased levels of CBP or SRC-1 can prevent inhibition of GC-mediated repression of NF-κB activity and NF-κB–mediated repression of GR activity (119). How this would be achieved in vivo is unclear, since a simple mechanism based on competition for limiting cofactors leads to the notion that activation of either transcription factor would ultimately downregulate any CBP-requiring promoter, which includes the majority of promoters examined to date. It may be instead that CBP alters the structural conformations of these proteins when both are bound to it, consequently modifying their transactivating potentials (119).

Two publications have shown that GR upregulates the protein levels of the NF-κB inhibitory protein I-κBα, resulting in repression (120,121). While this may provide an additional measure of control over the inflammatory response mediated by GR in some cells, the lack of global I-κBα upregulation and data from work with mutant receptors argue against this being the major mechanism of NF-κB–activated gene repression (122,123). Dimerization-deficient GR mutants and $GR^{dim/dim}$ mice incapable of transactivation and consequently incapable of I-κBα upregulation are still capable of transrepressing NF-κB activity (70,122). In concordance with this, dissociating GCs also retain the ability to repress NF-κB activity while losing their transactivation function and without inducing I-κBα (118,124). There may still be a role for I-κBα in GR-mediated transrepression of NF-κB, however, as recent advances have revealed there are many other possible levels of regulation of I-κBα (125–127).

## VI. Summary

The multifunctional role of GR has continued to attract the interest of researchers over the past 30 years. The anti-inflammatory properties of GR are of great biomedical interest and importance, and the characterization of the mechanisms employed by GR to mediate those effects remains a highly competitive and topical field of study. While much attention has been devoted to the anti-inflammatory properties of GR, evidence is mounting that there may be a pivotal role for the receptor in normal immune function (discussed in Ref. 125). Recent advances, such

as the development of $GR^{dim/dim}$ mice, have greatly enhanced our understanding of the relative importance of the transactivation and transrepression activities of GR. However, much work remains to fully characterize the role of the receptor in these mice. The discovery of ligands that can dissociate between the dual roles of the receptor and target GR interaction with individual factors is another useful development. Such specific ligands may be of great medical importance, since they may be able to eliminate or minimize undesirable side effects of long-term glucocorticoid treatment. As our knowledge improves it is hoped that more selective ligands may be developed, which can further discriminate among the modes of action of GR and thus provide better tools for the treatment of inflammatory and autoimmune diseases.

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## Discussion

**Dr. Hochhaus:** In the last years, a number of papers were published which demonstrated differences in the activities of certain GC on transrepression and transactivation. Are these differences likely to result in steroids with higher selectivity?

**Dr. Okret:** It is difficult to evaluate the selectivity of the reported GC with regard to transrepression and transactivation. Furthermore, we know very little about the relative contribution of transactivation versus transrepression for complex in vivo responses like the anti-inflammatory, osteoporotic, or antiproliferative effects of GC. However, I believe that new discriminating GC will show a higher selectivity with fewer side effects. A GC with transrepressing activity but no transactivating activity will most likely be efficient as an anti-inflammatory drug with less diabetogenic effect, since the first activity is generally believed mainly to involve transrepression, while transactivation of gluconeogenic enzymes in the liver is thought to be important for the latter effect.

**Dr. Edsbäcker:** Do you have any comments regarding the current status and trends regarding the β-subunit of the receptor? Does it exist, is it expressed to an increased extent in patients considered as nonresponders, and does it contribute to the nonresponsiveness?

**Dr. Okret:** We are not working with the GRβ. However, in the literature the possible effect of GRβ as an inhibitor of GRα activity is a matter of debate. According to my opinion, the crucial experiment, i.e., determination of the levels of endogenous GRβ versus GRα in one cell, is still missing. Before this is determined, it is difficult to draw any conclusion with regard to the contribution of GRβ to GC insensitivity.

**Dr. Hamid:** We recently reported the increased expression of GRβ immunoreactivity in steroid resistant asthma and in T cells in response to cytokine stimulation. We are currently using PCR to demonstrate the increased amount of GRβ mRNA compared to GRα in response to cytokine stimulation in T cells.

**Dr. Okret:** I cannot add more than that I responded to the question by Dr. Edsbäcker.

**Dr. Karin:** How did you measure repression of NFκB transcriptional activity through endogenous GR in U937 cells? Did you reintroduce GR mutants to receptor-negative U937 cells?

**Dr. Okret:** We measured repression of NFκB activity in the U937 cells by the endogenous GR by determining ICAM-1 expression. ICAM-1 expression was

repressed in these cells following GC treatment. It has previously been shown that GC-mediated repression of ICAM-1 transcription is mediated by the NFκB site in the ICAM-1 promoter (van de Stolpe et al. Am J. Respir Cell Mol Biol 1993; 8:340–347). We don't have access to GR-negative U937 cells and are not aware that such cells exist. Thus, we have not been able to perform the experiment of reintroducing GR in GR-negative U937 cells.

**Dr. Brattsand:** What is the turnover time of the activated GC receptor? Does it differ between the dimeric and monomeric forms? Does the ligand need to be metabolically stable all the time in the complex, or is it sufficient that the ligand just initiates the conformational changes?

**Dr. Okret:** Many years ago we determined the turnover rate of the GR in rat hepatoma cells (H4IIE). The half-life of the GR in the absence of ligand was around 25 hours, while in the presence of dexamethasone it was 12 hours. Whether there are differences in half-lives of the GR in different cell types, we do not know. Furthermore, we do not know whether there is a difference in the half-life of dimeric versus monomeric GR. This would be very difficult to determine in the cell. With regard to the fate of the ligand following binding and induction of the conformational change in the GR, this is to my knowledge not known. It is not known whether the ligand has to sit in the ligand-binding pocket all the time to maintain the conformational change in the GR or whether it can dissociate following induction of the structural changes. However, it is known that the structural changes involve movement of helix 12 in the ligand-binding domain to become a lid over the ligand-binding pocket, creating a surface for coactivator interaction. This "embedding" of the ligand results in a lower dissociation rate of ligand from the receptor.

**Dr. Schleimer:** There is an interesting counterregulation between GR and NFκB. Not only does GR antagonize NFκB by physical interaction, but it also induces IκB. As you pointed out, NFκB in turn can inhibit GR effects by the same interaction. To complete the symmetry, NFκB appears to be an inducer of GRβ. Over the next few years, we will be discussing the relationship between β-adrenergic agonists and steroids, as combination preparations are coming onto the market. Since the catalytic subunit of adenlyl cyclase is associated with, and necessary for, NFκB activation, I wonder how elevations of cAMP might alter NFκB activation and its influence on the actions of glucocorticoids? A related question is whether you have confirmed the results of Eickelman et al. showing ligand-independent activation of GR by salmeterol (or another β agonist)?

**Dr. Karin:** If cAMP potentiates NFκB activity, it may be more difficult to inhibit it by glucocorticoids. However, the clinical activators of NFκB that matter most for asthma—TNF and IL-1—don't have much effect on intracellular

levels of cAMP. On the other hand, the effect of cAMP on T-cell apoptosis could also be due to modification of signaling via the T-cell receptor, which has profound and complex effects on apoptosis.

**Dr. Brattsand:** We have studied how RU486 affects the cytokine blocking efficacy of the $\beta_2$-agonist formoterol in a bronchial epithelial cell model. While RU fully blocks the anticytokine effects off budesonide, it does not at all counteract the cytokine-modulating activity of formeterol. This suggests that the cytokine-modulating effect of $\beta_2$-agonists is not mediated by the GC receptor in these cells.

**Dr. Georas:** Regarding the GRβ-isoform, John Cidlowski has a recent paper showing that GRβ can bind DNA, and antagonize transacting effects of GRα (Oakley RH, Jewell CM, Yudt MR, Bofetiado DM, Cidlowski JA. The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. J Biol Chem 1999; 274:27857–27866). In the context of the GR using coactivators and possibly corepressors to regulate gene expression, what is the role of ligand binding for the GR to function? The current paradigm for many nuclear receptors is that ligand induces a conformational change favoring the association with coactivators over corepressors. How do you reconcile this with: (1) the need for nuclear translocation of the GR and (2) the observation that the GR can both activate and repress many genes?

**Dr. Okret:** As you mention, it is generally believed that the ligand induces a conformational change of nuclear receptors, which favors association with coactivators over corepressors. However, in the case of the GR, no interaction with classical corepressors like N-Cor or SMRT has been demonstrated. One can speculate that the requirement for corepressor binding to the GR is less important as an additional control level of GR activation exists, namely the requirement for nuclear translocation. In contrast to most other nuclear receptors, the GR is localized in the cytoplasm in the absence of ligand, and it only translocates to the nucleus following ligand binding. The role of the corepressor seems mainly to be to keep the receptor in an inactive state rather than to be involved in negative gene regulation. In contrast, competition for coactivators has been suggested to play a role in negative gene regulation. However, several observations exist that argue against this mechanism for negative gene regulation by the GR. Furthermore, data are also available that indicate that negative gene regulation may not require the same conformational changes in the receptor as positive gene regulation.

# 5

# Relationship of Dose- and Time-Dependent Corticosteroid Responses to Receptor Turnover

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## I. Introduction

The initial step in producing biological responses to administered corticosteroids is the diffusion of unbound drug from plasma into cells for interaction with cytosolic receptors. Some types of cells or tissues show responses rapidly, while others have a lag phase or slow onset of effect caused by a gene-mediated mechanism of action (1). Both types of responses last considerably beyond the time course of active drug in the system. For example, methylprednisolone, a moderately lipid-soluble compound, rapidly distributes into various cells and tissues and has a pharmacokinetic $t_{1/2}$ of 2–3 hours in humans and a duration of biological effects of 18–36 hours, depending on dose. An array of pharmacokinetic/pharmacodynamic (PK/PD) models have been proposed to rationalize and quantitate these response patterns in both animal and human systems. This chapter describes modeling efforts based on physiological principles that produce plausible methods of quantitating and predicting the effects of corticosteroids. The role of dose and timing or duration of drug administration in actual or expected pharmacological responses from corticosteroids will be emphasized.

## II. Dynamics of Rapid Steroid Effects

A family of relatively simple pharmacodynamic relationships was initially evolved (2,3) to describe the "rapid" or non–gene-mediated effects of corticosteroids on cell trafficking patterns of basophils (measured as whole blood histamine), T-helper cells, and other lymphocytes. It is assumed that corticosteroids cause an immediate change in the affinity of cells for distribution sites in an extravascular compartment. The decline in cell number, such as shown in Figure 1, is attributed to inhibition of cell movement from extravascular sites into blood ($k_{in}$); blood cell replenishment ($k_{out}$) occurs when steroid concentrations in plasma fall below the concentration (C) producing 50% inhibition ($IC_{50}$ value). The $IC_{50}$ value for methylprednisolone effects in humans is similar in magnitude to its $K_D$, or drug-receptor equilibrium dissociation constant. The type of equation used to quantitate indirect effects such as in Figure 1, where the drug inhibits $k_{in}$ is:

$$\frac{dR}{dt} = k_{in}\left(1 - \frac{I_{max} \cdot C}{IC_{50} + C}\right) - k_{out} \cdot R$$

where $I_{max}$ represents the maximal fractional inhibition of $k_{in}$ (3).

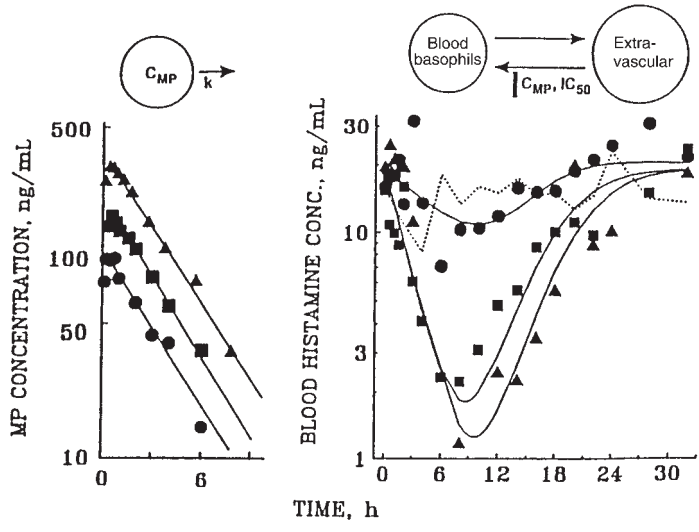


**Figure 1** Pharmacokinetic (left) and pharmacodynamic (right) models of cell trafficking (blood basophils measured as whole blood histamine) following doses of 10 mg (●), 20 mg (■), and 40 mg (▲) of methylprednisolone in one male subject. Equations for the suppression model (shown at the top) fitted baseline and all dose levels simultaneously. Symbols are experimental data, lines are fitted with model. (Adapted from Ref. 2.)

The models for basophil cell (4) and helper T-cell (5) trafficking have allowed for accurate quantitation of cell movement between blood and extravascular sites and permits extrapolation of effects to a wider range of steroid doses and administration methods. The helper T-cell model includes the complexity of circadian rhythm in baseline behavior of these cells. Similar equations and patterns are applicable to adrenal suppressive effects of methylprednisolone (6) and fluticasone propionate (7) with an added complication of an irregular circadian synthesis and secretion of cortisol, which governs the baseline conditions.

In considering optimal dosage regimens, the hypothesis was made that a "steroid-sparing" effect could be achieved by designing dosage regimens such that a loading dose occupies all of the receptors as they recycle following drug elimination. This was tested and confirmed in human studies (8) monitoring basophil trafficking and adrenal suppression. We studied the pharmacodynamic responses to single (40 mg) and divided (20 mg, 5 mg) bolus doses of methylprednisolone in healthy men. Divided dosing offered improved pharmacodynamic availabilities (ratio of AUC of effect) of 1.38 for whole blood histamine and 1.92 for cortisol suppression. Thus, by dosing methylprednisolone in a prolonged or divided fashion, it is possible to administer lower doses to achieve equivalent or better effects than large single doses. Unfortunately, this applies to both beneficial and adverse effects.

## III. Role of Administration Rate

Gobburu and Jusko (9) performed computer simulations and a limited literature review to assess the role of drug-delivery rate on responses expected for drugs with indirect mechanisms of action. Figure 2 shows the simulated pharmacokinetic profiles following the administration of a moderate dose of a hypothetical drug as either an intravenous bolus or an infusion over various durations (6, 12, 24, 48, 72 h). The infusion rates varied more than eightfold, allowing the importance of rate of drug delivery on the efficiency to be assessed. This dose produced concentrations just above an $IC_{50}$ of 100 ng/mL for long periods of time. The pharmacodynamic profiles after bolus and infusion dosing show their expected behavior with the response being inhibited. Figure 2 depicts a marked reduction of the response from the baseline value after administration of the bolus dose. The extended delivery produced a similar maximum response with a fourfold increase in the AUC of effect. An optimum regimen will consist of a modest loading dose followed by continuous delivery to keep receptors occupied.

## IV. Liposomal Methylprednisolone

A demonstration of a pronounced steroid-sparing phenomenon caused by extended delivery and local retention occurred in an evaluation of the kinetics, receptor

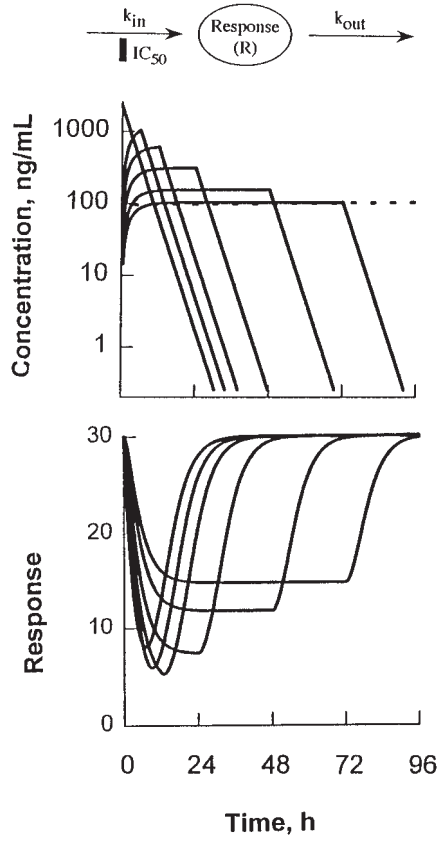


**Figure 2** Simulated time course of plasma drug concentrations (top) and inhibitory indirect responses (bottom) during and after infusions of a moderate dose of drug for the indicated time periods. (Adapted from Ref. 9.)

binding, and immunosuppression from solution versus liposomal formulations of methylprednisolone in rats (10). The latter produced marked tissue (liver and spleen) retention of the steroid and a 12-fold increase in receptor occupancy (Fig. 3). A pharmacodynamic measure of immunosuppression, inhibition of ex vivo rat splenocyte proliferation following stimulation with a mitogen, was augmented to an even greater degree by the liposomal drug. These studies show that prolonged local steroid concentrations can enhance steroid effects and offer promise that pharmacodynamic models will provide a basis for improved rationalization of steroid dosage regimens.

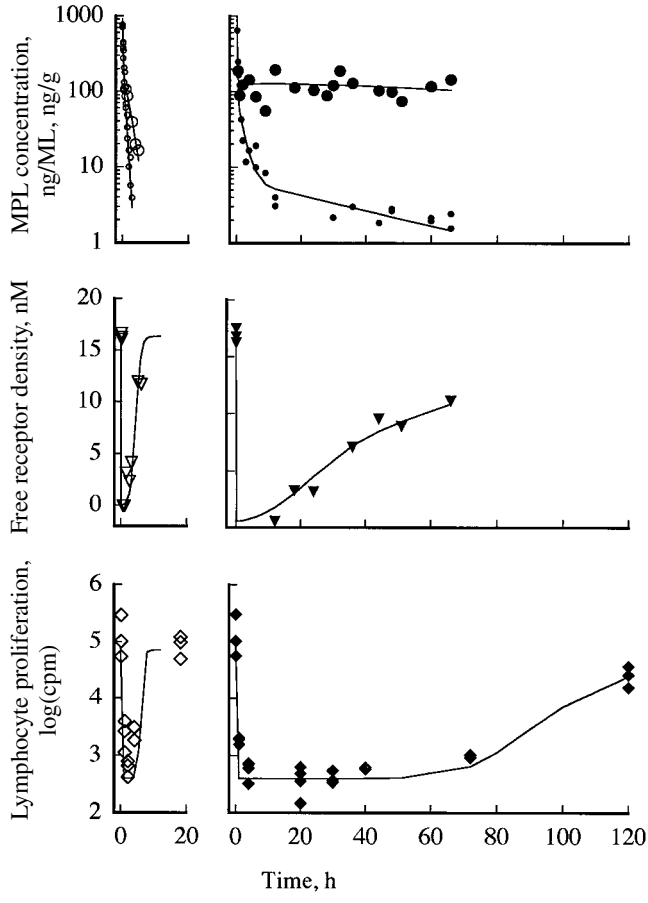


**Figure 3** Methylprednisolone concentrations as a function of time (upper panel) after 2 mg/kg iv dose in rat plasma (small circles) and spleen (large circles); receptor density in rat spleen (middle panel); and inhibition of splenocyte proliferation (bottom panel). Open symbols (left panels): drug solution. Closed symbols (right panels): liposomal formulation. Curves are produced by fitting of data to an appropriate pharmacokinetic/pharmacodynamic model. (Adapted from Ref. 10.)

## V. Drug Interactions

Corticosteroids exert their immunosuppressive properties partly by binding to intracellular steroid receptors and inhibiting the activities of transcription factors such as NF-κB (11,12). The inhibition of NF-κB results in an increase in an

NF-κB regulatory protein called IκB. IκB stabilizes NF-κB and prevents its entry into the nucleus of the cell, resulting a reduction in production of cytokines such as IL-1 and IL-2. With this blockage in synthesis of cytokines, T-cell proliferation in response to alloantigens is reduced. The net effect is inhibition of the inductive phase of cytotoxic T cells and prevention of graft rejection.

Sirolimus is a new macrolide immunosuppressive compound acting at the mid-late $G_1$ phase of the cell cycle through an original mechanism blocking transductional signals produced by the fixation of cytokines (e.g., IL-1, IL-2, and IL-6) to their membrane receptors (13,14). Sirolimus differs from cyclosporin A and steroids in its mode of action because at the $G_o$ phase, cyclosporin A acts by inhibiting IL-2 gene transcription and steroids act by decreasing cytokine (e.g., IL-1, -2, -6) and cell surface molecule (e.g., intercellular adhesion molecule-1, lymphocyte function–associated antigen-1) gene transcription. Thus, as prednisolone and sirolimus act through different mechanisms at the cytokine gene transcription or signal transduction levels, their combination may produce additive or synergistic therapeutic effects.

Drug interactions were studied using lectin-induced proliferation of isolated cell lymphocytes and whole blood lymphocytes from men and women as well as two-way mixed-lymphocyte reaction assays (15). Drug interactions were described with isobolograms and quantitated with the universal response surface approach for estimating the interaction parameter α (Fig. 4). All compounds inhibited more than 89% of control proliferative responses. In each assay, sirolimus was of similar or higher potency than prednisolone and 1.5-fold more potent in men than women. All combinations were profoundly synergistic ($\alpha >> 0$). These studies indicated that prednisolone and sirolimus synergistically interact in vitro, with

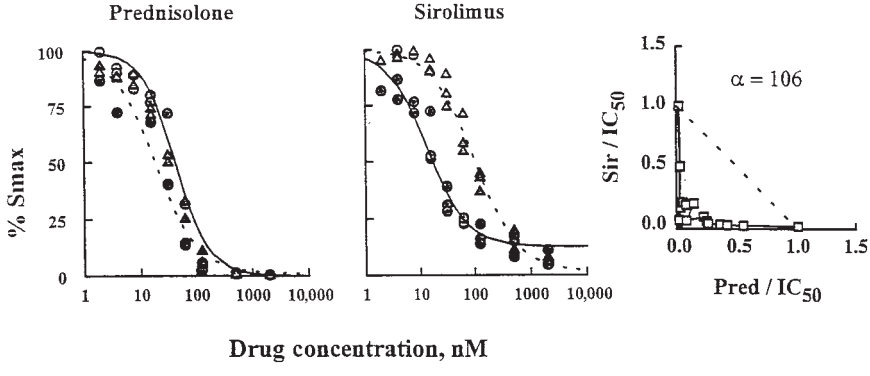


**Figure 4** Effects of prednisolone and sirolimus on inhibition of mitogen-stimulated human lymphocyte proliferation. The isobologram shows marked synergism when the drugs are included in various combinations. (Adapted from Ref. 15.)

gender and assay as additional factors, and that whole blood lymphocyte proliferation cultures are useful in assessing the nature and intensity of drug interactions.

## VI. Dynamics of Receptor Gene–Mediated Processes

The processes for glucocorticoid receptor (GR) regulation and receptor gene–-mediated effects of corticosteroids are depicted in Figure 5 and modeled as indicated in Figure 6. With their moderate lipophilicity, free corticosteroids easily diffuse into cells of target tissues. Our modeling assumes that free drug in plasma (D) is immediately accessible to cytosolic receptors. The receptors are also controlled by their synthesis ($k_{synGR}$) and degradation ($k_{dgrGR}$) rates. These steroids quickly bind to the glucocorticoid receptor located in the cytoplasm, and the complex immediately alters its configuration. Dissociation of heat-shock proteins (hsp) from the receptor occurs, including hsp 90, hsp 70, and hsp 56 (19). Without these heat-shock proteins, the steroid-receptor complex is subject to phosphorylation (20) and can translocate from the cytoplasm into the nucleus. Our models account for reversible ($k_{on}$, $k_{re}$) steroid-receptor binding and then first-order transfer ($k_r$) of the complex into nuclear-bound material [DR(N)].

In the nucleus, two units of the complex aggregate as a "dimer" (21). Zinc finger modules on the dimer bind to control sequences adjacent to the target gene. These control elements, which are palindromic repeats of a specific hexamer sequence, are called glucocorticoid-responsive elements (GRE) (22,23). The GRE is a transcription promotor element located 240–260 bases upstream from the starting point of transcription on the DNA template strand. With the interactions of RNA polymerase complexes, the steroid-receptor complex will activate the transcription of specific RNAs from the promoter. This is modeled with a distribution rate constant ($k_N$) between DR(N) and a transcription compartment (TC). In turn, a first-order transcription constant ($EF_1$) is used in the model to relate TC to the mRNA level of TAT. Finally, mRNA translocates to the cytoplasm (24), and it exists for a finite time governed by an elimination constant, $k_{dgr,\ TAT\ mRNA}$.

Expression of a specific protein occurs in the cytoplasm after the transcription of its mRNA. The mRNA is associated with ribosomal RNA (rRNA) for the translation of protein. The latter proceeds with the aid of transfer RNAs (tRNAs), which recognize the sequence codes on the mRNA template and carry specific amino acids as the materials to build the protein (25). The induced protein may be an enzyme (such as TAT) or have other regulatory functions (such as IκBα). The production ($EF_1$) and loss of TAT ($k_{dgr,TAT}$) were modeled as first-order constants.

Downregulation of GR mRNA after treatment with glucocorticoids has been studied in several in vitro systems. Two different mechanisms have been proposed. "Nuclear run-on" transcription techniques in liver nuclei isolated from ADX rats were used (26) to study transcription rates of GR mRNA. It was

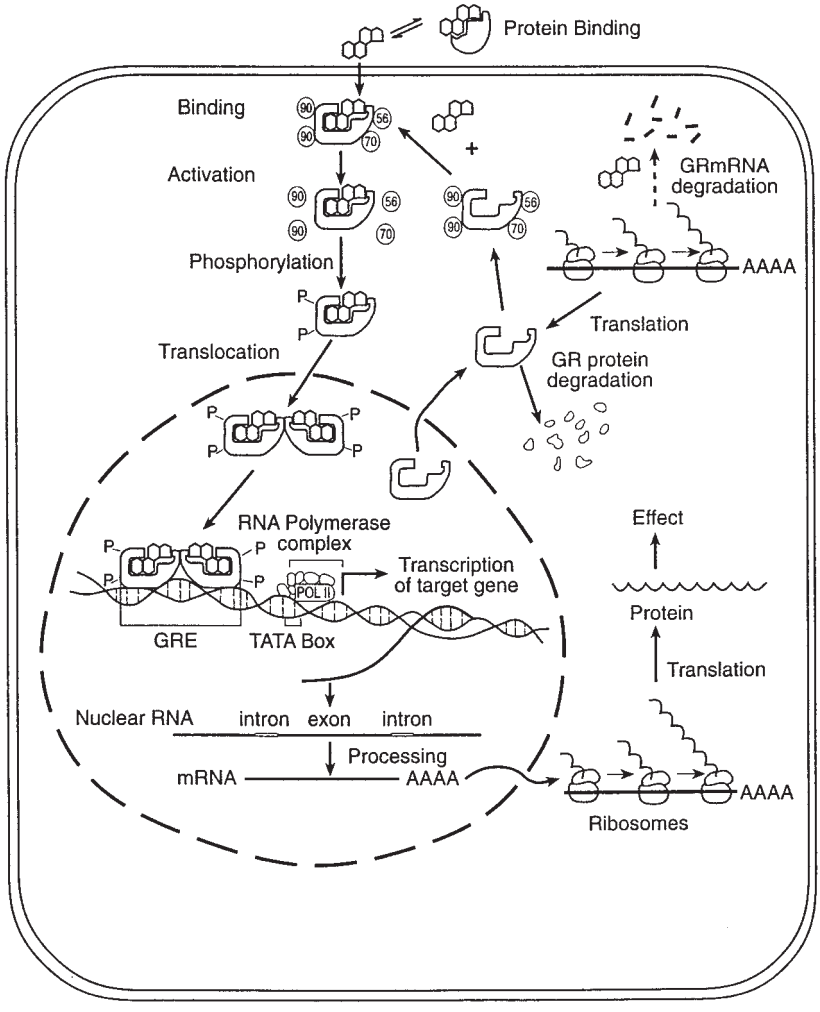


**Figure 5** Receptor gene–mediated pharmacodynamic model for corticosteroids. See text for details. (Adapted from Ref. 17.)

concluded that the downregulation of GR mRNA after treatment with dexamethasone was due to a decreased transcription rate for the messenger. The activated steroid-receptor complex in the nucleus may interact with its own gene and inhibit GR mRNA transcription ($k_{syn,GRmRNA}$) by interfering with the formation of a transcription initiation complex or physically blocking RNA chain elongation.

However, activated steroid-receptor complex in cytoplasm has been shown to decrease the stability of GR mRNA in mouse AT-20 cells (27). It was postu-

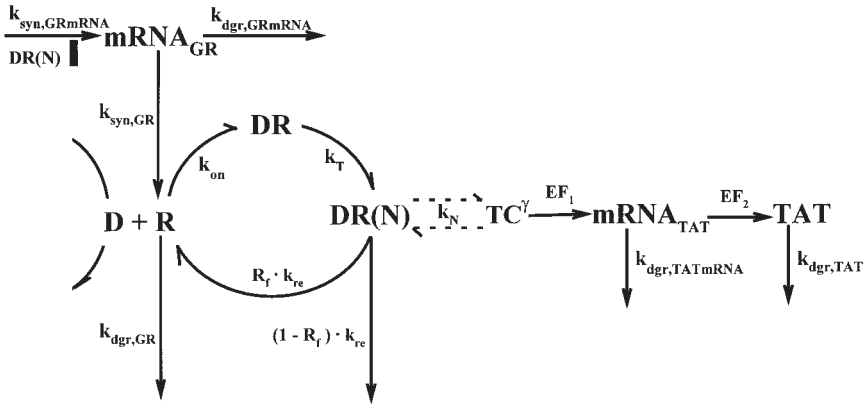


**Figure 6** Pharmacodynamic model of corticosteroid actions in rat liver. D, Steroid concentration at hepatic cytosol receptor site; R, free glucocorticoid receptor density; DR, steroid-receptor complex in the cytoplasm; DR(N), steroid-receptor complex in the nucleus; $mRNA_{GR}$, GR mRNA level; $k_{syn,GRmRNA}$, transcription rate of GR mRNA; $k_{dgr,GRmRNA}$, degradation rate of GR mRNA; $k_{syn,GR}$, translation factor for GR synthesis; $k_{dgr,GR}$, degradation rate of GR; $k_{on}$, association rate constant for steroid receptor binding; $k_T$, first-order rate constant for the translocation of steroid-receptor complex into the nucleus; $k_{re}$, overall turnover rate of DR(N); $R_f$, recycling fraction; TC, transcription compartment in which steroid-receptor complex initiates the transcription; $k_N$, distribution rate constant between DR(N) and TC; $\gamma$, a power term; $mRNA_{TAT}$, TAT mRNA level; $EF_1$, transcription factor for TAT mRNA induction; $k_{dgr,TATmRNA}$, degradation rate constant for TAT mRNA; TAT, TAT activity level; $EF_2$, translation factor for TAT induction; $k_{dgr,TAT}$, degradation rate constant for TAT.

lated that steroid-receptor complex may replace some binding proteins on GR mRNA, which are essential for the protection from RNAses (28). Steroid-receptor complex may therefore increase the degradation rate constant of the messenger ($k_{dgr,GRmRNA}$). Regardless of the mechanism of GR mRNA downregulation, the expression of new GR protein will be suppressed after glucocorticoid treatment due to downregulation of GR mRNA, which is the translation template for GR protein (16).

The fate of translocated steroid-receptor complex after the transcription of target genes is not yet well understood. Some of these complexes may return to the cytoplasm after they are dissociated from the GRE (16). This process is referred to as glucocorticoid receptor "recycling." While some of the receptors are degraded in the process $[(1\text{-}R_f)\ k_{re}]$, the rest of the them may be reassembled ($R_f \cdot k_{re}$) with heat-shock proteins and become active receptors. These receptors are ready to bind to steroids and reinitiate the whole process. Munck and Holbrook (29) employed this recycling theory and demonstrated rapid kinetic behavior with cyclic

models for glucocorticoid receptor complexes in rat thymus cells. Receptor recycling may play an important role in the recovery from GR downregulation, and the fraction recycled was captured as a constant, $R_f$.

## VII. Pharmacodynamic Studies

Tyrosine aminotransferase (TAT) is a hepatic enzyme and commonly employed biological maker used to study the delayed responses of corticosteroids. TAT induction in rat liver was modeled as a receptor-mediated effect of prednisolone in our first- and second-generation models (30–32). Quantitative Northern hybridization methods (33) allowed us to determine GR messenger RNA (mRNA) and TAT mRNA levels at different time points after corticosteroid treatment. This resulted in a third-generation model for corticosteroid pharmacodynamics in which the roles of GR mRNA in GR downregulation and TAT mRNA in TAT induction by MPL were examined (34). Our present fourth-generation model (35–37) is shown in Figure 6. Our experimental measurements can only capture cytoplasmic events, and processes occurring in the nucleus are modeled as a "black-box" based on a transit-compartment approach (38).

The model was applied and derived from the PK/PD data shown in Figure 7. Following a 50 mg/kg IV bolus dose of methylprednisolone in a group of adrenalectomized rats, the decline in MPL plasma concentrations was biexponential with a terminal half-life of about 0.6 hour. The steroid was undetectable by 7 hours. The profile of GR mRNA concentrations declined from a baseline of 25.8 fmol/g to the trough of 47% of the initial value and slowly returned to the baseline over 24–48 hours. It appeared that the steroid-receptor complex in the nucleus was able to suppress GR transcription for up to 10 hours postdoing. The time course of free GR showed an immediate decline after dosing, indicating that after steroid-receptor binding, the dissociation of hsp and translocation must be extremely rapid steps, which could be captured with a single rate constant ($k_T$). The recovery showed two phases with recovery from 0 to 30% of the baseline occurring in the first 10 hours. The second phase, which was parallel to the recovery of GR mRNA, was much slower and required 72 hours.

The first phase of GR recovery was modeled as coming from the recycling of DR(N), as suggested by Oakley and Cidlowski (16). The end of the first phase shown in Figure 7 was about 30% of the GR baseline value, which is lower than the estimated $R_f$ value (0.49), suggesting that about 40–50% of DR(N) will become a steroid-activatable form of GR again in cytoplasm. Since the first phase of GR recovery was within 10 hours after dosing, the MPL plasma concentration was still sufficient to form DR when free GR was recycled in the first few hours. These results suggest that some of the glucocorticoid receptors were involved in the entire cycle [forming DR, DR(N), initiating transcription, being recycled and reactivated in the cytoplasm] more than once before the receptor protein was degraded.

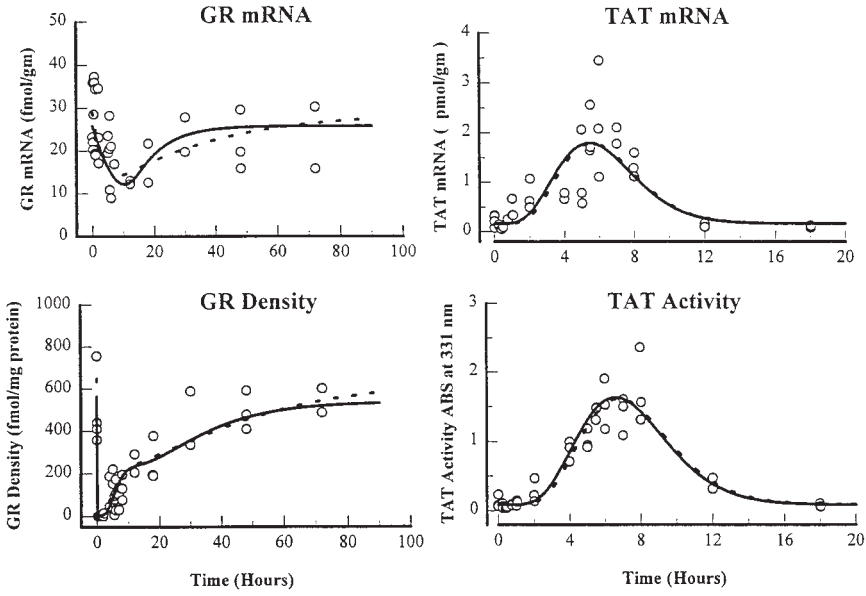


**Figure 7** Time course of responses to methylprednisolone after a 50 mg/kg iv bolus dose in rats: hepatic glucocorticoid receptor messenger RNA, free glucocorticoid receptor density, TAT mRNA, and TAT activity. Data points are experimental measurements and lines are fittings to the model shown in Figure 6. (Adapted from Ref. 36.)

The literature suggests (16) that the half-life of steroid-untreated GR mRNA is about 4–5 hours. Therefore, the estimated half-life of GR mRNA ($0.693/k_{dgr,GRmRNA}$) of 6 hours is reasonable. These results showed that assuming that activated steroid-receptor complex interferes with the transcription rate of GR mRNA is suitable for quantitating GR mRNA and GR downregulation in this analysis.

The induction of TAT mRNA in rat liver is shown in Figure 7. The TAT mRNA induction starts at about 1.5 hours, the peak appears at about 5.5 hours, and declines to baseline in 14 hours after MPL dosing. The transit compartment (TC) captures the delay through the $k_T$ and $k_N$ steps, while the $EF_l$ constant allows for a change in units as well as part of the rise in mRNA TAT concentrations. In addition, a power coefficient ($\gamma$) is used as a signal amplifier to control the sharpness of the mRNA TAT peak. A $\gamma$ value of 2.4 was found.

Following a similar pattern as TAT mRNA, the induction of TAT activity has a lag time of about 2 hours. The curve rises in parallel to TAT mRNA, reaches its maximum activity at about 7 hours, and declines to the baseline by about 18 hours postdosing. The decline of TAT is parallel to that of TAT mRNA.

The present fourth-generation PK/PD model for receptor gene–mediated corticosteroid effects is parsimonious in capturing a diverse array of steps in corticosteroid action with a minimum number of parameters, but it has predictive power and greater generality than for TAT induction. The published model was used to anticipate the modulation of responses from a second dose of methylprednisolone when given 24 hours after the first dose. Since the GR mRNA and free receptors are not fully recovered by 24 hours after a 50 mg/kg dose, the model predicted and experimentation confirmed that the TAT mRNA and TAT responses would be reduced following a second dose (36). This is a natural type of "tolerance" phenomenon.

Muscle tissue from the same rats used for the TAT analyses was employed to assess the effects of methylprednisolone on induction of mRNA and glutamine synthetase (GS) activity (37). It was surprising how similar the patterns of GR mRNA and free GR were in muscle and liver. The induction profile of GS mRNA and GS were also similar to that of TAT mRNA and TAT, but the former occurred over a slightly longer time frame.

## VIII. Dosage Regimen Simulations

The receptor gene–mediated PK/PD model was used further to assess the role of dose and delivery rate on receptor, mRNA, and TAT responses. Figure 8 shows the predicted effects of doses ranging from 5 to 100 mg/kg of methylprednisolone using parameters generated as described above. Clear differences in most response profiles are seen between the 5 and higher doses, but little change occurs between 20 and 100 mg/kg doses. Thus the system seems to be near capacity in this region. Figure 9 depicts simulations of responses to a 100 mg/kg dose infused over 1, 4, 8, and 12 hours. Receptor occupancy, in particular, is enhanced by slower drug delivery. The TAT response is essentially doubled when the drug is infused slowly. The initial high concentrations of corticosteroid after rapid administration cause part of the dose to be wasted as receptors become fully occupied. Improved efficacy is gained by extending the exposure of drug to receptors so that additional stimulus is gained as the receptors are recycled or regenerated. This concept was verified in an earlier experiment (39) showing that receptor and TAT profiles were similar when methylprednisolone was given as three 5 mg/kg doses at 1-hour intervals versus a single bolus of 25 mg/kg.

These types of studies provide useful insights into the role of dose and timing on selected short-term responses to corticosteroids in animal systems. Studies are underway to address the effects of chronic doses (long-term infusions) on these response systems. The general framework of these studies encompasses pharmacodynamics from a systems pharmacological perspective using tools of molecular biology and PK/PD modeling to assess in vivo drug responses in a reasonably un-

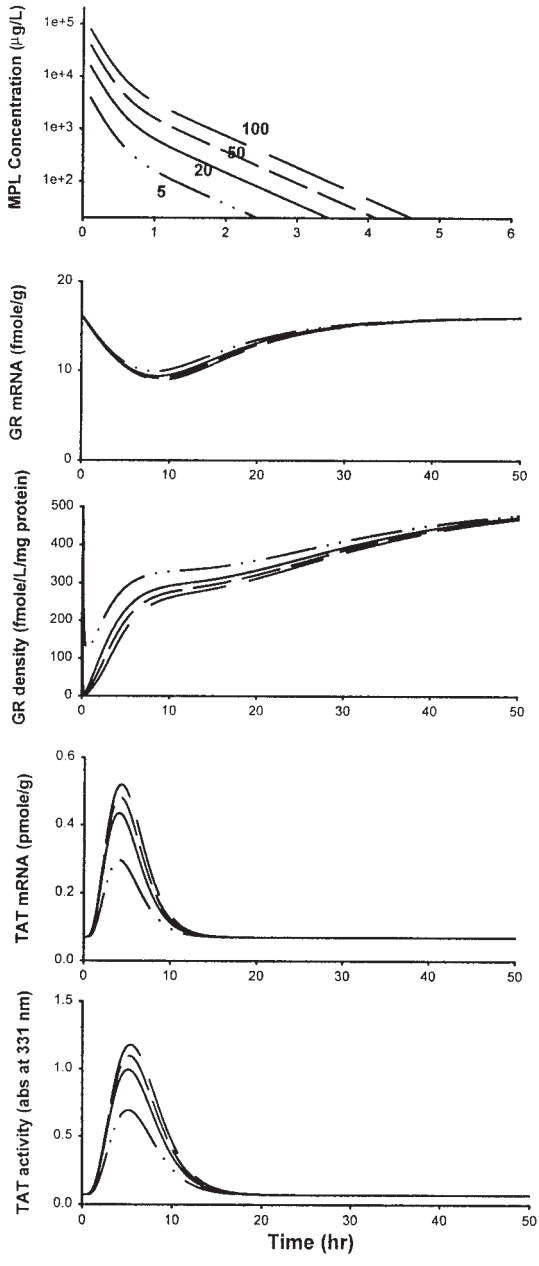


**Figure 8** Simulations of the PK/PD effects of dose (5–100 mg/kg) of methylprednisolone on the pharmacokinetics and indicated receptor mRNA, and TAT activities.

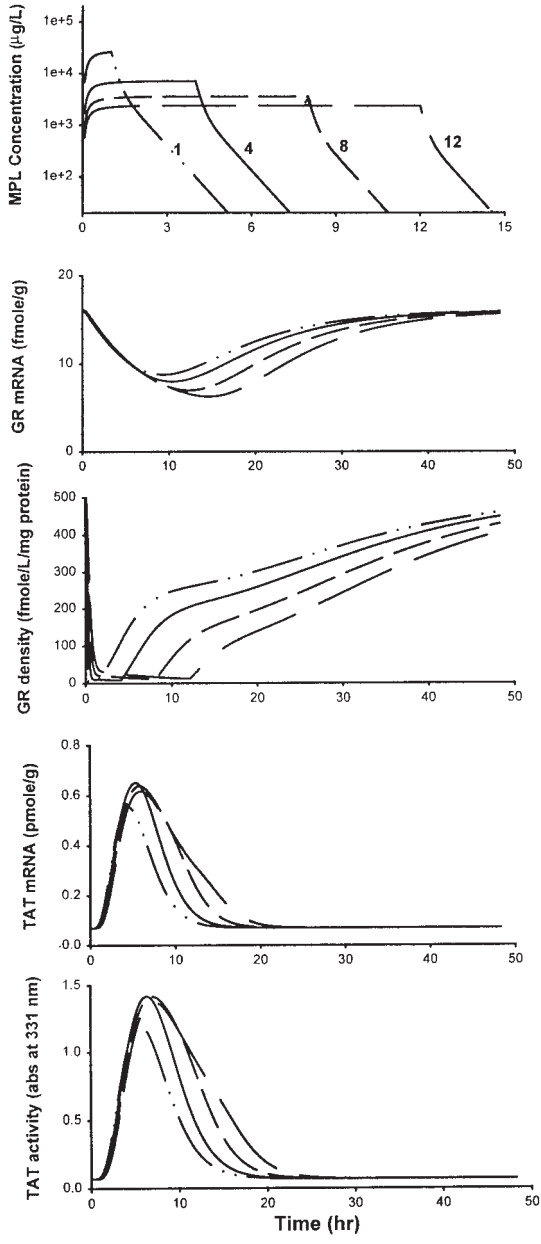


**Figure 9** Simulations of the PK/PD effects of alterations of delivery rate of methylprednisolone with a dose of 100 mg/kg infused over the indicated durations of time.

perturbed manner. The modeling and experimental approaches used for corticosteroids for animal and human responses have provided several conceptual advances in the area of mechanism-based PK/PD modeling including use of indirect response modeling (3), dealing with circadian rhythms (7), role of drug-receptor binding (30–36), signal transduction processes (38), enzyme induction, tolerance or downregulation (32,35), and a systems-analysis approach to interconnected complex processes (35).

## Acknowledgment

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## Discussion

**Dr. Edsbäcker:** Would you predict that plasma protein binding and nonspecific intracellular binding would greatly affect the predictions you can make from your MP models? As inhaled steroids generally have both a greater plasma protein binding and also a greater receptor affinity, this is of importance for the applicability of the model on the new lipophilic steroids.

**Dr. Jusko:** Generally in pharmacodynamics, and it applies with corticosteroids, the unbound drug in plasma serves as the driving force and equilibration component for drug access to cells and tissues. This is known as the "free drug hypothesis," which can be attributed to D. Riggs. Unbound drug concentrations, in turn, are governed by the intrinsic clearance processes responsible for drug elimination. Both plasma protein binding and nonspecific tissue binding are primary determinants of the volume of distribution. A larger volume of distribution produces a longer half-life (e.g., $t_{1/2} = 0.693$ V/CL), which would produce a longer duration of action of drugs with similar clearances.

**Dr. Derendorf:** Would you expect a gender-related difference in cortisol suppression for a high-affinity steroid such as ICS?

**Dr. Jusko:** We found offsetting gender differences in methylprednisolone clearance and sensitivity to cortisol suppression (Lew KH et al. Clin Pharmacol Ther 1993; 54:402), which produced similar net changes in cortisol concentrations. I am not aware of any similar findings for other corticosteroids, although the type of PK/PD modeling to uncover this is only of recent origin.

**Dr. Edsbäcker:** Regarding gender differences, we have made a meta-analysis of all in-house budesonide pharmacokinetic and cortisol supression data to look for effects of gender. No, or clinically insignificant, differences were found. In studies where oral contraceptives were allowed, a general elevation of baseline cortisol levels were found, but relative suppression by budesonide appeared similar in oral contraceptive users versus nonusers (Seidegard J, Simonsson M, Edsbäcker S. Clin Pharmacol Ther 2000; 68:13).

**Dr. Georas:** You have presented a beautiful model of induction of gene expression by GC, but of course a relevant question in asthma is the inhibition of gene expression. In that setting, keeping the number of GR molecules in the nucleus above a threshold value might be very important.

**Dr. Jusko:** For both gene induction and gene repression as well as a large array of other mechanisms, a common rule of thumb is that optimal pharmacological effects occur when drug concentrations are just above an $IC_{50}$, $EC_{50}$, KD, or other indicator of receptor or mediator sensitivity.

# 6

# Blockade of Chemokine Production/Function as an Example of Glucocorticoid Anti-inflammatory Actions

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## I. Overview of the Chemokine/Chemokine Receptor Superfamilies

Research in the field of chemokines has radically changed, over the last decade, the functional identity of this family of low molecular weight peptides. Initially identified as molecules that regulated leukocyte trafficking, their role in immunity and host defense has greatly expanded and has been found to be relevant in a variety of homeostatic and disease processes. Chemokines have now been recognized to play a role in such diverse conditions as atherosclerosis, AIDS, asthma, and in the immunopathology of tumors and transplants (1).

The relevance of members of the chemokine family as target of glucocorticoid anti-inflammatory action stems from the growing evidence on the crucial role that this class of molecules plays in the pathogenesis of many inflammatory reactions, due to their ability to act as potent chemoattractants and activators of specific subsets of leukocytes (2).

More than 40 members of this family of small, structurally related peptides as well as over 20 chemokine receptors have been identified and cloned, often through screening of expressed sequence tag databases (for extensive review, see Refs. 1–5). Chemokines are divided into four branches, or subfamilies, based upon

**Table 1** Human Chemokines: New Nomenclature Proposal

| Proposed | Current |
|---|---|
| 1. CXC chemokines | |
| CXCL1 | GROα/MGSA-α |
| CXCL2 | GROβ/MGSA-β |
| CXCL3 | GROγ/MGSA-γ |
| CXCL4 | PF-4 |
| CXCL5 | ENA-78 |
| CXCL6 | GCP-2 |
| CXCL7 | NAP-2 |
| CXCL8 | IL-8 |
| CXCL9 | Mig |
| CXCL10 | IP-10 |
| CXCL11 | I-TAC |
| CXCL12 | SDF-1α/β |
| CXCL13 | BLC/BCA-1 |
| CXCL14 | BRAK/bolekine |
| 2. CC chemokines | |
| CCL1 | I-309 |
| CCL2 | MCP1/MCAF |
| CCL3 | MIP-1α |
| CCL4 | MIP-1β |
| CCL5 | RANTES |
| (CCL6) | Unknown |
| CCL7 | MCP-3 |

the structural feature of the number and spacing of highly conserved cysteine residues present in their aminoacid sequence. These subfamilies are referred to as CC (or β), CXC (or α), C (or γ), and $CX_3C$ and share areas of highly conserved sequences. The absence or presence of an intervening amino acid between the first two of four conserved cysteines characterizes the CC and the CXC family, respectively (6). The C (or γ) subfamily includes lymphotactin, in which only two cysteines are conserved (7). Fractalkine is the only member of the $CX_3C$ subfamily and has unique structural features: it possesses a transmembrane domain linked to a CC-like domain via a long mucin-rich region, and it is the only membrane-bound chemokine (8). Molecules of the C-C chemokine subfamily, such as RANTES (regulated upon activation, normal T cell expressed and secreted), eotaxin, eotaxin-2, eotaxin-3, monocyte chemoattractant protein (MCP)-

**Table 1** Continued

| Proposed | Current |
|---|---|
| 2. CC chemokines (Cont'd) | |
| (CCL9/10) | Unknown |
| CCL11 | Eotaxin |
| (CCL12) | Unknown |
| CCL13 | MCP-4 |
| CCL14 | HCC-1 |
| CCL15 | HCC-2/Lkn-1/MIP-1δ |
| CCL16 | HCC-4/LEC |
| CCL17 | TARC |
| CCL18 | DC-K1/PARC/AMAC-1 |
| CCL19 | MIP-3β/ELC/exodus-3 |
| CCL20 | MIP-3α/LARC/exodus-1 |
| CCL21 | 6Ckine/SLC/exodus-2 |
| CCL22 | MDC/STCP-1 |
| CCL23 | MPIF-1 |
| CCL24 | Eotaxin-2/MPIF-2 |
| CCL25 | TECK |
| CCL26 | Eotaxin-3 |
| CCL27 | CTACK/ILC |
| 3. C chemokines | |
| XCL1 | Lymphotactin/SCM-1α/ATAC |
| XCL2 | SCM-1β |
| 4. $CX_3C$ chemokines | |
| CX3CL1 | Fractalkine |

*Source*: Ref. 1.

3, MCP-4, and monocyte-derived chemokine (MDC), are functionally characterized by potent and/or selective chemoattractant and activating properties toward eosinophils, basophils, monocytes, and T lymphocytes, while being very weak chemoattractants for neutrophils (9–18). The CXC subfamily is further subdivided according to the presence or absence of the tripeptide motif ELR (glutamic acid-leucine-arginine) in the amino terminus. ELR chemokines act as potent chemoattractants for neutrophils but not monocytes, and non-ELR chemokines, a small group constituted by IFN-γ–inducible protein (IP)-10, monokine induced by IFN-γ (MIG), platelet factor (PF)-4 and stromal cell–derived factor (SDF)-1, lack chemotactic activity on neutrophils but attract mononuclear cells. Recently, a new chemokine classification has been proposed based on the chemokine receptor nomenclature currently used (19), using the four receptor

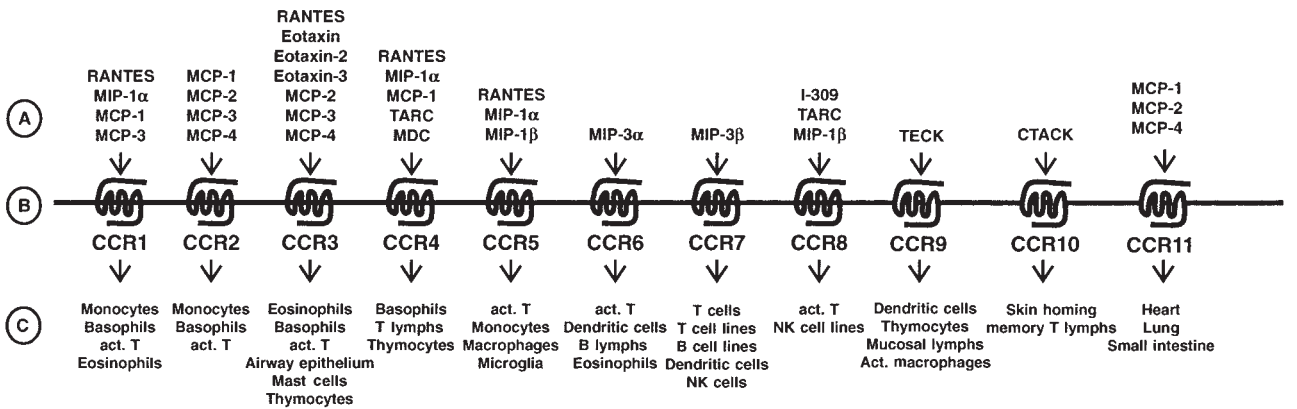


**Figure 1** Human C-C chemokine receptors (B), their ligands (A), and their cellular expression (C). act. T, Activated T lymphocytes; T lymphocytes, freshly isolated $CD4^+$ T lymphocytes.

subfamilies names—CC, CXC, XC, and C—followed by an L (as ligand) instead of an R (as receptor) and a number corresponding to that in use to designate the gene enoding each chemokine (1). Although in this chapter we have have referred to the chemokines using the current nomenclature, in Table 1 these denominations are paired with the new systematic name proposed by the new classification system.

Chemokine functions are mediated by binding to a complex network of seven transmembrane-spanning, G-protein–coupled receptors, the chemokine receptors (19), which are, for the most part, specific for the corresponding subfamily (5). The majority of the receptors for the CXC (CXCRs) and the CC (CCRs) subfamilies are shared by multiple chemokines, and many chemokines can bind to more than one receptor. For example, members of the CC chemokine family, the one mostly involved in allergic inflammation, differ in receptor usage, target cell specificity, and cellular sources (Fig. 1). RANTES can induce migration of cells expressing CCR1, CCR3, and CCR5, and MCP-4 can activate cells expressing CCR2 as well as CCR3. Eotaxin and eotaxin-2 appear to be the only CCR3-selective chemokines (20). Such variations in receptor utilization, and the ability of most of eosinophil-active CC chemokines to act on other cell targets, reveal a significant heterogeneity of their biological activity, with only partially overlapping functions (i.e., induction of eosinophil migration). On the other hand, such redundancy creates a challenge in the study of their function in vivo, since the full effect of a targeted inactivation of a specific chemokine or chemokine receptor gene may be masked by alternative chemokine pathways.

## II. The Role of Chemokines in Airway Allergic Inflammatory Diseases

Selective inflammatory cell recruitment is the result of a multi-step process in which chemokine-driven cell chemotaxis acts in concert with cytokine-induced selective priming of circulating leukocytes, as well as with upregulation of adhesion pathways governing leukocyte rolling, adhesion and transmigration through the endothelial layer. In a variety of chronic human inflammatory diseases, as well in animal models of inflammation, several studies have demonstrated the upregulated expression of a relatively specific subset of chemokines within the inflammatory site, which often correlated with the selective recruitment of distinct inflammatory cells types within the tissue sites (4). In the case of a chronic inflammatory allergic disease such as asthma, characterized by a predominant influx of eosinophils, as well as T lymphocytes, monocytes and basophils, the increased expression of several CC chemokines, such as MCP-1, MCP-4, RANTES, eotaxin and eotaxin-2 has been clearly established (2). In particular, expression of eotaxin

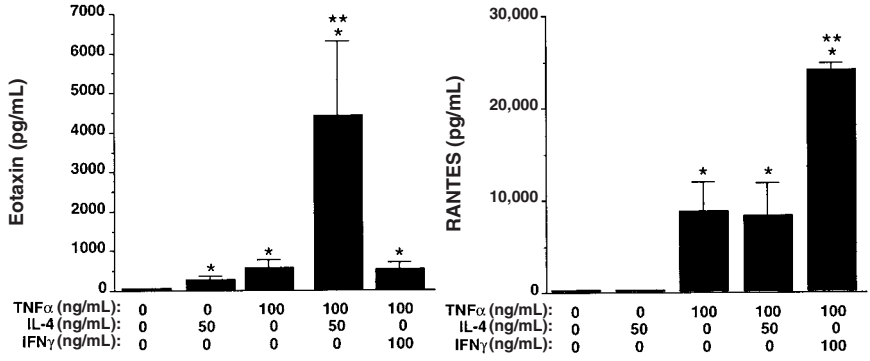


**Figure 2** Differential effect of IL-4 and IFNγ on C-C chemokine production in human airway epithelial cells. Eotaxin and RANTES levels in the supernatants of BEAS-2B cells treated for 18 hours with the indicated concentrations of TNFα and IL-4 ($n = 4-6$) and TNFα plus IFNγ ($n = 5$). *$p < 0.05$ when compared to chemokine levels in unstimulated cells: **$p < 0.05$ compared to TNFα-induced chemokine release. (From Ref. 38.)

in the airways has been strongly correlated with the presence of an eosinophilic infiltrate (21–23).

Chemokines are widely expressed in many tissues, either constitutively or following activation of the immune response. Upon stimulation in vitro with proinflammatory stimuli, such as TNFα or IL-1, a wide array of cells produce proinflammatory chemokines: circulating inflammatory cells, as well as resident cells, such as mast cells, dendritic cells, fibroblasts, epithelial cells, endothelial cells, and smooth muscle cells (2).

However, it is important to note that examination of specimens from inflamed tissues in animal models of allergic inflammation and in human subjects with respiratory allergy revealed a more narrow spectrum of cellular sources for chemokines, indicating that, in vivo, complex mechanisms regulate chemokine production in order to control, in a dynamic fashion, the leukocyte trafficking in homeostatic as well as in disease conditions.

Epithelial cells appear to be, both in animal models of allergic inflammation and in human diseases, one of the main sources of chemokine production in the airways (24). Extensive research in the last decade has broadened the role of epithelium from a "target" cell type being damaged as a result of inflammatory events, to an "effector" cell type, able to actively participate in the inflammatory response through the synthesis of numerous proinflammatory and immunomodulatory molecules: lipid and peptide mediators, adhesion molecules, catabolic enzymes and enzyme inhibitors, and, most remarkably, a wide array of cytokines and chemokines promoting the chemotaxis, recruitment, activation, and survival

of eosinophils and other inflammatory cells within tissue sites (25, 26). The presence of eosinophils and other infiltrating cells within the epithelial layer (27–29) was indeed suggestive that epithelial cells could act as a relevant source of chemoattractants. Immunohistochemistry and in situ hybridization studies on CC chemokine expression confirmed that epithelium is among the most intensely staining cell types, if not the most intensely staining cell type, in biopsies of both upper and lower airways of humans and mice (21,22,28,30–34). In addition, numerous in vitro studies have confirmed that airway epithelial cells produce substantial quantities of RANTES, eotaxin, and MCP-4 (14,35–37). Recent in vitro studies indicate that, despite the apparent redundancy of the repertoire of chemokines with overlapping functions produced by epithelial cells, there are striking differences in their specific profiles of activation. In particular, while TNFα induces RANTES, MCP-4, as well as eotaxin in human airway epithelial cells, the Th2 cytokine IL-4 leads to selective induction of eotaxin and MCP-4 expression and synergistically enhances TNFα-induced eotaxin expression. Conversely, the Th1 cytokine IFNγ potently and selectively upregulates RANTES expression induced by TNFα, but not eotaxin expression (Fig. 2) (38). Moreover, both IL-4 and IL-13 have been shown to further narrow the spectrum of epithelial-derived chemokines by downregulating IL-8 production in epithelial cells stimulated with TNFα (39).

Thus, it appears that during allergic inflammatory reactions the profile of cytokines released in the microenvironment might constitute an important regulatory signal for the expression of selective chemokine patterns from epithelium. A Th2 response, in which IL-4 and IL-13 are generated, will drive epithelial chemokine expression toward eotaxin and MCP-4, while a Th1 response, and IFNγ, will drive the response toward RANTES and IL-8. It can be envisioned that after antigen exposure, production of TNFα by macrophages and resident cells might induce the production of a broad, nonselective spectrum of chemoattractant signals from epithelium, leading to the recruitment of an heterogeneous leukocyte population comprising an early neutrophil component followed by lymphocytes, eosinophils, and monocytes. Once the inflammatory infiltrate includes adequate Th2 cells, the local production of IL-4 and IL-13, in coordination with release of TNFα from adjacent macrophages, might then induce the recruitment of a more selective, disease-specific inflammatory cell population by two parallel mechanisms: on one side, by downregulating the epithelial expression of some chemokines, such as IL-8 and RANTES, and at the same time potentiating the production of more eosinophilic chemokines such as eotaxin. These combined regulatory pathways would ultimately increase the selective influx of those effector cells, such as eosinophils and basophils, found in the airway mucosa in chronic allergic inflammation. In support of this hypothesis, recent studies of airway epithelium in asthmatic patients found that there is a close relationship between epithelial pro-

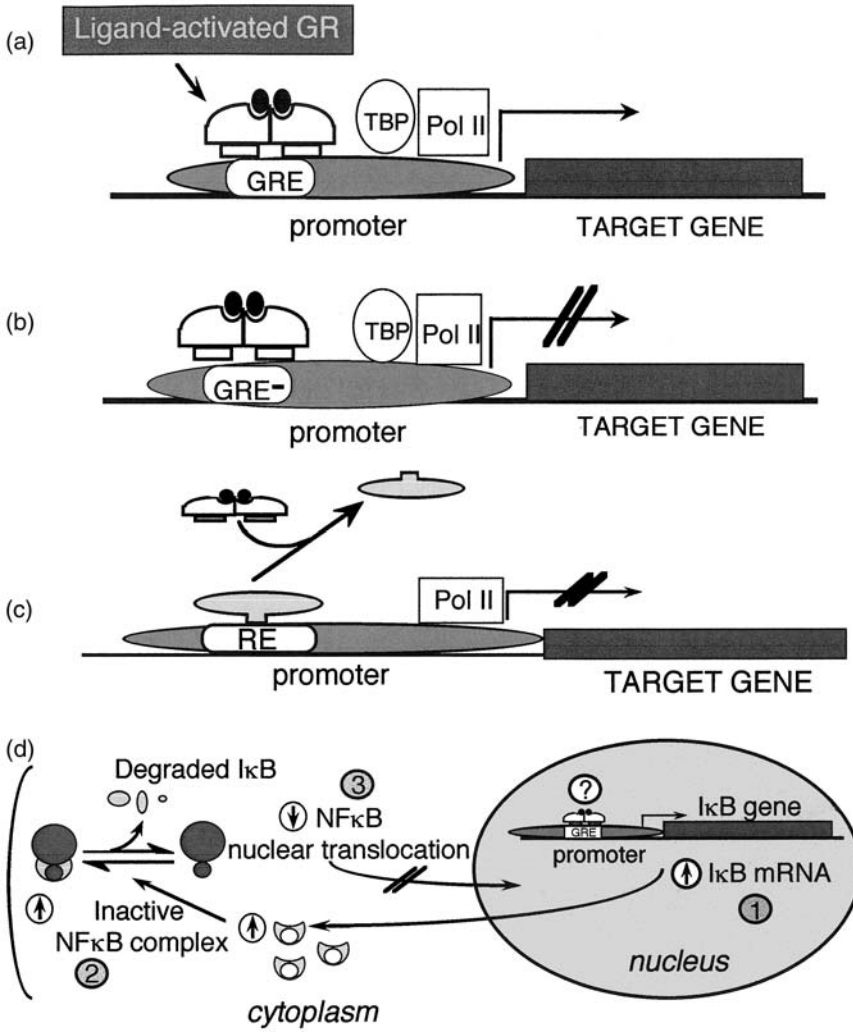


**Figure 3** Molecular mechanisms of glucocorticoid-mediated transcriptional regulation of inflammatory genes. Acting as a transcription factor, the ligand-activated glucocorticoid receptor (GR) modulates gene transcription either directly (a,b) or indirectly (c,d). (a) Direct target gene activation: transcription of the gene in question is initiated by binding to the GRE within the promoter. (b) Direct target gene repression: binding to the negative glucocorticoid response element (nGRE) has a repressive effect on the promoter activity of the target. (c) Indirect target gene repression: the inhibitory effect of glucocorticoids on gene transcription is indirect, resulting from either removal of transcription-activating factors, or (d) induction of transcription factor inhibitors.

duction of eotaxin and tissue eosinophilia (21), reinforcing the new, central role of epithelial cells in the mucosal chemokine network.

## III. Chemokine Expression as a Target of Glucocorticoid Action

It is now well established that the potent anti-inflammatory activity of glucocorticoids is due to a multifaceted mechanism of action, in which multiple and diverse metabolic functions are influenced by interference with a wide array of cellular and molecular pathways. The inhibition of the activation of genes involved in inflammation is now recognized as a key mechanism in glucocorticoid action, although it is not the only one; the targeted genes are involved in a great variety of functions, such as proinflammatory and immunomodulatory cytokines, enzymes, chemical mediators, plasma, and extracellular matrix components. The growing body of evidence indicating the crucial contribution of the mucosal chemokine network to the pathophysiology of airway allergic diseases makes this class of molecule an ideal target of glucocorticoid action.

After a brief overview of the molecular basis of glucocorticoid-induced gene regulation, we will evaluate some of the studies conducted so far analyzing the effect of glucocorticoids on chemokine expression.

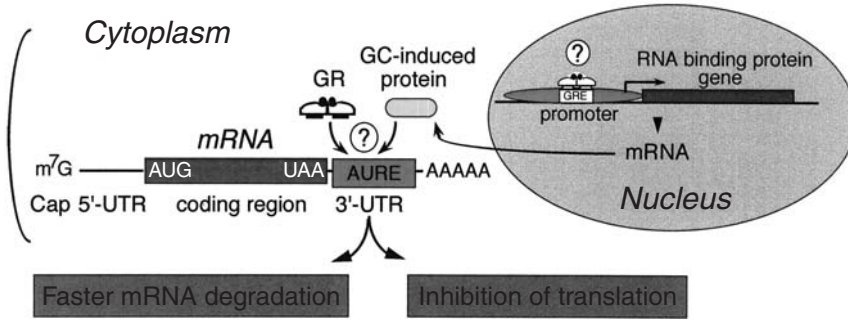


**Figure 4** Theoretical mechanism of glucocorticoid-mediated posttranscriptional gene regulation. The acceleration of the decay of the target gene mRNA by glucocorticoids may in some cases be mediated by ARE present in the 3′-UTR of the mature mRNA molecule. It is possible that destabilization may occur directly or through the synthesis of yet unidentified ARE-binding protein(s). Inhibition of kinase pathways controlling translation, also ARE-dependent, may be involved in inhibition of protein translation by glucocorticoids.

### A. Molecular Mechanisms of Glucocorticoid-Mediated Gene Regulation: An Overview

Glucocorticoids can interfere at different levels in the complex pathways leading to the expression of a gene. Repression can occur as a result of interference at a transcriptional level or as a consequence of mechanisms operating at posttranscriptional and even posttranslational levels (reviewed in Refs. 40,41).

Glucocorticoid-induced transcriptional regulation was first recognized to occur through DNA-dependent mechanisms, through direct binding of the ligand-activated glucocorticoid receptor (GR) to either positive or negative DNA glucocorticoid response elements (GREs and nGREs) in the promoter region of the targeted gene (42,43), either promoting the synthesis of anti-inflammatory genes (Fig. 3a) or preventing that of pro-inflammatory genes (Fig. 3b), respectively. Subsequently, it has been established that glucocorticoids can act in a DNA-independent fashion by engaging protein-protein interactions with other transcription factors, such as NFκB, AP-1, CREB, OCT-1, NF-IL-6, and others (44–46) (for more extensive review see Refs. 47, 48). The formation of these protein complexes prevents the interaction of transcription factors with their cognate binding sites within the promoter region of inflammatory genes, interfering with their ability to activate the expression of such genes (Fig. 3c). An alternative mechanism of glucocorticoid-mediated gene repression has been recently reported in T lymphocytes, where glucocorticoids have been shown to repress gene expression also by inducing the synthesis of IκB, an inhibitor of the transcription factor NFκB. This inhibitor binds NFκB in the cytoplasm and blocks the nuclear translocation of this transcription factor and the subsequent NFκB-dependent activation of inflammatory genes (49,50) (Fig. 3D) (49). A rise of the IκB level in the cell would retain NFκB in the cytoplasm and cause the relocation of NFκB from the nucleus to the cytoplasm, leading to termination of NFκB-mediated gene expression. To date a GRE has not been found in the IκB promoter. However, upregulation of IκB by glucocorticoids does not appear to play a role in other in vitro cell systems using endothelial cells and fibroblasts (51,52).

Glucocorticoids have also been found to inhibit gene expression using posttranscriptional mechanisms, by accelerating the degradation of mRNA molecules (Fig. 4). Regulation of many genes involved in inflammatory and immune responses can be rapidly achieved by stabilization/destabilization of their mRNAs, as demonstrated for G-CSF, GM-CSF, IL-1, IL-2, IL-3, and IL-6 (see Ref. 53 for review). Posttranscriptional gene regulation occurs via multiple mechanisms, such as the presence of regulatory sequences within the mRNA, the presence of cytoplasmic proteins interacting with mRNA, as well as induced changes in the secondary structure of the mRNA (53–55). Several sequences critical for mRNA stability have been identified in the 3′ end untranslated region (UTR) of mRNAs. The presence of adenylate/uridylate (AU)–rich elements (AREs) present in the 3′ UTR of an mRNA species has been clearly linked to acceleration of mRNA

turnover, presumably by acting as binding sites for mRNA-degrading proteins (56–60). The AREs consist of segments of 50–150 nucleotides containing multiple copies of the pentamer AUUUA and a high content of uridylate and adenylate residues. AREs have been shown to facilitate rapid deadenylation as the first step in mRNA degradation. However, other sequences structurally and functionally distinct from the ARE can also play a role in mRNA turnover (61).

Glucocorticoids have been shown to increase the degradation rate of mRNA encoding for IL-1β (60), GM-CSF (59), and IFNβ (62), and evidence indicates that AREs are necessary to mediate this glucocorticoid response. In cells transiently transfected with vectors expressing IFNβ, but carrying various deletions of the 3′UTR, glucocorticoids were able to inhibit IFNβ mRNA expression only in cells expressing the ARE-containing mRNA species (62). Other regulatory regions may be targets of glucocorticoid regulation, as proposed in the case of stabilization of the unspliced fibronectin mRNA, where glucocorticoid-responsive regulatory elements are thought to be located in the introns (63). Since glucocorticoid effects on mRNA stability can be protein synthesis–dependent (64–66), it is possible that glucocorticoids might induce the synthesis of proteins that decrease mRNA stability or translation. A wide array of distinct proteins binding to AREs, as well as to other *cis* elements in mRNAs, have been characterized (67). However, the identity of the glucocorticoid-dependent RNA-binding proteins is scarcely known. Treatment of a nontransformed $CD4^+$T-cell clone with dexamethasone-induced cell apoptosis in parallel with the appearance of the cytosolic binding proteins AU-A and AU-B (68). The 3′ UTRs of mRNAs also function as important regulatory elements of translational regulation: the ARE region in the 3′ UTR of TNFα was found to be necessary to mediate stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK)-mediated activation of TNFα translation in monocytes (69). Glucocorticoids can interfere with these pathways: dexamethasone was shown to inhibit TNFα translation by blocking the activity of the SAPK/JNK pathway in monocytes (70).

Glucocorticoids are able to modulate the expression of a gene even further downstream of transcriptional or posttranscriptional steps, by influencing posttranslational events. Translational processing accounts for a series of chemical modifications of gene products, such as site-specific cleavage, phosphorylation, glycosylation, or attachment of lipid components, which define the function and final location of the mature proteins. Glucocorticoids were shown to regulate the maturation of murine mammary tumor virus (MMTV) proteins in infected rat hepatoma cells by interfering with two posttranslational pathways: one controlling glycoprotein compartmentalization and processing, and a second controlling protein phosphorylation pathways (71). Posttranslational mechanisms also contributed to glucocorticoid-induced inhibition of inducible nitric oxide synthase (iNOS) expression in rat glomerular mesangial cells through the reduction of iNOS mRNA translation and increased degradation of iNOS protein (72).

### B. Chemokine Gene Expression: A Therapeutic Target and a Research Model for the Study of Molecular Mechanisms of Glucocorticoid Action

In vitro studies using different cell sources have revealed the characteristics of the pharmacological effects of glucocorticoids on chemokine production. The degree of inhibition appears to vary greatly, ranging from no effect to complete suppression of mRNA and protein production; the inhibitory efficacy is influenced by cell type and stimulus used, once again indicating that multiple pathways and mechanisms of gene suppression are involved in glucocorticoid activity (reviewed in Ref. 41).

Several investigators have chosen human airway epithelial cells as an in vitro model to characterize glucocorticoid effects on chemokine production (13,14,35,36,73,74). Epithelial cells are very rich in glucocorticoid receptors (75), and many proinflammatory genes have been found to be glucocorticoid-sensitive in these cells (25). Most importantly, airway epithelium is a major source of chemokines and a relevant target for topical glucocorticoids, the main therapeutic modality used for respiratory allergic diseases. Therefore, the local suppression of epithelial-derived chemokines may contribute significantly to the clinical efficacy of glucocorticoids by preventing chemokine-driven leukocyte infiltration within the airway mucosa. In these cells, the inhibitory effect on chemokines appears to be concentration-dependent, glucocorticoid-specific (35), and displays a rank order of potency for different glucocorticoids that resembles their anti-inflammatory activity in vivo (76,77). Studies on the molecular mechanisms of glucocorticoid activity on chemokine genes are still at an early stage but growing in number, and due to the relevance of the chemokine network in immunity they are likely to uncover novel and relevant molecular targets of glucocorticoid action.

Transcriptional inhibition by glucocorticoids has been found to occur via both DNA-dependent and -independent pathways in the case of IL-8 (78–81). With regard to eosinophil-active chemokines, the glucocorticoid budesonide inhibited eotaxin and RANTES promoter-driven reporter gene activity in a transiently transfected epithelial cell line, indicating that inhibitory mechanisms can occur at transcriptional level for these two chemokines (38). The molecular basis of such inhibitory activity is currently under investigation. A GRE is present in the promoter region of eotaxin, but its presence is not necessary for glucocorticoid inhibition. Furthermore, since both NFκB and AP-1 are necessary for the expression of several CC chemokines, including MCP-1 (82), RANTES (83), and eotaxin (79,80), it can be hypothesized that interference with AP-1-and NFκB-activating pathways, through protein-protein interactions of the ligand-activated GR, might play a role in the inhibitory mechanisms of glucocorticoids on the expression of chemokines from epithelium.

Recent data indicate that the Th2 cytokines IL-4 and IL-13 selectively induce eotaxin expression in epithelial cells and strongly potentiate that induced by

TNFα and that such induction is inhibited by cell treatment with budesonide (38). These data suggest that another potential glucocorticoid target is STAT6, a member of the STAT family of transcription factors that plays a crucial role in IL-4 and IL-13 signaling (84,85) and biological functions (86,87). In the eotaxin proximal promoter, a highly conserved binding site for STAT6 is located just 15 bp upstream of the TATA box and partially overlaps with a NF-κB element (79,80), suggesting a role for STAT6 in IL-4-induced eotaxin expression and also implying that cooperation between STAT-6 and NF-κB/Rel family members might mediate the synergistic effects of TNFα and IL-4 observed on eotaxin expression in BEAS-2B cells. Indeed, it has been recently shown that in BEAS-2B cells transiently transfected with an eotaxin promoter luciferase construct in which the STAT6 site is mutated, IL-4 fails to induce the reporter activity or to potentiate the induction by TNFα (88). Studies are presently undergoing to further define whether STAT-6 is a molecular target of glucocorticoid-mediated inhibition of eotaxin transcription.

Studies on the posttranscriptional regulation of chemokine mRNA half-life are still at an early stage, but they already indicate that chemokine mRNA turnover is influenced by cytokines and by glucocorticoids. Induction of IL-8 by IFNγ in monocytes, as well as its downregulation by IL-4, has been shown to occur via an increase or a decrease, respectively, in the stability of its mRNA (89,90). Induction of RANTES by respiratory syncytial virus is critically regulated at a posttrancriptional level in airway epithelial cells (91). Multiple AREs have been found in the 3′ end UTR of IL-8 (92), MCP-1 (93). MIP-1α (94), and eotaxin (37), but ARE are not present in the 3′UTR of RANTES (95) and MCP-4 (13). In the epithelial cell line BEAS-2B, budesonide induced a striking acceleration of eotaxin and MCP-4 mRNA decay while having no effect on RANTES mRNA half-life (35,38,73). Acceleration of IL-8 mRNA decay by glucocorticoids occurs in human fibroblasts (96) and appears to be the main regulatory mechanism of IL-8 suppression in human bone marrow stromal cells (97), although it did not seem to play a role in IL-8 inhibition by glucocorticoids in epithelial cells (98).

The presence of ARE in the 3′UTR may not be the only mechanism for glucocorticoid activity on posttranscriptional events. Lack of posttranscriptional effects by glucocorticoids on chemokine genes possessing ARE has been observed for MIP-1α mRNA in monocytes (99), which has four ARE in its 3′UTR (94), and for MCP-1 in HMC-1 cells (100); furthermore, budesonide increased decay of MCP-4 mRNA in epithelial cells (38), despite the lack of ARE in the 3′UTR of the MCP-4 transcript (13). Therefore, other sequences present in the 5′ or 3′UTR of chemokine mRNA species, or in intronic sequences of nuclear, immature RNA, or even in the coding region could function as binding sites of glucocorticoid-induced mRNA binding proteins.

Other pathways regulating translational activation of chemokine mRNA could be targeted by glucocorticoids. It has been recently shown that in an epithe-

lial cell line, IL-8 mRNA half-life is prolonged, after challenge with IL-1, by sequential activation of members of the p38 MAP kinase pathway, and that such stabilization is ARE dependent (101). p38 MAP kinase has also been recently recognized as important regulatory pathway for RANTES expression in airway epithelial cells (102,103). It would be of interest to ascertain if the p38 or any other tyrosine kinase-initiated signaling cascade involved in chemokine regulation could be inhibited by glucocorticoids.

## IV. Concluding Remarks

Given the relevance of the chemokine network in the pathogenesis of allergic inflammation, it is understandable why it has been considered, since its initial discovery and characterization, an important target for the anti-inflammatory therapy of allergic diseases. Evaluation of the effects of glucocorticoids on chemokine expression, both in vivo and in vitro, has shown that many genes of the C-C chemokine subfamily, which is more pathogenetically relevant in allergic diseases, are sensitive to the effects of glucocorticoids. The data so far generated and discussed in this chapter indicate that it is likely that the glucocorticoid effect on chemokines is mediated by multiple inhibitory mechanisms, acting at both transcriptional and posttranscriptional levels and contributing, to different degrees, to the suppression of each chemokine. The relevance of the epithelium as a major source of chemokines in the airways increases the likelihood that downregulation of these molecules by topical glucocorticoid treatment, in which epithelial cells are the most exposed to the drug, would have a significant role in the anti-inflammatory activity of the glucocorticoid therapy. Moreover, the complexity of the regulatory pathways of chemokine production, together with their glucocorticoid sensitivity, makes the study of chemokine expression an ideal experimental system for the identification of novel targets of glucocorticoid action.

More studies will be needed to fully uncover the molecular basis of the glucocorticoid effect on chemokines; at the same time, it will be important to establish how much chemokine inhibition plays a role in reducing the tissue recruitment of inflammatory cells and to what extent it influences the clinical outcome of glucocorticoid therapy in chronic allergic diseases.

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## Discussion

**Dr. Denburg:** What are the relative contributions of transcriptional and post-transcriptional events in the regulation of chemokines by corticosteroids?

**Dr. Stellato:** The occurrence of posttranscriptional regulation by glucocorticoids has been investigated for several chemokines, but studies exploring in detail the relative contribution of such mechanisms in comparison with those acting at trascriptional level in determining glucocorticoid's inhibitory activity are still at an early stage. Work done in this area on the effect of glucocorticoids on IL-8 production indicate that transcriptional and posttranscriptional regulatory mechanisms are affected by glucocorticoid treatment to different degrees according to the cell type: in human fibroblasts, glucocorticoids induce a significant acceleration of IL-8 mRNA decay; in human bone marrow stromal cells, posttranscriptional regulation appears to be the main mechanism of IL-8 suppression by glucocorticoids, since nuclear run-on experiments—performed in parallel with the mRNA stability assay—indicated that transcription of IL-8 was unaffected by glucocorticoids. In stark contrast, in primary epithelial cells, IL-8 inhibition by glucocorticoids was found not to be mediated by acceleration of mRNA decay; moreover, it is well known that the transcription factor NF-κB is a target of the repression of IL-8 by glucocorticoids. Such differences, in my opinion, may indicate that transcriptional and posttranscriptional regulatory processes are affecting the expression of a particular gene in a dynamic fashion, possibly according to changes in cell cycle, activation state, or other cell type–specific events, and the mechanism of glucocorticoid effect may consequently vary, affecting the regulatory event mostly driving gene expression in each particular case.

**Dr. Hamid:** Can steroids have any harmful effect by suppressing chemokines? It is likely that chemokines play a role in normal homeostatic and normal leukocyte trafficking.

**Dr. Stellato:** It is likely that the constitutive expression of chemokines is already under the influence of the physiological levels of endogenous steroids. It could be hypothesized that the factors regulating chemokine expression in homeostasis and in inflammatory condition may be different and that during inflammation chemokine expression is driven by glucocorticoid-sensitive mechanisms not in place in homeostatic conditions; alternatively, it is possible that during inflammation, the profile—or the levels—of molecules functioning as nuclear receptor coactivators (i.e., CREB-binding protein/p300) may change and become a major target of glucocorticoid action.

**Dr. Busse:** These are very interesting observations. Are there data that indicate the signal transduction processes are different or distinct for RANTES and

eotaxin? Second, IFNγ has been shown to inhibit eosinophil recruitment in animals. Do your observations suggest that IFNγ inhibition of eotaxin means this chemokine is the principal chemokine in the recruitment of eosinophils? Third, what is known about the different chemokine receptors on different cells like the eosinophils?

**Dr. Stellato:** Rothenberg's group have reported that there are differences between eotaxin and RANTES in their ability to induce internalization of CCR3 in human eosinophils, but I do not recall any reports on differences in downstream signaling events. The redundancy of the chemokine network makes the task of identifying the key player for eosinophil recruitment a difficult one. Based on the in vivo data on eotaxin expression in allergen challenge models and in diseases such as asthma, expression of eotaxin is the only one clearly showing a correlation with the eosinophil influx. Eosinophils have been reported to express CCR1 and CCR3; experiments in animal models suggest that CCR3 is the major player in governing chemokine-driven eosinophil chemotaxis. Eosinophils also express CXCR2, which binds multiple CXC chemokines.

**Dr. Brattsand:** A popular theory today is that the adverse steroid effects are mediated over GRE-mediated upregulation via the receptor dimer, while the major anti-inflammations are mediated by repression probably via the monomeric receptor. Do you know whether the very interesting AUUUA mechanism requires protein synthesis? Can you test this in the knockouts lacking the dimeric form of GC receptor?

**Dr. Stellato:** Although I have not tested the protein synthesis requirements of the effect of budesonide on eotaxin mRNA half-life, in the literature it is reported that the effect of glucocorticoids on mRNA decay can be protein synthesis–dependent, suggesting that glucocorticoids can induce the synthesis of proteins, that can in turn influence mRNA stability or translation.

**Dr. O'Byrne:** Does eotaxin regulate its own receptor? Does eotaxin have effects on any other cells apart from the eosinophil?

**Dr. Stellato:** In human eosinophils, engagement of CCR3 by eotaxin or RANTES leads to prolonged receptor internalization and subsequent cellular desensitization. I do not think it is known whether CCR3 ligands are regulating the expression of CCR3 at the mRNA level, but we are planning to study this issue focusing on the CCR3 expression we found on epithelial cells. Expression of CCR3 has also been reported on human basophils, a subsets of Th2 lymphocytes, astroglial cells, and mast cells, and shown to mediate chemotaxis of these cells. The functional role of CCR3 on structural cells such as epithelial cells is still not fully understood. Expression of chemokine receptors on several other structural cells, such as endothelial cells and smooth muscle cells,

is being increasingly recognized and has been associated with cell proliferation and chemotaxis. It is possible that chemokine receptors in structural cells may participate not only in the local chemokine network during inflammatory processes, but also in mechanisms of tissue repair or act as viral cell surface receptors.

# 7

# Newly Recognized Glucocorticoid Targets

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## I. Introduction

Synthetic glucocorticoids (GC) have been in use for the control of inflammatory diseases for over a half century. Although their utility has not been diminished by the lack of knowledge of their mechanism of action, insight into the mechanism(s) of GC action has steadily advanced (1–3). It has recently become clear that GC owe their efficacy to a mechanistically diverse and coordinated targeting of immune and inflammatory processes (1,2). The diversity of GC molecular targets comes in no small measure as a result of the fact that GC are endogenous hormones that regulate inflammation and have highly evolved effects. These actions of endogenous GC represent a balancing act in which excessive inflammation must be regulated without serious compromise of the protective actions of the immune and inflammatory response (4). The GC literature is now so large that is nearly impossible to comprehensively review the actions of these fascinating hormones. The purpose of this chapter is to discuss newly recognized and potentially important targets or intermediaries of the anti-inflammatory effects of GC; we have intentionally avoided discussions of the GC targets considered at length in previous reviews by us and others.

**Table 1** Transcription Factors Known to Interact with the Glucocorticoid Receptor

| Factor | Function (Ref.) |
|---|---|
| General basal transcriptional machinery (TFIID) | (144) |
| TBP | TATA-binding protein (22,145) |
| NF-κB | Inducer of inflammatory genes (146) |
| AP-1 | Inducer of inflammatory genes (147,148) |
| STAT6 | Inducer of Th2-associated genes; inhibition of GR-induced reporter construct in mouse T-cell line (184) |
| STAT5a and b | Prolactin/growth hormone responses (149) |
| STAT3 | Response to several inflammatory cytokines (150,151) |
| GATA-1 | Hematopoietic and inflammatory responses (152) |
| Egr-1 | Synergism to activate phenylethanolamine-*N*-transferase gene (153) |
| AP-2 | Synergism to activate phenylethanolamine-*N*-transferase gene (153) |
| HNF3 | Activation of the phosphoenolpyruvate carboxykinase gene (154) |
| Oct-1 and -2 | Synergism with GR to activate MMTV promoter and many cellular genes (155,156) |
| NF-IL6 | Synergism with GR to activate $alpha_1$-acid glycoprotein gene (157) |
| CREB | Interaction with GR in controlling PEPCK gene expression (158) |

## II. Glucocorticoids and Regulation of Gene Expression

### A. Overview

With the discovery that the GC receptor complex can activate gene expression, the anti-inflammatory actions of glucocorticoids were hypothesized to be due to the induction of anti-inflammatory genes (5). This prompted a widespread search for anti-inflammatory genes which are induced by GC and may act as mediators of GC action. These studies led to the discovery of some GC-induced anti-inflammatory proteins, including lipocortin, secretory leukocyte inhibitory protein, and soluble cytokine receptors (6,7) (see below). It is also clear that GC suppress the expression of a host of proinflammatory genes, notably the cytokine families (8–10). The number of proinflammatory genes that have been discovered is now enormous, including large numbers of cytokines, hematopoietic growth factors, chemokines, inflammatory enzymes, etc. An extensive list of proinflammatory proteins whose expression is inhibited by GC has been published elsewhere (10). Studies of the molecular mechanisms of GC inhibition of gene expression demonstrated that in many cases it results from direct interaction of the GC receptor complex with transcription activating factors responsible for inducing the gene in question (11). The physical interaction of GR with transcription factors can mediate both transactivation and transrepression in a case-dependent fashion. A list of

the transcription factors with which the GR has been shown to associate is provided in Table 1 (see also Ref. 12). As more genes that are regulated by these factors are identified, a new wave of potential GC targets will emerge.

Recognition that GC are good inhibitors of the expression of numerous proinflammatory proteins has contributed to a widespread belief that GC action relies heavily upon inhibition of the expression of such proteins. From this grew the exciting possibility that selective GC could be developed that had these transrepressive effects (i.e., prevent expression of proinflammatory proteins), but which are devoid of transactivating effects (i.e., mediated via GC receptor binding to GRE). The value of this approach stems from the hypothesis that the undesirable side effects of GC, for example, reduced integrity of bone or skin, HPA suppression, gluconeogenic effects, etc., result from GRE-mediated effects, while the desirable anti-inflammatory effects result from transrepression. Although there remains considerable interest in this concept, the number of recognized or putative GC-induced anti-inflammatory proteins has grown (see Table 2). Based solely on the extent of this list, we can speculate that removing the gene-activating properties of GC by drug design may compromise their anti-inflammatory effects to some extent. When added to the likelihood that transrepression may be responsible for some GC side effects, the potential for success of this strategy is uncertain.

The goal of this review is to discuss newly recognized and potential glucocorticoid targets or effector molecules. The molecules to be considered include both glucocorticoid-induced and glucocorticoid-suppressed gene products. The molecular mechanisms of the influences of GC on gene expression, both positive and negative, have been reviewed in detail elsewhere (2,11,13) (see also Chap. 24).

### B. Glucocorticoids and Chromatin: Histone Modifications

There is increasing evidence that glucocorticoids and other members of the nuclear receptor family can have fundamental effects on chromatin structure. An overview of chromatin structure is followed by a general discussion of glucocorticoid effects below. Nuclear chromatin is composed of DNA compacted via winding around histone proteins to form nucleosome structures. The nucleosome is made up of a core histone octamer (two copies of histones 2A, 2B, 3, and 4) and histone H1, which binds to the linker DNA between adjacent nucleosomes (see Fig. 1). Two types of chromatin can be visualized after staining nuclei with Giemsa: lightly staining transcriptionally active euchromatin and darkly staining transcriptionally silenced or repressed heterochromatin, usually found near the edges of the nucleus (14). The extent to which the DNA is complexed to the nucleosomes is dependent on posttranslational modifications of the core histone proteins, H3 and H4. Histones possess flexible N-terminal tail domains, rich in lysine residues, making them very basic and positively charged at physiological pH. The negative charge of the sugar-phosphate backbone of DNA is presumed to electrostatically interact with these lysine side chains, thus tightly compacting the DNA

**Table 2** Putative Inhibitory Genes Induced by Glucocortocoids

| | Comment (Ref.) |
|---|---|
| 1. Cell surface receptors | |
| CTLA-4 | Dex potentiates expression in T cells (73) |
| PECAM-1 | GRE consensus sequence in promoter (85) |
| RM3/1 antigen | CD 163 (scavenger receptor) upregulated by GC on human monocytes and macrophages (62) |
| IL-1R type 2 | GC induce expression (159) |
| IL-10R | Increased expression in response to GC in skin (160) |
| Mannose receptor, CD16 and CD32 | Dex upregulates expression on human dendritic cells (161) |
| GITR | GC-induced TNFR-related protein in mouse which inhibits T-cell apoptosis (70,162) |
| VIP receptor/VIP | High-affinity VIP-R increases in response to GC in human mononuclear leukocytes (163); VIP mRNA enhanced in GC-treated rat cerebral cortex (164) |
| 2. Soluble receptors/ antagonists | |
| IL-1R | Decoy receptor induced by GC (165) |
| IL-4R | GC increase levels in children with seasonal allergic rhinitis (166) |
| IL-6sR | GC induce in prostate cancer cell lines |
| IL-1R antagonists | GC induce in human keratinocytes (168) |
| 3. Signaling/intracellular inhibitory molecules | |
| SOCS-2 | JAK/STAT pathway inhibitor: GC potentiate expression in cultured hepatocytes (169) |
| CIS | JAK/STAT pathway inhibitor: GC potentiate expression in cultured hepatocytes (169) |
| GILZ | GC-induced anti-apoptotic transcription factor in thymocytes and T cells (170) |

around the nucleosomes. Acetylation of the lysine side chains by the action of histone acetyltransferases (HATs) adds a sterically bulky group to the histone tails, and neutralizes the positive charge, relaxing the DNA from the nucleosome (15) and providing access for transcription factors. Acetylation of the core histones has thus been closely linked to transcriptional activation (16–19).

HAT activity can be detected in numerous coactivator proteins as well as in the basal transcriptional machinery itself. Of particular relevance to the discussion here are p300 and CBP (CREB-binding protein), two related coactivator mole-

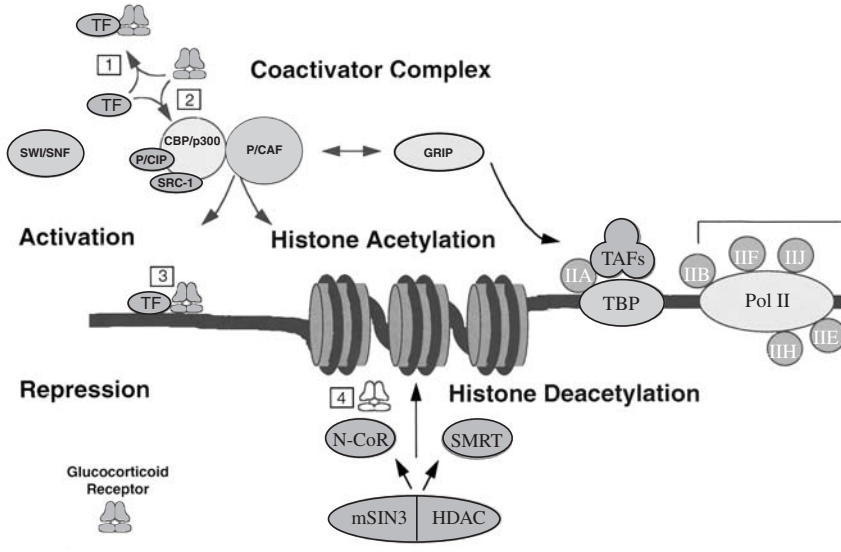


**Figure 1** Regulation of gene expression by GR and/or other nuclear receptors and interactions with multiple coactivator and corepressor complexes. CBP/p300 potentially link GR with the core machinery to activate transcription, after chromatin remodeling by the SWI/SNF complex. [1], GR can interact directly with several transcription factors (TF—see text) to enhance or inhibit transcription. [2], Competition for coactivator protein complexes such as CBP/p300 between GR and TF with opposing actions may shape the outcome of gene expression. GR interactions with a variety of coactivators have been described (see text). [3], GR may also interact with TF once bound to the DNA to enhance or inhibit responses. [4], Induction of expression of HDACs or activation of HDAC or corepressor function by GR is a mechanism by which GR could mediate inhibition of gene expression. The corepressor complexes, mSIN3, N-CoR, and SMRT, are linked to unliganded GR to repress transcription in the presence of liganded GR. GR is shown as a dimer in the figure, although some effects may be mediated by monomeric receptor.

cules with intrinsic HAT activity. These coactivators are known to interact directly with the GR and are essential for nuclear receptor function (20,21). The tau-1 activation domain of GR has been shown to interact with the C-terminal region of CBP (22). The ability of ligand-bound GR to bind HATs may be important for transcription of glucocorticoid-activated genes. In addition to direct interaction with CBP, nuclear hormone receptors can bind CBP through members of the p160 and p140 coactivator family, some of which also contain HAT activity, in a ligand-dependent fashion (see Fig. 1 and Table 3). These include steroid receptor coactivator-1 (SRC-1), ACTR, (human) transcription factor intermediary factor-2

**Table 3** Important Coactivator Proteins That Interact with the Nuclear Receptor Family

| Coactivator | HAT activity | Species | Comment | Known interaction with GR | Ref. |
|---|---|---|---|---|---|
| p300/CBP | Yes | Human | Integrators that mediate transcription of multiple signal transduction pathways | Yes | 20,22 |
| p/CAF | Yes | Human | p300/CBP-associated factor; required for transcription of many genes | | 171 |
| *p160 family* | | | | | |
| NCoA-1 | | Mouse | Highly related to p/CIP | | 172 |
| SRC-1 | Yes | Human | | Yes | 22,173 |
| GRIP-1 | | Mouse | Partial homology to SRC-1; also known as NCoA-2; TIF-2 (probable human ortholog) | Yes | 23 |
| p/CIP | | Mouse | Complexed with CBP; required for transcriptional activity of nuclear receptors and other p300/CBP-dependent transcription factors (also known as RAC-3, AIB1, ACTR, and TRAM-1) | | 172 |
| ACTR | Yes | Human | (see p/CIP above) | | |
| TRAM-1 | | Human | Homologous to SRC-1/TIF-2; binds TR and other nuclear receptors; a novel co-activator that interacts with nuclear receptors outside AF-2 region, cf. SRC-1/TIF-2 | | 174 |
| *Other coactivators* | | | | | |
| RIP140 | | Human | Complex effects on positive and negative gene regulation by GR | Yes | 175 |
| ASC-2 | | Human | Amplified in cancer; coactivator for many steroid receptors and p300/CBP; TFIIA, TBP (TATA-binding protein) and SRC-1 | Yes | 176 |
| AIB1 | | Human | Amplified in breast and ovarian cancers; a steroid receptor coactivator | | 177 |
| RAC-3 | | Human | Related to SRC-1/TIF-2; interacts with several liganded receptors | | 178 |
| HMG-1 and -2 proteins | | Rat and cow | GR coactivator; enhances sequence-specific DNA binding of GR | Yes | 179 |
| 14-3-3 eta | | Human | GR coactivator; regulatory role in GR-mediated signal pathways | Yes | 180 |
| GRIP 170 | | Human | GR coactivator; enhances GR induction of promoter activity | Yes | 181 |
| hRPF1 | | Human | GR coactivator; link between activated GR and general transcription apparatus | Yes | 182 |
| RAP 46 | | Human | GR coactivator; identified by screening expression library with GR | Yes | 183 |

(TIF-2), and (its mouse ortholog) glucocorticoid receptor interacting protein-1 (GRIP-1) (23). The LXXLL motif found in SRC-1, CBP, and others is necessary and sufficient to mediate binding to liganded nuclear receptors (24). While many of these coactivators are able to interact with several nuclear receptors, others such as GRIP and TRAP appear to be relatively receptor selective (25). It is also important to mention that CBP can interact with several transcription factors important in inflammation, including AP-1, NF-κB, and STAT proteins. Competition between transcription factors with opposing functions, such as NF-κB and GR, for sites on CBP might decide the final outcome of transcription in a gene-specific way (2) (see Fig. 1). Such competition can theoretically lead to reciprocal repression of glucocorticoid responses in situations where the transcription factor is overexpressed. The finding that a number of other nuclear receptors interact with transcription factors within the transcriptional machinery in a ligand-dependent fashion suggests that this may be a generalized mechanism of nuclear receptor action (26–28).

Deacetylation reverses the above phenomena and is mediated by histone deacetylases (HDACs). Deacetylated histones have been associated with transcriptionally inactive chromatin (29). Nuclear receptors can bind corepressor molecules including nuclear receptor corepressor (N-CoR) and SMRT (silencing mediator for retinoid and thyroid hormone receptors), which then recruit large repressor complexes containing mSin3 and HDACs to bring about transcriptional repression (30,31) (see Fig. 1). It is notable that N-CoR and SMRT have been shown to associate with the retinoic acid receptor and the thyroid receptor in the unliganded state. It has not yet been determined whether any of these corepressors can associate with the GR, but as more corepressors are identified and characterized, it is likely that GR-interacting corepressors will be identified (2). Interestingly, studies by Ito et al. have shown that IL-1β induces acetylation of histones K8 and K12 and that dexamethasone inhibits this acetylation (32). They also show that GR can reduce histone acetylation both by directly inhibiting CBP-associated HAT activity as well as by recruiting HDAC2. These authors concluded that histone acetylation is an important level of control of inflammatory gene expression at which glucocorticoids act. Although the precise roles that glucocorticoids play in the recruitment of HATs and HDACs are still unclear, modification of histone structure must be considered to be an important mechanism of regulation of gene expression by glucocorticoids.

### C. Glucocorticoids and Chromatin: Chromatin Remodeling Complexes

Chromatin is further subjected to remodeling by large, multiprotein, ATP-dependent remodeling machines. These proteins disrupt nucleosomes in vitro and are candidates for complexes that cause chromatin decondensation during gene induction. Steroid receptors are able to interact with repressed nucleopro-

tein templates and to recruit the necessary proteins for such chromatin remodeling (33). The remodeling complexes first described in yeast are the SWI/SNF proteins, which couple ATP hydrolysis to nucleosomal remodeling at diverse promoters to facilitate interactions of basal transcription factors with these promoters. Unlike HATs, SWI/SNF complexes do not covalently modify histones but rather catalyze uncoupling of ionic interactions between histones and their substrate DNA. Human SWI/SNF homologs have been found to enhance the activation functions of GR, ER, and RAR (34–36). The glucocorticoid receptor can interact with the human SWI/SNF machine, which requires the presence of a GRE in the chromatinized template (37). However, how and whether glucocorticoid receptor is targeted to specific chromatinized regions of DNA in this process in living cells is still an open question.

### D. GC Effect on Phosphatases

Many of the responses to inflammatory cytokines involve signal transduction pathways which are dependent on phosphorylation events for activation of those pathways. There are some studies that indicate that glucocorticoids might exert inhibitory effects on these pathways by phosphatase activation. Activation of phosphatases might occur at the transcriptional level or by activating the phosphatases directly. Glucocorticoids have been reported to regulate $Ca^{2+}$-mediated pathways of T-cell activation by inhibition of the multifunctional $Ca^{2+}$/calmodulin kinase (CaM kinase II) by direct interaction with the kinase and by induction of protein phosphatase 2A and/or 1 activity (38). Dexamethasone increased cellular acid phosphatase activity in antigen-induced rat leukemic cells (39). It has been proposed that glucocorticoid suppression of phospholipase $A_2$ (PLA2) activity stimulated by $Ca^{2+}$ ionophores is mediated by glucocorticoid induction of Ser/Thr protein phosphatases, which inhibit PLA2 activity (40). The activity of type I protein phosphatases has been shown to be regulated by glucocorticoids (reviewed in Refs. 41 and 42). Protein dephosphorylation is an essential step for glucocorticoid-induced apoptosis in T-cell hybridomas, suggesting that glucocorticoids may be inducing the expression and/or activity of protein phosphatase for cell death to occur (43). It remains to be established how important phosphatase activation is as a glucocorticoid mechanism.

## III. Regulation of Immune and Inflammatory Responses by Glucocorticoids

In this section we discuss some newly recognized actions of glucocorticoids on inflammatory cells. We have omitted eosinophils, basophils, and mast cells, which have been reviewed elsewhere (1,44). Thus, this discussion focuses on monocytes, macrophages, dendritic cells, and other antigen-presenting cells (APC) and T lym-

phocytes. B lymphocytes are generally viewed as not being particularly GC responsive, at least with regard to immunoglobulin production (in fact, GC enhance antibody responses in vitro). However, B lymphocytes also perform antigen-presenting functions, and some of the recently recognized effects of GC on APC may also apply to B cells.

### A. Monocytes, Macrophages, and Dendritic Cells

New information on migratory patterns of these cells, mechanisms of antigen processing, and presentation and co-stimuli for antigen presentation is accumulating at an astounding rate (45,46). The effects of GC on several functions of dendritic cells or alveolar macrophages have been explored. For instance, the cytokine IL-12 has over the last few years been recognized to be an important product of macrophages, which induces IFN$\gamma$ and profoundly regulates T-cell activation (47). Glucocorticoids have been found to be potent and effective inhibitors of IL-12 production from human dendritic cells and/or macrophages (48–51). While some effects of GC on mediators such as IL-12 or IFN have led investigators to propose that GC selectively inhibit Th1-mediated responses, it must be borne in mind that GC are also effective inhibitors of the production of cytokines which polarize T cells toward Th2, including IL-4 and IL-13 (52–54), making conclusions about whether GC favor Th1 or Th2 polarization difficult. Recognition of the existence of subsets of dendritic cells that selectively induce TH1 and TH2 cells should make possible studies to determine whether GC have polarizing actions on antigen-presenting cells (55).

Several studies have identified profound inhibitory or modulatory effects of GC on steps of terminal differentiation and activation [including expression of cytokines and cell surface molecules such as B7.1 and B7.2 (CD80 and CD86)] in mononuclear cells (56,57). In a potentially important series of studies, Brokaw et al. showed that treatment of rats with GC led to a rapid fall in $Ia^+$ dendritic cells, to approximately 25–30% of the resting levels (58). Using the TUNEL assay, these investigators showed that this effect of GC was due to massive apoptosis of airway dendritic cells. Such findings may explain the observations of Burke and Poulter and collaborators several years ago showing that GC treatment of asthmatics leads to a reduction in HLA-$DR^+$ cells in the airways of asthmatics (59).

Glucocorticoids also exert some stimulatory effects on phagocytic cells. A recent study has shown that GC potentiate the phagocytosis of apoptotic eosinophils and neutrophils by human monocyte–derived macrophages, a potentially important anti-inflammatory effect of GC (60). A related and previously unrecognized anti-inflammatory effect of glucocorticoids may be to promote the local recruitment and differentiation of monocytes into phagocytic macrophages. For instance, recent studies by Penton-Rol et al. have shown a selective increase of the chemokine receptor CCR2, which mediates monocyte movement in response to MCP proteins (61). Other studies have shown that GC induce RM3/1,

a CD163-like scavenger receptor that may be involved in anti-inflammatory effects of recruited monocytes (62). In summary, GC have several newly recognized effects on phagocytic cells in the airways. While they may diminish the number of antigen-presenting cells such as activated macrophages or dendritic cells, as determined by expression of B7 and/or class II MHC molecules, GC may simultaneously increase the number or phagocytic activity of monocyte-derived macrophages involved in resolution of an allergic inflammatory response and initiating the wound-healing response.

### B. T Lymphocytes

A number of effects of GC on T lymphocytes have been recognized recently. Past studies have shown conclusively that GC inhibit production of a host of cytokines from T lymphocytes and thereby lead to inhibition of T-cell proliferation, cytotoxic T-cell activity, and other T-cell responses (63). Endogenous glucocorticoids, which are synthesized in the thymus as well as the adrenal gland, can profoundly influence T-cell differentiation and apoptosis (63). Recently, GC have been shown to upregulate the chemokine receptor CXCR4 (64). This receptor is involved in mediating the response to SDF-1, a chemokine thought to be important for T-lymphocyte trafficking and as a costimulatory molecule (65,66). A host of chemokine/receptor pairs have been found to mediate lymphocyte trafficking to lymph nodes, germinal centers, and tissue sites (67,68). Classical studies by Haynes and Fauci demonstrated that GC differentially regulate the movement of recirculating vs. nonrecirculating lymphocytes (69). As lymphocytes become activated, they lose some chemokine receptors that mediate their trafficking to lymph nodes (e.g., CCR7) and gain a host of receptors that mediate their migration into peripheral tissue sites, such as lung, skin, etc. (67,68). It seems likely that glucocorticoids will be found to modulate expression of numerous chemokine receptors and that modulation of lymphocyte chemokine receptor expression is an important mechanism by which glucocorticoids regulate the trafficking pattern of blood lymphocytes.

Recent studies by Nocentini et al. identified a GC-induced protein referred to as GITR, a GC-induced TNF receptor family–related gene. This protein blocks T-cell receptor–induced apoptosis and may lead to prolonged survival of T cells (70). This observation has to be reconciled with numerous studies showing that GC can induce apoptosis, especially in thymocytes. It is now clear that GC effects on lymphocyte viability are influenced by the state of stimulation and differentiation of the cell (63). Other recent studies have shown that GC downregulate granzyme B (71). This is an enzyme involved in T-cell killing and may, to some extent, explain earlier observations that GC inhibit CTL function (72). Finally, an interesting recent study has shown that GC cause a dramatic potentiation of CTLA-4 expression (73). CTLA-4 contains an immunotyrosine inhibitory motif and has been hypothe-

sized by Bluestone et al. to be centrally involved in T-cell tolerance (74,75). Induction by GC of CTLA-4 could therefore potentiate tolerogenic responses of T cells.

## IV. Effects of Glucocorticoids on Fluid Dynamics in the Airways

Antigen challenge and allergic airways disease are both associated with movements of fluids into the mucosal tissue, as well as into the lumen of the airways. GC have long been known to be effective in reducing both intralumenal plasma exudation as well as secretion of mucus into the airways. Considerable progress has been made in the study of the dynamics of fluid flow across both endothelial and epithelial barriers. While it is beyond the scope of this chapter to discuss these advances in detail, several that are likely to have relevance to the therapeutic effects of GC will be mentioned.

### A. Vascular Endothelial Cells

One of the hallmarks of inflammation is the exudation of plasma proteins into the extravascular space. Although GC are excellent inhibitors of vascular leak induced in vivo in humans or experimental animals by diverse stimuli such as high altitude, endotoxin, ethanol, recombinant IL-2, lipid mediators, and other stimuli, it is not known whether this effect results from a direct action on endothelial cells or is an indirect effect (76–82). GC do reduce vascular leak in the skin following challenge with mediators thought to work directly on the endothelium such as histamine or bradykinin (83). Recently, using endothelial cells from a variety of sources, a number of interesting effects of GC on endothelial cell phenotype or function have been identified, suggesting that GC have some direct effects on endothelial cells. GC have been found to increase tight junctions and decrease intracellular gaps in cultured endothelial cells associated with an increase in the junction-associated protein ZO-1 (84). Another junctional protein, PECAM-1 (CD31), may also be induced by GC, as it contains a GRE in the proximal promoter region (85). PECAM-1 is an ubiquitous adhesion molecule thought to be involved in transendothelial migration of leukocytes and maintaining junction integrity. Interestingly, PECAM-1 is an ITIM-containing transmembrane protein and thus may have some heretofore unrecognized regulatory effects in endothelial cells (86). Study of the influence of ligation of PECAM-1 on endothelial function, especially after treatment with GC, seems worthwhile.

Several other recent findings are relevant to the antipermeability effects of GC. Glucocorticoids have been found to decrease histamine receptors on vascular endothelial cells, which could, in part, explain suppression of histamine-induced vascular leak (87). Glucocorticoids have also been shown to inhibit the action of components of neurogenic vascular responses, including CGRP/substance P/

neurokinin A (88–91). Also of potential relevance to the effects of GC on vascular leak are recent studies showing that GC are potent inhibitors of the production of vascular endothelial growth factor (VEGF) (92–95). VEGF was originally known as vasopermeability-inducing factor (VPF), as it is a potent inducer of vascular leak. Not only do glucocorticoids inhibit production of VEGF, but they have also been found to inhibit the activation of the kinases JNK and ERK by VEGF in endothelial cells (96). The effects of GC on VEGF production and function are summarized in Figure 2. Other targets of GC action have been identified in endothelial cells. Recent studies have shown that GC can decrease endothelial NO synthase expression (eNOS), and they increase osteopontin, which itself can cause decreased iNOS (96–98). Perhaps most important with regard to fluid dynamics in the airways are studies by King et al. showing that GC have profound effects on the expression of aquaporin 1 without any effects on aquaporin 3, 4, or 5 (99,100). The aquaporins are water channels that are often expressed in selected tissues and are felt to be extremely important in regulating fluid dynamics in a variety of tissues, including lung. Glucocorticoid induction of aquaporin 1 is found in the late gestational period and may be important for the rapid changes in fluid handling in the airways at the time of delivery of the newborn (99,100). The importance of GC modulation of aquaporins in disease or in homeostasis in adults has not yet been established. A summary of the distribution of expression of the aquaporins in the airways is presented in Figure 3.

### B. Airway Epithelium

Glucocorticoids are widely perceived to be powerful suppressors of bronchorrhea and mucus production in patients during asthmatic attacks. While older in vitro studies suggested that GC can inhibit mucus secretion in airway tissue, relatively little is known about the mechanisms of these effects (101–103). Epithelial cells

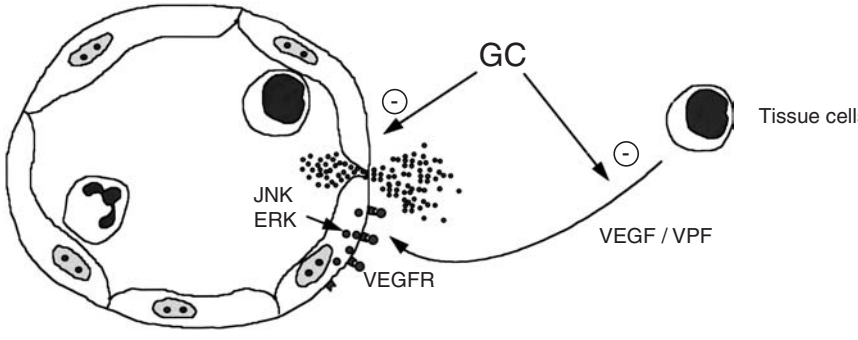


**Figure 2** GC inhibit both the production and action of VEGF (see text).

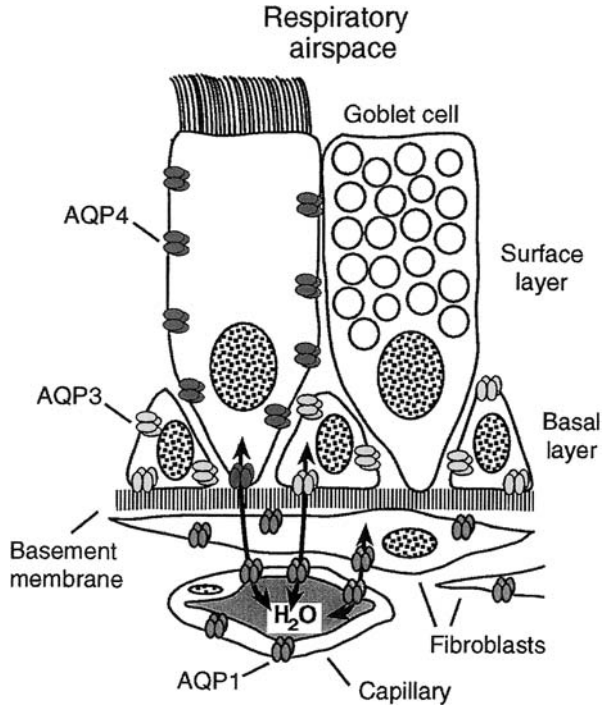


**Figure 3** Distribution of aquaporin proteins in the airways. Note that expression of AQ1 is highly regulated by GC (see text). (From Ref. 100.)

are now known to be important targets of GC, and a variety of epithelial-expressed inflammatory proteins have been shown to be suppressed by GC, including numerous chemokines, iNOS, GM-CSF, etc. (104). Glucocorticoids inhibit fluid flux across airway epithelium in vivo and in vitro. Using mammary epithelial cells, an elegant series of studies has demonstrated that GC change the junctional organization of the cells, causing remodeling of tight and adherens junctions and changes in transepithelial electrical resistance reflecting sealing of tight junctions (105). In this process, GC induce ZO-1 expression and recruit the junctional proteins ZO-1 and β-catenin as well as F-actin to the junctional region (105). Effects of GC on components of surfactant and airway mucus are complex. Glucocorticoids selectively decrease the expression of the surfactant protein SP-A1 but not SP-A2 or SP-B (106–108). The functional consequence of selective surfactant protein regulation is not clear. Glucocorticoids also increase fatty acid synthase, which is involved in producing the lipid component of surfactant (109,110). Indeed, one of the remarkable effects of GC in premature infants is an enhancement

of surfactant production sufficient to prevent the respiratory distress syndrome of neonates if the mother is treated with GC before premature delivery (111). While some of these effects may have to do with GC actions on aquaporins (see above), others are likely due to increases in surfactant production. With respect to mucus, GC have been found to decrease MUC-2 and MUC-5ac, two of the important mucoproteins produced by mucus-secreting cells in the airways (112).

## V. Glucocorticoid Effects on Bone

Due to the effects of systemic GC on formation and turnover of bone, numerous groups have studied the effects of GC on the cell types involved in maintaining bone architecture. Recent findings have demonstrated both stage- and species-specific effects of GC on osteoclasts, osteoblasts, and their progenitors (113–117). Although administration of high doses of oral GC causes bone resorption in humans and many other species, GC actually cause increases in bone mass in rats, making interpretation of some of the literature difficult (118). Using human cells, glucocorticoids have been shown to inhibit osteoblast maturation as well as induce apoptosis of mature osteoblasts, reducing osteoblast numbers and activity (113–116). A decrease in the $TGF\beta_1$ receptors in osteoblasts by GC may contribute to these effects (119). GC have also been found to increase numbers of osteoclasts (113,117,120). They may do so in part by increasing osteoprotegerin ligand, a mediator felt by some to be the final effector in osteoclast generation (120,121). GC also decrease osteoprotegerin, the soluble form of a TNF family receptor that can neutralize the osteoclast-stimulating properties of osteoprotegerin ligand (120, 121). A similar reciprocal effect of GC on ligands and soluble binding factors has been found in the case of IGF-1 and IGFBP-rp1 (122). Glucocorticoids have been implicated in space flight–induced osteoporosis; a study performed on the space shuttle has shown a 3- to 10-fold increase in IGFbp3 in space and a 30–70% decrease in IGFbp5, associated with a 3- to 8-fold increase in GC receptor in cultured bone cells. The increase of GC receptor was proposed to mediate some of the bone remodeling observed in astronauts during prolonged space travel (123). Various reports have indicated an influence of GC on other regulators of the formation of bone, including oncostatin M (124), TIMP-3 (124), parathyroid hormone (125), galectin 3 (126), and calcitonin (127).

## VI. Somatostatin

Somatostatin is a hormone that regulates inflammation as well as endocrinological functions such as growth hormone secretion (128). Glucocorticoids have been found to increase somatostatin in many tissues and cells, including brain, gastrointestinal tract, and macrophages, perhaps via a GRE in the somatostatin promoter (128–130). The well-known suppression of growth hormone release, and

growth, by GC is thought to be mediated via induction of somatostatin release (128). Along with increases in the release of somatostatin, GC also are found to increase the expression of some somatostatin receptors, e.g., SSTR2 (129). SSTR2a mediates an inhibitory effect of somatostatin on IFNγ release (131). Thus, GC may inhibit IFNγ release in part by increasing the receptor for somatostatin as well as increasing the production of somatostatin. Somatostatin receptors are also found to activate PTP phosphatases, which downregulate MAP kinase pathways (128). Thus, activation of somatostatin pathways may be another mechanism by which GC suppress inflammation (132). Evidence has been provided that somatostatin can inhibit inflammation by antidromic nerve stimulation (133).

## VII. Miscellaneous Targets

As time goes by, the spectrum of molecules recognized to be important in inflammation and regulated by GC continues to grow. Glucocorticoids have been shown to inhibit or enhance stem cell factor release in a case-dependent way (134). Since stem cell factor has been shown to be involved in activation of mast cells, this may be an indirect way by which GC can regulate mast cell function. Glucocorticoids have been shown to inhibit the action and the expression of TGFβ (135). Since TGFβ is thought to be important in airway remodeling in asthma, some of the GC antiremodeling effects may be related to this action. It has been proposed that MIF is a glucocorticoid-induced modulator of cytokine production (136). Glucocorticoids have been found to be essential for formation of the adrenal catecholamine epinephrine by virtue of a GC requirement for expression of the synthetic enzymes PNMT and secretogranin (137). Glucocorticoids have also been shown to inhibit cell surface expression of the inflammatory cytokine LIF as well as to inhibit expression of IL-4Rα chain, a receptor component known to be important in differentiation of T cells as well as mediation of allergic inflammation (138). Finally, based on the presence of GREs in putative promoter sequences, there is reason to believe that GC may regulate the function of a number of molecules, which are potentially involved in allergic inflammation, including kallikrein-binding protein (a serpin) (139), prostacyclin synthase (140), the thromboxane receptor (141), the M1 muscarinic receptor (142), and TGFβ (143).

## VIII. Conclusions

While this review is clearly not comprehensive, we hope we have achieved our goal of providing some information on recently recognized targets of GC action either in vitro or in vivo. We have attempted to focus attention on those likely to be important in the regulation of inflammation of the airways and look forward to

future studies that will help better delineate the relevance of these new molecular targets to the broad antiasthmatic activity of GC.

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## Discussion

**Dr. Seale:** You mentioned lipocortin almost as a "throwaway" line at the end. Is that the appropriate perspective?

**Dr. Schleimer:** Although lipocortin was once thought to be a mediator or transducer of steroid effects, it is now not widely believed to play such a role.

**Dr. Inman:** Does the effect of GC that increases monocyte recruitment also affect the balance between macrophages and dendritic cells in terms of antigen presentation?

**Dr. Schleimer:** This is an interesting question, which I can't answer well. Glucocorticoids decrease dendritic cell numbers in the lungs; alveolar macrophage numbers are not changed. Since dendritic cells are far superior as antigen-presenting cells, the effect on dendritic cell numbers may be of particular relevance.

**Dr. Georas:** Another recently identified GC-induced gene with direct relevance to asthma is the M2 muscarinic receptor. David Jacoby at Hopkins has unpublished data that this receptor, which inhibits acetylcholine release and thus has a bronchoprotective effect, is increased by steroids in cultured parasympathetic neurons (Jacoby DB, Yost BL, Kumaravel B, Chan-Li Y, Xiao HQ, Kawashima K, Fryer AD. Glucocorticoid treatment increases inhibitory m(2) muscarinic receptor expression and function in the airways. Am J Respir Cell Mol Biol 2001; 24(4):485–491).

**Dr. Szefler:** Are there different patterns of chemokines related to allergic and nonallergic features of asthma?

**Dr. Schleimer:** I don't know of studies comparing chemokine patterns in atopic versus nonatopic asthmatics. Pathology studies indicate that the cell recruitment pattern is similar, however.

**Dr. Stellato:** I thought that endothelial cells were unresponsive to GC, since the expression of many inflammatory genes (adhesion molecules, chemokines) was not suppressed in vitro by GC treatment. You showed that many genes are indeed inhibited in endothelial cells by GC. Could you comment on that?

**Dr. Schleimer:** As you know, cultured human umbilical vein endothelial cells respond to inflammatory stimuli by expressing adhesion molecules and releasing cytokines and mediators. In many laboratories, GC have failed to inhibit these responses. Since there are numerous reports showing inhibitory effects of GC on similar responses of endothelial cells from animals or from other tissue locations in humans, this may be explained by the low numbers of glucocorticoid receptors in umbilical vein endothelial cells. More studies are clearly needed in this area.

**Dr. Persson:** As you describe so well, steroid targets are exceedingly and increasingly complex; this information affects drug research. If complexity is required (for efficacy), current drug discovery work (i.e., technologies focusing on single targets) will fail to produce "efficacious" drugs (indeed, due to the nonspecificity the steroid drugs could not have been "discovered" today). Actions/targets included in the list of steroid mechanisms may, inferentially, not be worth pursuing as single targets for innovative drugs. This is especially so for those steroid effects that are potently induced in vivo. What is the use of novel antidendritic drugs if in inhaled steroid–treated airways dendritic cells have already been abolished? In fact, target validation seems to be poorly developed compared to the rate of appearance of novel proposed targets; for example, steroids clearly induce eosinophil apoptosis in vitro, and this mechanism has received major attention and acceptance. However, this action has not been seen in vivo in airway tissues. (Indeed, apoptotic eosinophils are conspicuously absent in the blood or in perfused asthmatic or rhinitic airway tissues.) Finally, it would be interesting to learn about your current priorities among actions/targets? For example, if VEGF inhibition is an important aspect, then we should perhaps not delay in examining VEGF antagonists in asthma.

**Dr. Schleimer:** You raise an important and disturbing point. Of the targets under development now, several are known to be blocked by steroids, including IL-4, IL-5, CCR3 agonists (eotaxins, MCPs, etc.), and IL-13. Steroids don't significantly reduce IgE levels, though. In most cases, the steroid effect is incomplete, however, and a single target inhibitor that ablates the pathway could exceed the steroid effect on that particular target. Since the global allergic response may require several elements, it is not unreasonable to hope that blocking a single element can block the overall response. As you well know, eosinophil apoptosis in vivo is a point of controversy in the field, which needs to be resolved. There are no compelling data to suggest that VEGF is a particularly attractive target in asthma, although recent studies from Australia indicate that angiogenesis may be increased in asthma.

# Part Four

## DETERMINANTS OF AIRWAY–LUNG SELECTIVITY

# 8

# Aerosol Delivery Devices and Airways/Lung Deposition

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## I. Introduction

Sufficient local concentration of drug can be achieved on airway surfaces throughout the lung by using the inhaled route. A number of factors determine the dose of drug deposited on airway/lung surfaces drug and the overall therapeutic effect (1–5). These are the physical characteristics of the aerosol produced, the patient inhalation variables, and the extent of their airways/lung disease. Additional factors are the distribution of target sites in the lung and local pharmacokinetics for the particular drug. Knowing the actual dose inhaled from a delivery system for an observed response is extremely useful information. It allows a comparison of different drugs within the same category by measuring their relative potency using a more accurate estimate of dose deposited than label claim (6,7). Additionally, a more precise assessment of the relative performance of delivery systems used for a specific drug therapy can be made (8–10), providing information to guide the physician in choosing a delivery system.

A recent review from Selroos et al. (11) described in vitro doses and lung deposition values measured from pressurized metered dose inhalers (pMDIs) with or without spacers, dry powder inhalers (DPIs), and nebulizers. These were compared with in vivo responses, not necessarily in the same subjects who participated

in the deposition studies, but with a variety of radiotracer, pharmacokinetic, and pharmacodynamic studies using the same or different drugs. This overview highlights key features of several delivery devices used to provide aerosol therapy and discusses how to define the dose available for inhalation from each system in order to more accurately predict and compare clinical outcomes.

Issues that are important when discussing aerosol drug delivery but that will not be discussed in detail in this chapter are patient compliance or adherence with taking medications, ergonomics, and economics. Ease of use of the various inhaler systems is an important consideration when designing an inhaler. If patients are not able to load doses easily, prepare the inhaler quickly, or take their doses with certainty, it is likely that adherence to their therapy will be poor. Having to follow a complex treatment schedule or deal with instructions that the patient or caregiver cannot readily follow will further compromise the therapy. Other considerations may be the costs associated with delivering inhaled medications to patients both in-hospital and out-of-hospital. These costs will vary with the setting, the options available, and the type of drug prescribed.

## II. Aerosol Delivery Systems

The choice of systems producing therapeutic aerosols is currently limited to three main classes: 1) pneumatic (jet) and ultrasonic nebulization for providing continuous or intermittent aerosols of liquid solutions or suspensions, 2) pMDIs with or without an attached spacer (S) or holding chamber (HC), and 3) DPIs. The latter two systems are used for dispensing metered drug doses, although metered doses of drugs in liquid form are provided from some of the newer inhalers (12). Within these three categories there are a variety of devices that provide aerosols with mostly similar, but sometimes very different characteristics and, hence, different amounts of useful aerosol provided to the patient for inhalation (13–15). It is known that the variable efficiency of production of aerosol among inhalers within the same device category may require the prescription of different doses of a drug (11,16,17).

Some inhaler devices have been in use for 50 years or more. Other hardware, such as spacers and valved holding chambers, have been used as add-on devices for pMDIs for approximately the last 25 years. New designs and improvements upon existing designs have occurred in all of the above three categories and particularly in the last decade with the recognition that aerosols can be used to carry medication into the deep lung. While the aerosol route is the preferred method for treating airways/lung disease, the technology has recently been applied to the treatment of some systemic diseases, such as diabetes (18). One outcome of this development is the number of innovative inhalers available for generating respirable aerosols (19). Treatment with aerosols of proteins, peptides, and anal-

gesics requires highly efficient delivery of small particles, as for these therapies to be effective, the drugs need to be deposited in the very peripheral airways for rapid absorption into the circulation. Ease of use, portability, and patient compliance considerations, such as dose counters and integrated electronic management systems to track treatments and treatment schedules, are being incorporated into some new inhaler designs. As systems become more sophisticated, with better control over aerosol generation, one should expect the dose of therapy delivered to the lung to be more precise and the aerosol to have the size characteristics for optimal lower respiratory tract deposition. Despite improved technology, the varying breathing patterns and the differences in oropharyngeal and airway geometries cause variations in the inhaled dose between infants, children, adults, and elderly patients (20–22), which will continue to lead to altered clinical responses between patient populations.

### A. Nebulizers and MDLIs

Over the last 8–10 years, major innovations have occurred in the delivery of wet aerosols. The most sophisticated of these systems are designs for metered dose liquid inhalers (MDLIs) (12) that mimic the action of pMDIs in that a reproducible, unit dose of drug is released with one to two actuations. The main features of these systems include self-generation of aerosol (23–25), production of low-velocity aerosols (24,25), breath actuation (25–27), and electronic management of delivered doses and treatment schedules (26,27). Lung deposition has been shown to range from 31 to 70%, greater than from most current inhalers (12, 24,26,28–30). Other improvements upon jet nebulizers—e.g., increasing drug output by air entrainment through rather than across the nebulizer, controlling the dose inhaled (31), and decreasing drug wastage by reducing or eliminating aerosol generation during the patient's expiratory phase (32,33)—have produced a number of devices with increased efficiencies for delivering therapy.

However, the standard and likely most commonly used jet nebulizer is the constant output design, run by compressed air or oxygen with supplemental air drawn in across the top of the nebulizer, diluting the solution or suspension aerosol produced within the nebulizer as it exits towards the patient and thereby decreasing the inhaled dose (34). The patient can synchronize inhalation with actuation to avoid wastage of drug aerosolized during expiration by placing a thumb-control orifice in the compressed air line. Treatment times are, however, lengthened to completely aerosolize the reservoir contents and the dose inhaled is increased. These systems can also become breath-actuated (breath-synchronized systems) by pulsing the airflow to the jet orifice with a dosimeter. The length of time the aerosol is delivered during inspiration significantly influences the dose inhaled in children (34,35). Using breath synchronization to provide budesonide suspension aerosol over the full inspiratory breath proved to be a more successful delivery

technique than generating the aerosol continuously over the entire breath cycle. Breath-enhanced nebulizers direct supplemental air through the nebulizer across the venturi, sweeping out more of the available aerosol and providing an increased output of drug (36). With use of internal valves, the output of these nebulizers can be reduced to the jet flow output, thus decreasing drug wastage during exhalation as with breath-synchronization nebulizer circuits (37).

The median particle size of most jet nebulizer aerosols ranges from 2 to 6 μm (2,13). The particle size can be further reduced by placing baffles within the nebulizer, using one-way valves in the mouthpiece or increasing the length of tubing between the nebulizer and the patient (38). However, these additions result in drug loss within the circuit, reducing the amount of aerosol available to the patient. The losses are variable, and therefore, it is not possible to deliver precise doses to patients.

During operation of a jet nebulizer, solvent evaporates, resulting in a decrease in the temperature of the reservoir solution and a progressive increase in the concentration of drug in the reservoir and in the aerosol droplets produced (39,40). The rate at which the solution undergoes increased concentration is affected by the jet flow rate. A noticeable reduction in drug output and an increase in the size of the aerosol droplets produced occur if the changes in concentration are marked (40). Other factors affecting aerosol output and particle size are the driving pressure or the flow rate of compressed air applied to the jet. The higher the pressure or flow rate, the greater the output over time in terms of total solution aerosolized (13,14,40–43).

Unit doses loaded into nebulizers are five- to sixfold greater than doses per actuation for pMDIs. These high doses are required for equivalent efficacy (44), although local side effects may be greater (45). In vitro measurements of total drug available from a nebulizer will be greater because of the starting dose (46), but when deposition is expressed as a percentage of the reservoir dose, the lower delivery efficiency will be obvious. Standard jet nebulizers deposit from 2 to 12% of the reservoir contents in the lung (47); breath-enhanced nebulizers are more efficient and can double the deposition efficiency of the older designs. As shown in Figure 1, much greater deposition has been measured for some MDLIs, with values up to 78% recorded by imaging the lung. These are encouraging results for using the aerosol route with therapies such as insulin.

### B. pMDIs

Doses released from pMDIs contain active drug in micronized powder form or in solution, plus surfactant, co-solvents, and propellants (48). There may be other excipients in the formulation such as flavoring agents. The characteristics of an aerosol produced from a pMDI spray in terms of particle size characteristics and spray pattern are influenced mainly by the vapor pressure of the canister, deter-

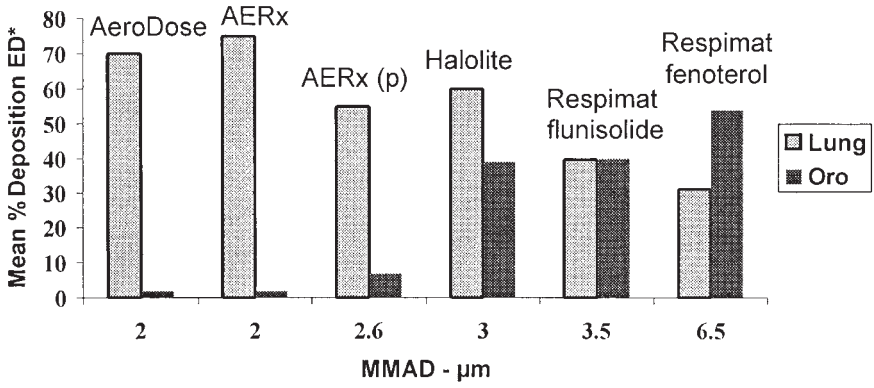



**Figure 1** Relationship between the aerosol mass median aerodynamic diameter (MMAD) and deposition to the lung and oropharynx for metered dose liquid inhalers (MDLI). The finer the aerosol, the more deposition to the lower respiratory tract with less deposited in the mouth and throat. AERx (Aradigm, Hayward, CA); AeroDose (AeroGen, Sunnyvale, CA); AERx prototype (Aradigm, Hayward, CA); Halolite (Aradigm, Hayward, CA); flunisolide Respimat® (Boehringer Ingelheim, Germany); fenoterol Respimat® (Boehringer Ingelheim, Germany). *Emitted dose. (Data from Ref. 12.)

mined by the propellant mix (49). The propellants provide the energy source to disperse drugs into a small size aerosol capable of penetrating to the target tissue. The size and velocity of the pressurized aerosol affect the deposition of these drugs in the lung (50–52).

The dose reproducibility and the amount of drug released per actuation over the life of the canister are also a function of the design of the metering valve (53). With chlorofluorocarbon (CFC) products, storage conditions, valve orientation during long-term and short-term storage, as well as ambient temperature have all been shown to result in in vitro dose variability (54). With replacement propellant hydrofluorocarbon (HFC) products, dose reproducibility has been shown to be high throughout the life of the canister, and moreover, the emitted dose appears to be unaffected by storage, temperature, or valve orientation (52). The volume of the metering valve, which varies from 30 to 100 µL, determines the amount of drug released per actuation of the pMDI. Increasing the metering volume will cause increased loss of drug on the actuator mouthpiece because of the lower rate of evaporation of the greater amount of propellent released (48,55).

Atomization of the liquid stream released from a pMDI on actuation begins instantly as the propellants "flash" or vaporize and proceeds through continued evaporation of the propellants (50,56); aerosol production takes approximately 20 ms. The velocity of the liquid spray on ejection from the CFC pMDI is about 15 m/s, rapidly decreasing to approximately 7 m/s within 0.1 s as the spray cloud

forms, decelerates, and moves away from actuator orifice (57). This high-velocity jet causes approximately 80% of the dose to impact in the oropharynx, particularly when the canister is fired with the actuator mouthpiece inside the mouth. This impaction is reduced with some HFA formulations due to their lower spray velocity (58). The local deposition from steroids can lead to local irritation such as hoarseness and sore throat, but the incidence is low (59–62). Other side effects reported in small numbers of patients have been a reduction in $FEV_1$ due to the lubricant or surfactant present in the formulation (62) and candidiasis (61,62). Holding the canister outside the wide open mouth provides a space for the spray to deccelerate as the propellants, both CFC and HFA, evaporate, enhancing the capacity to entrain the aerosol into the inspiratory airstream. An advantage of the open-mouth technique is that less propellant is inhaled and the aerosol is finer. Using the open-mouth method for inhaling the spray, coupled with a low inspiratory flow rate, can result in a doubling of the dose delivered to the lower respiratory tract in adults from approximately 7% to 14% (63). However, the open-mouth technique is difficult for many patients to master, particularly children. Furthermore, drug continually deposited on the face or in the eyes can result in additional problems over time and particularly with inhalation of high doses of steroids and anticholinergic drugs. As discussed below, spacers are the preferred alternative for inhaling these drugs. In comparison, the deposition of QVAR, the solution beclomethasone dipropionate pMDI, resulting from reformulating this steroid in HFA134a has been measured in four independent laboratories and found to be approximately 52% of the emitted dose (ex-actuator) for both healthy volunteers and patients with asthma (64–67). This represents a 2.5-fold increase compared to the CFC suspension product (65) and correlates well with clinical outcomes measured in a number of studies (68,69).

### C. Spacers

An important function of spacers is the selective removal of nonrespirable particles of the pMDI spray through impaction of the fast-moving spray on spacer walls and valves. In general, the particle size of a pMDI suspension aerosol exiting a spacer is decreased by approximately 25% while the fraction containing particles less than 5 μm in diameter is increased (70). With valved holding chambers (HC), this fraction can be augmented by further evaporation of propellant from the aerosol in the finite time between actuation and inhalation; the increase in this fraction appears to depend on the pMDI formulation (71). While the dose of a drug available at a spacer exit can vary for different spacers (72), a result of formulation factors, design differences, and spacer volume which can range from 15 to 750 mL, the particle size distribution is similar (2,73). In vitro measurements of emitted dose have shown that not all drugs can be used with all spacers (74), requiring measurements of the doses available to ensure sufficient drug is avail-

able to the user. Static charge on plastic spacer walls reduces the dose available for inhalation (75,76). The loss can be recovered by washing with a mild detergent or priming the spacer before use with several doses of drug (77). Like the detergent, this reduces the electrostatic charge by leaving a thin layer of surfactant on the walls. Emitted doses have been shown to be higher with antistatic treatment of plastic spacers and lung deposition greater (77,78) and comparable to metal spacers, which do not carry a electrostatic charge. Despite these findings, it has recently been demonstrated in a crossover study in children that clinical effects are not markedly improved when using static-free spacers. Salbutamol was inhaled from plastic spacers holding an electrostic charge and then from the same spacers with their charge removed. A further comparison was made to the Nebuchamber, the metal spacer from AstraZeneca (AstraZeneca, Sweden). No significant difference in peak flow was noted between static and static-free spacers or the metal spacer (79,80).

An advantage of valved spacers is that patients can easily inhale the aerosol using a low inspiratory flow rate. This, coupled with the finer aerosol available from the spacer, helps promote deposition in the lung and enhanced clinical response (81). Using gamma scintigraphy (82,83) and pharmacokinetic studies (84, 85), lung deposition from pMDIs with spacers has been shown to be the same or greater than from the pMDI alone, between 5 and 35%, with oropharyngeal deposition markedly reduced to approximately 4–15%. Using a spacer with beclomethasone dipropionate decreased systemic absorption of the drug and produced fewer side effects compared to inhaling the drug from a dry powder inhaler (86). While there may be a greater amount of drug deposited in the lung with some larger spacer devices (83), clinically there appears to be little advantage to using spacer devices greater than 150 mL in volume (70). Open-tube (OT) spacers and reverse-flow (RF) designs, devices in which the pMDI is positioned close to the mouth and fired in the direction away from the patient, require the patient to synchronize inhalation with actuation. Failure to coordinate these two maneuvers will reduce the drug deposited in the lung compared to using a valved holding chamber (87). Similarly, too rapid an inhalation from an OT spacer has been shown to decrease deposition by approximately 30% (88), resulting in a reduced clinical effect (89).

Face masks with and without expiratory valves and coupled to valved holding chambers are widely used to deliver pMDI aerosols to children. The resistance of both valves needs to be sufficiently low to allow them to open and close with their low tidal volumes and flow rates (90,91). The face mask must provide a proper seal to the child's face to avoid loss of dose, an important consideration whether they are inhaling pMDI aerosols (92) or aerosols from nebulizers (35). The tidal volume:spacer volume ratio should also be considered when using spacers with young children, particularly infants. Drug is less concentrated in a

large-volume spacer and decreasing with time due to sedimentation to spacer walls. This may mean that only a small amount of aerosol is inhaled, even allowing for 30 s of tidal breathing through the device (93).

### D. DPIs

In contrast to pMDIs, DPIs do not require propellants; they are breath-actuated, thus eliminating the need for synchronization of inhalation with actuation. DPIs may allow greater formulation flexibility and do not require the same physical and chemical stability of drug, compared with suspension- or solution-based pMDIs (48). They can be classified according to their means of storing and providing the drug, i.e., as single capsules, in a bulk reservoir, or as multi–single unit dose devices (MUSD) (Table 1). The latter can take the form of blisters, blister tape, capsules, or multichambered cassettes (94,95). With all types of DPIs, some drug remains in the storage medium following dosing. The amount of drug available per actuation should account for this loss and be sufficient to achieve a clinical response. With the exception of budesonide and terbutaline sulfate in the Turbuhaler (AstraZeneca, Sweden), most powder devices require a lactose carrier to allow the powder dose to flow out of the inhaler (95).

DPIs that rely on the patient's inspiratory effort to dispense the dose are often referred to as passive or patient-driven devices as opposed to power-assisted or active DPIs. The advantage of passive devices are that they are breath-actuated and do not require an energy source to generate the aerosol, such as propellants in the pMDI, electrical energy, or compressed air. However, because they are dependent on the patient's inspiratory flow rate to dispense the drug powder, there can be differences in lung delivery efficiencies within and between DPIs and, ultimately, clinical response. Active or powered devices designed to be independent of patient effort require a holding chamber to contain the powder released from the device, and, as with pMDI spacers/holding chambers (S/HC), this results in some drug being lost in the chamber. The Spiros DPI (Dura Pharmaceuticals, San Diego, CA) is an exception, combining both passive and active design features. While electrically driven, it is also a breath-actuated DPI, dispensing powder with the initiation of inhalation.

Further differentiation between DPIs is based on the specific resistance of the device (96), determined by its geometry, and which, in turn, governs the maximal inspiratory flow rate (IFR) that can be drawn through the device and hence the optimal delivery of powder aerosol to the lung. The range of specific resistance values for current designs is approximately 0.02–0.2 $(cmH_2O/L.s^{-1})^{1/2}$. High resistance decreases the ability to draw air through the inhaler, but use at the optimal IFR for the inhaler will deliver more drug (17,97). For the DPIs shown in Table 1, there is a threefold variation in lung deposition between DPIs, from 12–37% of the emitted dose. Deposition appears to be lower for DPIs with lower

**Table 1** Lung Deposition from Various DPIs at the Optimal Operating IFR

| DPI | Dose storage | Specific[a] resistance | Optimal inspiratory flow rate (L/min) | Drug | Subjects | Lung deposition (mean % emitted dose) |
|---|---|---|---|---|---|---|
| Spiros® (3,154)<br>Dura Pharmaceuticals, San Diego, CA | MUSD<br>Cassette | High | 15 | Salbutamol<br>Beclomethasone dipropionate | 10 normals<br>10 normals | 37.4<br>41.0 |
| Pulvinal (155)<br>Chiesi, Parma, Italy | Reservoir | Medium | 60 | Salbutamol | 10 normals | 15.1 |
| Easyhaler® (156)<br>Orion Farmos, Kuopio, Finland | Reservoir | High | 60 | Salbutamol | 10 normals | 28.9 |
| Turbuhaler® (82,158)<br>AstraZeneca, Lund, Sweden | Reservoir | High | 60<br>73 | Salbutamol<br>Budesonide | 8 normals<br>8 asthmatics<br>8 normals | 23.2<br>26.1 |
| Clickhaler® (157,158)<br>ML Laboratories, St. Albans, UK | Reservoir | High | 57<br>73 | Beclomethasone dipropionate<br>Budesonide | 8 normals<br>8 normals | 29.8<br>26.8 |
| Diskus (159,160)<br>GlaxoSmithKline, UK | MUSD<br>Tape | Medium | 60 | Fluticasone propionate | n/a normals<br>n/a asthmatics | 16.6[b]<br>11.9[b] |
| Taifun (161)<br>Leiras OY, Turku, Finland | Reservoir | | 30 | Budesonide | 10 normals | 34.3 |
| Ultrahaler® (162)<br>Fisons, Loughborough, UK | Reservoir | Low | 75 | Nedocromil | 12 normals | 13.3 |

n/a, not available.

[a]High = >0.1; medium = 0.05–0.01; low = <0.05 (cm $H_2O$/L.$s^{-1}$)$^{1/2}$.

[b]Assessed by pharmacokinetic measurement.

specific resistances, and the values are much lower than for the MDLIs described earlier. DPI oropharyngeal doses are approximately 60% for all designs.

## III. Characterization of an Aerosol Dose from a Delivery System

### A. Nominal/Emitted Dose

The aerosolized dose of drug available at the exit or mouthpiece of a nebulizer or inhaler, either the pMDI or DPI, is defined as the emitted dose (ED) (Fig. 2), and reflects the loss of drug on the actuator mouthpiece or in the inhaler or spacer. This value is less than the package unit drug dose, termed the nominal dose or label claim (LC). In the United States the label claim is the emitted dose, while in Canada and Europe the LC is the unit dose loaded into the inhaler, also termed the metered dose. The emitted aerosol can be fractionated into fine (<4.7 μm diameter) and coarse (>4.7 μm diameter) particles. The doses of drug carried by these particles are termed the fine particle dose and coarse particle dose. The inhaled dose is equal to the emitted dose, provided nothing is inserted between the inhaler exit and the mouth to capture some of the aerosol. Part of the inhaled dose deposits

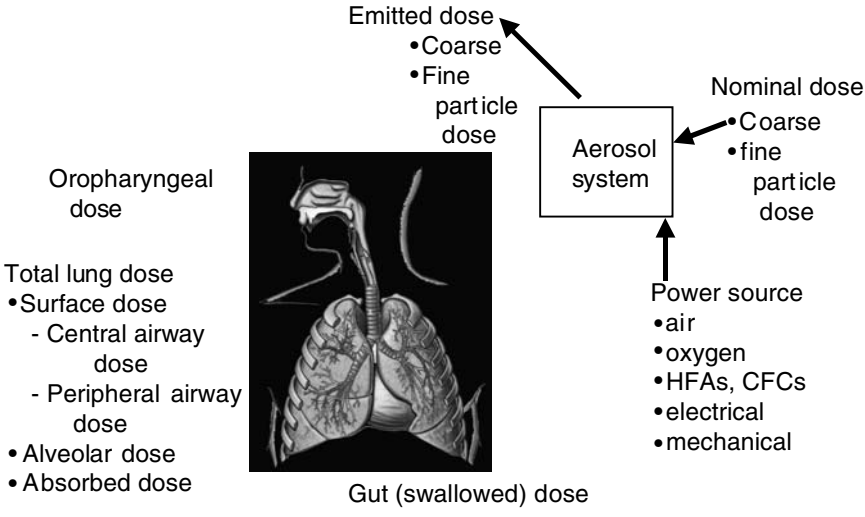



**Figure 2** Categorization of doses of an aerosol from a delivery system and the possible discrimination of these doses in the lung.

in the oropharynx, and the balance is distributed in the lung on airway surfaces. Some of the deposited dose is absorbed through the airways/lung, part is cleared by mucociliary action, and part may be retained in the lung. Each of these doses contributes in part to the clinical efficacy but also to any adverse effects experienced by the patient.

The difference between the nominal dose and the emitted dose reflects losses on system hardware, for example, nebulizer walls, tubing and mouthpieces, plastic parts of DPIs, blister packaging surfaces, metering valves, pMDI actuator mouthpieces, and spacers. The extent of these losses can be measured using chemical assays and can, in some systems, substantially reduce the nominal dose by up to 70%, when, for example, a spacer is used with a pMDI. Mouthpiece actuator losses vary from 5 to 20%. The quantity of drug used to fill the inhaler reservoir, a term that applies to nebulizers, metered dose inhalers, or dry powder inhalers, is the total amount of drug available for inhalation from the inhaler. Again, a portion of this total dose is unavailable for aerosolization and thus inhalation. The term "dead volume" is often applied to that portion of drug not nebulized from jet or ultrasonic nebulizers, often representing 20% or more of the total (43). With pMDIs and DPIs, there is usually an 10–20% overfill in the amount of drug loaded into the bulk reservoir to guarantee that the total number of doses specified on the package are available to the patient. To normalize delivery performance between inhalers dispensing different formulations of the same drug, a change in the nominal unit dose can be made by the pharmaceutical company. This would occur during development as changes to an already marketed formulation would require, as a minimum, confirming bridging studies for reapproval. This circumvents inherent differences between systems without compromising treatment as patients are switched from one type of delivery system to another (Table 2) (17). Thus, while the dose of drug provided from one inhaler system may be greater or less than that from an alternative device, the clinical responses can still be the same.

### B. Aerosol Characteristics/Particle Properties

Therapeutic aerosols are heterodisperse, either spherical in shape if produced from a pure solution or nonspherical if a suspension, and with a range of physical diameters and shapes (98). Large particles (>10 m) deposit ex-lung, unless particle density and/or inspiratory flow rates are manipulated to circumvent the physical size constraints (99,100). Particles <10 μm deposit in the mouth, trachea, and airways throughout the lung. Site of deposition is mainly a function of particle size, but the distribution of the deposited aerosol is also dependent on air flow rate or air velocity and airway caliber (101–103). The physics of particle deposition and the influence on deposition seen with inhalation of therapeutic aerosols under various conditions are well described in the literature (104–107).

**Table 2** Comparison of Fine Particle Fractions and Fine Particle Doses of Salbutamol Delivered from Various Inhalers as % Label Claim

| Inhaler | Nominal dose (μg/ actuation) | FPF (%LC < 4.7 μm at 28.3 Lpm), mean(SD) | FPD (μg < 4.7 μm at 28.3 Lpm), mean(SD) | FPF (%LC < 4.7 μm at 60 Lpm), mean(SD) | FPD (g < 4.7 μm at 60 Lpm), mean(SD) |
|---|---|---|---|---|---|
| pMDI | 100 | 47.7(4.7) | 47.7(4.7) | 49.6(4.3) | 49.6(4.3) |
| PMDI + Volumatic | 100 | 68.9(12.2) | 68.9(12.2) | 58.8(6.6) | 58.8(6.6) |
| Diskus | 200 | 23.3(0.9) | 46.6(1.8) | 31.6(0.9) | 63.2(1.8) |
| Diskhaler | 200 | 20.4(8.6) | 40.8(17.2) | 26.9(5.7) | 53.8(11.4) |
| Turbuhaler | 100 | 13.3(1.6) | 13.3(1.6) | 27.6(8.0) | 27.6(8.0) |
| Turbuhaler | 50 | 9.7(3.2) | 4.9(1.6) | 23.3(3.0) | 11.7(1.5) |

FPF = Fine particle fraction; FPD = fine particle dose; LC = label claim.
*Source*: Data from Ref. 17.

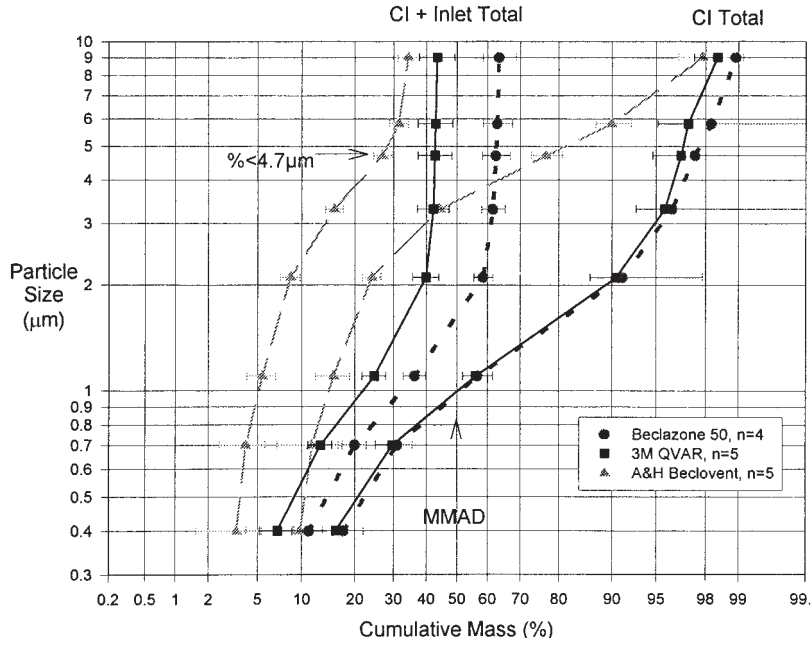



**Figure 3** Cumulative mass distributions for pMDI aerosols of Beclazone 50, QVAR, and Beclovent. Curves are shown for the emitted dose ex-actuator (CI + inlet total) and the dose that is only deposited in the impactor (CI total). The mass median aerodynamic diameter (MMAD) of the aerosols deposited in the impactor is read from the CI curves at 50% of the cumulative mass and is 0.99 μm for Beclazone 50 (Baker-Waterford, Ireland), 1.0 μm for QVAR (3M Pharmaceuticals, St. Paul, MN), and 3.3 μm for Beclovent (Allen and Hanbury, Research Triangle Park, NC). The fine particle fraction (% $<$ 4.7 μm) of the emitted dose is read from the CI + Inlet total curves and used to calculate the values shown in Table 3.

Defining an aerosol in terms of its Stokes equivalent diameter corrects for differences in size and shape within the aerosol. By further normalizing this diameter to that of a water droplet ($\varrho$ = 1.0 g/cc) with the same settling velocity (aerodynamic equivalent diameter), a comparison of the behavior in the lung of different aerosol products with different densities can be made, independent of the type of drug aerosolized or inhaler used (98). The mass median aerodynamic diameter (MMAD) of the aerosol is a statistic from the size (frequency) distribution data characterizing the aerosol under kinetic conditions in terms of its mass (Fig. 3). The MMAD means that 50% of the mass of the aerosol resides in particles less than the MMAD and 50% in particles greater than the MMAD. Both the MMAD and its (geometric) standard deviation (GSD), a measure of the het-

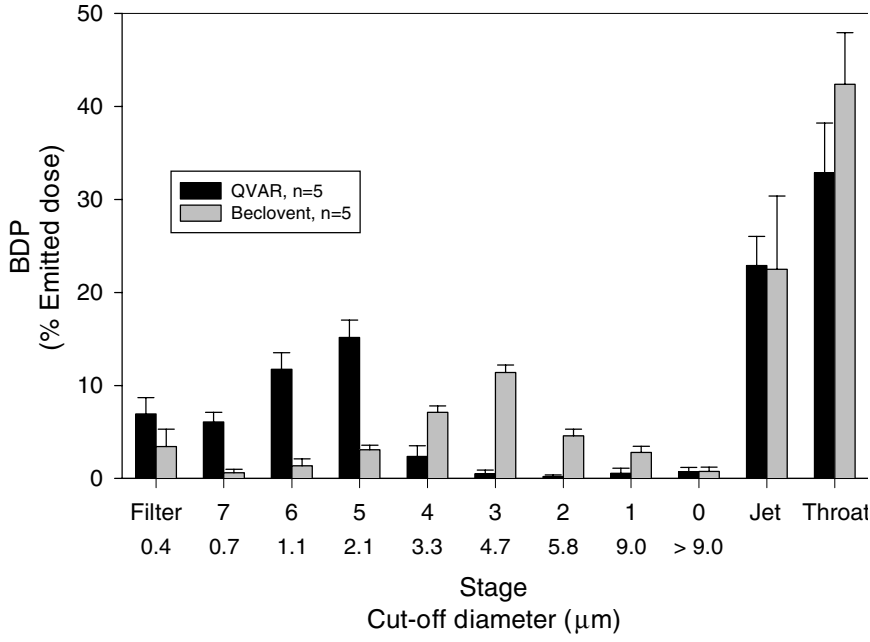



**Figure 4** Histogram illustrating the % of the emitted dose deposited on the individual cascade impactor (CI) stages, jet and throat (inlet) for QVAR (3M Pharmaceuticals, St. Paul, MN) and Beclovent (Allen and Hanbury, Research Triangle Park, NC). The height of the bars indicates the differences in amounts on the stages.

erogeneity of the aerosol, are predictors for the site of deposition in the lung as well as indicating the dose or collective amount of drug (mass) carried by the aerosol. Size classification of the aerosol in terms of its aerodynamic behavior is performed using cascade impactors, multistage liquid impingers, and optical systems, with chemical assay of the drug a major advantage of the impactor/impinger techniques. While time-consuming and labor-intensive compared to the laser techniques, the ability to quantify the amount of drug carried by aerosol particles of a specific size is useful for interpreting the resulting lung deposition patterns and clinical effects of the inhaled dose (Fig. 4). Sizing data from light scattering instruments gives the median diameter of the aerosol, with the assumption that all particles in the aerosol being tested are spherical and of unit density. This information predicts the site of deposition of aerosol in the lung but says nothing of its drug content (108). The accuracy in determining these in vitro doses is increased if the impactor/impinger flow rates used to sample the aerosol are matched to the

optimal performance of the inhaler and the patient's IFR (97). As shown in Table 2 (17), the fine particle fraction [FPF (% < 4.7 μm)] measured for the Turbuhaler at 28.3 Lpm was less than that measured at 60 Lpm, the manufacturer's recommended flow rate for optimal in vitro performance and patient use. As with a number of DPIs, deposition is flow dependent (12,109): a lower inhalation flow rate delivers a lower dose of drug to the lung. Decreased plasma levels of terbutaline were measured when this bronchodilator was inhaled at 34 Lpm from the Turbuhaler, in line with the decreased fine particle dose (110).

Air flow patterns within the lung additionally affect movement and behavior of inhaled aerosol particles or droplets. In the normal lung, laminar flow occurs in distal, peripheral airways, at approximately the sixth generation of airway (111). Airway narrowing due to constriction, edema, and/or secretions causes airstream velocities to increase resulting in turbulent flow and augmented deposition of larger particles, mainly at airway bifurcations (101,111). Thus, in airways disease, as resistance to airflow increases, deposition of particles becomes more proximal with less of the inhaled drug dose available to the distal lung for therapy. Similar effects are seen if the patient hyperventilates, with drug impaction increased in the oropharynx and on large airways.

### C. Partitioning of an Aerosol: Fine and Coarse Particle Fractions, Fine and Coarse Particle Drug:Mass Ratios

In addition to the MMAD and geometric standard deviation (GSD), a third parameter, the fine particle fraction (FPF), or the percentage of particles within the aerosol that are <5 μm (4.7 μm) or 6 μm (5.8 μm) in diameter, is being used more frequently to describe the quality of an aerosol and its potential usefulness for targeting and delivering sufficient quantities of drug to the peripheral airways. The 4.7 μm cut-off diameter to define the FPF is accepted as the standard, although 5.8 μm has also been used for a number of years as these particles do deposit in the lung, but on larger, more proximal airways. The fine particle dose (FPD) is calculated as the fine particle fraction multiplied by the emitted dose (ED) of drug available at the inhaler exit ($FPD = FPF_{\%<4.7\mu m} \times ED \times 100\%$). The counterpart is the coarse particle fraction ($CPF = CPF_{\%>4.7\mu m}$) and resulting coarse particle dose ($CPD = CPF_{\%>4.7\mu m} \times ED \times 100\%$), or that quantity of the aerosol contained in particles > 4.7μm, which would preferentially deposit in the mouth and throat and on large central airways. In general, the percentage of particles with increasing likelihood for depositing in the distal lung increases as the FPF increases, also indicating that the aerosol has a smaller mass median aerodynamic diameter. While more drug is carried in larger droplets or particles, the probability of particles larger than 6 μm depositing in the lower respiratory tract decreases with an increasing CPF. Submicrometer droplets, <1 μm in diameter and present in in-

creasing numbers in ethanolic pressurized steroid formulations, such as HFA134a BDP (QVAR, 3M Pharmaceuticals, St. Paul, MN), are retained less in the lung but, due to their size and the vast number of submicrometer droplets in the aerosol, can penetrate into the pulmonary regions, even in the presence of airflow obstruction (83,83a). An extrafine particle fraction (EFPF) and dose (EFPD) has been defined for those aerosols whose distributions contain a majority of particles $<1$ μm in diameter. Approximately 20% of these extrafine pMDI aerosols will be exhaled, as aerosols of this diameter behave as a gas and, indeed, are widely used to measure lung ventilation in patients suspected of having a pulmonary embolism (112).

### D. Use of Dose Ratios to Compare Inhaler Performance Delivering the Same Drug

To calculate the FPDs for the inhalers shown in Table 2, a measurement of the emitted dose is required. With the results of the FPD calculation, one can compare delivery efficiencies and estimate the amount of drug deposited in the lung for the various aerosol systems. As mentioned above, the ED is the dose available at the mouth and is less than the nominal dose strength due to losses in the system; the label claim (LC) is the amount of drug available ex-actuator and would be the same dose as the nominal strength provided there is no other hardware inserted between the inhaler actuator and the mouth. In Table 2, the values for the FPFs for the pMDI + Volumatic spacer are expressed as %LC of the pMDI dose available ex-spacer. Due to impaction of large particles on spacer walls and valves and evaporation of the propellant, an increase in FPF of the aerosol ex-spacer occurs compared to the pMDI alone (17,70). To calculate the FPD ex-spacer, a value for the ED ex-spacer needs to be provided or the FPF given is for the aerosol ex-spacer. For example, if losses in the Volumatic are of the order of 50% of the nominal dose, the ED available at the mouth would be approximately 50 μg and the FPD approximately 30 μg. It can be seen that at the higher sampling flow rate of 60 Lpm, the FPF for the pMDI aerosol available from the spacer decreased, reflecting a loss of fines resulting from increased impaction of aerosol on the spacer valve and walls.

Further support for understanding inhaler performance and drug delivery to the lung has been demonstrated by Wilson et al. (10). They compared systemic effects from fluticasone propionate (FP) inhaled as a powder from the Diskus (GlaxoSmithKline, United Kingdom) and as a pressurized aerosol using the Volumatic large-volume spacer. Each system administered the same nominal dose of FP. Indicators of adrenal suppression (overnight and early morning urinary cortisol/creatinine excretion, 8 a.m. serum cortisol levels) as measures of lung bioavailability showed significantly greater systemic activity for the pMDI + spacer than for the DPI, suggesting greater absorption of drug from the peripheral lung when the pMDI was given via the spacer. For reasons mentioned earlier in this chapter

and well documented in the literature, the particle size of an aerosol exiting a spacer is typically finer than from pMDIs used without a spacer or from DPIs. A direct result of this decrease in particle size is an increase in aerosol deposited in the peripheral lung, potentially giving rise to greater systemic absorption—the outcome documented by Wilson in his comparison of inhalers. Additionally and depending upon the type of spacer or DPI used, overall deposition efficiencies may or may not be comparable. The results from Wilson can easily be explained by the combined differences in aerosol quality, namely, a lower fine particle dose and lower in vivo deposition efficiency for the Diskus compared to the pMDI and Volumatic. Combining these two factors, namely, aerosol size of the drug with inhaler deposition efficiency, makes possible a more accurate estimate of absolute doses deposited (regional or total) and a prediction of differences in the kinetics and overall response to the drug. Wilson and colleagues concluded that an understanding of delivery system performance is important to the physician when considering switching a patient from one inhaler to another. Similarly, in a study in stable but symptomatic asthmatics, Chapman and colleagues demonstrated that equivalent responses to salbutamol inhaled via the Turbuhaler could be obtained at one-half the dose prescribed via the pMDI, explained by the different dosing efficiencies of the inhaler systems (113). By combining the in vitro estimate of FPD with the known in vivo deposition efficiencies for the two delivery systems, a similar fine-particle drug dose will be deposited in the lung and, not surprisingly, give rise to the same clinical response. This result is similar to the one obtained in children treated with budesonide inhaled via the pMDI with the Nebuhaler or from the Turbuhaler at half the dose. The results showed that their asthma was well controlled when treated with the lower dose of budesonide through the Turbuhaler (114). A twofold difference in deposition has been measured for budesonide via the Turbuhaler compared to the pMDI alone in healthy adults (9), while in adult asthmatics the addition of the Nebuhaler to budesonide pMDI increased lung deposition by approximately 45% compared to the Turbuhaler (82). It is possible that the clinical observations made in children in the above study (114) would be different in the adult.

### E. Use of Dose Ratios to Compare Different Formulations of Salbutamol

An example of how the aerosol size fractions can differentiate between formulations that give the same clinical outcome at prescribed doses can be illustrated with a comparison of the reformulated salbutamol pMDI, Airomir (3M Pharmaceuticals, St. Paul, MN) to its counterpart CFC Ventolin. The particle size distribution of Airomir versus Ventolin has been shown to be the same (58,115), with the mass median aerodynamic diameter (MMAD) of Airomir measured by cascade impaction as 2.69 μm versus 2.62 μm for Ventolin. The emitted doses ex-

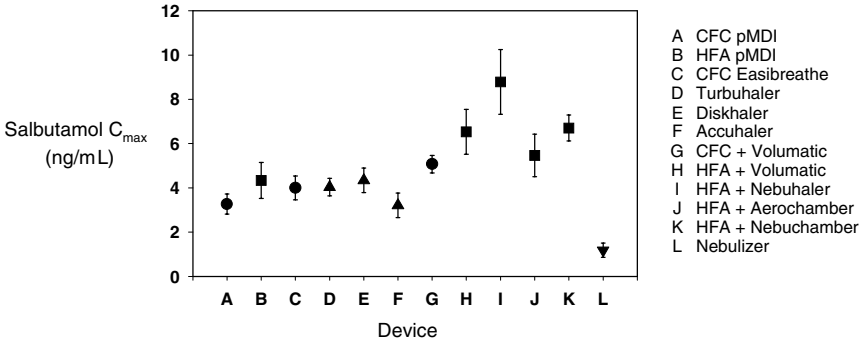



**Figure 5** Data from normal subjects showing plasma salbutamol levels ($C_{max}$) obtained after inhalation of salbutamol aerosol from a variety of inhalers (A–L). Values for HFA salbutamol (Airomir, 3M Pharmaceuticals, UK) without (B) and with spacers (H–K) are significantly greater than from inhalation of CFC pMDI aerosol (A). (Data from Ref. 116.)

actuator were found to be less for Airomir than Ventolin (85.3 ± 5.4μg vs. 96.9 ± 6.9 μg; $p < 0.05$), but the FPF of the emitted dose ex-actuator, defined as the percentage contained in particles less than 5.8 μm in diameter, was greater for Airomir (65.5% vs. 41.4%; $p < 0.05$) (115). Because the FPF is greater for Airomir, the fine particle dose is 54.2 μg compared to 40.3 μg for Ventolin. The increased serum levels for Airomir, measured by Lipworth and colleagues in healthy volunteers and compared to those following inhalation of Ventolin, can be seen in Figure 5 (116). In a separate in vitro filter study, emitted doses and particle sizing were measured for Airomir used with several spacers of different volumes (115). The fine particle dose was increased compared to that for Ventolin through the spacers and was comparable between the three spacers: approximately 70 μg of Airomir was measured at the mouthpiece of the 145 mL Aerochamber (Trudell Medical International, Canada), the 330 mL Babyhaler (GlaxoSmithKline, UK), and the metal 280 mL Nebuchamber (Astra Zeneca, Sweden) (115). The in vitro data shown in Figure 6 (115) for Airomir + AC and + NES are also reflected in the pharmacokinetic (pk) results of Lipworth in Figure 5 (116), supporting greater total deposition in the lung compared to Airomir alone. Clinically, there appears to be no difference in either short-term or long-term effects on pulmonary function between Airomir and Ventolin (117–120). However, studies comparing doses below the minimum nominal dose of 100 μg should be undertaken to differentiate the bronchodilator response to these two aerosols, avoiding the plateau of the dose-response curve where responses are likely to be muted.

Not surprisingly, and as shown by these few examples, the fine particle con-

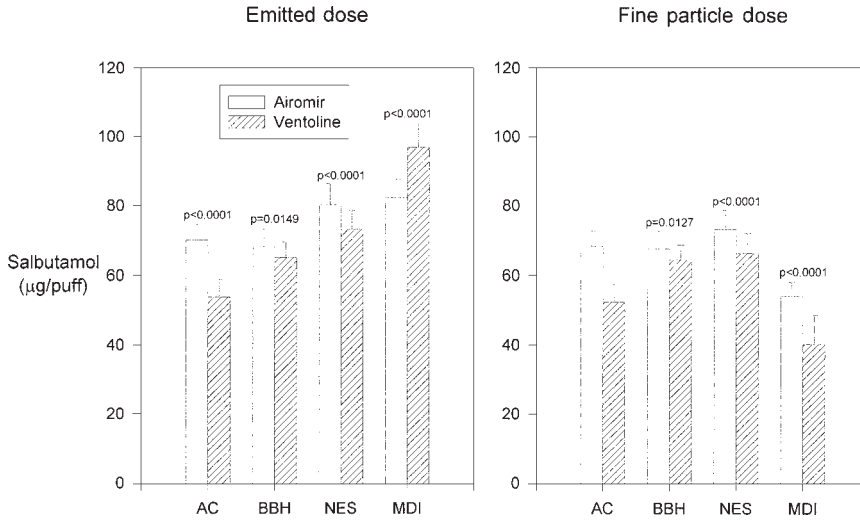



**Figure 6** Data showing emitted doses (μg/puff) and fine particle doses (μg $<$ 4.7 μm /puff) of salbutamol from (open bars) Airomir (3M Pharmaceuticals, St. Paul, MN) and (hatched bars) Ventoline pMDI (GlaxoSmithKline, France) alone and through three valved spacers: Aerochamber (AC)(TMI, Canada), BabyHaler (BBH) (GlaxoSmithKline, UK), and Nebuchamber (NES) (AstraZeneca, Sweden) (*t*-test or Mann-Whitney rank sum test; $n$ = 30). With the exception of the ED from the pMDIs alone, the EDs and FPDs through the three spacers are greater for HFA Airomir compared to CFC Ventoline. The higher doses may lead to a greater clinical effect when the HFA formulation is inhaled. (Data from Ref. 115.)

tent of a therapeutic aerosol greatly influences the systemic uptake of a drug, perhaps more so than the overall response to the drug. The presence of inflammatory cells in the distal lung in airways $<$2 mm (121) strongly suggests that fine particle steroid aerosols should be targeted to this area of the lung, despite the potential for increased side effects. The distribution of particles to the distal lung, that is, beyond the seventh generation of airway, increases as particle size decreases (4), but shifts to more proximal airways will occur with increased turbulence due to airway narrowing (105). As aerosols are heterodisperse, deposition on more central airways is unavoidable and may be preferred as therapy is then applied throughout the lung.

Although the potential for absorption of drug from the peripheral airways is greater due to the larger surface area, the rate of absorption will also be determined by other factors such as the molecular weight of the drug, the depth of the mucus layer, ciliary function, and the integrity of the airways (121a). Not discussed here

**Table 3** Comparison of Fine Particle Dose and Coarse Particle Dose Ratios for 2 HFA-BDPs (QVAR, BZ50) and a CFC-BDP[a]

| F-ratio[b] | QVAR/BV50 | BZ50/QVAR | BZ50/BV50 |
|---|---|---|---|
| FPD-ratio = $F_f$ | 17.62 μg/12.61 μg = 1.40 | 25.85 μg/17.62 μg = 1.47 | 25.85 μg/12.61 μg = 2.05 |
| CPD-ratio = $F_c$ | 23.39 μg/33.91 μg = 0.69 | 15.29 μg/23.39 μg = 0.65 | 15.29 μg/33.91 μg = 0.45 |
| I = $F_f/F_c$ | 1.40/0.69 = 2.03 | 1.47/0.65 = 2.24 | 2.05/0.45 = 4.55 |

FPD = Fine particle dose; CPD = coarse particle dose; I = Index of Aerosol Quality; BZ50 = Beclazone 50 (Baker-Waterford, Ireland).
[a]The nominal strength for all three pMDIs is 50 μg/actuation. Beclovent from GlaxoSmithKline, Mississauga, Ontario, Canada; Beclovent from Allen and Hanbury, Research Triangle Park, NC.
[b]Calculated from Anderson Cascade Impactor (ACI) data.
*Source*: Refs. 122,126.

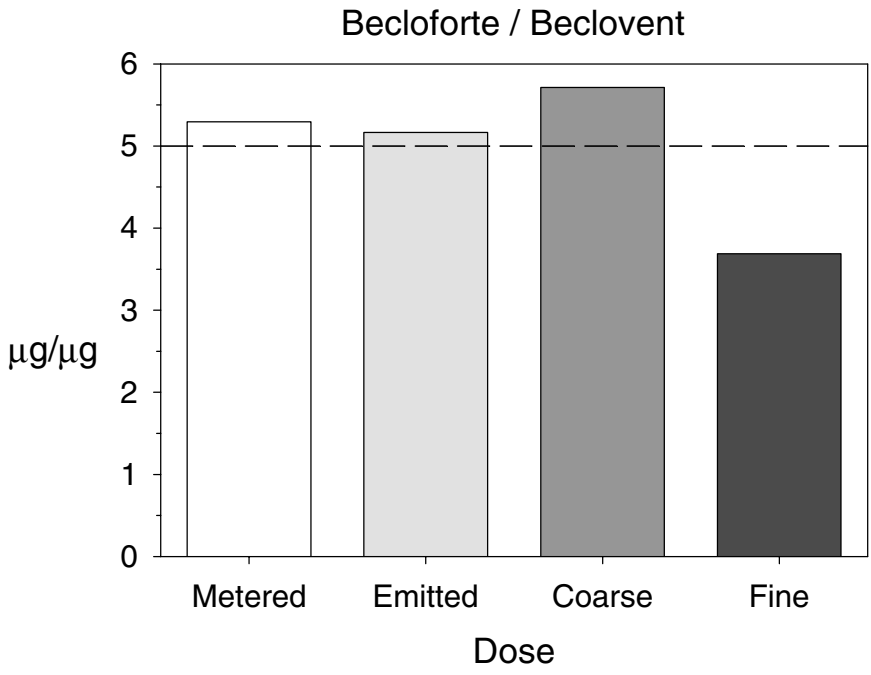



**Figure 7** Comparison of doses for two strengths of the same drug: Becloforte (250 g/puff) and Beclovent (50 µg/puff). While the fivefold difference in concentration of weight is seen in the metered and emitted doses, Becloforte has a greater coarse particle dose and a lower fine particle dose compared to Beclovent. Thus the distribution in the lung of Becloforte aerosol may be more proximal than for Beclovent. (Data from Ref. 122.)

is the role of the bronchial circulation in providing drug, absorbed from large particles deposited on more proximal sites in the lung, to airways $<2$ mm in diameter. Whether the small airway response to therapy would be enhanced if the lung was loaded with a large-particle steroid aerosol is not known.

### F. Use of Dose Ratios to Compare Different Formulations of Beclomethasone Diproprionate

FPFs and FPDs are given in Table 3 for QVAR and Beclovent (BV), two 50 µg/puff inhalers of the same drug but formulated in different propellants (122,123, 126). The latter is a CFC suspension and the former is a HFA134a solution of beclomethasone dipropionate (BDP). The F-ratios are an indication of the in vitro equivalence or lack of equivalence of the aerosols for depositing in the lung ($F_f$) and the oropharynx ($F_c$). The Index of Aerosol Quality (I) is the combined effect

of the two F-ratios and represents the overall extent of the in vitro differences between the formulations. It is useful to compare the FPD and CPD values for these two drugs as the ratios show the difference in quality of the aerosol following reformulation. Overall, a twofold change in the fine and coarse aerosol content was effected, as can be seen in the ratios of the FPD and CPD values ($F_f = 1.40$ and $F_c = 0.69$), favoring the fine component of the HFA aerosol. The values indicate that the mass contained in fine droplets is 40% greater for QVAR compared to BV, while the mass of coarse droplets decreased by 31%. Overall, there is a twofold difference in the aerosol quality of QVAR compared to BV as seen in the I value and a slightly greater (3-fold) difference for the MMADs (Fig. 3). In adult asthmatics, lung deposition with QVAR has been shown to be two- to threefold greater compared to BV (65), with parallel changes in clinical response (68).

These ratios can also be used to compare different dose strengths, but one should not expect a linear increase in deposited dose for a higher strength of the same drug. Figure 7 shows the dose ratios for Becloforte (BF, GlaxoSmithKline, Ware, UK), 250 μg/puff pMDI of BDP, compared to Beclovent (BV). It can be seen that while the ED and metered dose (ex-valve) values are approximately 5:1, the CPD ratio is greater and the FPD ratio smaller (122). The increase in MMAD for BF is a direct consequence of these changes to the aerosol, and while patients

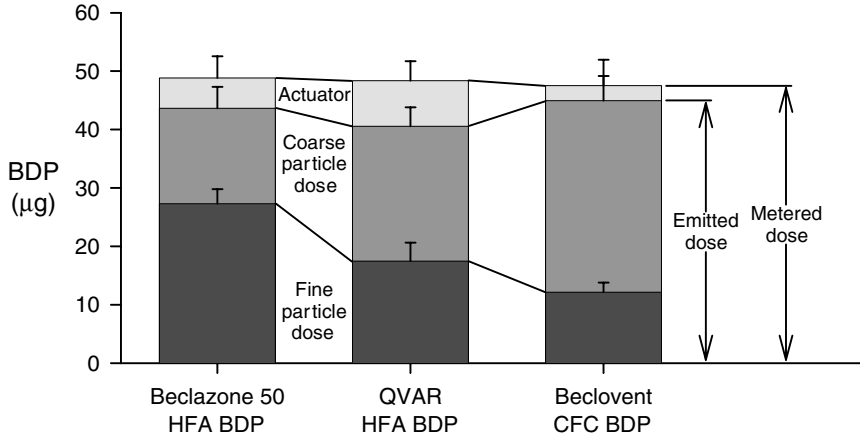



**Figure 8** Illustration of the doses for three pMDI formulations with a nominal dose of 50 μg of beclomethasone dipropionate/puff. Beclazone 50 and QVAR are HFA solution aerosols, with a finer MMAD compared to Beclovent, the CFC pMDI. While the EDs are similar, differences in the CPDs and FPDs were measured between all three inhalers. Deposition following inhalation of Beclazone 50 would be predicted to be more peripheral than from the other two formulations. (Data from Ref. 123.)

will inhale an aerosol with five times the weight of drug per actuation, the site of deposition in the lung may be more proximal than for BV.

### G. Use of Dose Ratios to Compare a Generic Inhaler (Beclazone) to the Innovator (QVAR)

A second example for the use of these dose ratios is in the in vitro comparison of two BDP solution HFA pMDIs, QVAR and Beclazone 50 (BZ50, Baker-Waterford, Ireland) (123). The ratios of the EDs, FPFs, and CPFs and the corresponding doses are shown in Table 3. The EDs are the same for both formulations, but BZ50 has almost 50% more aerosol-containing drug in the fine particle range. In the United Kingdom, QVAR has been approved on a 1:2.5 dose ratio to Beclovent; in Ireland has BZ50 been approved on a 1:1 ratio. From the in vitro doses shown in Table 3 and plotted in Figure 8, it appears that these two HFA BDP formulations are different and that inhalation of BZ50 may give a greater dose of BDP to the lungs compared to QVAR. Thus a 1:1 substitution of BZ50 for CFC-BDP may not be appropriate. Two published clinical trials have compared CFC-free Beclazone to a CFC BDP product, also from Norton Healthcare Ltd, United Kingdom, and showed equivalence between the two aerosols (124,125). It could be argued that the study designs, subjects enrolled, and doses tested were such that differences in response would be difficult to discern. Needless to say, there is some confusion on the part of physicians as to how to transition these two HFA aerosols.

## IV. Effect of Particle Size and Inspiratory Flow Rate on the Emitted Dose

The most advantageous size for a therapeutic aerosol is one with a MMAD between 1 and 5 μm, and most of the currently marketed inhalers produce aerosols within this range. Attempts have been made to tailor clinical aerosols, making them more uniform in size and hence targeted to specific airways. Trials testing inhalation of equivalent inhaled doses of monodisperse albuterol aerosols in moderate to severe asthmatic patients resulted in maximum changes in lung function with particles of 2.8 μm MMAD compared to those 1.5 and 5.0 μm in diameter (127,128). However, not surprisingly, no difference could be seen in bronchodilator response when compared with the heterodisperse pMDI CFC aerosol, perhaps because the airway surface dose of drug achieved was the same for both aerosols and sufficient drug was deposited at specific receptor sites with both systems to effect a similar bronchodilatation (129).

Increasing the air flow rate used to inhale an aerosol can increase the dose dispensed from an inhaler (130), but it also can reduce the drug dose inhaled into the lower respiratory tract, preferentially depositing drug onto central airways (63,131,132). Responses may change with this altered deposition pattern (8,11).

However, it is difficult to differentiate the influence of the topographical distribution of aerosolized drug on the clinical response from the effect due to the amount of drug inhaled, particularly when sufficient drug is prescribed as a single dose and the plateau of the dose-response curve is readily achieved with this dose. A number of studies in both children and adults have measured the effect of IFR on lung deposition and clinical response from pMDIs and DPIs; in general, results indicate a flow dependency in both deposition and response (107).

The in vitro measurements of emitted dose, FPF, CPF, and EFPF of the dispensed aerosol are all affected by the air drawn through the inhaler (133). The air flow acts to disperse the dose and additionally, if a powder, to fluidize and deaggregate the powder aliquot. As seen in Table 2, the above fractions will increase or decrease, depending on the in vitro test flow rate or the in vivo IFR used during a patient inhalation manoeuvre. For passive DPIs, the critical step in dispensing the dose is the process of deaggregating the powder. These types of DPI rely on the effort extended by the patient via the inspiratory breath to dispense the powder from the device (134). Too poor an effort (low IFR) will not fully aerosolize the powder, resulting in a large particle aerosol with a higher CPF (and lower FPF). As shown by the data in Table 2 for the Turbuhaler, not using the optimal flow rate translates into a lower dispensed dose and FPD, less drug inhaled into the lung, and potentially, a reduced response (135).

The higher IFR used for pMDI delivery of budesonide, coupled with the larger MMAD and CPF of the pMDI aerosol, may explain the reduced lung deposition compared to inhaling the pMDI dose from a valved holding chamber, the Nebuhaler (AstraZeneca, Sweden), using a lower IFR (82). In an attempt to discriminate responses based on site of deposition, effected by manipulating both the inspiratory flow rate and particle size, Ruffin and coworkers performed a series of experiments delivering radiolabeled agonist and antagonist aerosols, targeted to central or peripheral regions of the lung in asthmatic patients (136,137). Delivering histamine predominantly to the central airways required 10- to 15-fold less drug to cause a 15% fall in $FEV_1$ compared to peripherally deposited histamine. These results illustrated that sufficient surface concentration of drug was obtained centrally for histamine using a much lower dose, but enough to trigger the required response. However, the effects on $FEV_1$ delivering isoprenaline to central or peripheral airways pretreated with either peripherally or centrally deposited propranolol were mixed (137), perhaps because the deposition patterns for the aerosol were not sufficiently discriminatory in all the subjects studied.

## V. Measuring Aerosol Lung Dose and Distribution

There are a number of ways to measure deposition of particles or droplets in the lung. The information obtained can be used to estimate the dose of drug deposited

in the lung and, with radiotracers and imaging, the dose at particular (airway) sites in the lung. Theoretical calculations or empirical models provide guidance as to what may occur in vivo, although it is difficult to accurately model the many conditions affecting delivery, deposition, retention and absorption of aerosol in the lung and, additionally, have the results fully predict outcomes in human subjects with or without lung disease. Experimental data obtained using in vitro and in vivo animal models, in vivo radioisotope studies, and pharmacokinetic studies (116, 138,139) provide a good indication as to where drug is deposited in the lung. However, airway geometry, patterns of breathing, and lung function vary between subjects and over age groups, as do the methods used to obtain and analyze deposition data. As a result, the deposition data obtained from different laboratories may vary for the same drug due to the combination of using different methods for preparing the radiolabeled drug, varying inclusion criteria for the subjects, different standardization practices for the delivery of the aerosol, and various imaging techniques and data analysis.

Methods to detect the distribution of an inhaled radiolabeled aerosol consist of both nonimaging and imaging techniques, the latter being either two-dimensional (2D planar) or three-dimensional (3D) [single photon emission computed tomography (SPECT) or positron emission tomography (PET)]. Much of the above deposition data has also been generated by pharmacokinetic investigations, which, when combined with charcoal blocking of the intestinal uptake and with parallel intravenous studies, give a good estimation of total airways/lung uptake. (The design and results of these types of studies are reported in Chapters 9, 10, and 12, respectively, in this text.) Several human studies have been designed to investigate the correlation of deposition outcomes measured with imaging and pharmacokinetic samples collected at the same time in the same subject exposed to radiolabeled budesonide fom the Turbuhaler. The data from Borgström (110a) showed that the two methods gave similar results for *total* deposition. The advantage of scintigraphy is that it provides a detailed visual image of where drug deposits in the lung and that regional information can be obtained. Drug delivery to the lung has also been assessed using indirect pharmacokinetic methods. This approach involves measuring drug levels in urine (84) or plasma (85) 30 minutes after inhalation, before significant quantities have been absorbed from the GI tract. The results are dependent on the sensitivity of the drug assay and often require inhalation of large doses of drug for reliable sampling. Blocking with charcoal is viewed as not necessary and would, in any case, not be representative of what would occur in the clinical setting. The results provide only an indirect rather than an absolute measure of lung dose. However, these types of studies have been used extensively to compare devices or inhalation techniques.

Two-dimensional imaging is used by a number of laboratories to measure the distribution of deposited dose and calculate the inhaled dose from a variety of inhalers and drugs. The lung however, is a three-dimensional structure and with

two-dimensional planar imaging, the distribution of the radiotracer can only be viewed in two dimensions. The contribution from overlapping small airways in the hilar region has been shown to be considerable, resulting in an overestimation of both "central" airway and peripheral deposition (140). Using three-dimensional techniques such as SPECT and PET can reduce this error, as they allow a more accurate measurement of the dose deposited within the lung (141–143). Other issues in imaging a three-dimensional object in two dimensions that impact on the measurement of deposited dose are the system resolution (4–6 mm for PET and 6–12 mm for SPECT and two-dimensional planar cameras) and the correction for attenuation of the deposited radioactivity in the lung by the chest wall or in the oropharynx and larynx by bone, cartilage, and tissue (144). The former reduction in signal is nonlinear, particularly if the aerosol deposition is nonuniform, as would occur in disease (145). Several methods have been used for estimating attenuation factors employing both internal and external sources of radioactivity (146–149). While a universal factor can be applied to all in vivo data, it is truly specific to the subject being imaged. Additionally, it is a function of the parameters of the imaging system, that is, the combined camera/collimator resolution and sensitivity. Not correcting the imaging (emission) data for tissue attenuation underestimates the absolute dose measured (149). Tomography, both PET and SPECT, overcomes these issues. With PET in particular, the acquisition of a transmission scan immediately following the PET scan and with the subject still under the scanner provides an accurate geometric outline of each lung slice. The correction for tissue attenuation of radioactivity can then be applied specifically to each voxel of each emission slice. To define regions of interest, e.g., central and peripheral lung regions, on either PET or SPECT images, the transmission slice is overlayed onto the emission slice enabling the peripheral or outer lung border for that individual slice to be delineated, providing the boundary of the lung from which the peripheral region of interest is drawn (150). The process is repeated for each lung slice and the radioactive counts within each slice summed to give either the total and regional doses deposited or the dose per slice versus distance through the lung (144).

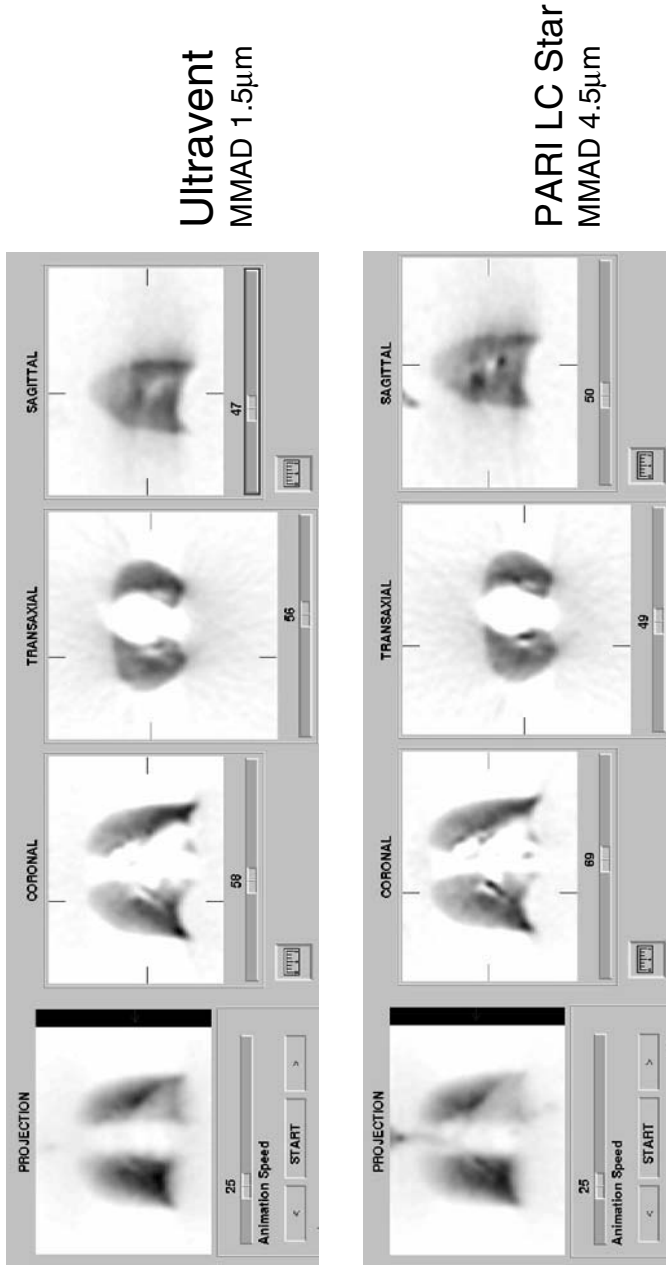
Tomography with inhaled SPECT and PET tracers are increasingly being used as investigative tools to measure lung dose and distribution from nebulizers, pMDIs, and DPIs as these imaging techniques provide greater accuracy in measuring drug distribution in the lung (144,151). The PET scans in Figure 9 show the projection views and an image of one slice of lung from each of the three planes (coronal, transaxial, and sagittal) in a normal subject (A) and a subject with cystic fibrosis (B) following inhalation of 4.5 and 1.5 μm $^{18}$FDG aerosols generated from a Pari LC Star (Pari, Germany) and an Ultravent (Mallinckrodt, St. Louis, MO) jet nebulizer, respectively (152). The projection view (Figs. 9, 10) is the summation of all slices in the coronal plane and would be equivalent to what would be acquired with the two-dimensional gamma camera. It can be seen that the deposi-

tion detail provided in the single coronal slice is diluted in the projection view. With two-dimensional imaging, the calculation of total and regional deposited dose is made using the data essentially contained in the "projection" view, whereas with PET and SPECT there is the additional ability to "peel off" lung and calculate doses per slice of lung tissue. In the selected transaxial slices from the apical, middle, and basal regions shown in Figure 11 from this CF subject, it can be seen that while there is considerable impaction of the 1.5 μm fine aerosol in the lung, the very anterior and posterior areas of the right midlung are well ventilated and could receive drug. This information would not be readily seen in two dimensions. In rotating the projection view (Fig. 11), one can see that what appears to be central deposition of radioactivity in the right lung is, in fact, located in the posterior and basal regions. Better discrimination of the sites of impaction are also seen in the left lung. This three-dimensional visualization and quantification of how much of a drug is deposited and where can help the physician see whether a sufficient amount of an inhaled therapy would be able to successfully target specific areas in the lung. Furthermore, regional analysis of deposition from a PET or SPECT scan can be calculated for each slice and plotted versus distance through the lung, a feature not possible with two-dimensional imaging. Of added interest with this imaging technique is the option to label a drug directly with a PET emitter (143,151,153). The data will then give a picture of where the drug is in the lung, the total and regional dose deposited, and the drug's fate over time.

## VI. Considerations for Future Investigations

What determines the dose of a drug inhaled into the airways/lung? Some of the factors are obvious—inhaler design, formulation, quality of the aerosol produced from the inhaler, inhalation techniques, and airway/lung disease status. As shown in Figure 1, the aerosol available at the mouth for inhalation, the emitted dose ex-delivery system, can be divided into two main components—coarse and fine. The divisions, based on theoretical calculations and experimental models of deposition and particle size, can be finer, but given the available clinical measurement tools, suit the purpose for relating deposition to efficacy.

What is the ideal size distribution for a corticosteroid aerosol? The distribution of particle sizes from an inhaler needs to be such that the doses delivered provide maximal efficacy with few or no side effects. The aerosol must be fine enough to achieve the target, that is, sufficient mass of drug deposited at sites of inflammation, but not so fine that the particles are not retained in the lung—a balance between physics, physiology, and formulation. Clearly, with the physical and clinical observations to date we have not attained this goal. To provide this information, perhaps the starting point for deposition studies investigating the influence of particle size on response should be to use the minimum dose that


Ultravent
MMAD 1.5µm
PROJECTION
CORONAL
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SAGITTAL
25
Animation Speed
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PARI LC Star
MMAD 4.5µm
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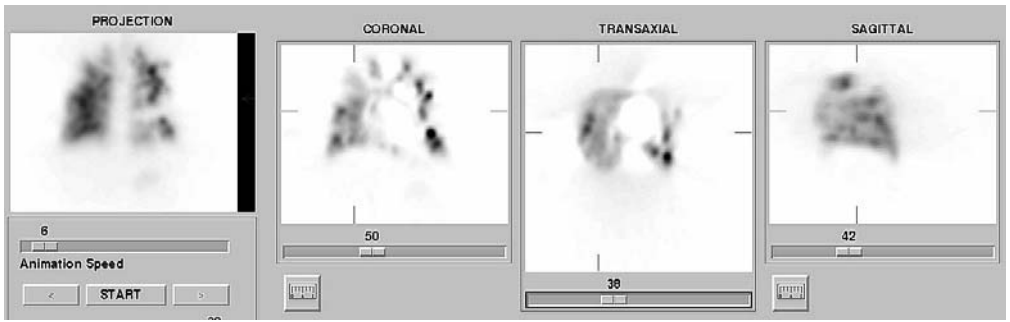
50

(a)




(b)

**Figure 9** Selected slices from PET images acquired in a normal subject (a) and a subject with cystic fibrosis (b) following inhalation of a 1.5 μm aerosol of $^{18}$FDG from the Ultravent jet nebulizer in comparison to a 4.5 μm aerosol from the PARI LC Star jet nebulizer. Deposition is similar for the two aerosols in the three planes (coronal, transaxial, and sagittal) in both subjects, but more hot spots are evident with the larger aerosol. Information in the projection view is not as detailed as that available from the individual slices.

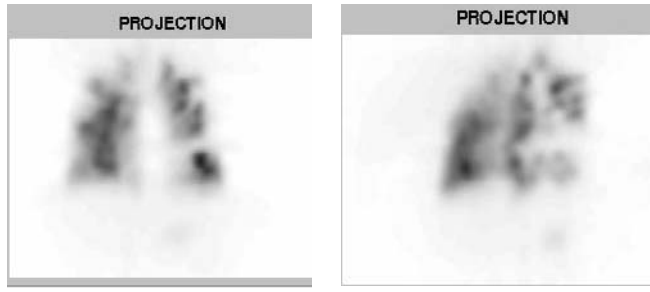



**Figure 10** Projection view from a PET scan for one subject with cystic fibrosis. Rotation of the projection view, shown on the right, indicates that the location of aerosol deposited in both the right and left lung is posterior and basal, with some impaction of aerosol in the anterior of the left lung. This information is not apparent in the "head-on" projection view shown on the left. (From Ref. 152.)

achieves some measure of clinical effect. Added doses may enable deposition changes initiated by the previous (lower) dose to be factored into the observations and help better differentiate the response.

What is the distribution of the emitted dose in the lung? The airways filter the inhaled aerosol, depositing particles on airway surfaces according to droplet/particle size, airflow dynamics, and airway size. The dose carried by large particles is greater than that carried by smaller particles, but large particles deposit on large, proximal airways unless the air flow pattern is markedly altered—not a realistic exercise for clinical applications. Aerosol size can be reduced using spacers, usually providing the same or an increased fine particle dose compared to the original aerosol. While calculations for this type of application were not fully provided in this chapter, use of the equations is straightforward. Unfortunately, the inhalers presently available preclude creating an aerosol with the right "mix" of particles to target specific sites in the lung, but future possibilities exist for these types of developments. A balance also needs to be made between steroid efficacy and side effects, the latter mainly due to systemic absorption of the fine aerosol dose.

Can we define the relationship between inflammatory changes in the lung, changes in lung function, and the various dose fractions of an inhaled drug? The latter are provided by in vitro measurements and these have certain limitations. With imaging, an in vivo estimate of large airway/small airway dose can be made. Can differences be detected in in vivo responses to the fine particle dose versus the coarse particle dose? Are the clinical data sufficiently exact to provide good correlations between dose distribution in the lung and response? Perhaps the use of the fine and coarse dose values should define the dose in the dose-response curves

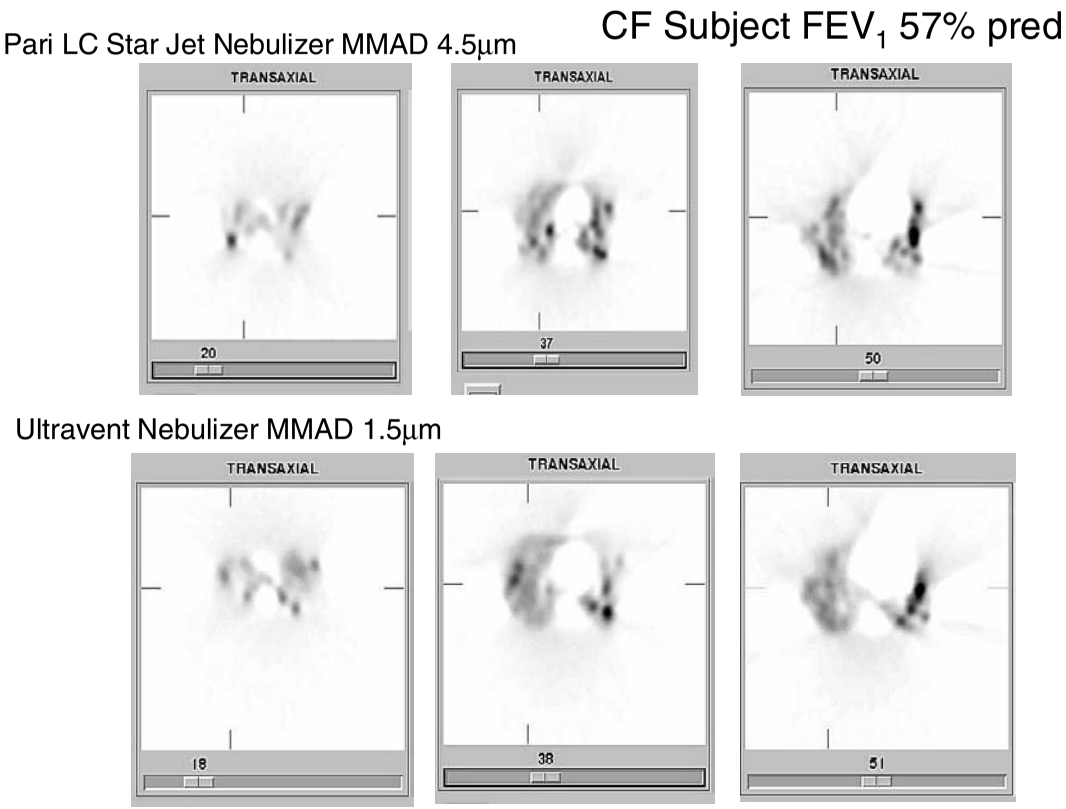



**Figure 11** Examples of deposition in three (of a possible 80) slices from the transaxial plane for a subject with cystic fibrosis after inhaling aerosols of two different particle sizes, 4.5 μm and 1.5 μm. The images show the detail that is obtained with three-dimensional imaging and indicates the differences in deposition within the lung, information not possible to obtain with planar two-dimensional imaging.

obtained in pharmacodynamic studies, rather than using nominal doses, as this may provide a more accurate comparison of the assesment of bioequivalence or relative potency between inhaled drugs from different delivery systems. The results presented here seem to suggest that links can be made to clinical observations, and further investigations should be encouraged and supported.

However, study designs need to combine deposition measurements with response, in the same subject, made when the test aerosol is given. Patient status varies from day to day, and we do not yet have the clinical tools to precisely measure the effects of small changes in airway caliber on pulmonary function to allow a separation of these measurements in time. In addition, better control of the delivery of the test aerosols needs to be built into study protocols to minimize the inherent variability in breathing patterns. While not realistic clinically as yet, results from controlled studies give more accurate results.

Can conditions be set now for the development of aerosols that can be directed more precisely to the disease target—the "magic bullet" theory? Or are the inhaled steroids/delivery systems currently available for treating asthma broad enough in their coverage of the lung that more than adequate treatment is readily achieved? The findings discussed in this chapter suggest otherwise. We need to broaden our knowledge of aerosol delivery, not only for inhaled steroids, but for all drugs used to treat asthma and other lung diseases using the inhaled route.

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## Discussion

**Dr. Jeffery:** Your results are exciting for many reasons, but I particularly like your demonstration of the marked differences in deposition pattern of particles in the normal and the asthmatic. Does your experience of differing sites of particle deposition provide us with clues as to which airway sites—large, central, or peripheral airways—and alveoli are most important in producing the clinical expression of asthma? Do we really need to target small airways, or is central deposition sufficient (particularly as alveolar deposition will give rise to unwanted systemic absorption)?

**Prof. Dolovich:** We are only just beginning to look at site of deposition and response using three-dimensional imaging. There is some clinical evidence for inhaled β-agonists showing a greater response with a finer aerosol but no information for steroids measuring deposition and clinical outcomes in parallel in the same subjects in the same trial. Given the weight of evidence that inflammation is present in "small" airways, it would seem prudent to treat the small airways with topical steroid, that is, with steroid aerosol inhaled and deposited at the site of inflammation. Given the locale of 2 mm airways, i.e., distal to the seventh generation of airway, fine aerosols $< 5$ μm MMAD should target these airways successfully. The location of the maximal deposition for a particular size of aerosol shifts distally into the lung as the aerosol becomes finer, but there will always be particles deposited on airway surfaces on either side of this peak. So the central airways, while perhaps not being specifically targeted with the particular size of aerosol used to treat the small airway, will nonetheless be exposed to the therapy in varying amounts. There is some evidence to suggest that aerosol deposited in central airways is transported to the small airways by the bronchial circulation. If the inhaled steroid was deposited only in the central airways, thereby treating the small airway indirectly, there could be an increased systemic exposure, and this may give rise to greater side effects.

**Dr. Hamid:** I just want to follow on Dr. Jeffery's question, as the slides you showed are from our study. We in fact demonstrated that there is marked inflammation in small airways in those patients. There were more eosinophils in small airways compared to larger airways. Although these patients were on inhaled steroids, it suggests that we need to deliver the drug more peripherally to achieve a maximum effect. A number of studies have confirmed these observations using transbronchial biopsies of postmortem tissue.

**Dr. Hargreave:** Is the total dose the same or different with airflow obstruction?

**Prof. Dolovich:** For metered doses of drug, the total dose inhaled will be similar, but the distribution of that dose in the lung would be different, being concentrated at points of airway narrowing due either to increased mucus secretion

or airway edema. For DPIs, though, if the pressure drop across the inhaler is increased, there could be a greater amount of drug decanted from the inhaler.

**Dr. Hochhaus:** Your results are very exciting, demonstrating the need for further evaluating how regional deposition affects pulmonary selectivity. I would like to add that in addition to the these effects, other biopharmaceutical factors should also be considered. There has been a development towards solution-based inhalation delivery systems. Despite higher deposition efficiency, these devices might be less beneficial, as the pulmonary residence time of drug given as solution might be too short to induce distinct pulmonary selectivity. I will talk tommorrrow about some of these findings.

**Dr. Jeffery:** If P450 activity has the potential to inactivate steroid, then the airway site of deposition in man is of importance. The cellular make-up of the lining epithelium differs markedly depending upon airway generation. In the large airways, there are secretory (goblet) cells, whereas in the terminal bronchioli there is, normally, a scarcity of goblet cells and a predominance of Clara cells, which I believe have marked P450 activity. There is a similar difference in the rat used experimentally and in the mouse. Nearly all nonciliated cells of the peripheral airways are Clara in type. I would predict that small airways may deactivate steroid moreso than large ones.

# 9

# Uptake, Retention, and Biotransformation of Corticosteroids in the Lung and Airways

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## I. Introduction

Inhaled corticosteroids (iCSs) are the basis of modern asthma treatment, and most of the commercially available corticosteroid (CS) formulations are highly efficient, while causing no clinically important systemic side effects in a majority of patients. Although there is evidence of some extrapulmonary antiinflammatory action of iCSs (see Chap. 11), the primary therapeutic effect of topically applied CSs in the airways originates from a local antiinflammatory action (1–3). Therefore, the combination of delivery system and CS will determine the therapeutic outcome and usefulness of the treatment: the site, extent, and distribution of the deposited dose are factors primarily governed by the performance of the inhaler; the dissolution, clearance and uptake from the airways, the affinity to the corticosteroid receptor, the residence time in the vicinity of the receptors, the local metabolism, and the systemic absorption are factors governed by the intrinsic physicochemical and pharmacological properties of the CS itself. The choice of corticosteroid formulation for a certain asthma patient should be based on knowledge of both the drug and the inhaler, combined with an understanding of individual patient factors such as inhalation technique, age, disease severity, preference, and expected compliance.

The purpose of this chapter is to highlight the pharmacokinetic factors determining the local therapeutic effect of inhaled steroids, focusing on (1) site of deposition, (2) rate and extent of uptake, (3) airway and pulmonary retention, including interactions with the corticosteroid receptor, and (4) local biotransformation. If not optimum, these factors, alone or in combination, can sometimes be overcome by an increase in dose, albeit at the expense of reduced airway selectivity. To put airway selectivity into a clinical context, the review will finally discuss the consequences of these local pharmacokinetic properties on overall benefit versus risk ratios of inhaled steroid formulations.

## II. Site of Deposition

The therapeutic effect of an inhaled antiasthma drug formulation is linked to the amount taken up by the target organ, which in turn is dependent on the amount actually deposited there. This has been clearly shown for both β-agonists (4,5) and ipratropium (6). For iCSs, the relationship between therapeutic effect and airway deposition is not as clear-cut as for β-agonists, probably because of a more complex mode of action and a substantial lag time between dosing and effect. That a relationship exists has, however, been suggested in studies with budesonide and beclomethasone dipropionate (7,8).

Drug delivery to the airways is critically dependent upon the inhaled fine particle dose, and a particle size of 5 μm is considered to be the maximum for appropriate airway delivery of particles having a unit density. For isoproterenol, greater improvement in lung function was achieved when inhaling monodisperse 2.5 μm particles than after inhaling the same total dose of 5 μm particles (9). Terbutaline sulphate particles larger than 5 μm did not improve lung function in asthmatics (10). Interestingly, Zanen et al. reported that the response to β-agonists is also reduced when the particles get even smaller: Monodisperse 2.8 μm salbutamol particles improved lung function more than did 1.5 and 5 μm particles in patients with mild (11) and severe (12) asthma. Probably, the intermediate size particles result in greatest deposition at β-receptor–dense sites at affected parts of the airways.

Airway deposition of inhaled corticosteroid products varies greatly, as discussed in the previous chapter. Depending on the asthma severity, inflammation may involve both central and peripheral airways as well as the lung parenchyma (13,14). Hence, it is difficult to determine which level of deposition within the lung is the most important when considering iCS treatment. In asthmatics who were well trained in their inhalation technique, administration of budesonide as a chlorofluorocarbon (CFC) suspension via a pressurized metered dose inhaler (pMDI) resulted in similar (without spacer) or greater (with Nebuhaler® spacer) peripheral deposition than administration via Turbuhaler® (15). The total lung deposition for Turbuhaler was, however, about twice that for a pMDI without spacer and about the same as for a pMDI with spacer. The more peripheral deposition from a pMDI was,

however, not translated into a greater therapeutic effect. On the contrary, in a study using a downtitration design, budesonide via Turbuhaler was as effective as twice the dose given via a pMDI with Nebuhaler in asthmatic children (7). Interestingly, it appears that airway deposition of budesonide via Turbuhaler is much more peripheral in healthy subjects (16) than in patients with asthma (15).

BDP delivered via a pMDI hydrofluoroalkane (HFA) solution resulted in considerably greater total lung deposition than via the pMDI CFC suspension formulation (17). In addition, the fraction of the lung dose that was deposited in the peripheral airways appeared to be greater with HFA than with CFC. This was true for patients with asthma as well as for healthy subjects (17). The improved BDP delivery resulted in improved lung function, and the potency ratio of improvement in $FEV_1$ was 2.6 (8). Small airways tended to improve to a greater extent with the HFA than the CFC formulation, as the potency ratio for $FEF_{25-75}$, which is indicative of peripheral airway function, was slightly greater (3.2) than the $FEV_1$ ratio. In addition, when assessing peripheral air trapping by high-resolution computer tomography, it appeared that the HFA had a greater effect than the CFC formulation (18).

The use of nebulized corticosteroids is increasing and is considered to be a convenient alternative in severely ill patients and for young children, patient groups in which the inhalation technique may be suboptimal and the peripheral dose penetration may be less efficient. For many of the new high-performance nebulizers, the generation of very small particles ($<2$ μm) is more efficient than for the dry powder inhalers (DPIs) and pMDIs. Also, with the new "intelligent" nebulizers, with which aerosol can be generated during any part of the respiratory cycle, and the new breath-actuated inhalers, particles may be directed towards specific parts of the bronchial tree (19).

More studies are needed to clarify the relationship between regional deposition of inhaled corticosteroids and therapeutic outcome. Although studies have shown a correlation between central airway recruitment of inflammatory cells and bronchial hyperreactivity in mild asthma (20), it appears that alveolar influx of inflammatory cells is a prominent feature when lung function is more severely compromised (14). Hence, while patients with mild asthma may benefit just by local treatment of hyperreactive and narrowed central airways, the more severely ill patient may need total exposure of the lungs by the corticosteroid to achieve the maximum therapeutic effect. The most convenient alternative in severe asthma may be inhaled corticosteroid preceded or combined with a bronchodilatating β-agonist: airway deposition of budesonide in patients with asthma improved significantly following pretreatment with terbutaline (21).

## III. Rate of Dissolution and Absorption

Water solubility differs between different inhaled steroids (Table 1). While a clinically relevant dose (200 μg) of fluticasone propionate (FP) or beclomethasone

**Table 1** Water Solubility of Corticosteroids at 37°C and Dissolution Time in Human Bronchial Fluid In Vitro

| | Water solubility (μg/mL) | Dissolution time (human bronchial fluid in vitro) |
|---|---|---|
| Flunisolide | 140 | <2 min |
| Triamcinolone acetonide | 21 | Not determined |
| Budesonide | 16 | 6 min |
| Beclomethasone 17-propionate | 15.5 | Not determined |
| Fluticasone 17-propionate | 0.14 | >8 h |
| Beclomethasone dipropionate | 0.13 | >5 h |

*Source*: Adapted from Ref. 102.

dipropionate (BDP) requires at least 2 L of water to dissolve, the same amount of the less hydrophobic steroid flunisolide would need only 1.5 mL. Water solubility is reflected in the different dissolution times in human bronchial fluid in vitro.

Pharmacokinetic studies in humans have shown that budesonide is rapidly absorbed after oral inhalation: $T_{max}$ after oral inhalation via Turbuhaler is about 20 minutes; mean absorption time about 40 minutes (22). FP has a slower rate of absorption: $T_{max}$ after oral inhalation via Accuhaler® or pMDI is about 2 hours; mean absorption time is 6–8 hours (22), probably as a result of the protracted dissolution. This may be advantageous, because drug retention at the target site is an important determinant of airway selectivity (23). However, high water solubility will limit the impact of mucociliary clearance (see below) and by that increase the rate and extent of pulmonary uptake. This will increase intracellular accessibility and cytosolic receptor site concentrations. High water solubility is also generally associated with a smaller volume of body distribution and less peripheral tissue retention. This in turn should reduce the risk of accumulation and systemic effects. Hence, therapeutic efficacy cannot easily be predicted from lipophilicity alone.

## IV. Mucociliary Clearance

The nose and airways act as a primary defense against foreign particles and aerosolized pollutants. In healthy subjects, most of the inhaled foreign particles will be transported to the pharynx via mucociliary clearance (MCC), normally within 6 and certainly within 24 hours. Inhaled drugs encounter the same fate and will, if not readily dissolved, be cleared from ciliated airways. As the therapeutic aim of inhaled drugs is topical, the mucosal exposure and pharmacological effect will hereby be reduced.

Mucus is produced in submucosal glands and goblet and Clara cells, and the normal mucus film in ciliated airways is about 5–10 μm thick. The mucus blanket

moves upward at about 1 mm/min in small peripheral airways but as quickly as 2 cm/min in the trachea (24). Eventually, particles deposited in the mucus reach the pharynx, where they are swallowed. In asthma, MCC is generally reduced. In acutely ill patients requiring hospitalization, MCC was strongly inhibited, but was normalized at discharge (25). In patients with stable asthma, several studies have demonstrated only subtle mucociliary impairment relative to healthy subjects (26,27). In mild asthma and asthma in remission, reductions in MCC of about 25% have been noted. The inhibitory effect of some inflammatory mediators, such as leukotriene $D_4$ (26), may contribute to the reduced clearance. Smooth muscle hyperplasia, evidenced in bronchial biopsies, correlates to a reduction in MCC (28). Sleep reduces MCC by two thirds to three quarters (29). Inhaled terbutaline appeared to normalize MCC in mild asthmatics (30), as did high doses of oral prednisolone in stable asthmatics (31). Inhaled CSs have not been shown to affect MCC, although some preservatives in nasal CS formulations have been suggested to have a mild ciliotoxic effect (32).

The lipophilic CSs appear to be more affected by MCC than the more rapidly dissolved CSs. Following 14 days of treatment with BDP, which is highly lipophilic, in a CFC suspension pMDI, bronchoalveolar lavage (BAL) concentrations of the primary metabolite beclomethasone-17β-monopropionate (BMP) at 90–120 minutes after the last dose were significantly higher than following treatment with BDP in an HFA solution pPMDI (33). These findings were initially unexpected, given the high lung deposition of the BDP HFA solution, but were explained in terms of much more prompt dissolution in lung fluid and less central deposition following the HFA formulation. This led to a more rapid absorption and less MCC than for the CFC formulation. Similarly, the systemic exposure to BDP was almost 10 times greater after inhalation via Spiros®, a DPI with improved airway delivery characteristics, than after inhalation via a conventional CFC pMDI (34). The difference in airway deposition between the two inhalers appeared to be no greater than fivefold. Again, a more central deposition of BDP via the pMDI would lead to a greater loss by MCC, in this way further increasing the difference between the two formulations in pulmonary uptake.

Mucociliary clearance also appears to significantly reduce pulmonary uptake of fluticasone propionate (FP) in patients with moderate to severe asthma. Lung deposition, approximated by the systemic availability, following inhalation via pMDI was on average 10% in moderate to severe asthmatics versus 21% in healthy subjects (35). Intravenous kinetics were virtually identical in the two populations, implying that there was no difference in basic pharmacokinetic properties. A reduction in systemic availability by about half in patients versus healthy subjects was also suggested for the dry powder formulations of FP (36). In patients with asthma treated with a single dose of FP (37), a close correlation between cortisol suppression and pretreatment lung function was observed (Fig. 1), implying that pulmonary uptake of FP increases with decreasing disease severity.

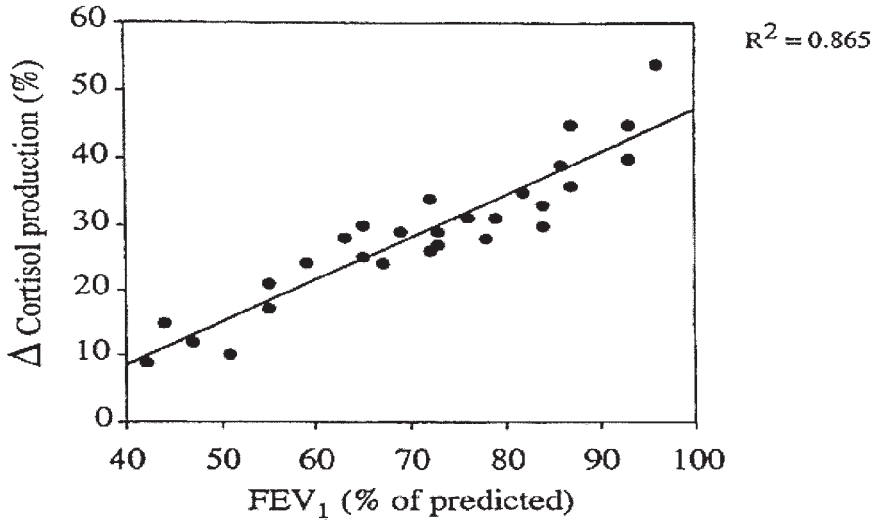


**Figure 1** Correlation between $FEV_1$ just before inhalation of FP and the fall in cortisol production after a single 500 μg dose of FP via Diskhaler in patients with asthma. (Data from Ref. 37.)

When comparing mild steroid-naïve asthmatics and healthy subjects, there were no differences between the two groups in either plasma concentrations or cortisol suppression following administration of FP via Accuhaler or budesonide via Turbuhaler (22). Interestingly, the difference in cortisol suppression for FP given by Accuhaler between moderate asthmatics and healthy subjects was confirmed in a study by Harrison and Tattersfield, but could not be shown for budesonide via Turbuhaler (Fig. 2) (38). Taken together, these data suggest that FP, but not budesonide, is subject to substantial MCC. For a drug with an extended dissolution time in mucus, this will affect overall lung uptake. When disease is more severe, deposition will become more central, resulting in even greater MC transport and, thus, an even lower total lung uptake.

In this context, it may be of interest to point out that the systemic uptake of intranasal CSs is probably even more limited by MCC than orally inhaled CSs. Although nasal MCC appears to be slightly reduced in rhinitic patients compared with healthy subjects because of differences in mucus rheology, the transport from proximal parts of the nose to the pharynx takes only about 10 minutes (39). The water-soluble CSs with high rates of mucosal dissolution, such as flunisolide, show substantial nasal uptake—systemic availability after administration of a nasal aqueous suspension was 49% (40). The nasal uptake of the more lipophilic CS FP is very much lower, and systemic availabilities of 2% or less were reported (41,42). Similar low uptake was suggested for mometasone furoate (MF) nasal spray (42,43).

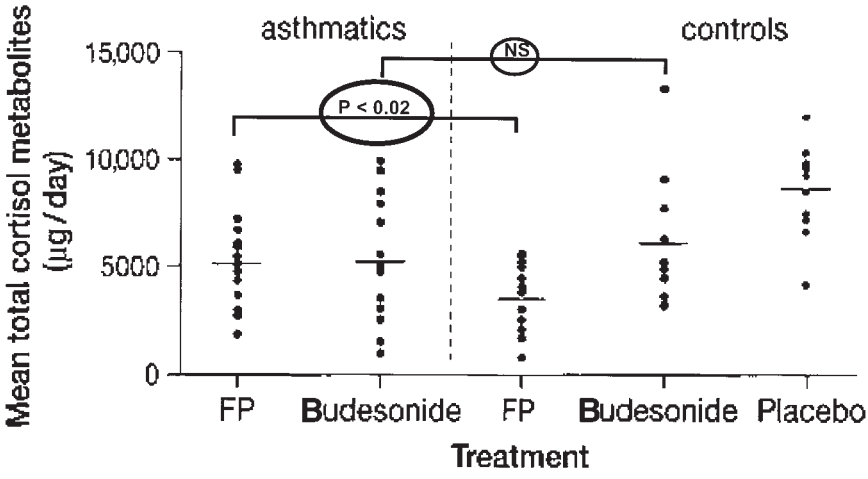


**Figure 2** Individual and total cortisol metabolites after 7-day treatment with FP 1500 µg/day or budesonide 1600 µg/day in 30 asthmatics (mean $FEV_1$ 2.0 L, 60% predicted) and 45 healthy controls. (Adapted from Ref. 38.)

## V. Airway and Lung Uptake and Retention

There is no evidence to suggest that the mode of CS action differs between endogenous CSs and exogenously supplied CSs. Neither does the mode of anti-inflammatory action of systemically utilized synthetic CSs appear to differ from that of the more potent iCSs used for local action. However, receptor kinetics (see Sec. VII) and cellular uptake and retention differ considerably between CSs. This will have an impact on the onset, duration, and magnitude of clinical effect and may also affect the selectivity between the airway and the systemic effects.

In contrast to the systemic steroids, the topically potent CSs used for inhalation treatment show a substantial uptake in the airways (Fig. 3). Peak tracheal concentrations of tritium-labeled budesonide, FP, and BDP were almost 100-fold greater than those after hydrocortisone and dexamethasone after a 10-minute tracheal superfusion in rats (44).

Measured peak levels were obtained in the first sample (10 min after stopping perfusion). The subsequent retention of radioactivity was substantially greater for budesonide and FP than for the systemic steroids. Radioactivity corresponding to BDP declined at a rate resembling that of dexamethasone (DEX) and hydrocortisone (HC), which may be explained by the extensive biotransformation to the much less lipophilic monopropionate and the free alcohol (see below) occurring in the airways. Lipophilicity as an important factor in the retention was suggested by Rohdewald et al. (45) and Esmailpour et al. (46). They showed that the uptake

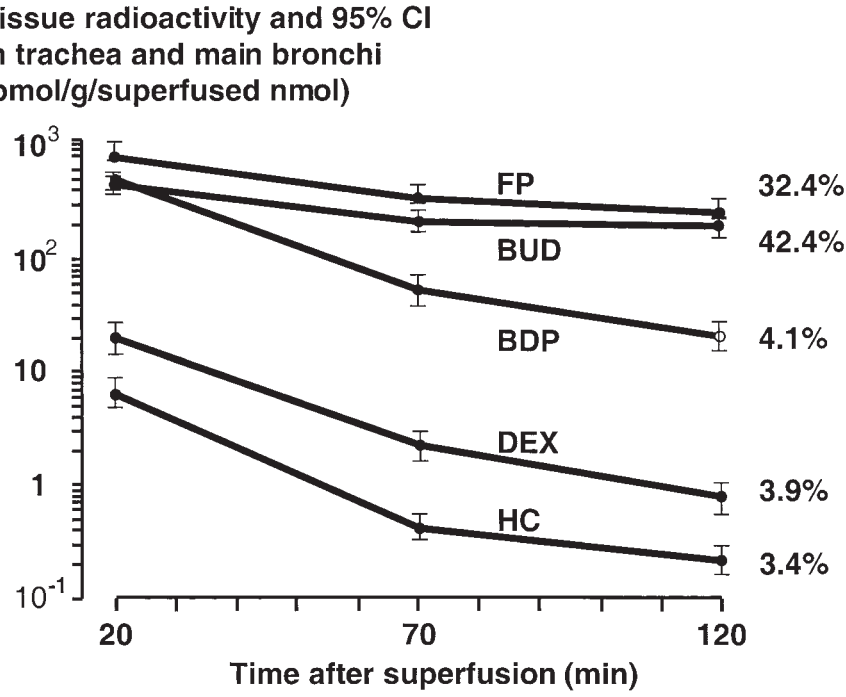


**Figure 3** Tissue radioactivity in perfused sections of trachea after 10-minute perfusion with $^{3}$H-corticosteroid solutions, followed by saline perfusion between 10–120 minute. Values are geometric means, with 95% confidence intervals ($N = 3–5$), for each GCS and time point. The percentage values express the ratio of tissue radioactivity at 120 minute to that at 20 minute. (Data from Ref. 44.)

of CSs from buffer into human lung or nasal tissue in vitro reached equilibrium in 5–20 minutes and was greatest for FP followed by BDP > BMP > budesonide > flunisolide > hydrocortisone, which essentially reflects the relative lipophilicity of these agents. Following reequilibration of the CS-containing lung samples with plasma, the same order of retention was found as for tissue uptake. The relative uptake of CSs was assessed by analysis of remaining amounts in buffer and plasma. The fact that budesonide was relatively little taken up by the tissue in vitro stands in contrast to the in vivo findings discussed below. Confounding factors in the in vitro experiments may include nonspecific adsorption of dissolved steroid to incubation flasks and lack of cofactors for esterification (budesonide forms fatty acid esters; see Sec. VI).

The uptake in lung tissue of systemic CSs is generally comparable to that in other well-perfused organs, such as heart, liver, and kidney (47). After intravenous

dosing in the rabbit, the ratio between tissue and unbound plasma concentrations (i.e., the partition coefficient) of prednisolone in the lung, liver, and kidney was about three in each organ. Neither do the pharmacokinetics in airway fluids appear to differ substantially from those in plasma after systemic dosing. Following intravenous administration of hydrocortisone in humans, Braude and Rebuck (48) showed a strong correlation and an approximate 1:1 ratio between levels in plasma and in lung epithelial lining fluid. After intravenous injection of various water-soluble CSs in healthy subjects, the bronchoalveolar lavage/plasma pseudo-equilibrium was reached at 10 minute and ranged between 0.4 and 1.3 for methylprednisolone, prednisolone, dexamethasone, and triamcinolone, being highest for prednisolone and lowest for triamcinolone (49).

The cellular localization of the tissue-bound CS fraction has been little studied in the lung. For $^{3}$H-dexamethasone incubated in vitro with mouse lung tissue samples, radioactivity was chiefly localized to alveolar type II cells, bronchiolar and arteriolar smooth muscle cells, fibroblasts, and endothelial cells of the pulmonary vasculature (50).

The multidrug resistance (MDR) gene–encoded proteins phosphoglycoprotein (P-gp) and multidrug resistance–associated protein (MRP) represent a family of drug efflux proteins that convey multidrug resistance to cells in which they are expressed. Many cells express these proteins, and although lung cells have been relatively little studied in this respect, both P-gp and MRP are present in human alveolar epithelial cells (A549). MRP is also found in human airway epithelial cells (Calu-1) (51). Some of the systemically active steroids are substrates and can also act as antagonists towards P-gp–mediated efflux. For example, dexamethasone levels in the brain relative to the blood are five times higher in P-gp knockout mice than in controls (52). In cells expressing P-gp, the accumulation of dexamethasone and cortisol was reduced by as much as 50% compared with corresponding cells not expressing this efflux protein (53). It has been suggested that the more hydrophilic (systemic) steroids are better transported and are poorer antagonists of these efflux proteins than the more hydrophobic (topical) steroids (53). The P-gp ligands cyclosporin A and FK 506 potentiated dexamethasone-induced chloramphenicol acyl transferase reporter gene transcription in mouse fibroblasts to a greater extent than the more lipophilic topical CS triamcinolone acetonide (54). Also, verapamile affected dexamethasone but not triamcinolone acetonide–induced transcription in the same in vitro system. Recently, budesonide was shown to inhibit P-gp and MRP in human alveolar epithelial (A549) cells, but only at extreme concentrations (1–100 mM) (51). Interestingly RU-486, a potent corticosteroid receptor antagonist, is a strong inhibitor of P-gp activity (55).

Hence, systemically utilized CSs appear to equilibrate rapidly with lung parenchyma and airways and show no greater affinity for the respiratory organs than for other well-perfused organs. It is likely that P-gp, which is present in most cells, limits the cellular uptake of systemic CSs, particularly the more hydrophilic ones.

The lipophilic CSs, on the other hand, seem to be much less affected by these efflux proteins.

## VI. Corticosteroid Esterification

Fatty acid esters of steroids, discovered in the mid-1970s (56), represented a new and unique class of conjugates, distinctly separate from the classical polar sulfate and glucuronide conjugates. Esterification of cholesterol, estrogens, and mineralo- and glucocorticoids has subsequently been discovered (57) and shown to serve as a hormone-storing process (58) and to prolong hormonal response (59). Esterification is an important part of the cellular uptake, transport, and storage of cholesterol destined for steroid hormone synthesis, where cholesteryl esters are internalized via lipoprotein receptors and stored in the Golgi apparatus as lipid droplets (60). P-gp plays a central role, being required for esterification of low density lipoprotein–derived cholesterol (61). Only corticosterone and 5-dihydrocorticosterone among the natural and none among the synthetic corticosteroids have previously been shown to form C-21 esters. 11-Dehydrocorticosterone, which is abundant in many tissues of the rat, does not appear to form esters. These data suggest that the CS esterification mechanism is relatively stereoselective.

### A. Identification and Biochemistry of Fatty Acid Esters of Budesonide

The rapid pulmonary uptake of budesonide combined with a significant retention within the airways and lung parenchyma, shown in the mid and late 1980s (62), remained unexplained for about 10 years. From relatively recent kinetical and biochemical studies, it became clear that a significant portion of budesonide, and to a much lesser extent triamcinolone acetonide, are intracellularly esterified (44, 63,64). After 6-hour incubation of normal human bronchial epithelial cells with $^{3}$H-budesonide, 93% of the total cellular budesonide was in the form of fatty acid esters (64). Fatty acid esterification of budesonide was also demonstrated in other biological systems, including liver and lung microsomes from the rat, human blood monocytes, human colonic carcinoma (CACO-2) cells, and rat fibroblasts (R-1 cells) (63). Hydrocortisone appears to follow the same biotransformation pathway, but here hydrolysis is much more prompt and little ester is therefore detected in tissue samples (A. Tunek, personal communication). FP, BDP, and probably other CSs lacking the C-21-hydroxy group do not form fatty acid esters (63).

Several fatty acid esters of budesonide have been identified. Budesonide is esterified in the 21-position, predominantly with oleic acid, but also with palmitoleic, palmitic, linoleic, and arachidonic acids (Fig. 4).

The formation of the esters is dependent on CoA and ATP, and by adding various fatty acids to cell incubates, the relative amount of each fatty acid ester can

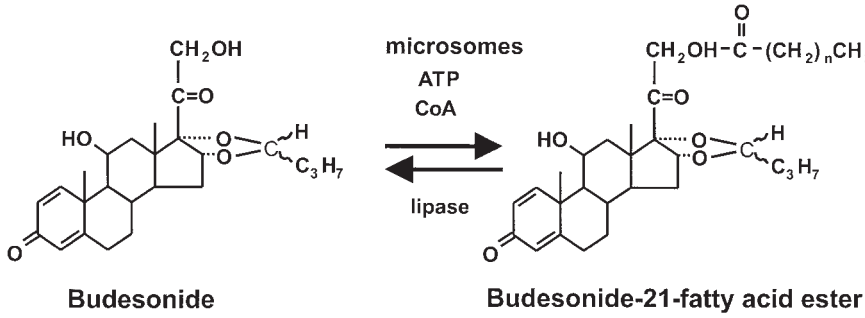


**Figure 4** Formation and hydrolysis of budesonide-21-fatty acid esters. (From Ref. 63.)

be changed. The acyl-cholesterol CoA transferase (ACAT) inhibitor cyclandelate significantly reduced the intracellular content of esterified budesonide (by about 80% in a rat fibroblast cell system), suggesting that ACAT is an important esterification enzyme for budesonide. Esters could be hydrolyzed back to intact budesonide by porcine pancreatic lipase and bovine pancreatic cholesterol esterase.

The reversible esterification process appears to promote an inactive intracellular storing pool of intact budesonide. Blocking of budesonide binding to the corticosteroid receptor by the corticosteroid antagonist mifepristone (RU-486) resulted in a relative increase in intracellular budesonide esters (65,66). Blocking of ACAT reduced the cellular content of intact budesonide much less than that of esterified drug. These data suggest that corticosteroid receptor binding is little affected by esterification. Unlike budesonide, the budesonide esters have little affinity for the GCS receptor and are between 500 and 10,000 times more lipophilic than budesonide itself (44).

In the airways, budesonide esterification is prompt and extensive. Only 20 minutes after inhalation or intratracheal instillation of tritium-labeled budesonide in rats, radioactivity bound to the trachea and main bronchi expressed to about 80% fatty acid conjugates of budesonide (44). After tracheal perfusion with tritium-labeled budesonide, the maximum radioactivity was localized to the mucosal and serosal compartments of the trachea (44). Much less esterification occurs in striated muscle, even after local injection of budesonide into an adjacent muscle. Twenty minutes after intramuscular injection, only 12% of muscle radioactivity originated from budesonide esters, while at the same time there was considerably greater proportion in the lung and trachea (approximately a 1:1 ratio of budesonide/budesonide ester) (67). These experiments indicate that budesonide is considerably more prone to forming esters in the trachea and lung than in striated muscle.

An inflammatory response does not appear to affect the fatty acid esterification of budesonide: lipopolysaccaride-evoked lung inflammation did not affect the

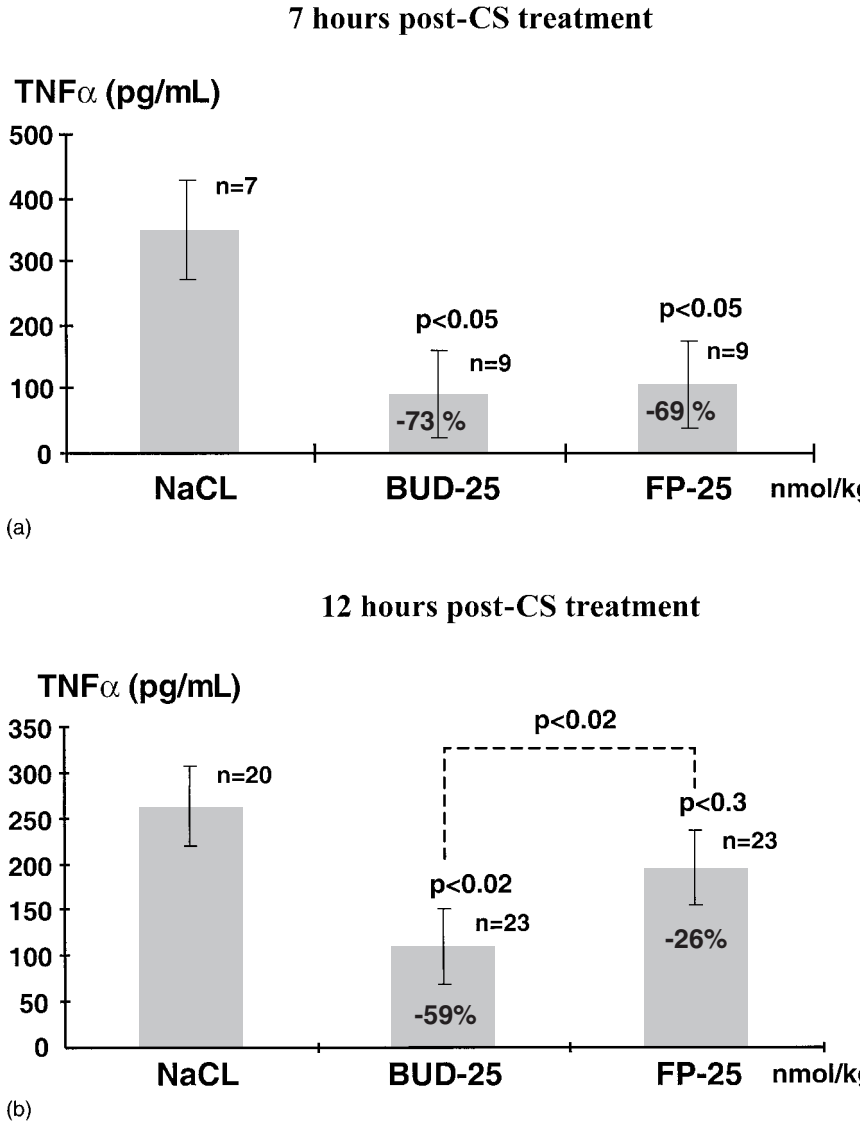


**Figure 5** Inhibition of bronchoalveolar lavage content of TNF by intratracheal budesonide and fluticasone vs. control (saline). Rats were treated with single doses of 25 nmol/kg corticosteroid suspension by instillation followed by lipopolysacharide (LPS), 100 μg (50 μL) in solution, 1 or 6 hours later. Six hours after LPS instillation, bronchoalveolar concentrations of TNF were determined by ELISA. (a) Effects of budesonide and fluticasone at 7 hours. (b) Effects of budesonide and fluticasone at 12 hours. (Data from Ref. 71.)

propensity of the airways to form budesonide esters in vivo (68). In addition, pretreatment of normal human bronchial epithelial cells with TNF-α did not affect the relative proportion of fatty acid esters (69).

### B. Duration of Action of Budesonide

Fatty acid esterification of budesonide prolongs anti-inflammatory action by increasing the dwell time within the airways. The anti-inflammatory effects of budesonide and FP were investigated in a steroid-sensitive, transcription factor AP-1 mediated in vitro model, using transfected rat fibroblasts with β-galactosidase activity as readout (64). Experiments were performed either during continuous 24-hour incubation or during pulse exposure followed by extensive washing and further incubation without CS up to 24 hours. The latter experiment was performed to mimic the clinically more relevant situation of intermittent airway exposure. Using a 30-minute CS pulse, washing, and continued incubation without CS for 24 hours, pretreatment of cells with the ACAT inhibitor cyclandelate significantly reduced (by more than half) the budesonide-induced downregulation of the AP-1–mediated effect (70). The total levels of budesonide esters were simultaneously reduced by 80% compared with the same experiment performed without cyclandelate. Cyclandelate did not affect the cellular content of FP in this model (65). Budesonide was significantly less potent than FP during continuous 24-hour exposure over a wide concentration range. However, following a 6-hour CS pulse, washing, and subsequent incubation without CS for 18 hours, FP lost most of its downregulating effect, while that of budesonide remained almost intact (64). Taken together, the long effect duration following a pulse of budesonide in this cell model may be explained in terms of prompt formation of esters, which are retained in the cell, and from which intact budesonide is slowly regained.

A similar prolongation of anti-inflammatory effect for budesonide relative to FP has been shown to occur in rat airways in vivo (71). Budesonide or FP at a dose of 25 nmol/kg b.wt. (11–13 μg/kg b.wt.) or vehicle (100 μL saline) was intratracheally instilled in rats, followed 1 or 6 hours later by lipopolysaccaride (LPS). LPS induces an inflammatory reaction, where TNF-α (and other proinflammatory cytokines) are released. At 6 hours after LPS instillation, the trachea and main bronchi were lavaged ex vivo with buffer and TNF-α was measured (by ELISA). While budesonide and FP were equally effective at 7 hours, at 12 hours budesonide was significantly more effective than FP in reducing the release of TNF- α (Fig. 5) (71).

### C. Human Pharmacology

The extensive uptake of budesonide and FP in airway cells in vitro and in animal airways and lungs in vivo has been confirmed in humans. Drug concentrations

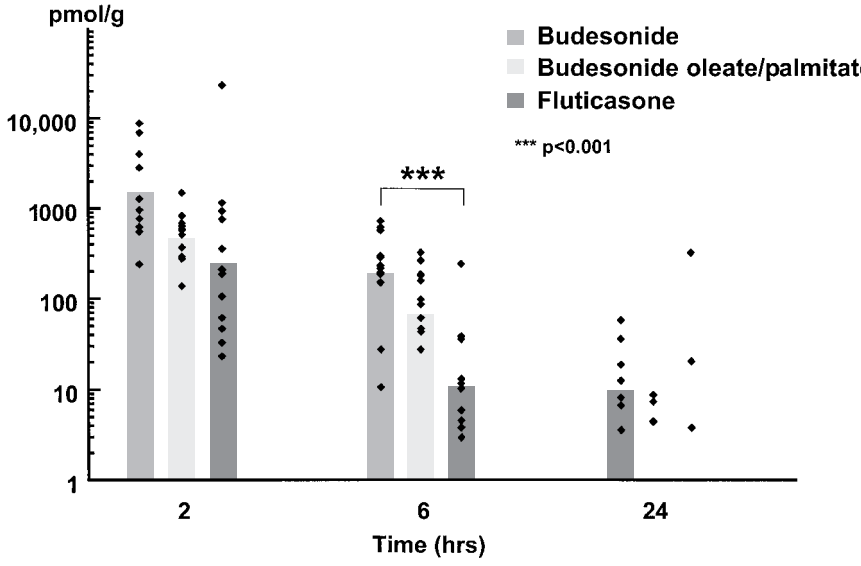


**Figure 6** Nasal biopsy concentrations of budesonide, budesonide esters (oleate + palmitate), and fluticasone propionate after administration of single nasal doses of budesonide nasal spray (two puffs per nostril, total dose 256 μg) and fluticasone nasal spray (two puffs per nostril, total dose 200 μg) to 24 healthy subjects. Biopsies were taken from each subject at two time points, just before dosing and at 2, 6, or 24 hours postdose, in a randomized fashion, giving a total af 12 samples per time point. No analyte was above limit of quantification in samples obtained before dosing. All data points that were above limit of quantitation are depicted in the figure. (Data from Ref. 73.)

were found to be severalfold higher in airways than in plasma after oral inhalation and nasal administration of budesonide and FP (46,72,73). The presence of fatty acid esters has been shown in vivo in human lung and nasal samples (73,74). Central and peripheral lung tissue was obtained from seven patients undergoing lung resection surgery. All patients received a single 1600 μg dose of budesonide via Turbuhaler at varying time points (3.5–14.8 h) preoperatively. Budesonide and budesonide fatty acid esters, predominantly oleate, were found in lung tissue samples from all patients. The concentrations were about the same for the sum of budesonide fatty acid esters (range 0.9–17.2 pmol/g wet tissue) as for budesonide itself (range 0.7–19.3 pmol/g wet tissue). The ratio between budesonide and budesonide oleate concentrations was similar in central and peripheral lung samples.

In nasal biopsy samples, budesonide esters were present in substantial amounts after single 256 μg doses in 24 healthy subjects (Fig. 6) (73). Again, at the time points studied (2, 6, and 24 hours after a single dose), budesonide oleate

was the dominating ester (>90% of total esters), with a concentration corresponding to about half that of intact budesonide. The variability in budesonide-oleate concentrations was less than that of unesterified budesonide at all time points, suggesting that ester formation is relatively stable among individuals.

This study also revealed that intact budesonide was present in significantly higher amounts and was retained longer than FP in the nose: the dose-corrected ratio between intact budesonide and FP was 3.5 at 2 hours and 13.7 at 6 hours. At 24 hours, budesonide and FP were detected in 67 and 25% of the biopsies, respectively. It may thus be concluded from this study that tissue retention is not a simple reflexion of the lipophilicity of the intact steroid.

## VII. Biotransformation of CSs, Other Than Esterification, in Airways and Lung

All current iCSs are inactivated mainly by biotransformation in the liver, most commonly by oxidative metabolism via CYP3A4. Recent data suggest that the gut mucosa also contributes to this oxidative metabolism (75). The expression of CYP3A in the lungs is low. The only cells containing any substantial amounts of CYPs are the nonciliated bronchiolar epithelial cells (Clara cells) and the alveolar type II cells. Alveolar macrophages appear devoid of CYP activity (76,77). The constitutive levels are much lower in lung than in liver, and indeed much lower in humans than in other species: human lung contains only about 1% as much constitutive CYP as rabbit lung, and this low level comprises CYPs with other substrate specificities: CYP1A1 (which metabolizes polyaromatic hydrocarbons), CYP2E1 (substrate chlorzoxazone and ethanol), CYP2F1 (skatole), CYP3A (many xenobiotics), CYP4B (fatty acids), and CYP5 (thromboxane synthase) (78). Among the CYP3As, CYP3A5 is the predominant CYP3A in the lung, and 3A4 is expressed in about 20% of individuals in lung tissues (bronchial and alveolar epithelium and alveolar macrophages) (79,80). In primary human bronchial epithelial cells, gene expression for CYPs 1A1, 1B1, 2B7, 2E1, 4B1 but not 3A4 was found (77).

Budesonide is not metabolized in the human lung 9000 $\times$ *g* supernatant fraction in vitro (81), and the same holds true for FP, flunisolide, and triamcinolone acetonide (P. Andersson, personal communication). Neither does budesonide appear to be oxidatively metabolized in the nose (82). The metabolism of inhaled MF remains to be studied. The very low systemic availability claimed after both oral inhalation (0.96% via DPI, less via MDI) and nasal administration could imply that MF undergoes local metabolism. Indeed, a large number of minor tritium-labeled metabolites were separated after inhalation of $^{3}$H-MF via a DPI (83). None were identified, and it remains to be shown whether any of the tentative metabolites have pharmacological activity, as suggested by Isogai et al. (84).

**Table 2** In Vitro Metabolism of Beclomethasone Dipropionate and Beclomethasone Monopropionate in Human Liver and Lung Homogenates as Well as in Human Plasma and Blood

| | Half-lives (mean [SD]) | |
|---|---|---|
| Incubation media | BDP | 17-BMP |
| Liver ($n = 5$) | 2.6 [0.2] min | 3.7 [0.2] h |
| Lung | 35 [2] min ($n = 35$) | 3.5 [0.2] h ($n = 60$) |
| Plasma ($n = 6$) | 10.9 [0.02] h | 3.3 [0.04] h |
| Blood ($n = 5$) | 3.3 [0.06] h | 27 [0.5] h |

*Source*: Data from Ref. 86.

At present, data are also lacking regarding any local metabolism of the investigational CSs ciclesonide and loteprednole etabonate in the airways.

Some conjugation enzymes, such as glutathione transferases and sulfotransferases, but not glucuronosyl transferases, are present in human bronchial epithelial cells and alveolar macrophage cells (77). In vitro, budesonide is biotransformed by liver and lung sulfotransferases (85). There was a significant gender difference in hepatic sulfation of budesonide, with testosterone being a potent inhibitor of this biotransformation pathway. In lung, budesonide sulfotransferase activity was one-fifth that in liver and was similar in smokers and nonsmokers. The extent of hepatic and lung sulfation in vivo remains to be shown but is probably limited, as there is no apparent gender difference in either the pharmacokinetics or systemic pharmacodynamics of budesonide (AstraZeneca, data on file).

Esterase enzymes show high activity in the lung, and BDP is hydrolyzed to beclomethasone-17β-monopropionate (BMP) in homogenates of human lung at a relatively high rate (see Table 2) (81,86). As BDP has low but BMP a very high receptor affinity, this hydrolysis represents an activation step. In addition, solubility is much increased by the hydrolysis, which may affect the rate of pulmonary uptake and circumvent MCC. Interestingly, in vitro BMP is further metabolized to beclomethasone alcohol at a similar rate in the lung, plasma, and liver but substantially slower in the blood (Table 2). The slow metabolism of 17 β-BMP in the blood compared with in plasma may be a result of cellular uptake and intracellular "protection" from plasma esterases.

In the human lung in vitro, hydrocortisone is relatively readily metabolized to cortisone by 11-β-hydroxysteroid dehydrogenase (11 β-HSD). The potent 11 β-HSD inhibitors glycyrrhetinic acid and glycyrrhizin have anti-inflammatory properties, possibly due to the local inhibition of this hydrocortisone-to-cortisone in-

activation step (87). Synthetic CSs are, however, not known to be metabolized via this pathway.

## VIII. Kinetics of Receptor Binding and Cellular Response

The current understanding of corticosteroid action includes extracellular transport via binding proteins in blood (albumin and transcortin), free diffusion over the lipid bilayers of cell membranes, and subsequent binding to cytoplasmatic corticosteroid receptors (88). There is some evidence to suggest that CS entry into cells involves specific membrane-associated receptors distinct from the classical intracellular CS receptors (89), but this has hitherto been described primarily in neuronal tissues (90,91). The corticosteroid-receptor complex becomes activated by relatively poorly understood mechanisms, including conformational changes, dissociation from heat-shock proteins, and hyperphosphorylation. Activated receptors then translocate into the nucleus and bind as homodimers to specific DNA sequences. Two forms of the human corticosteroid receptor are known to exist, hGRα and hGRβ (92), where the former appears to be localized to the cell nucleus independent of the presence of any ligand. The role of hGRα is currently under debate (93), and it has been speculated that it acts as a negative regulator of hGRβ transactivation, and that the tissue- and cell-specific balance between the two receptor forms may modulate the responsiveness of tissues to CSs (94). However, the hGRα seems to be much less expressed than the hGRβ form (95).

Following exposure of human lung tissue samples to a CS at 20°C, receptor association starts quickly and is complete after 40–60 minutes, irrespective of the steroid tested (96). As dissociation is much slower, the half–life of the receptor complex is substantial, about 10 hours for FP, 5 hours for budesonide, and less for the weaker CSs (Table 3). However, these half-lives have been estimated under nonphysiological conditions in vitro and cannot be translated into corresponding in vivo half-lives. The subsequent steps of corticosteroid-receptor complex activation and nuclear binding have also been timed and are rapid: under physiological conditions in rat thymus cells at 37°C, the half-lives of activation and nuclear binding were 30–60 and 10 s, respectively (97). Transcription and posttranscriptional events are generally the rate-limiting factors, and the final, receptor-mediated, genomic corticosteroid effects generally take 6–12 hours to reach a maximum (98). The rapid effects of CSs (89) cannot be explained by these pathways, but whether they are of any major therapeutic importance remains to be shown.

Corticosteroid receptors are relatively evenly distributed throughout the human lung, with no apparent difference between healthy subjects and asthmatics. The amount of CS receptor–binding sites in human bronchial epithelial cells is approximately 30 fmol/mg protein ($2.6 \times 10^4$ sites/cell) (99) and appears to

**Table 3** Kinetic Constants of Corticosteroid-Receptor Complexes in Cytosolic Preparations of Human Lung Tissue at 20°C

| Corticosteroid | $k_1$ (L/[nmol × min]) × $10^5$ | $k_{-1}$ (L/min) × $10^{-4}$ | $K_d$ (nmol/L) | $t_{1/2}$ (h) |
|---|---|---|---|---|
| Dexamethasone | 4.8 | 88 | 18.3 | 1.3 |
| | 12.5 | 117 | 9.4 | 1.0 |
| | 9.6 | 109 | 11.3 | 1.0 |
| Methylprednisolone | 5.5 | 236 | 42.9 | 0.5 |
| Triamcinolone acetonide | 4.9 | 30 | 6.1 | 3.9 |
| Fluticasone propionate | 23.9 | 12 | 0.49 | 10.1 |
| | 27.4 | 15 | 0.55 | 7.7 |
| Budesonide | 18.9 | 25 | 1.3 | 4.6 |

$k_1$ = Association rate constant; $k_{-1}$ = dissociation rate constant; $K_d$ = equilibrium dissociation constant; $t_{1/2}$ = half-life of the receptor complex.
*Source*: Adapted from Ref. 102.

be slightly higher in the alveolar walls and vascular endothelium of the lung (100). From these data, assuming a similar distribution of receptors throughout the lung and that about one third of lung tissue consists of protein, the receptor concentration can be estimated at 10–20 nmol/L. CSs bind with high affinity to the corticosteroid receptor, and equilibrium dissociation constants ($K_d$) have been estimated to range from about 0.5 nmol/L for FP to about 10 nmol/L for dexamethasone (Table 3). For MF, the $K_d$ may be even lower (101). The $K_d$ can be directly translated into the concentration of free (non–tissue-bound) CS needed to saturate the receptor by 50%. Hence, at equilibrium with 50% saturated receptors, about 1 nmol of a CS with a $K_d$ of 1 nmol/L is unbound and 5–10 nmol bound to the receptor per liter of lung tissue (assuming a receptor concentration of 10–20 nmol/L). So, theoretically, relatively little (5–10 μg/L in the above calculations) of the drug reaching the airways is needed to saturate the receptor. Any surplus will then either be retained unspecifically in the tissues, unchanged or as biotransformation products (e.g., budesonide oleate), or be absorbed into the systemic circulation. However, if a highly potent CS is given at a sufficiently low dose, a substantial fraction of the given dose will occupy airway/lung CS receptors. This will leave less to be promptly absorbed, which may lead to systemic "spill-over" concentrations that are below the linear, steep part of the concentration-time curve for systemic effects. This will improve airway selectivity, as illustrated in Figure 7.

Anti-inflammatory effects appear to be related to receptor affinity in most cell systems in vitro (102), although there is seldom a strict correlation. In activated human airway epithelial cells, FP was 10 times more potent than budesonide

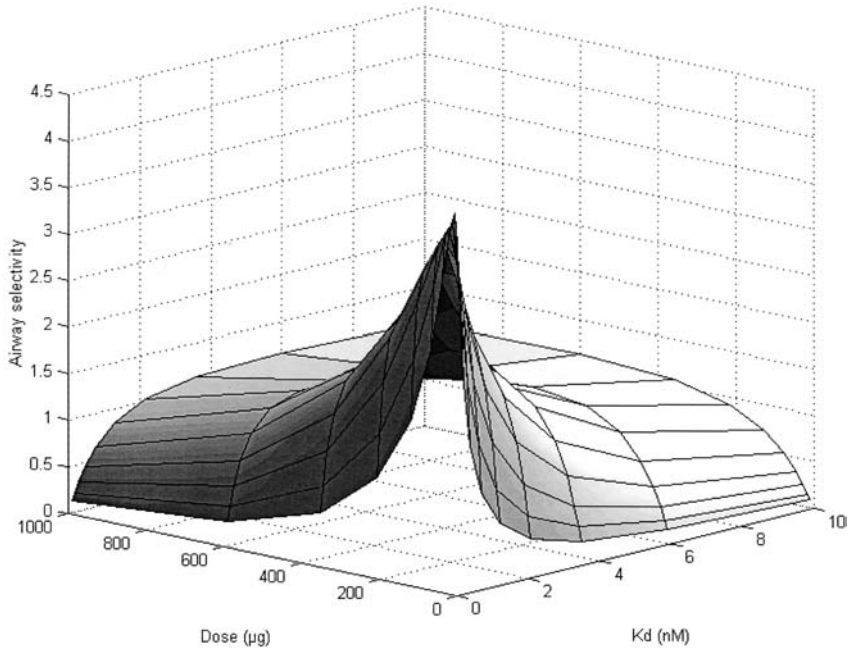


**Figure 7** Airway selectivity as a function of receptor affinity ($K_d$) and dose. Pharmacokinetic compartment model adopted from Hochhaus (23), with the following assumptions: two corticosteroid concentration [CS] compartments (lung and systemic); inhalation of CS powder twice daily at steady state (after 40 doses) with lung deposition = 30%; rapid rate of dissolution ($T_{1/2diss}$ = 18 min) and systemic absorption from lung ($T_{1/2abs}$ = 4 min); mucociliary clearance, $T_{1/2muc}$ = 4.2 h; oral availability = 10%; clearance from systemic compartment only = 60 L/h; $V_{lung}$ = 2 L; $V_{syst}$ = 150 L. Pharmacodynamic model based on receptor binding in lung and systemic compartments, where binding to the CS receptor over 12 hours equals effect, and airway selectivity equals the difference between 12-hour integrated effects in the lung and the systemic receptor compartments at steady state. Total corticosteroid receptor concentration ([R]+[CSR]) throughout the body = 15 nM; $V_{lung}$ = 2 L for corticosteroid and receptor; $V_{syst}$ = 70 L for receptor; $K_d = k_{-1}/k_1$ (according to Table 3 above); $k_{-1} = 1 \times 10^{-4}$ [$min^{-1}$]; rate of receptor binding = $k_{-1}$[CS][R]; rate of receptor unbinding = $k_{-1}$[SR]. Simulations were made in MATLAB®.

at inhibiting cytokine release (103), but equally potent at inhibiting eicosanoid output (104). The difference between FP and dexamethasone at inhibiting AP-1 binding to DNA (a 15-fold difference) was similar to the difference in their relative receptor binding affinity (about 18-fold) (105). Differences between the two CSs at inhibiting binding to NF-κB and inhibiting the release of GM-CSF was,

however, much greater—about 200-fold. Different cell systems also show different sensitivity to CS treatment: human monocytes, for example, are much more sensitive than human alveolar macrophages (106). Possible reasons for the divergence in results include differences in experimental set-up and readouts between studies, differences in solubilities, cellular uptake, and biotransformation between CSs, and differences in the presence of critical cofactors. In vitro pulse experiments should better reflect the clinical situation of intermittent dosing, uptake, retention, and elimination. Results from such studies tend to give results very different from those obtained with the continuous incubations used in the great majority of in vitro studies (64).

Taken together, the actual binding of the CS to the receptor and the subsequent nuclear translocation are rapid processes, the transcription and receptor dissociation slower. Whether any of these processes affect the overall retention of the CS within the tissue in vivo remains to be shown, although after inhalation only a minor portion of the dose deposited in the airways interacts with the receptor at any given time. This explains why unspecific binding via lipophilic interactions rather than receptor affinity is so important for total tissue uptake and by that its retention. A low receptor affinity can generally be compensated for by increasing the dose (23), and therapeutic efficacy hereby remains unaffected. However, for extremely potent CSs at low absolute doses, an improvement in airway selectivity may be gained by the receptors acting as a "sink," subtracting a substantial portion of drug, which otherwise would have been promptly absorbed into the systemic circulation. A necessary prerequisite for this to occur is that other pharmacokinetic properties, such as distribution volume and clearance, remain unchanged, which is seldom the case when comparing the receptor kinetics of different CS formulations.

## IX. Clinical Implications

Therapeutic efficacy (i.e., maximum possible therapeutic effect, irrespective of dose) appears to be similar or identical for the commercially available inhaled CS formulations: no well-controlled double-blind clinical study has to date convincingly shown better efficacy of one CS formulation over another at maximum tolerable doses, in spite of large differences between different inhaled CS formulations regarding their receptor affinity and their uptake, retention, and biotransformation in the lung and airways. Hence, it appears that differences in local pharmacokinetic properties between different CS formulations can, at least to some extent, be compensated for by adjusting the dose and/or dosing frequency. The development of improved iCSs within the pharmaceutical industry has been very much focused on increasing potency, and indeed the two most recent iCSs on the market, FP and MF, have the hitherto highest affinities for the corticosteroid

receptor. Although there is some evidence that a higher receptor affinity per se can be translated into a higher therapeutic potency, many other local pharmacokinetic factors will also affect this relationship and probably to a greater extent: lung deposition, MCC, airway retention, and local biotransformation are some that have been discussed above. In addition, optimizing these factors may improve *airway selectivity*, as the overall dose needed to achieve a certain therapeutic effect will be reduced, which in turn will reduce systemic spillover and side effects. Added to this is the cumulation risk at steady state, which is more pronounced for the lipophilic CSs. Hence, improved airway selectivity is probably the most important consequence of both the recent and also the forthcoming pharmaceutical and pharmacological efforts in iCS development.

Mucociliary clearance appears to affect the uptake of slowly dissolving CSs. The clinical implication of this finding is that the time available for absorption of the bulk of drug deposited in the large airways is restricted and that the relatively small fraction deposited peripherally will be absorbed at more central sites. Hence, as the inflammatory events associated with asthma afflict both central and peripheral airways (13), a more rapid absorption could be advantageous from an airway exposure point of view. In addition, as patients improve following iCS treatment, deposition appears to become more peripheral because of normalization of the large airways. For lipophilic CSs, this will increase the systemic burden to levels normally encountered in healthy subjects, a factor that needs to be taken into consideration when titrating iCS doses following disease improvement. Thus, for the most lipophilic CSs, systemic effects may not be reduced in proportion to the dose reduction in patients improving from severe airway obstruction. The adverse effects of MCC on lipophilic CS uptake could be reduced by evening dosing, as MCC is strongly reduced during the night (29). Combination treatment with β-agonists probably improves deposition in the small airways, but will also normalize a pathologically reduced MCC (30). The impact of central versus peripheral airway deposition and diurnal, pathological, and pharmacological changes in MCC on the therapeutic outcome of iCSs largely remains to be investigated.

No local biotransformation in the lung, except esterification and ester hydrolysis, appears to have any clinical significance for the currently available inhaled CS formulations. BDP is relatively rapidly hydrolyzed in both the lung and liver to the pharmacologically active BMP and more slowly to the less active beclomethasone alcohol (B). It is likely that the balance between esterase activities in lung and blood, with processes of activation (i.e., hydrolysis of BDP to BMP) and deactivation (further hydrolysis of BMP to B), as well as changes in rate of uptake due to the differences in lipophilicity between BDP, BMP, and B, may affect the therapeutic outcome and systemic absorption in the individual patient. In addition, it is not known whether esterase activity is affected by inflammation. If the inactivation step (i.e., BMP hydrolysis or oxidation) occurs at the same rate in the lung

as in the liver and plasma in vivo as it does in vitro (Table 2), BMP exposure in the systemic circulation will be independent of route of absorption, whether it is the lungs or the gut (the latter derived from the swallowed fraction of an inhaled dose). The clinical consequence of this finding is that, for BDP/BMP, airway selectivity is not affected per se by liver first-pass metabolism. This is in contrast to most other iCSs, where liver first-pass metabolism of the swallowed fraction *reduces* the overall systemic exposure and the risk of systemic side effects. Systemic availability data to confirm these in vitro findings are essentially lacking, although a recent study in children with asthma by Agertoft et al. (107) indicated that the oral availability of BMP was almost 70%, which is considerably higher than previously suggested (108).

The latency time of CSs can be short, as lung function improves in chronic stable asthmatics already within a few hours after a single dose of oral or inhaled corticosteroids (109,110). The maximum effect was achieved within 12 hours but appeared 1–3 hours earlier following inhalation. The slower rate of uptake after oral dosing may explain these findings. Although bronchial hyperreactivity generally takes several weeks of antiasthmatic treatment to improve dramatically, already 6 hours after a single dose in patients with asthma there was a small improvement in $PC_{20}$ (111). The onset of effects on inflammatory markers can also be detected soon after a single dose: a single high dose of nebulized budesonide significantly reduced exhaled NO at 6 hours in patients with asthma (112). Hence, inhibition of inflammatory cell activation can be shown after a single dose of an iCS, but slower events such as tissue repair and normalization in tissues of leukocyte content and distribution may require much longer treatment time. Hence, for full anti-inflammatory effect, particularly in severe asthma, several weeks of regular treatment are needed. No data on onset of antiasthmatic action are yet available regarding the highly lipophilic, slowly dissolving CSs, but it is likely that, if anything, onset will be slower because of later mucosal access. Similarly, there is no evidence of any clinically relevant differences between different iCS formulations regarding the time needed to induce the complete array of anti-inflammatory actions.

Airway uptake and retention are required for sufficient duration of clinical action. As noncompliance probably is the most important single reason for treatment failure (113), extended duration of pharmacological action is an important property, which counteracts any negative consequences of lost doses and/or allow once-daily dosing. In rhinitis, most topical CSs are used once daily, but only budesonide is as yet approved in mild to moderate asthma as a once-daily regimen (114,115). Budesonide's extended duration of anti-inflammatory action is most probably a result of its unique fatty acid esterification. Human PK/PD modeling has also suggested that the formation of budesonide esters is a mechanism by which not only the duration of action, but also the topical selectivity of budeso-

nide may be increased (116). The high initial concentrations of budesonide in airways and lung lead to initial corticosteroid receptor saturation, but also to prompt formation of a large "first-pass" budesonide ester pool in the airways. The preclinical data clearly showed that this increases duration of action as compared to a situation in which ester formation is inhibited. However, the esterification is reversible, and budesonide is released into the systemic circulation in its moderately lipophilic and readily cleared intact form. This will limit a general tissue retention of active drug. Different tissues have varying ability to esterify—airways/lung high and striated muscle low capacity (67). Together these features contribute to the favorable benefit vs. risk ratio of budesonide in the clinical setting.

The pharmacokinetics of the most lipophilic iCSs, with their slow uptake and a tendency for accumulation, would suggest an extended duration of action. This seems to be the case for MF, which given once daily in the morning at a daily dose of 400 μg was as efficacious as 200 μg twice daily in patients with moderate persistent asthma (117). For FP, at a daily dose of 100 μg there was an equal effect in children with stable mild to moderate asthma with a once- or twice-daily regimen (118). However, with FP 200 μg daily in adults with symptomatic mild to moderate asthma, a once-daily regimen was not as efficient as a twice-daily regimen (119), which is surprising given the high lipophilicity and long MAT for this drug. The differences in systemic pharmacokinetic properties between different iCSs and its influence on the systemic pharmacodynamic effects will be discussed in other chapters of this book.

## X. Some Unanswered Questions

Relatively much is known about lipophilicity, rate of dissolution, affinity to the CS receptor, intracellular dwell time, biotransformation, and elimination of available iCS formulations. Much less is known about trans- and intracellular trafficking and storage. It is likely that several unspecific cellular binding sites exist having different capacities, as well as affinities, in, e.g., the cell membrane, endoplasmic reticulum, lysosomes, and Golgi apparatus. In this context, reversible fatty acid esterification is a newly discovered mode of intracellular storage of iCSs. Also, it should be borne in mind that in the steady-state treatment situation a true equilibrium is never reached, but rather a series of drug concentration gradients, which are dependent on absolute doses and dosing frequencies. Free drug will thus continuously flow between different intracellular compartments and between intracellular compartments and extracellular sites. Here, a critical aspect is whether the intracellular availability of the steroid is primarily governed by cytosolic free concentrations or whether the drug can flow directly from lipid-rich sites to interact with effector/receptor loci—this occurs for cholesterol and is not an unlikely

mechanism also for CSs. Finally, most of above issues may be relevant also in the rest of the body, and it is important to understand the balance between different pharmacokinetic and pharmacodynamic properties in the airways and lungs versus the rest of the body to exploit the full potential of treatment for the individual asthma patient.

## XI. Conclusion

Optimization of the pharmacokinetics of inhaled corticosteroids has led to a highly efficacious and safe treatment of most asthmatic patients. The physicochemical properties and the delivery characteristics will together affect the overall performance of the individual iCS formulation. Pharmacokinetic factors of importance for maximum topical activity of the iCSs (apart from dose) include its exposure at the airway and lung targets, its intrinsic activity, its local biotransformation, and its intracellular retention at the target site. Retention is improved by binding to high-capacity intracellular sites in adition to the high-affinity but low-capacity receptor sites. Prolonged intraluminal deposition may also help, but at least in the more central airways this contributes to tissue uptake for only a few hours due to mucociliary clearance. Lung and airway biotransformation occurs to a relatively limited extent for the currently available iCSs and is primarily confined to ester formation and hydrolysis. Reversible esterification is a newly discovered intracellular pathway for budesonide, which prolongs retention and duration of therapeutic action. Much is known about the binding of the CS to the CS receptor and the initial events following binding and activation of the CS-receptor complex. Less is, however, currently known about the subsequent events leading to the cascade of anti-inflammatory effects, and, particularly, the intracellular trafficking and storage of iCSs at site of action and at extrapulmonary sites.

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## Discussion

**Dr. Jeffery:** If P450 activity has the potential to inactivate steroid, then the airway site of deposition in man is of importance. The cellular make-up of the lining epithelium differs markedly depending upon airway generation. In the large airways, there are secretory (goblet) cells, whereas in the terminal bronchioli there is, normally, a scarcity of goblet cells and a predominance of Clara cells, which I believe have marked P450 activity. There is a similar difference in the rat used experimentally and in the mouse. Nearly all nonciliated cells are Clara in type. I would predict that small airways may deactivate steroid more so than large. Is there any evidence to support or refute this?

**Dr. Edsbäcker:** In investigations in vitro or ex vivo with budesonide, we found no metabolites from oxidative metabolism in peripheral lung tissue. As far as I know, there is no evidence that differences exist in the metabolic pathways or extent of metabolism in central or peripheral lung tissue for any of the available inhaled steroids, including BDP.

**Prof. Dolovich:** If you could deliver steroid only in peripheral airways ($\sim$2 mm in diameter), would you expect a different rate of absorption from these airways compared to larger, more proximal ones such as you have shown in the trachea?

**Dr. Edsbäcker:** Yes, you would expect systemic absorption to be more rapid in the periphery for several reasons: first, to reach the periphery, particles need to be smaller, and the smaller the particles the more rapid is the rate of dissolution. Second, there is less mucus and a thinner and more vascularized epithelium to penetrate in the periphery. Mucociliary clearance, which is significant in the central airways, will also hinder a rapid absorption of lipophilic steroids. Finally, the intracellular retention of budesonide via esterification is particularly pronounced in the airways, which will hinder part of the delivered dose to be promptly absorbed. This of course only applies to steroids forming such esters.

**Question (unknown):** Can changes in the physicochemical properties of bronchial mucus secondary to inflammatory or infectious processes affect significantly the absorption and airway biotransformation of corticosteroids?

**Dr. Edsbäcker:** There is little evidence to suggest that this is the case. The paper by Weiner I referred to in my presentation suggests that the more severe the disease, the less is the systemic uptake of fluticasone. It is likely that a major explanation of this finding is differences in regional deposition, so that the more severe your asthma is, the more central deposition will become. A central deposition, in turn, will make the steroid more susceptible for mucociliary clearance (MCC), particularly the more lipophilic and by that more slowly absorbed steroids. Impairment of MCC is relatively subtle in asthmatics and will likely have

little effect per se on the uptake. Whether other pathological changes in the mucus have any significant influence on uptake remains to be investigated.

**Dr. Newman:** Both budesonide and flunisolide have been shown to be as effective when dosed once a day as opposed to twice a day. On the other hand, fluticasone is not as effective when dosed once a day as twice. Is this consistent with the esterification pattern for these three inhaled steroids?

**Dr. Edsbäcker:** I think that the esterification of budesonide in airways and lung contributes to its relatively long duration of action and efficacy as a once-daily treatment regimen in mild to moderate asthma. There has been some reports on a once daily efficacy also for flunisolide, but whether this is a result of esterification remains to be shown. As we have shown some esterification to occur for triamcinolone acetonide, I would assume that some esterification may occur also for flunisolide having a similar molecular structure as triamcinolone acetonide. The extent of this formation is unknown, but appears less than for budesonide. Fluticasone, for sure, does not form esters, but here its high lipophilicity probably contributes to some prolongation of effect duration. However, the clinical data supporting a once daily treatment regimen for fluticasone are weak.

**Dr. Denburg:** What are the relative contributions of nonepithelial cells (e.g., eosinophils) to airways tissue uptake, retention, and metabolism of ICSs?

**Dr. Edsbäcker:** This evidently depends on the relative infiltration of these inflammatory cells, which in turn is linked to disease severity. Interestingly, there appear to be differences between various white blood cells in their esterification capacity, in that monocytes form more and granulocytes form less esters, but overall there is no reason to believe that the nonepithelial cells in any marked way should differ from epithelial cells regarding steroid uptake and retention.

# 10

# Systemic Disposition and Effects of Inhaled Corticosteroids

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## I. Introduction

With the introduction of modern corticosteroids with improved therapeutic ratios, the treatment of asthma has been significantly improved. The improvement can be mainly attributed to optimized pharmacokinetic properties. It is the goal of inhaled corticosteroid therapy to produce long-lasting therapeutic effects at the pulmonary target site with minimized oral bioavailability and minimized systemic side effects by rapid clearance of absorbed drug (1). Unless stated otherwise, the pulmonary target site refers to the central and peripheral areas of the lung including the tracheobronchial region.

Immediately following inhalation, 10 – 40% of the dose is deposited in the lung, while the majority (up to 90%) impacts on the oropharyngeal region and is swallowed (Fig.1). Following absorption from the gastrointestinal tract, the drug passes through the liver before entry into the systemic circulation. All commonly used inhaled corticosteroids, particularly budesonide and fluticasone propionate, are metabolized during their first pass through the liver and thus, following oral absorption, enter the systemic circulation mostly as inactive metabolites. Drugs that are not efficiently inactivated during first-pass metabolism are able to enter the systemic circulation unchanged, resulting in extrapulmonary effects (most of

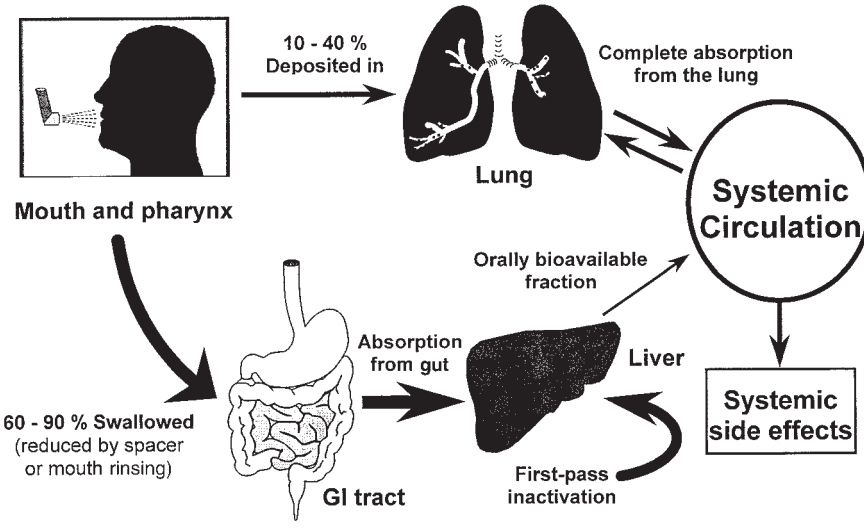


**Figure 1** Input pharmacokinetics of corticosteroids after inhaled administration. The majority of administered drug is deposited in mouth and pharynx and subsequently swallowed. After absorption from the gastrointestinal tract, the drug is inactivated by first-pass metabolism in the liver, and the amount reaching the systemic circulation is determined by the oral bioavailability. Only the drug deposited in the lung exerts the desired local activity. However, drug deposited in the lung is completely absorbed and contributes to the drug concentration in the systemic circulation.

which are unwanted). It is important to note that the fraction of the dose delivered to the lung will also be absorbed into the systemic circulation. Only dissolved drug is able to reach the intracellular steroid receptors in the lung, following which the absorption is rapid (2).

At present there are five compounds used to varying degrees in different countries for corticosteroid inhalation treatment: triamcinolone acetonide (TAA), flunisolide (FLU), beclomethasone dipropionate (BDP), budesonide (BUD), and fluticasone propionate (FP) (Fig. 2). The physicochemical modifications of the basic steroid structure from one compound to another confer differences in pharmacokinetic and pharmacodynamic properties that produce clinically significant difference (3). The objective of this chapter was to compare and contrast the pharmacokinetic (PK) and pharmacodynamic (PD) properties of these compounds and to assess the predictability of their systemic bioactivity using a PK/PD modeling approach.

## II. PK/PD Properties of Inhaled Corticosteroids

Table 1 lists the PK/PD properties of the currently used inhaled steroids.

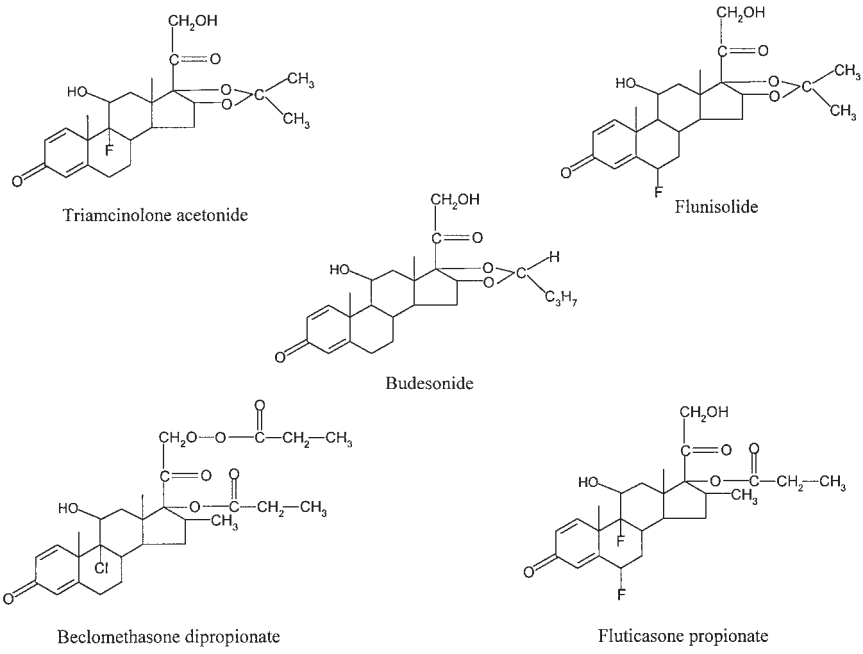


**Figure 2** Structures of commercially used inhaled corticosteroids.

### A. Relative Receptor Affinity

Most inhaled steroids are used in their pharmacologically active form, with BDP being a notable exception. BDP is a prodrug that first needs to be activated by hydrolysis. The active form of BDP is the respective monoester, beclomethasone-17-monopropionate (17-BMP). Unlike other corticosteroids, 17-BMP also forms an active metabolite, beclomethasone (B) (4). With respect to their receptor affinity relative to dexamethasone (RRA = 100), FP has the highest affinity (RRA = 1800) among the currently used inhaled steroids followed by 17-BMP (RRA = 1022), BUD (RRA = 935), TAA (RRA = 233), FLU (RRA = 190), and B (RRA = 73) (1,5). In practical terms, this means that a 10-fold higher unbound concentration of FLU at the receptor site is needed to produce the same degree of receptor occupancy as FP. This fact also makes clear why inhaled corticosteroids should never be compared on the basis of equal weight doses, but only in terms of their equipotent doses.

### B. Plasma Protein Binding

Because only the free, unbound drug is able to interact with the corticosteroid receptor, it is important to convert measured plasma or serum concentrations to the respective unbound concentrations. All inhaled corticosteroids show moderate to

**Table 1** Pharmacokinetic and Pharmacodynamic Parameters of Inhaled Corticosteroids

| Drug | FLU | TAA | BUD | BDP | 17-BMP | FP |
|---|---|---|---|---|---|---|
| Oral F (%) | 7–20 | 23 | 6–11[a] | 15–20 | 72[b] | <1 |
| Inh F (%) | 39 (MDI) | 22–25 (MDI) | 26 (MDI), 38 (TBH) | 25 (CFC) | 27 (CFC) 70 (HFA) | 26 (MDI), 12–17 (DPI) |
| $f_u$ (%) | 20 | 29 | 12 | 13 | NA | 10 |
| CL (L/h) | 58 | 37 | 84, 67 (22S), 117 (22R) | 230 | 54[c] | 69 |
| $Vd_{ss}$ (L) | 96 | 103 | 183, 245 (22S), 425 (22R) | NA | 84[c] | 318 |
| IV $t_{1/2}$ (h) | 1.6 | 2.0 | 2.8, 2.7 (22S), 2.7 (22R) | 0.1–0.5 | 0.6–1.7 | 7–13 |
| Inh $t_{1/2}$ (h) | 1.6 | 3.6 | 3.0 | 0.1 | 1.5–6.5 | 11–14.4 |
| MRT-IV (h) | 1.7[d] | 2.7 | 2.2 | NA | 1.6 | 4.9 |
| MAT (h) | <1 | 2.9 | 0.3–1.8 (TBH), 0.8–2.6 (MDI) | NA | NA | 5–9 |
| RRA (Dex = 100) | 190 | 233 | 935 | NA | 1022 | 1800 |
| Ref. | 1,7,11,13, 19,28 | 1,6,12,17 | 1,8,18,25, 38,40 | 1,3,13,14, 21,26,27, 32,34 | 1,3,5,13, 14,21,26, 27,32,34 | 1,10,15,16, 20,24,29, 30,31 |

Abbreviations: FLU = flunisolide; TAA = triamcinolone acetonide; BUD = budesonide; BDP = beclomethasone dipropionate; 17-BMP = beclomethasone-17-monopropionate; FP = fluticasone propionate; Oral F = oral bioavailability; Inh F = overall systemic bioavailability after inhalation; $f_u$ = fraction unbound; CL = clearance; $Vd_{ss}$ = volume of distribution at steady state; IV $t_{1/2}$ = elimination half-life; Inh $t_{1/2}$ = terminal half-life after inhalation; MRT-IV = mean residence time after intravenous administration; MAT = mean absorption time after inhalation; RRA = relative receptor affinity (Dex = dexamethasone); MDI = metered dose inhaler; DPI = dry powder inhaler; TBH = Turbuhaler; CFC = chlorofluorocarbon propellant–based MDI; HFA = hydrofluoroalkane-134a propellant–based MDI; NA = not available.

[a] Radiolabeled budesonide.

[b] In rats.

[c] Assuming complete conversion after intravenous administration of BDP.

[d] Calculated from $Vd_{ss}$ and CL after intravenous administration.

high levels of protein binding. TAA has the lowest plasma protein binding (71%) (6) followed by FLU (80%) (7), BUD (88%) (8), and FP (91%) (Glaxo Wellcome Inc., 1996). BDP has been reported to be 87% bound to plasma protein (9), but no data are available for 17-BMP.

### C. Oral Bioavailability

Inhaled corticosteroids are intended to provide localized therapy with immediate drug activity at the site of delivery in the lungs. However, it is well known that the greater part of an inhaled dose is swallowed and therefore available for undesired oral absorption, resulting in unwanted systemic effects. Hence, an ideal inhaled corticosteroid should have minimum oral bioavailability. This goal has been achieved in the case of FP, which has an oral bioavailability of less than 1% (10). The absorbed fraction of the other inhaled corticosteroids after oral intake is greater: 6–11% for BUD (8,11), 7–20% for FLU (12,13), 23% for TAA (14), and 15–20% for BDP (15). No data are available for 17-BMP in humans, but a 72% oral bioavailability has been reported in rats (16).

### D. Pulmonary Bioavailability

In general, corticosteroids are absorbed well from the lungs. Indeed, it can be assumed that all drug available at the receptor site in the lungs will be absorbed. Corticosteroids administered by inhalation can therefore be detected in the blood, although the blood corticosteroid concentration represents the sum of pulmonary and orally absorbed fractions. For this reason it is difficult to separately assess the pulmonary bioavailability of those inhaled corticosteroids that also undergo significant oral absorption. Oral absorption of FP is negligible, and hence its pulmonary bioavailability can be calculated with greater confidence. Indeed the pulmonary bioavailability can be calculated to range between 10 and 30% for FP, depending on the inhalation device (17,18). The overall systemic availabilities after inhalation in healthy subjects are reported to be 22–25% for TAA (14,19) delivered via a metered dose inhaler (MDI), 26% via MDI and 38% via Turbuhaler for BUD (20), 39% via MDI for FLU (21), 26% via MDI and 12–17% via the dry powder inhalers for FP (17), and 25% via MDI for BDP (3). With the global phasing out of chlorofluorocarbon (CFC) propellant–based MDIs, hydrofluoroalkane-134a (HFA)–based formulations have been recently developed for corticosteroids. For FP, it has been reported that lung deposition is similar with the HFA and the CFC formulations, whereas for BDP, the HFA-based product Qvar™ is reported to provide a significantly higher respirable fraction (60%) compared to the CFC-based MDI (22,23). The use of integrated spacer devices such as in the Azmacort® formulation for TAA has been shown to provide respirable fractions exceeding 60% (24). Recently, the deposition of FLU has been reported to be significantly improved (39% respirable fraction compared to 10% with conventional

MDIs) with the use of a novel, mechanically driven, liquid droplet inhaler device RESPIMAT (25).

### E. Systemic Clearance

One of the most important properties of inhaled corticosteroids is their clearance after absorption, which minimizes the systemic side effects. In theory, the faster the systemic clearance, the higher the therapeutic index (1). All of the currently used inhaled corticosteroids show rapid systemic clearance that is of similar magnitude: 84 L/h for BUD (8), 69 L/h for FP (26), 58 L/h for FLU (12), and 37 L/h for TAA (14). Budesonide is a 1:1 mixture of the epimers 22 S and 22 R, which are reported to have different clearance rates, 67 and 117 L/h, respectively (27). Nevertheless, these values are approximately the same as the rate of hepatic blood flow, which would be the maximum clearance rate possible for hepatically metabolized drugs. Indeed even with an increased hepatic extraction efficiency, this value could not be increased because the maximum clearance rate would be achieved when all of the drug supplied by liver blood flow was removed. In this scenario, these so-called high-extraction drugs are removed by the liver at a rate that is equivalent to liver blood flow. Only BDP has been reported to have a systemic clearance greater than hepatic blood flow (230 L/h), indicating extrahepatic metabolism (28). However, in this case the metabolic reaction does not result in the formation of an inactive metabolite and therefore termination of systemic activity, but in the formation of the extremely potent metabolite 17-BMP. The clearance rate of 17-BMP, assuming complete conversion after intravenous administration of BDP, is reported to be 54 L/h (29).

### F. Volume of Distribution

The volume of distribution is a pharmacokinetic parameter that allows quantification of tissue distribution. The larger its value, the greater the amount of drug located inside the peripheral body compartments. However, a large volume of distribution does not necessarily indicate greater pharmacological activity in the peripheral body compartments because most of the drug is present in its pharmacologically inactive, bound form. The active, unbound drug concentration at steady state is independent of volume and depends only on clearance and degree of protein binding. Since there are different ways of calculating volume of distribution, comparison of literature values must be done with great care. The volume of distribution at steady state ($Vd_{ss}$) for FP was reported to be 318 L (26) quite in agreement with its high lipophilicity. $Vd_{ss}$ was reported to be 183 L for BUD (20), 103 L for TAA (14) 96 L for FLU (7), and 84 L for 17-BMP (29). The epimers of BUD also exhibit significantly different distribution patterns with reported $Vd_{area}$ (terminal phase apparent volume of distribution) values of 245 and 425L for the 22S and 22R epimers, respectively (27).

### G. Elimination Half-life

The elimination half-life of any drug is a secondary pharmacokinetic parameter that is dependent on the rate of systemic clearance and the volume of distribution. The elimination half-life quantifies how rapidly the plasma concentration changes but does not indicate the magnitude of this concentration. As a result of its large volume of distribution, FP has the longest elimination half-life of 7–13 hours, as measured after intravenous administration (26,30,31). The elimination half-lives of BUD, TAA, and FLU are reported to be 2.8 (8), 2.0 (14), and 1.6 h (21,32), and those of BDP and 17-BMP after intravenous administration of BDP were recently reported to be 0.1–0.5 and 0.6–1.7 h (15,28,29), respectively. The epimers of BUD have identical elimination half-lives (2.7 h), even though they exhibit different distribution and clearance characteristics (27).

### H. Terminal Half-life After Inhalation

The terminal half-life after inhalation can differ from the true elimination half-life after intravenous administration if absorption is slow and if it is the overall rate-limiting step ("flip-flop pharmacokinetics"). Hence a slower terminal elimination half-life after inhalation than after intravenous administration indicates slow absorption. This may be the case for TAA since the terminal half-life after inhalation of 3.6 hours was found to be longer than that after intravenous administration (2.0 h) (14). In the case of 17-BMP, the evidence is rather inconclusive. A 0.1-hour terminal half-life has been reported for BDP, whereas values ranging between 1.5 and 6.5 hours have been reported for 17-BMP (15,29,33). In a recent study with subjects receiving BDP by intravenous as well as by inhaled administration, the mean terminal half-life of 17-BMP after inhalation of BDP was found to be longer (2.7 h, range: 2.2–3.7) than after intravenous administration (1.7 h), indicating a possibility of slow absorption (29). For the other drugs, the terminal half-lives after intravenous and inhaled administrations have been found to be similar: 10–14 hours for FP (30,31,34), 3 hours for BUD (20), and 1.6 hour for FLU (32). In such cases, a parameter such as the mean absorption time is a better indicator of the absorption rate than the terminal half-life. Moreover, accurate determination of the terminal half-life depends on the sensitivity of measurement of blood drug concentrations. Thus, low levels of drug in the lung may remain undetected, resulting in apparently low residence and absorption times.

### I. Pulmonary Residence Time

One of the predominant factors responsible for achieving pulmonary selectivity is the pulmonary residence time. Pulmonary residence time is determined by the release rate of the inhaled particle from an inhaled solid (powder) or an alternative delivery system such as liposomes, the absorption rate of dissolved drug across pulmonary membranes, and the mucociliary clearance, which removes drug

particles from the upper portions of the lung (1). The absorption across membranes is a rapid process for lipophilic glucocorticoids, and hence the dissolution rate of a glucocorticoid powder is the main determinant for controlling the pulmonary residence time (2). One parameter that is useful in estimating the duration of pulmonary retention of inhaled steroids is the mean absorption time (MAT), which denotes the average time it takes for a molecule of the drug to get absorbed into the systemic circulation. The longer the MAT, the greater the pulmonary residence. The reported MATs (calculated as the difference between the mean residence times after inhaled and intravenous administrations) are 5–9 hours for FP (18,31,34), 2.9 hours for TAA (14), and 0.3–1.8 hour for BUD (20). No data are available for FLU, BDP, or 17-BMP. The prolonged pulmonary residence of FP as indicated by the relatively long mean absorption time is consistent with its very low aqueous solubility (0.04 $\mu g.mL^{-1}$) compared to other inhaled corticosteroids (28). As with Vd, residence time in the lung is not necessarily indicative of pharmacological activity, as the latter depends on whether or not the drug is in the unbound form. However, a long residence time in the lung most likely indicates a longer availability for topical release and hence activity.

Recent studies have shown that the formation of esters that act as a depot for the active corticosteroid in the lung may be an alternative mechanism for prolonging the pulmonary residence. For example, the formation of several fatty acid conjugates of BUD in the lung has been shown in vivo (35). This topic is addressed in more detail in this book in Chapter 21.

### J. Accumulation

Accumulation is the term used to describe the increase in plasma drug concentration that may occur during multiple-dose administration until a steady state is reached. The accumulation time is a function of the terminal elimination half-life of the drug. As a general rule, it takes approximately four to five half-lives to reach steady state. In the case of FP, this is equivalent to about 2 days (31,34). Steady state is reached in about half a day in the case of BUD and TAA, within 8 hours for FLU, and in about a day for BDP and its metabolites (i.e., after the first dose) (36,37). The magnitude of the steady-state plasma concentration, however, is independent of the half-life and is only a function of systemic clearance. For instance, it will take longer to reach steady state for FP than for BUD. However, for equal amounts of drug absorbed, the resulting average steady-state concentrations will be quite similar. Because of the shorter half-life, BUD plasma concentrations will exhibit greater fluctuation.

### K. Pharmacokinetics in Asthmatics

Most studies evaluating the clinical pharmacokinetics of inhaled steroids have been performed in healthy volunteers. Differences in drug exposure, especially

lung deposition due to altered airway caliber, between asthmatics and healthy volunteers have been characterized primarily using dose titration studies involving asthma control as the primary outcome. Thus, pharmacokinetic information in the target group, patients with varying degree of respiratory disease, is scarce.

In a study with 13 patients with mild to moderate asthma ($FEV_1$ 50–80% predicted), 500 μg FP b.i.d. was inhaled from Diskhaler™ and Diskus™ dry powder devices, respectively. In both groups, comparable FP plasma concentration profiles were obtained after 4 weeks of treatment with no statistically significant differences between the devices, evaluated by comparison of $C_{max}$ and AUC values (38). However, AUC and $C_{max}$ were lower in the asthmatic patients than in healthy subjects, probably due to impaired inhalation and deposition of the administered dose in the lung. Falcoz and coworkers reported a geometric mean for $C_{max}$ and AUC during multiple dosing therapy from Diskhaler™ of 0.190 ng/mL and 1.124 ng/mL.h for healthy subjects, and of 0.120 ng/mL and 0.412 ng/mL.h for asthmatics, respectively, when normalized for a common dose of 500 μg FP b.i.d. (17,39). In a second study, including 10 patients with mild-to-moderate asthma dosed with 100 and 500 μg FP b.i.d. from Diskhaler™ over 4 weeks, FP plasma concentrations were also lower than in healthy subjects (39). Jusko and Harding investigated FP in 118 asthmatics ($FEV_1 \geq 50\%$ predicted) receiving either 250, 500, 750, or 1000 μg FP for 28 days, inhaled from pMDI (40). Plasma concentration time courses showed considerable variability, but the mean AUC was proportional to the administered dose.

In a 4-week study involving 33 mild asthmatics ($FEV_1 \geq 65\%$ predicted), the dose proportionality of three different doses of BUD (400, 800, and 1600 μg twice daily) inhaled via Turbuhaler® was evaluated (41). BUD was found to exhibit linear pharmacokinetics, both within and up to twice the maximum of the clinically recommended dose range. The dose-normalized AUC and $C_{max}$ values obtained in the study were similar to those obtained in 24 healthy volunteers receiving 800 μg doses via Turbuhaler®, suggesting that mild asthmatics have similar kinetics as in the healthy (41,42). These results are consistent with studies that have reported comparable lung deposition in asthmatics ($50 < FEV_1$: <92% predicted) as well as in healthy subjects after inhalation via the MDI and Turbuhaler formulations (20,43). In a study with six asthmatic children aged 10–13 years ($FEV_1 \geq 75\%$ predicted), the clearance of BUD (after adjustment in body surface areas) was found to be 50% higher than that found in adults (44).

The single and steady-state pharmacokinetics of BDP delivered via CFC and HFA pressurized metered dose inhalers was investigated in 43 steroid-naïve asthmatic patients ($FEV_1$ 60% predicted) over 14 days (37). No clinically meaningful difference was observed in total beclomethasone pharmacokinetics (representing the sum of BDP, 17-BMP, beclomethasone, and other metabolites) between single and multiple doses over 14 days.

Zaborny and coworkers investigated the pharmacokinetics of TAA in

moderately severe asthmatics and found it to be linear over a dose range of 400–1600 μg when inhaled via the Azmacort® MDI device (45). The reported AUC and $C_{max}$ values were consistent with those obtained in another study involving 12 healthy volunteers receiving 2 mg via the Azmacort® inhaler (14,45). Steady-state pharmacokinetic data for TAA and FLU in asthmatics are not available in the literature.

## III. PK/PD Modeling of Cortisol Suppression

### A. Cortisol Suppression as a Surrogate Marker for Systemic Activity of Inhaled Steroids

Since inhaled steroids are absorbed into the systemic circulation after topical administration, they exert systemic effects. The most significant systemic adverse effects of inhaled steroids include growth suppression, reduction in bone density, and cataracts. Unfortunately, measures of these effects would need monitoring a large number of patients for a relatively long period of time (i.e., years). Endogenous cortisol suppression, although not shown to directly correlate with clinical effect, has been shown in a number of studies to be a reliable surrogate marker for estimating the systemic activity of inhaled steroids. The hypothalamic-pituitary-adrenal (HPA) axis is exquisitely sensitive to the presence of exogenous corticosteroids, is relatively easy to assess, and therefore has proven to be a convenient target for assessing the systemic activity of inhaled steroids.

There are a number of methods available for assessing adrenal function. They can be categorized into either basal hormone measurements or stimulation tests. Test procedures for basal cortisol release include measurements of morning cortisol plasma concentrations, integrated plasma cortisol concentrations (ICC), and urinary free cortisol excretion. Stimulation tests include the ACTH stimulation test, the CRH test, the insulin (tolerance) stress test, and the metyrapone test (46). Single measurements of morning cortisol are an insensitive method for the assessment of HPA activity due to the circadian rhythm of cortisol release and great intraindividual variations in cortisol levels during the phase of daily peak concentrations in the early morning. The more accurate and sensitive measurements of HPA axis function are the integrated cortisol levels (cortisol AUC) by multiple blood sampling at frequent intervals over up to 24 hours and the ACTH stimulation test (46,47).

Under clinical settings, the degree of endogenous cortisol suppression (CCS), when using the ICC method, is usually reported as the difference in the areas under the curve (calculated using the trapezoidal rule) between the placebo and the drug-treated groups over a 24-hour time period. This approach, however, is only a descriptive method and has no predictive value. In other words, extrapolations of systemic drug effect to other clinical situations would require informa-

tion from a large number of clinical studies because there are significant differences in cortisol suppression depending on the dose, inhaler device, patient population, duration, frequency, timing, route of administration, and relative potency of the administered corticosteroid. To address this issue, a PK/PD modeling approach that is both descriptive as well as predictive has been developed.

### B. PK/PD Modeling Approach

Endogenous cortisol concentrations in plasma follow a circadian rhythm. Peak concentrations are reached in the morning between 6 and 10 a.m., through concentrations at night between 8 p.m. and 2 a.m. (48–50). Several PK/PD-modeling approaches have been launched to describe the daily rhythm in the plasma concentration time course of endogenous cortisol and its suppression after administration of exogenous corticosteroids (6,51–56). A clinically valuable, integrated, $E_{max}$-based PK/PD model has been developed to describe the cumulative suppression of endogenous cortisol release (CCS) caused by exogenous corticosteroids (57).

The model describes the daily cortisol release ($R_C$ in concentration/time) at baseline situation with two straight lines. For the time between the maximum cortisol release ($t_{max}$) and the minimum cortisol release ($t_{min}$), $R_C$ decreases in a linear fashion from the maximum release rate ($R_{max}$ in amount/time) at time $t_{max}$ to approximately 0 at time $t_{min}$:

$$R_C = \frac{R_{max}}{Vd^{Cort} \cdot (t_{max} - t_{min} - 24)} \cdot t - \frac{R_{max} \cdot t_{min}}{Vd^{Cort} \cdot (t_{max} - t_{min} - 24)} \quad (1)$$

where $Vd^{Cort}$ is the volume of distribution of cortisol and t is the time after cortisol monitoring was initiated ($t_0$ = 8 a.m.).

For the time between $t_{min}$ and $t_{max}$, $R_C$ increases according to:

$$R_C = \frac{R_{max}}{Vd^{Cort} \cdot (t_{max} - t_{min})} \cdot t - \frac{R_{max} \cdot t_{min}}{Vd^{Cort} \cdot (t_{max} - t_{min})} \quad (2)$$

The resulting change in cortisol plasma concentrations ($C_{Cort}$) at baseline situation is then described by Eq. (3), where $k_e^{Cort}$ is the first-order elimination rate constant for cortisol.

$$\frac{dC_{Cort}}{dt} = R_C - k_e^{Cort} \cdot C_{Cort} \quad (3)$$

Based on Eq. (3), an indirect response model is then formulated to characterize the suppression of endogenous cortisol concentrations during exogenous corticosteroid therapy, thereby relating the corticosteroid concentrations to the effect on cortisol release according to:

$$\frac{dC_{Cort}}{dt} = R_C \cdot \left(1 - \frac{E_{max} \cdot C}{EC_{50} + C}\right) - k_e^{Cort} \cdot C_{Cort} \quad (4)$$

where $E_{max}$ is the maximum suppressive effect, $EC_{50}$ is the corticosteroid plasma concentration that produces half of $E_{max}$, and C is the unbound plasma concentration of the exogenous corticosteroid whose systemic disposition could be described using either a one-compartment (TAA) or two-compartment (FLU, BUD, and FP) body model with first-order absorption. Since the maximum possible effect is complete suppression of cortisol release, $E_{max}$ is fixed at 1. The effect of exogenous corticosteroids on endogenous cortisol suppression is then quantified according to:

$$\%CCS = \frac{AUC^{Base,24} - AUC^{Therapy,24}}{AUC^{Base,24}} \quad (5)$$

where %CCS is the difference between the areas under the curve between the placebo and the drug-treated groups, $AUC^{Base,24}$ is the area under the cortisol plasma concentration time curve over a 24-hour interval for the placebo group and $AUC^{Therapy,24}$ is the area under the curve over the same time period for the drug-treated group. A graphical representation of the calculation of %CCS after single and multiple dosing is shown in Figure 3.

### C. Predictive Power of the PK/PD Model

It has been mathematically shown that the estimation of CCS with the trapezoidal rule as well as with the PK/PD model will theoretically result in the same values for %CCS, thereby allowing comparisons between %CCS values measured in clinical studies and those simulated using the PK/PD procedure (57). However, the PK/PD-based approach provides the advantage that it is not limited to clinically determined data, but allows predictions beyond the existing data set for other dosing regimens. The model has been used to simulate different clinical situations involving different drugs (FP, BUD, TAA, and FLU), doses, and devices (MDI, DPI, etc.) and has been shown to predict cumulative cortisol suppression reported in several published clinical studies with good accuracy (58). BDP was not included in the study due to lack of information on 17-BMP. Figure 4 shows the correlation between model-predicted CCS and measured CCS values for the four drugs after inhalation ($r = 0.84$). The predictions correlate fairly well with the measured data for all four drugs irrespective of the inhaler device or dose.

After single doses, CCS increased with increasing dose for all four corticosteroids. Equal administered amounts of the four drugs, however, produced different degrees of CCS according to their pharmacokinetic and pharmacodynamic properties in the order TAA < FLU < BUD < FP (59). The described model was able to adequately quantify the differences in cumulative suppressive effects when

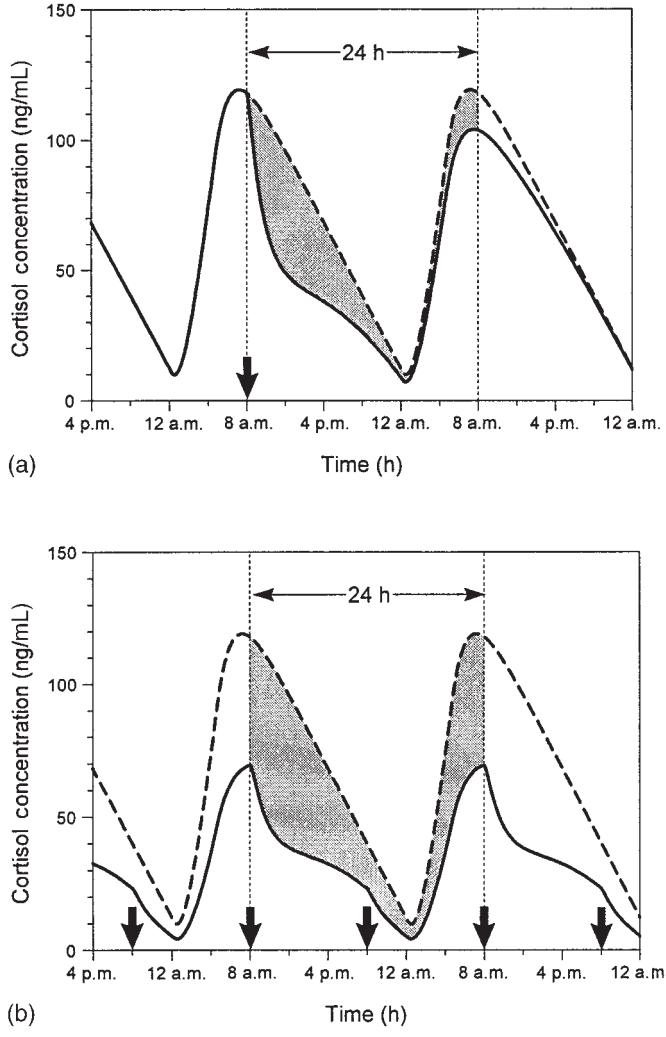


**Figure 3** Area (shaded) between the plasma concentration-time profiles of endogenous cortisol at baseline (– – –) and after exogenous corticosteroid administration (——) that corresponds to the amount of suppressed cortisol within 24 hours after (a) single dose given at 8 a.m. (⬇) and (b) steady-state b.i.d. doses given at 8 a.m. and 8 p.m.

inhaled via different inhalers. For example, the model-predicted values of 40% and 25% CCS after a single dose of 1 mg FP inhaled via MDI and Diskhaler™, respectively, were consistent with the reported values (54,60,61). Similar differences between Turbuhaler and MDI were also accounted for by the model, but in

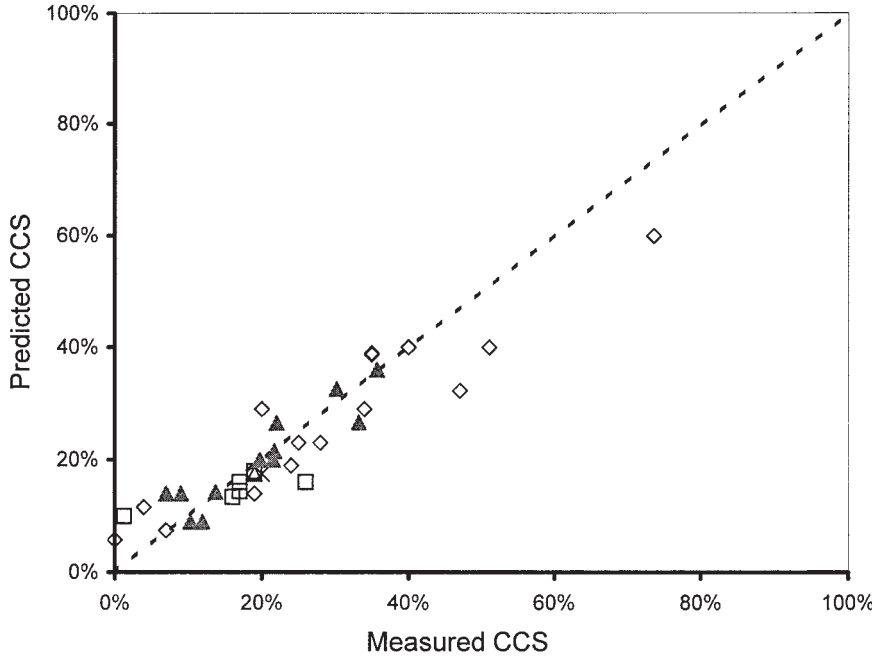


**Figure 4** Correlation between cumulative cortisol suppression (CCS) measured in clinical studies and predicted by simulation of the respective situation for FP (◇), BUD (□), FLU (▲) and TAA (×) with the PK/PD approach ($r = 0.84$). The dotted line describes the ideal situation, where measured and predicted CCS would be identical.

this case the suppression was slightly higher for Turbuhaler® after a single dose of BUD than for MDI (60,62–64).

Using simulations, it has been previously shown for FP and FLU that dose timing, especially for a single dose, is a pivotal influential factor that determines the extent of cortisol suppression (65). The approach was also extended for TAA and BUD for both single dose (i.e., the first dose of the treatment) as well as steady-state situations (i.e., twice-daily administration during steady state) in order to evaluate the diurnal variation in CCS. Figure 5 shows the relationship between administration time and CCS after inhalation of single doses of 500 and 1000 μg of TAA, BUD, and FLU and 250 and 500 μg of FP. A circadian pattern in CCS was observed with maximum CCS when the drugs were administered in the early morning around 3–4 a.m. and minimum CCS when administered in the afternoon hours between 4 and 7 p.m. This pattern is a result of the temporal arrangement of the systemic drug activity in relation to endogenous cortisol release. On the one hand, CCS reaches its maximum if the time of maximum cortisol

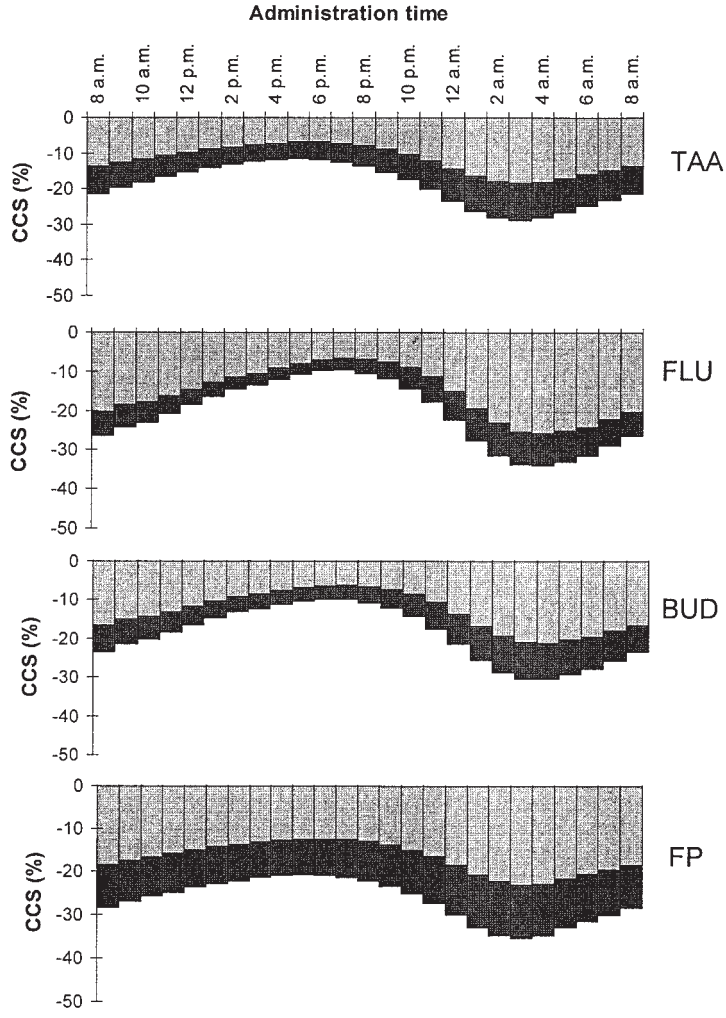


**Figure 5** Relationship between daily administration time and cumulative cortisol suppression (CCS%) compared with baseline within 24 hours after inhalation of single doses of 500 µg (gray) and 1000 µg (black) of TAA, FLU, and BUD and 250 µg (gray) and 500 µg (black) of FP.

release in the early morning falls within the period of high systemic activity. Conversely, CCS is minimized if the period of high systemic activity is located around the time of minimum cortisol release in the late evening, several hours before the release maximum. Because the period of systemic activity is modulated by the

corticosteroid's terminal half-life, it is shorter for corticosteroids with shorter terminal half-lives, such as FLU (1.6 h), BUD (3.0 h), and TAA (3.6 h), and longer for long terminal half-life drugs such as FP (11.7 h). Hence, in order to minimize systemic activity during the period of increased endogenous cortisol release (i.e., in the early morning), the optimum administration time was slightly shifted from late afternoon around 7 p.m. for FLU to early afternoon at 4 p.m. for FP. Despite exhibiting similar diurnal rhythms, the degree of fluctuation of CCS, however, was much less pronounced for FP compared to the other drugs. This is readily observed in Figure 6, which relates the administration time with CCS after administration of equipotent doses of FP, FLU, TAA, and BUD, determined by calculating the dose delivered from an MDI, using literature values of overall systemic bioavailability, that caused 25% CCS when administered at 8 a.m. CCS caused by a single dose of BUD or FLU is minimized twofold or more if administered at 8 p.m. instead of 8 a.m., whereas the change in CCS after a FP dose at 8 p.m. is relatively insignificant (approximately 10%) compared to the 8 a.m. dose. These observations might have a profound effect on the comparability of clinical studies regarding the systemic activity of these steroids. For example, inhalation of single doses of 500 μg FP and 1000 μg FLU causes similar CCS (26.3% vs. 26.6%) when administered at 8 a.m. However, when given at 8 p.m., CCS remains similar after

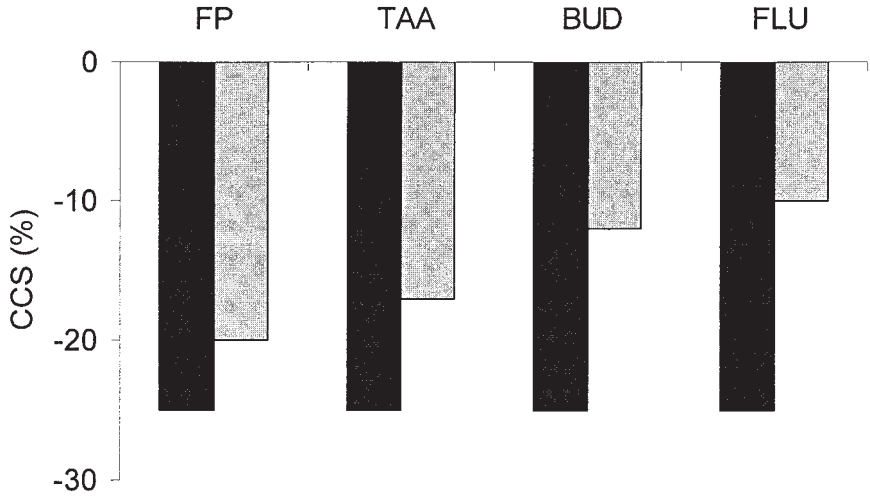


**Figure 6** Relationship between administration time and cumulative cortisol suppression (CCS%) after inhalation of single doses of FP (400 μg), TAA (1350 μg), BUD (1150 μg), and FLU (850 μg) via their respective MDIs. These doses are equipotent when administered at 8 a.m. (black) but show significant differences in CCS when administered at 8 p.m. (gray).

the FP dose (23.7%) but is more than halved after the FLU dose (10.8%). Hence, without considering the administration time, one might conclude that the HPA suppression of both drugs at the given dose level is either equivalent or in a 2:1 ratio.

Since multiple rather than single dosing regimens are more commonly used for inhaled steroids, results derived at steady-state conditions are more clinically relevant than those obtained from single dose studies. A similar approach as for single dosing was used to assess the time dependency of CCS during multiple dosing at steady state with the dosing regimen following a circadian periodicity, i.e., doses and dosing times identical for each day (e.g., 500 μg given at 8 a.m. and at 8 p.m.). Simulations were performed for 500 and 1000 μg steady-state b.i.d. doses of BUD, TAA, and FLU and 250 and 500 μg doses for FP inhaled at various combinations of times (e.g., 8 a.m. and 8 p.m., 9 a.m. and 9 p.m., etc.). The results indicate that the systemic activity of inhaled steroids during multiple b.i.d. dosing is most likely not influenced by the times of administration (Fig. 7). However, for studies that evaluate CCS after the last dose of a multiple dosing regimen, this inference would no longer be valid. For example, given a multiple-dose study, different degrees of CCS may be observed for the same dose of the drug depending on whether the time of the last dose falls in the morning or in the evening. This in turn could lead to inconsistent conclusions similar to single dose studies discussed earlier. It has to be mentioned that the simulations in this study assume no diurnal variation of drug-specific PK/PD parameters and no changes with time.

Figure 8 shows the dose-response (%CCS) relationships for the four steroids when inhaled via their respective MDIs during a twice-daily steady-state regimen. It is interesting to note that in spite of the identical mechanism of action, the slopes of the respective dose-response curves are not identical for the four steroids. This is due to the fact that a dose-response curve integrates both the pharmacokinetic and pharmacodynamic properties of the investigated compound. Whereas the slopes of the concentration-response curves are identical for the four steroids, their pharmacokinetic parameters differ. This results in the observed differences in the slopes of their dose-response curves.

Since the therapeutic safety of inhaled steroids is predominantly governed by the magnitude of their systemic activity, the method also allows assessing the benefit-to-risk ratio of inhaled corticosteroids if additional data on efficacy are considered. Special attention must be given to the delivery devices whenever these dose-response relationships are evaluated. These predictive capabilities may be used to optimize dosing regimens as well as to compare different corticosteroids with regard to cumulative systemic activity without or with a reduced number of clinical trials.

The clinical relevance of subnormal HPA-axis function in asthmatics using inhaled corticosteroids is not clear. Moderate reduction of cortisol release merely reflects the physiological feedback inhibition, and therefore the normal response mechanism of the HPA axis towards corticosteroid exposure, rather than a clinically significant change in body functioning. Furthermore, due to the presence of

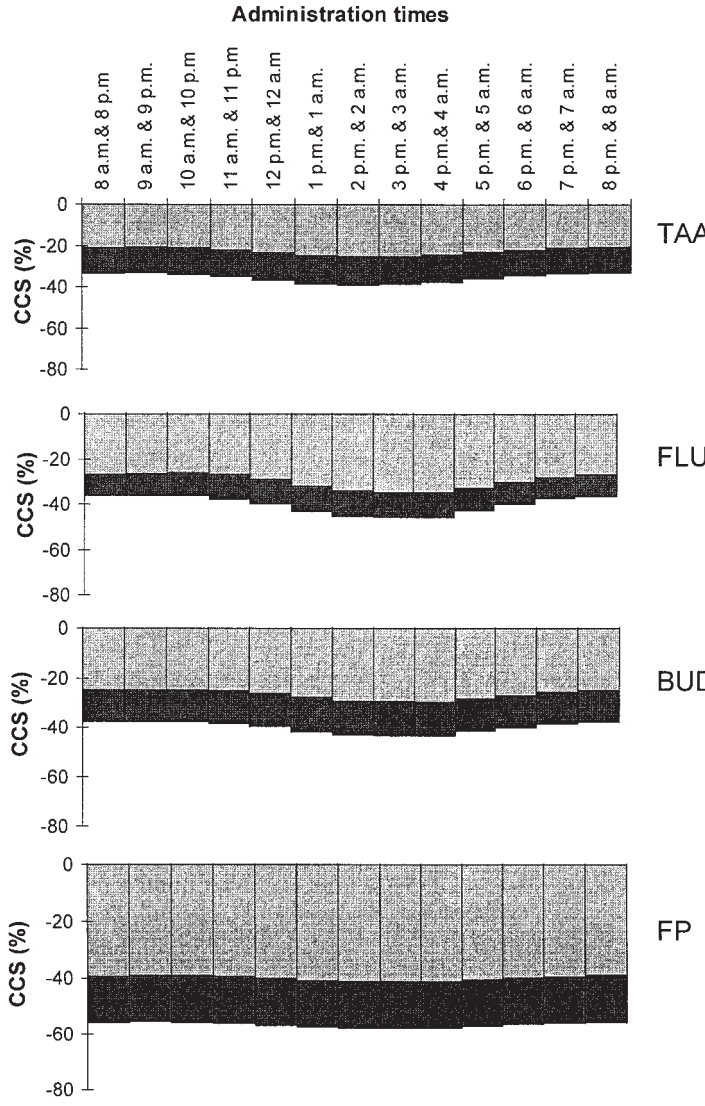


**Figure 7** Relationship between daily administration times of a b.i.d. regimen and cumulative cortisol suppression compared with baseline during inhalation of steady-state doses of 500 µg (gray) and 1000 µg (black) of TAA, FLU, and BUD and 250 µg (gray) and 500 µg (black) of FP.

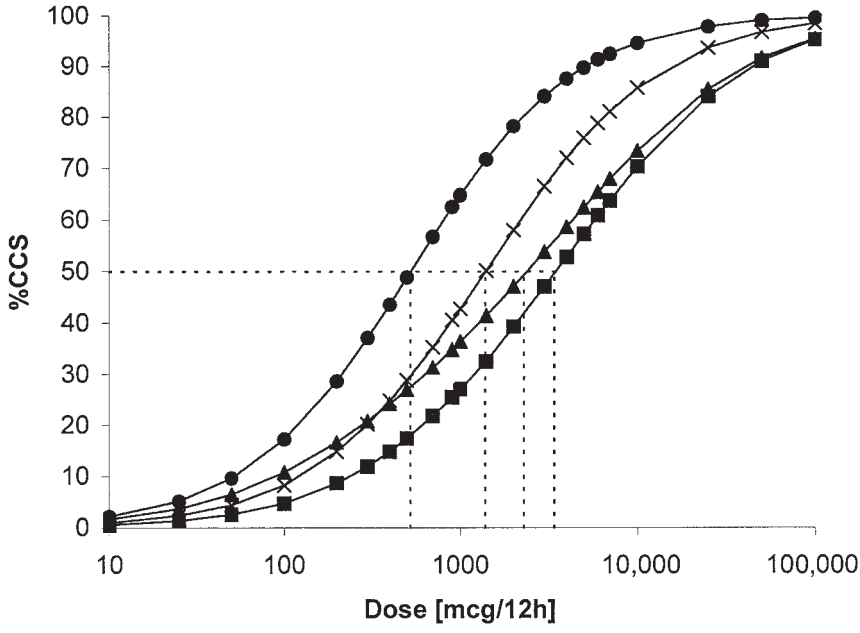


**Figure 8** Dose-response (%CCS) relationships for FP (—●—), TAA (—×—), FLU (—▲—), and BUD (—■—) during a steady-state twice-daily regimen, delivered via MDI. The dotted lines highlight the difference in the $ED_{50}$ values (dose administered every 12 hours that caused 50% CCS) between the drugs when delivered via their respective commercial MDI devices.

the exogenous steroid, total corticosteroid activity in the body may still remain within the physiological range. Thus, changes in HPA-axis function based on laboratory findings are not necessarily predictive of clinically significant events on skin, bone, eye, adrenal gland, growth, etc. and should rather be interpreted as markers for total systemic activity.

## IV. Conclusions

A comparison of the pharmacokinetic and pharmacodynamic properties of inhaled corticosteroids currently used in medical practice clearly reveals significant differences between these compounds. Although all of these agents show rapid systemic clearance after absorption, there are differences in oral bioavailability and absorption rate after inhalation. Absorption rate is probably more relevant because it reflects pulmonary residence time after inhalation and therefore duration of availability in the lungs. Initial attempts at mathematical deconvolution to

estimate pulmonary residence time have resulted in significant differences between agents. Long residence times have been calculated for FP and TAA, but BUD and FLU appear to disappear rapidly. More studies are needed to evaluate the pulmonary residence of BDP and 17-BMP. These properties appear to be related to pulmonary solubility, which appears to be the rate-limiting step in pulmonary absorption. However, more detailed studies are needed to further elucidate the exact local events at the various pulmonary levels that follow inhaled corticosteroid administration.

There is little doubt that the use of inhaled corticosteroids has vastly improved the benefit-risk ratio for the preventive treatment of asthma. However, the trend toward the earlier use of inhaled corticosteroids, particularly in children, makes it even more important to critically appraise their potential for producing systemic adverse effects during long-term administration.

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## Discussion

**Dr. Edsbäcker:** Thank you for a nice and clear presentation. There are, however, a few remarks I want to make, based on your data: 1) First of all, you claim that fluticasone shows flip-flop kinetics after inhalation, based on data from different studies. However, all studies where the kinetics of inhaled and i.v. fluticasone have been compared in the same study suggest that systemic distribution rather than rate of uptake from the lungs is the rate limiting step in the elimination of inhaled drug (Thorsson L, Dahlström K, Edsbäcker S, Källen A, Paulson J, Wiren JE. Pharmacokinetics and systemic effects of inhaled fluticasone propionate in healthy subjects. Br J Clin Pharmacol 1997; 43:155–61; Källèn A, Thorsson L. The elimination rate of fluticasone propionate is not governed by uptake rate from the airways. Eur Respir J 1999; 14 (suppl 30):197s). 2) Your cortisol suppression model predicts elegantly the suppression which can be expected after various steroid treatment regimens. However, most of these simulations were made following single doses, in spite of the fact that several of the more lipophilic steroids clearly accumulate at steady state, and by that would affect HPA more than after a single dose. 3) When you presented both your experimental as well as your modeling data on different steroids, the cortisol suppressive effect were compared using different doses: hence, half the dose of fluticasone was compared with a full dose of budesonide, flunisolide, and triamcinolone acetonide. What was the logic behind this?

**Dr. Derendorf:** 1) The half-life of fluticasone propionate after inhalation has been reported in some studies to be longer than reported i.v. half-lives. However, in the studies you mentioned there was no difference in the terminal half-lives. So, I am not sure if there really is flip-flop in this case or not. However, this is not really a critical issue. In any case, all studies have shown a long mean absorption time of fluticasone propionate after inhalation. This noncompartmental parameter (MAT) is a much more robust number and safer to interpret than the meaning of terminal half-lives. 2) We have applied our model to a number of single and multiple dose cases. It could be shown that the model was able to give reasonable predictions in either case. We have recently published an interactive spreadsheet that allows the user to enter the steroid, device, dose, and time of dosing to predict cortisol effects after both single and multiple dosing (AAPS Pharmsci. at http://www.pharmsci.org, 2(3), article 22, 2000). 3) The model allows one to calculate cortisol effects for any dose you want. The data I presented were from an experimental study where we compared half the dose of FP with the full dose of budesonide. Our studies with flunisolide and triamcinolone acetonide were performed on even higher doses. The doses for budesonide and fluticasone propionate were chosen based on clinical and laboratory findings that on a microgram-to-microgram basis. Fluticasone propionate is

approximately twice as potent as budesonide in the treatment of asthma (Barnes et al., Respir Med 1998; 92:95–104).

**Dr. O'Byrne:** Are the effects of inhaled glucocorticoids on adrenal suppression measured at steady state at 5 days the same when measured at steady state at 6 months or less?

**Dr. Derendorf:** Unfortunately, detailed long-term studies that have looked at 24-hour serum cortisol after 6 months of treatment are not available.

**Dr. Seale:** There is a threefold difference in the percentage of unbound drug between the steroids, which you have discussed. How important is the free drug concentration in terms of systemic effects of these different steroids?

**Dr. Derendorf:** For inhaled corticosteroids these differences are important since they directly translate into the resulting unbound concentrations. The more protein binding, the lower the unbound concentrations. Clearance is not affected by protein binding since inhaled corticosteroids represent flow-limited high extraction drugs.

**Dr. Pedersen:** There seems to be a discrepancy between the rapid appearance of BUD in the blood in the pharmacokinetic studies and the lung retention data presented by Dr. Edsbäcker. How do you explain that? Is it because the fraction which is retained in the lung is so low that it is not detected by the pharmacokinetic method?

**Dr. Derendorf:** Our calculations have shown that the largest part of the absorbed budesonide dose enters the bloodstream very quickly. We cannot exclude that a very small fraction of the dose is retained in the lung for a longer time, but we also do not have any evidence from our data that this is the case.

# 11

# Extrapulmonary Effects of Inhaled Corticosteroids

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## I. Introduction

While inhaled corticosteroids (IS) have been developed and designed in order to minimize or eliminate systemic effects, this has not to date been fully achievable. As outlined in several chapters in this volume, IS, even those with the lowest systemic bioavailability, still exert effects outside of the airways. Recent understanding of the process of allergic inflammation in the airways has generated the notion that upper and lower airway allergic disease, such as rhinitis and asthma, are expressions of a systemic inflammatory process. Given this, some of the systemic actions of IS may in fact be desirable and beneficial, especially if these target systemic processes involved in the development of rhinitis and asthma. This chapter will highlight the evidence that asthma is part of a systemic disease process and review the evidence for the extrapulmonary beneficial effects of IS in this process.

## II. Evidence for a Systemic Allergic Airways Inflammatory Disease

Rhinitis and asthma are linked closely epidemiologically, as has been known for quite some time and emphasized in recent surveys (ISAAC study) (1). Patients with seasonal allergic rhinitis without asthma symptoms can, as a group, be shown to have increased bronchial hyperresponsiveness (BHR) (2–5). During the course of seasonal allergen exposure, patients with allergic rhinitis can be shown to have increased numbers of inflammatory cells (eosinophils and mast cells) in the lower airways, even without symptoms of lower airway disease (6,7). Longstanding observations from our laboratory have indicated fluctuations of eosinophil/basophil (Eo/B) progenitors in the peripheral blood in patients with seasonal allergic rhinitis (8,9), parallelling changes that are recognized in mature eosinophils and basophils in circulation. The finding of Eo/B progenitor decreases during the height of seasonal allergen exposure led us to hypothesize the concept of "in situ hemopoiesis" to explain a process of systemic activation of hemopoietic mechanisms, in which communication among bone marrow peripheral blood and airway tissue compartments contributed to the allergic inflammatory process at a systemic level (10–12). Indeed, allergen challenge to the lower airways in atopic asthmatics elicits an immediate rise in Eo/B progenitors, especially in subjects with late phase responses (13,14). These original observations have been buttressed by a series of investigations demonstrating conclusively that: exacerbations of asthma are attended by increases in peripheral blood Eo/B progenitors (14); allergen challenge to dogs or mice with allergic airways inflammation elicits bone marrow progenitor responses (15–17), with trafficking of bone marrow cells to the airway either in pure upper or lower airway disease (18); and allergen challenge to atopic asthmatics upregulates the high affinity receptor for IL-5 on bone marrow progenitors (IL-5Rα) (19), beginning a process of eosinophil differentiation that is likely driven by the release of a serum hemopoietic factor—as we have found in the canine model (16)—which acts on the bone marrow and initiates a series of systemic events. It is these events that IS may target in a beneficial manner in asthma.

## III. Effects of IS In Vivo on Progenitors in Asthma and Rhinitis

There are several animal and human studies now indicating that IS may exert extrapulmonary effects on hemopoietic mechanisms initiated in the bone marrow, which contribute to disease pathogenesis. First, controlled, stepwise withdrawal of IS in asthmatic subjects, sufficient to provoke a mild exacerbation, leads to an immediate rise in Eo/B progenitors in the peripheral blood (14,20); this rise can be suppressed with progenitor levels restored to normal in circulation by reintro-

duction of IS at therapeutic doses that treat the exacerbation (14,20). IS given to patients with chronic cough and eosinophilic bronchitis likewise suppress the ambiently elevated levels of Eo/B progenitors in blood, in parallel with a beneficial effect on the cough symptom (21). Second, in canine studies that utilize an *Ascaris suum*–induced bronchial hyperresponsiveness as a model of allergic airways inflammation and asthma, IS in doses sufficient to achieve remission of airways inflammation and physiological changes suppress the allergen-induced upregulation of bone marrow myeloid progenitors (15); this is seen in conjunction with suppression in vitro of a serum hemopoietic activity released after allergen inhalation (16) and at doses of IS that can be shown to achieve plasma and bone marrow levels of corticosteroid at concentrations expected to exert effects on inflammatory cells and their progenitors (M. Inman et al., unpublished) (Table 1).

In this latter model, studies of the effects of serum containing hemopoietic activity have revealed a synergy between upregulation of the marrow and the increased release of hemopoietins into circulation (M. Inman et al., unpublished), both of which are targeted by IS. Intravenous administration of corticosteroids to achieve the same levels in blood and marrow that are achieved with IS can be shown to suppress both the systemic (progenitor) and tissue (airways) response (M. Inman et al., unpublished). Similar studies by Toogood et al. (22) comparing oral corticosteroids to IS have concluded that the topical route is the one that optimally controls symptoms; however such studies, done with several corticosteroid preparations (22,23), have not measured systemic inflammatory responses such as bone marrow or peripheral blood progenitors.

Other in vivo studies we have recently completed may shed further light on the effects of IS on a systemic, bone marrow–related process in asthma and

**Table 1** Effects of Inhaled Steroids on Progenitors In Vivo

| |
|---|
| Suppress Eo/B progenitor rise in asthma exacerbations |
| Suppress Eo/B progenitors in eosinophilic bronchitis and chronic cough |
| Prevent allergen-induced increases in peripheral blood Eo/B progenitors in atopic asthmatics |
| Suppress GM-CSF localization in developing peripheral blood Eo/B progenitors |
| Prevent allergen-induced increases in canine bone marrow myeloid progenitors in a model of bronchial hyperresponsiveness |
| Suppress the systemic release of a serum hemopoietic activity acting on canine myeloid progenitors |
| Decrease baseline levels of bone marrow Eo/B progenitors and IL-5R$\alpha^+$ cells in atopic asthmatics |
| Do not prevent (1 week treatment) the upregulation of IL-5R$\alpha^+$ on bone marrow allergen-induced progenitors in atopic asthmatics |

rhinitis. Allergen challenge in human atopic asthmatic subjects can be shown to upregulate IL-5Rα on increased numbers of $CD34^+$ pluripotent bone marrow progenitors within 24 hours (19,24); this is attended by an increased IL-5 sensitivity of Eo/B progenitors in the bone marrow in those who develop a dual response to allergen provocation (25). At the same time, Eo/B colony-forming units (CFU), a functional readout of a later stage progenitor than the $CD34^+$ population, are also increased following allergen challenge (19). Both Eo/B-CFU and $CD34^+$IL-5 $R\alpha^+$ cell increases after allergen are not suppressed by pretreatment for a week with budesonide 400 μg bid (25); however, baseline levels of $CD34^+$IL-$3R\alpha^+$ as well as $CD34^+$ and IL-$5R\alpha^+$ cells are decreased on treatment with IS (25). Very recent observations have revealed, in consonant with our previous studies, that peripheral blood Eo/B-CFU fall on treatment with IS at these doses (Fig. 1) and that the cytokine profile induced in developing Eo/B-CFU is also suppressed by IS (G.M. Gauvreau et al., unpublished). These studies indicate that different com-

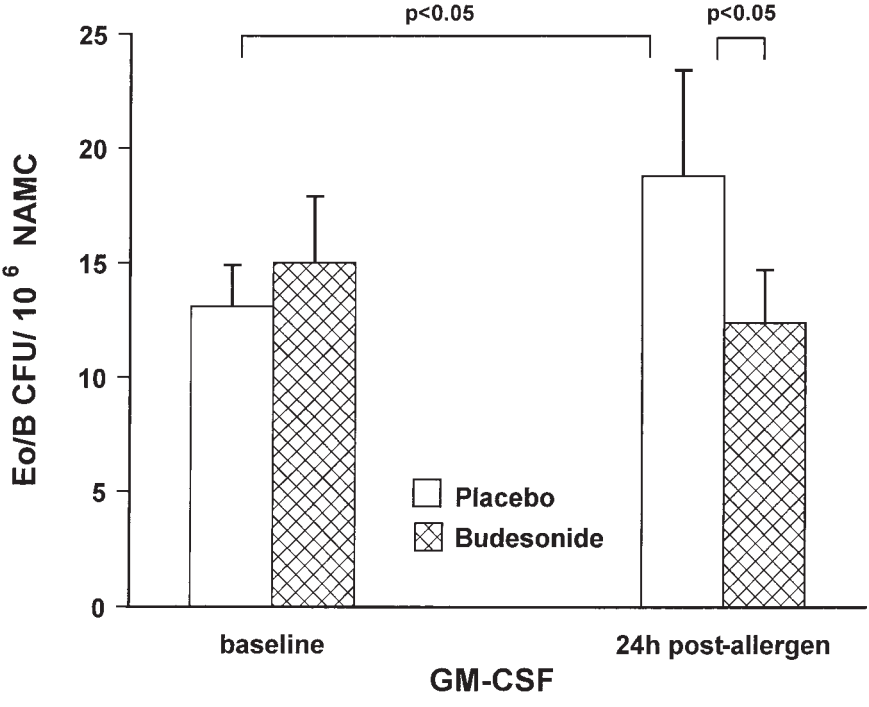


**Figure 1** Effect of placebo or budesonide (200 μg b.i.d. for 1 week) on allergen-induced Eo/B colony-forming cells. A significant ($p < 0.05$) suppression was observed after treatment with IS.

partments of the systemic hemopoietic response to airway allergen provocation respond differentially to IS. Whether this is a reflection of the dose of IS, length of therapy, or other properties of the cells and the different compartments remains to be examined. Nonetheless, these studies emphasize that IS do exert effects on even subtle molecular changes in eosinophil differentiation that are relevant to the development of asthma and rhinitis. We have recently also found that in a murine model of ovalbumin-induced airway inflammation, marked by increases in bone marrow eosinophil progenitors that parallel the development of airway physiological changes (17), IS exert suppressive effects on the hemopoietic response that are limited to a certain time period following allergen provocation (H. Shen et al., unpublished).

## IV. The Effects of IS In Vitro and Ex Vivo on Hemopoietic Responses

Studies of the effects of corticosteroids ex vivo and in vitro on hemopoietic responses (Table 2) have revealed that certain progenitor populations are exquisitely sensitive to IS concentrations achieved systemically in vivo. For example, the myeloid progenitor cell line, HL-60, is inducible to Eo/B lineage differentiation (26); this can be suppressed by $10^{-7}$–$10^{-8}$ M budesonide. Similarly at $10^{-7}$–$10^{-9}$ M budesonide in vitro, peripheral blood Eo/B-CFU are decreased or abolished (27). However, in parallel with in vivo observations related to differential effects of IS on bone marrow and peripheral blood compartments, recent evidence we have obtained revealed an upregulation of IL-5–dependent Eo/B-CFU in vitro in the presence of budesonide at $10^{-7}$–$10^{-8}$ M(28). Moreover, bone marrow obtained from atopic asthmatics, showing upregulation of $CD34^{+}IL\text{-}5R\alpha^{+}$ cells, exhibits increased sensitivity to IL-5 ex vivo when taken after treatment with budesonide rather than placebo (R. Sehmi et al., unpublished). These studies indicate: (1) that the bone marrow progenitor compartment responds differently from the peripheral blood compartment to IS; and (2) increased IL-5 responsiveness of bone marrow progenitors may require a more prolonged treatment with IS in vivo to be fully suppressed.

**Table 2** Effects of Inhaled Steroids on Progenitors Ex Vivo and In Vitro

| |
|---|
| Suppress canine myeloid differentiation in vitro |
| Suppress eosinophil differentiation of the myeloid cell line, HL-60 |
| Suppress peripheral blood Eo/B progenitor differentiation in vitro |
| Enhance IL-5–mediated bone marrow Eo/B progenitor differentiation in vitro |

## V. Mechanisms of IS Effects on Hemopoietic Processes

Recent studies by J. Tavernier et al. (unpublished) indicate that IL-5 itself, rather than IL-3 or GM-CSF, regulates the expression of its own high affinity receptor, IL-5Rα, on differentiated eosinophils from cord blood $CD34^+$ progenitors. We have demonstrated that IL-5 can upregulate IL-5Rα on $CD34^+$ cells themselves in 24-hour in vitro cultures (R. Sehmi et al., unpublished). It is not clear whether or how IS, given at doses aimed to achieve the physiological concentrations that can exert effects on progenitors, regulate IL-5Rα on progenitors. It would be important to investigate this process more fully, since the extrapulmonary beneficial effects of IS may target this interaction, and this may be important in the long-term downregulation of the eosinophilic inflammatory response that appears to emanate, at least in part, from bone marrow–derived mechanisms.

Further, the question of mobilization of Eo/B progenitors from the marrow, their movement through the circulation, and arrival and presence in the tissue compartment needs to be more fully delineated in relation to the beneficial effects of IS systemically. We and others now have evidence that $CD34^+$ progenitors reside in upper (29) and lower airways tissue, with IL-5Rα upregulation on these cells in the bronchial mucosa (30); an inverse relationship between IL-5Rα and $FEV_1$ has been demonstrated, suggesting that this is an important mechanism to be targeted by IS (30). Intranasal corticosteroids appear to upregulate the numbers of $CD34^+$ cells in nasal polyps, implying a block in differentiation (29), but whether or not changes in IL-5Rα occur on these cells after topical steroid treatment to the airway needs to be further studied both in the upper and lower compartments.

Finally, the cooperation between IL-5 and eotaxin in mobilizing bone marrow eosinophils and their progenitors into the circulation and in localizing eosinophil responses in the airways (31,32) needs to be examined further in relation to the effects of IS. It would be important to determine whether progenitors responsive to eotaxin bearing the receptor CCR3 specific for this chemokine are affected by IS systemically. Recent evidence we have obtained suggests the acquisition of CCR3 on bone marrow progenitors in kinetically relevant fashion after allergen provocation (33), indicating that this may also be a target for extrapulmonary effects of IS. Most likely, both IL-5–dependent and eotaxin-dependent mechanisms eliciting hemopoietic responses need to be targeted by IS in order to achieve remission of symptoms and long-term therapeutic benefits.

Finally, the question of chronic versus acute therapy with IS in relation to the extrapulmonary effects needs to be studied further. How much IS, and for how long, is required to fully suppress the upregulation of $CD34^+$ IL-$5R\alpha^+$ cells in the bone marrow is a question that can be addressed in long-term studies. Data from our mouse model of eosinophilic airways inflammation indicate that there are prolonged effects of the initial eosinophilic response on airway physiology (unpub-

lished); presumably insufficient doses of IS, with chronic upregulation of IL-5Rα and CCR3 on bone marrow progenitors, would allow for continued eosinophil differentiation and ingress into tissues as both mature and immature cells, leading to further airway changes and possibly remodeling. It is conceivable, therefore, that newer generations of IS need to be reexamined for their systemic bioavailability and potency profiles, with the aim of achieving some beneficial extrapulmonary effects and not only tissue-specific anti-inflammatory effects. In vivo dose-response studies in which levels of steroid achieved in plasma and in tissue can be related to biological effects in vitro could be of help in future studies. More importantly, studies of the effects (if any) of "soft" steroids of varying metabolic action and stability on hemopoietic mechanisms need to be considered. It is quite possible that the combination of direct tissue (airways) effects and systemic (bone marrow) actions of inhaled corticosteroids is what is necessary to achieve the full clinical benefit of these medications. A corollary of this is that combination therapy of IS with cytokine or leukotriene antagonists may be more capable of exerting effects on hemopoietic processes than IS alone.

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## Discussion

**Dr. O'Byrne:** Is the lack of efficacy of the soft (esterase-sensitive) steroids due to inability to reach the airway cell?

**Dr. Denburg:** I would speculate that, in order to have a sustained effect on chronic eosinophilic inflammation, direct or indirect effects on the bone marrow are necessary. Soft steroids will lack these effects.

**Dr. Busse:** To try to determine the effect of inhaled steroids on local (airway) versus systemic (circulating) cells, we evaluated IL-5 generation from airway cells after antigen challenge. Airway, but not circulating, cells had increased IL-5 generation. This response was blocked by inhaled steroids; there was no effect in circulating cells. Do you think that this represents differences of progenitor CD34 cells versus mature cells?

**Dr. Denburg:** It is quite possible that differences between mature eosinophils and their progenitors exist with regard to IL-5 receptor regulation by IL-5. Tavernier has, however, also shown that IL-5 upregulates IL-5R on maturing eosinophils.

**Dr. Schleimer:** It's worth noting that Minshall et al. have shown that airway exposure to allergen increases IL-5–expressing T cells in the bone marrow, suggesting that the communication between lung and bone marrow can occur via cells as well as mediators.

**Dr. Hamid:** Do you think that the effect of inhibitory steroids is particularly due to its effect on local eosinophil differentiation for the proposed cell in the lung?

**Dr. Denburg:** It is likely that an effect on progenitors in tissues also occurs. We have seen an increase in $CD34^+$ cells in nasal polyps after intranasal steroids, implying a block in differentiation (Kim et al., Am J Respir Cell Mol Biol, 1999; 20:388–397).

# 12

# Factors Involved in the Pulmonary Targeting of Inhaled Glucocorticoids

## The Use of Pharmacokinetic/Dynamic Simulations

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### I. Introduction

Despite the development of new antiasthmatic drugs, inhaled glucocorticoids remain important in the therapy of asthma. Beclomethasone dipropionate, the prototype of the first generation of inhaled glucocorticoids, was successfully introduced without taking into consideration major targeting strategies. The second and third generations of inhaled glucocorticoids, with budesonide and fluticasone propionate as the main representatives, incorporated a number of rationale design features into the drug development process that significantly improved pulmonary selectivity (in the following, the term "pulmonary" is used synonymously for describing central and peripheral areas of the lung including the tracheobronchial region, unless stated otherwise). It is undisputed that further improvements in pulmonary selectivity of glucocorticoids will be difficult and will not occur in quantum leaps but in discrete steps. Therefore, if further improvements in pulmonary selectivity are to be realized, it is necessary to fully comprehend the requirements for pulmonary targeting, while at the same time, suitable methods to monitor the

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local and systemic effects are needed to assess these properties on a quantitative level.

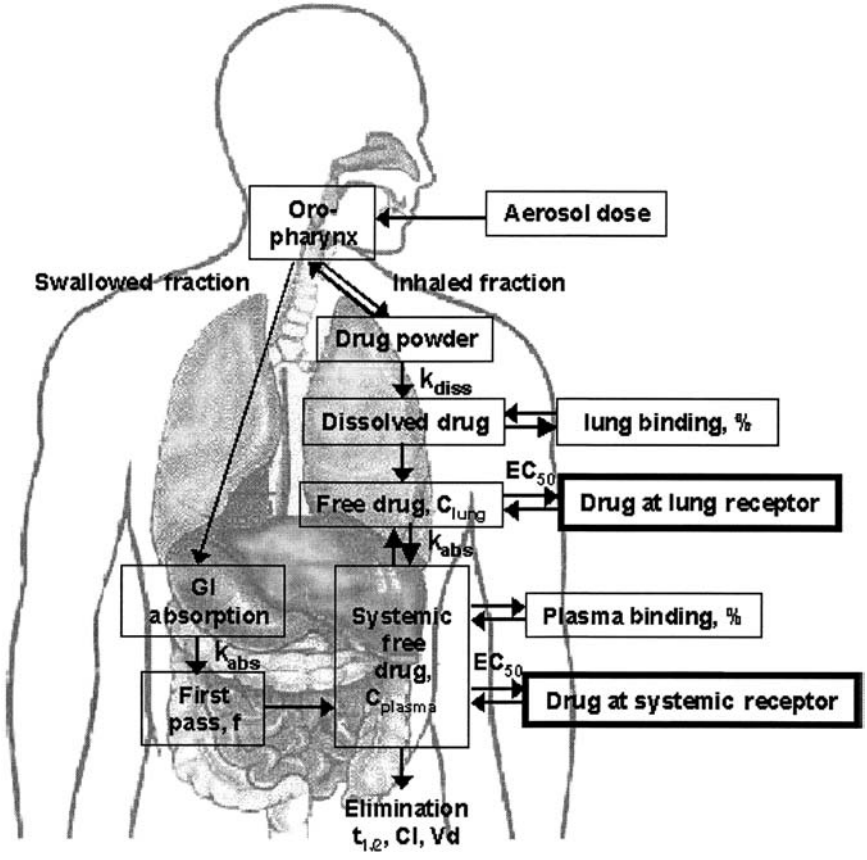
## II. Assessment of Pulmonary Selectivity

From a clinical pharmacologist's point of view it would be desirable to assess pulmonary selectivity of glucocorticoids in asthmatics by monitoring simultaneously both the pulmonary effects and systemic side effects. This assessment has been successful for $\beta_2$-adrenergic drugs due to the availability of strong surrogate markers for systemic and pulmonary effects (1). However, such studies are more difficult for inhaled glucocorticoids. Although there are strong surrogate markers available for the quantification of systemic effects, such as the suppression of blood lymphocytes and plasma cortisol, descriptors of pulmonary effects are relatively weak, as indicated by the absence of dose-response relationships often observed in such studies. This combination of hard systemic markers and soft local markers makes clinical studies assessing pulmonary selectivity contingent on the design of the study. It will be of particular interest to note the results of more carefully designed clinical studies, such as the NHLBI Asthma Clinical Research Network Initiative, which uses optimized respiratory endpoints [e.g., effects on exercise-induced asthma (2)] in conjunction with controlled assessment of systemic effects.

### A. Pharmacokinetic Models

Because of the above-mentioned challenges, several theoretical approaches have been considered to assess the significance of various drug properties on respiratory selectivity in early drug development. Independently, Byron and Gonda developed the first detailed pharmacokinetic models for the fate of an inhaled glucocorticoid in the lung (3–5). These simulations were based on the understanding that respiratory selectivity of an inhaled glucocorticoid will depend on respiratory pharmacokinetic drug properties. Gonda's and Byron's pioneering work focussed attention on the desired properties of optimized delivery forms. In particular, Gonda stressed the importance of pulmonary pharmacokinetics on the "duration of effective drug levels" and the toxicological potential of accumulation of slow-release drug delivery systems. While both models were able to simulate and predict the pharmacodynamically relevant drug levels in the central and peripheral parts of the lung, they were not suitable to describe the degree of pulmonary targeting as they were lacking the systemic component of drug action.

Building on Byron's and Gonda's work by incorporating pharmacokinetic/pharmacodynamic relationships, as well as a descriptor of systemic side effects, new models of pulmonary targeting were developed that related the pharmacologically relevant descriptors of an inhaled glucocorticoid to its pulmonary selectivity (6). This was done by predicting the degree of receptor occupancies with time in the lung and the systemic circulation, using the difference between the two




**Scheme 1** Diagram describing the PK/PD model used within simulations of pulmonary targeting.

curves as the marker for pulmonary targeting. The model (Scheme 1) considered pulmonary related factors such as the efficiency of pulmonary drug deposition, mucociliary transport rate in the central regions of the lung, pulmonary drug release and absorption rates, and the interaction of the steroid with pulmonary receptors. The factors responsible for the degree of the systemic effects (as expressed in the degree of occupancy of systemic receptors) include the amount of swallowed drug, oral bioavailability, the systemic drug levels available from pulmonary absorption, systemic clearance, protein binding, volume of distribution, and receptor affinity (Scheme 1; Table 1). Currently, such models are unable to predict actual pulmonary selectivity of clinically relevant inhaled glucocorticoid because some lung-related parameters such as local tissue binding and drug concentration gradients are unknown. However, current models have been very helpful in as-

**Table 1** Important Pulmonary Targeting Factors

| Pulmonary components | Systemic components |
|---|---|
| Pulmonary deposition efficiency | Oral bioavailability |
| Location of pulmonary deposition | Clearance |
| Pulmonary residence time (dissolution rate and other factors) | Volume of distribution |
| Pulmonary absorption rate | Plasma protein binding |
| Lung tissue binding | Tissue binding |
| Pulmonary pharmacodynamic drug characteristics | Systemic pharmacodynamic drug characteristics |

sessing the relevance of pharmacokinetic and pharmacodynamic properties on pulmonary targeting. These relevant factors (Table 1) will be discussed in the following sections based on the PK/PD model of Scheme 1 (simulations are presented for b.i.d. dosing at steady state).

### B. Affinity to the Glucocorticoid Receptor

Other chapters in this book summarize recent progress in understanding the complex mode of action of inhaled glucocorticoids. A significant body of literature suggests that the receptor-binding affinity of a glucocorticoid correlates with its activity at the site of action. Good correlations between the receptor-binding affinity and the activity in tests systems not affected by pharmacokinetic properties have been found for a number of pharmacological parameters. These include topical anti-inflammatory properties (7–9), activity in skin blanching (10), and modulation of the activity of enzymatic systems in cell culture, such as tyrosine aminotransferase (11). Thus, the receptor-binding affinity of glucocorticoids and the degree of receptor occupancy has been used as a predictor for its pharmacological activity at the site of action (12). Glucocorticoids are highly conserved in the body, and local antiasthmatic effects and unwanted side effects are mediated through the same receptor (13). Despite this observation, a high receptor affinity was previously seen beneficial for improved selectivity of inhaled glucocorticoids (13). More recent models show that differences in receptor affinity can be overcome by selecting the appropriate dose (6). This is shown in Figure 1a and b. where identical pulmonary and systemic receptor occupancies are observed when a drug with a three times lower receptor affinity is given at three times the dose. This suggests that the receptor affinity is not important for determining the pulmonary selectivity as long as the necessary dose can be inhaled. Hence, pharmacokinetic properties are more important than the pharmacodynamic potency for achieving pulmonary selectivity (6). This bold statement might have to be revisited if the reported dissociation between the activity for specific genomic and

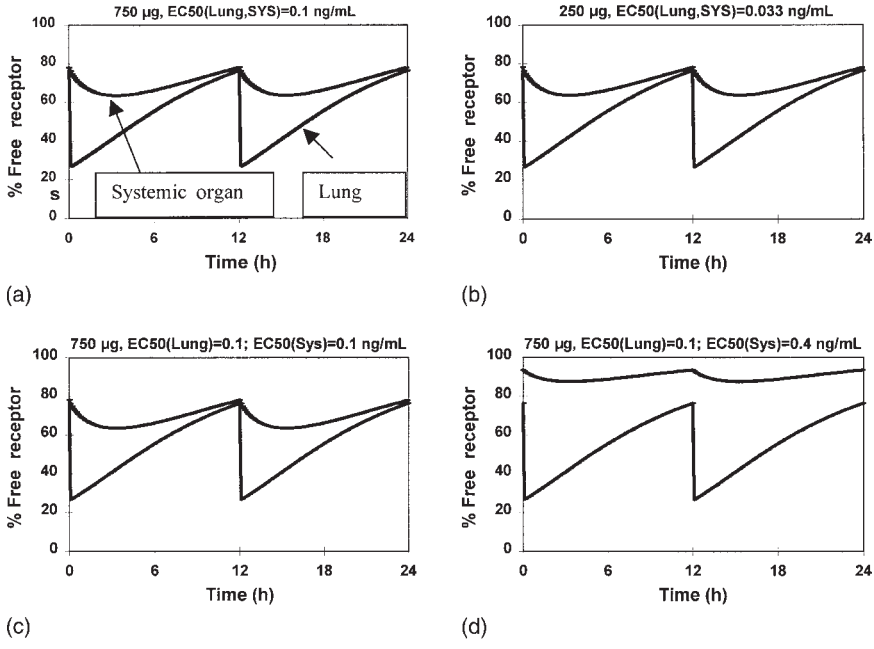



**Figure 1** Effect of receptor affinity on pulmonary (lower line) and systemic (upper line) receptor occupancies. Simulations are shown for the situation that receptor affinities are identical for lung and systemic organs. (a and b) Differences in receptor binding affinity can be overcome by adjusting the dose. There are no differences in selectivity between situations a and b. (c and d) In the case that systemic side effects are mediated via less sensitive mechanisms (d), pulmonary selectivity can be achieved on the pharmacodynamic level (compare c and d).

nongenomic effects [transactivation and AP-1 transrepression (14)] leads to the development of new glucocorticoids with increased effect/risk ratio. In this case, respiratory selectivity may be improved by identifying glucocorticoids with a higher intrinsic activity towards desired antiasthmatic pathways but low activity for nonasthmatic systemic side effects (Fig. 1c and d), although the development of such drugs will be challenging.

### C. Oral Bioavailability

The systemic load of an inhaled formulation is related to the combined effect of the drug absorbed via the pulmonary and the oral routes. Considering the high degree of orally impacted and swallowed drug (commonly >70% of the dose), a significant amount of the delivered dose is available for oral absorption. Ultimately, this portion of the drug, if entering the systemic circulation, will reduce

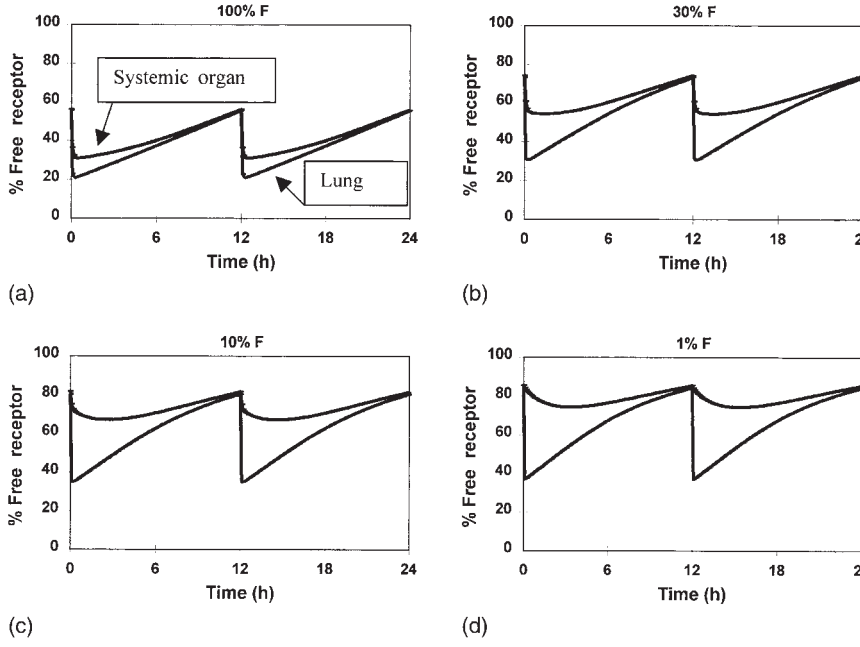



**Figure 2** Effect of oral bioavailability on pulmonary (lower line) and systemic (upper line) receptor occupancies. Simulations are shown for 100, 30, 10, and 1% oral bioavailability.

the pulmonary selectivity due to the related systemic effects. Thus, an optimal drug candidate should show the lowest possible oral bioavailability (Fig. 2). It should be stressed that, even in this case, systemic effects will still be observed (Fig. 2) because drug absorbed from the lung will also induce systemic side effects. Although this is rather obvious, publications and marketing departments often improperly relate "zero" oral bioavailability to "zero" systemic side effects. While hepatic clearance values of most inhaled glucocorticoids are close to the liver blood flow, oral bioavailabilities of most commercially available glucocorticoids are low. Fluticasone propionate (15,16) shows the lowest oral bioavailability ($<1\%$), not only because of its high intrinsic clearance, but also due to poor absorption from the gastrointestinal tract (15,16). Bioavailabilities of approximately 1% have also been reported for the newer glucocorticoids mometasone furoate and loteprednol etabonate (17) while higher values have been reported for budesonide [6–11% (18)], flunisolide [7–20% (19,20)], and triamcinolone acetonide [23% (21)]. The only major exception with substantial oral bioavailability is beclomethasone dipropionate, whose active metabolite beclomethasone monopropionate shows oral bioavailabilities of 70% in rats (22). Within this context, it has

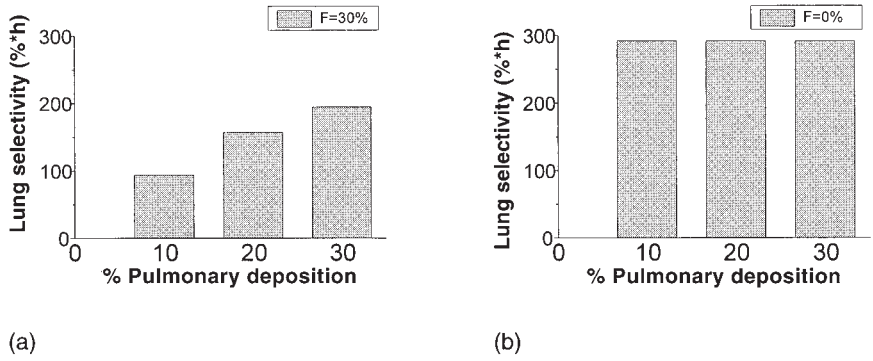



**Figure 3** Effect of pulmonary deposition efficiency on pulmonary selectivity (difference between cumulative pulmonary and systemic receptor occupancies). Simulations are shown for 30, 10, and 1% pulmonary deposition for a drug with 30% oral bioavailability F (a) and 0% oral bioavailability F (b). Note that the dose reaching the lung was set to be the same in all cases.

been stated that oral bioavailabilities of 25% or less should not induce clinically relevant systemic side effects, as long as the pulmonary deposition is large (23). Under these conditions the amount of drug reaching the systemic circulation through oral absorption is relatively small compared to the fraction of drug reaching the systemic circulation through pulmonary absorption. New drugs in development, however, will have to meet the requirement of close to zero oral bioavailabilities in order to be competitive in the market.

### D. Delivery Systems, Deposition Ratio, and Regional Lung Deposition

The delivery device determines not only how much drug and in what region of the lung (central or peripheral) it is deposited, but also the amount available for absorption in the gastrointestinal tract. While for a long time the pulmonary deposition efficiency of metered-dose and dry-powder inhalers were in the range of 10–30%, newer devices have shown improvements in pulmonary deposition up to 40% (24–30).

From a drug-targeting point of view, high pulmonary deposition is important for a drug with high oral bioavailability because the amount of drug entering the systemic circulation through oral absorption will be responsible for distinct systemic side effects (Fig. 3). In contrast, the pulmonary selectivity of a drug with low oral bioavailability will not be significantly affected by the pulmonary deposition efficiency of the device because the orally deposited drug will not be absorbed. Thus, pulmonary selectivity is mainly independent of the pulmonary

deposition efficiency. In this case, however, increased pulmonary deposition will allow a dose reduction and a potential cost-saving.

The possibility of targeting central and alveolar lung regions has increased the potential to further improve targeting by delivering the drug to relevant regions of the airway. However, future studies need to focus on the geographical aspects of glucocorticoid lung effects in order to identify the regions important for glucocorticoid asthma therapy.

As stated previously, pulmonary deposition efficiency is not crucial for local targeting of drugs with close to zero oral bioavailabilities. However, further improved deposition efficiencies of new inhalation devices are needed for the pulmonary delivery of highly active drugs for systemic use. The use of such devices for glucocorticoid inhalation therapy might decrease the high variability of deposition generally observed for glucocorticoids with the older devices.

### E. Systemic Pharmacokinetic Parameters

Once a glucocorticoid molecule reaches the systemic circulation, through absorption via either the lung or the GI tract, it is able to induce systemic side effects through interaction with the systemic glucocorticoid receptors. The systemic pharmacokinetic parameters potentially affecting pulmonary selectivity are clearance, volume of distribution, and fraction unbound in plasma ($f_u$) and fraction unbound in tissue ($f_{uT}$).

#### *Clearance*

Clearance determines the overall systemic elimination rate or cumulative drug concentration with time in the body and, most notably, regulates the systemic drug exposure (AUC). Figure 4 depicts the relationships between clearance and the degree of pulmonary targeting (see also Fig. 7a). Not surprisingly, these simulations reveal that an increase in systemic clearance enhances the pulmonary selectivity, thereby supporting the statements that inhaled glucocorticoids possess the highest clearance possible (13). Budesonide, fluticasone propionate, and other inhaled glucocorticoids are highly metabolized in the liver. The clearance of such high extraction drugs is independent of the plasma protein binding and close to the liver blood flow. Therefore, further efforts to develop new steroids with increased intrinsic hepatic clearance is unnecessary, as such steroids will not be cleared more efficiently. PK/PD simulations support the development of new drugs with extrahepatic elimination (22,31). In this case, the challenge for such developments is to identify enzymatic systems that rapidly inactivate the drug in blood but not in the lung. Figure 5 simulates this situation by describing scenarios in which three glucocorticoids differ in their lung stability. While drug A, the steroid with the highest lung stability and distinct systemic clearance of 300 L/h, shows distinct targeting, targeting gets lost for drugs with the same high systemic clearance but

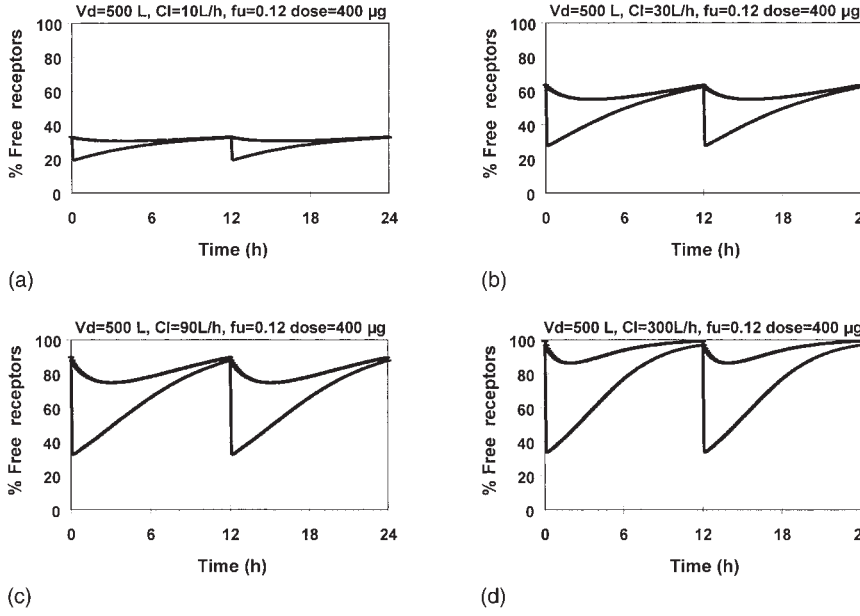



**Figure 4** Effect of systemic clearance on pulmonary (lower line) and systemic (upper line) receptor occupancies. Simulations are shown for clearance values of 10, 30, 90, and 300 L/h.

decreased lung stability (drugs B and C, lung half-life of 1 or 0.1 h). Thus, glucocorticoid soft drugs with a high extrahepatic clearance in the blood need to be stable in the lung tissue. So far the identification of enzyme systems in the blood that are not in the lung has been a challenge. A new glucocorticoid lactone, metabolized by the serum enzyme paraoxonase, appeared to meet this requirement of high pulmonary stability (32) in in vitro studies, but also failed in clinical studies.

### *Volume of Distribution/Distribution Processes*

The second parameter determining the half-life of a drug is the volume of distribution. The volume of distribution ($V_d$) is the parameter indicating the extent of tissue distribution. For lipophilic drugs, which are able to enter most of the tissue compartments (with a volume of the tissues being defined as $V_T$), the volume of distribution $V_d$ is determined by the drug's plasma protein ($f_u$) and tissue binding ($f_{uT}$) by the equation:

$$V_d = V_p + V_T \cdot \frac{f_u}{f_{uT}}$$

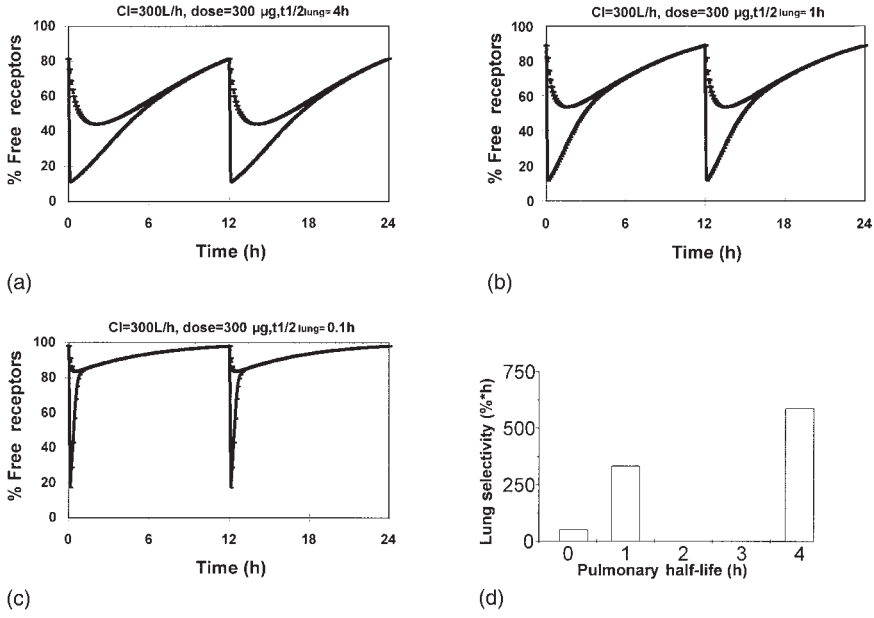



**Figure 5** Effect of pulmonary metabolism on pulmonary (lower line) and systemic (upper line) receptor occupancies. Simulations are shown for a systemic clearance of 300 L/h. Pulmonary stability differs in simulation a–c. The bar graph in d summarizes the results.

where $V_p$ is the volume of plasma. Thus, a drug that shows a stronger tissue binding than plasma protein binding will have a larger volume of distribution.

Current inhaled glucocorticoids exhibit pronounced plasma protein binding [budesonide 88% (18), fluticasone propionate 90–99% (33,34), flunisolide 80% (35), and triamcinolone acetonide 71% (36)]. Using this information, the calculated overall fraction of free drug in the body can be calculated from the $V_d$. This parameter ranges for inhaled glucocorticoids from 12% for triamcinolone acetonide to 1.3% for fluticasone propionate (assuming $f_u = 0.1$). This indicates that only a small fraction of the overall drug is available to interact with systemic and local receptor sites and that this fraction varies greatly among inhaled glucocorticoids.

It was of interest to evaluate the importance of the volume of distribution to lung selectivity. As in all simulations, data were simulated for the steady-state situation. If two drugs have the same clearance (high extraction drug, no effect of $f_u$ on clearance) but differ in the $V_d$ due to differences in tissue binding, plasma levels directly after dosing will be higher for the drug with the smaller $V_d$ (less pronounced tissue binding; Fig. 6a, b). However, the half-life of the drug with the larger $V_d$ will be longer, because most of the drug will be in the tissues and cannot be metabolized in the liver. Consequently, plasma levels at later time points

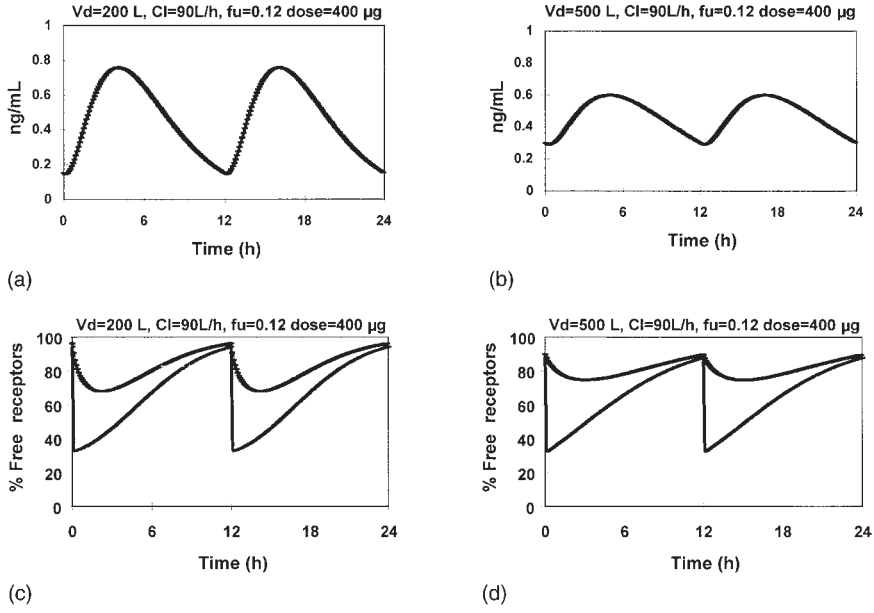



**Figure 6** Effect of volume of distribution on pulmonary (lower line) and systemic (upper line) receptor occupancies. Plasma concentration time profiles are shown for a volume of distribution of 200 L (a) and 500 L (b). Corresponding pulmonary (lower line) and systemic (upper line) receptor occupancies are shown in c and d.

will be higher for the larger $V_d$ drug (Fig. 6a, b). Assuming plasma levels are related to the systemic effects and that $f_u$ is the same for the two hypothetical drugs, one can predict that the systemic effects of these two drugs will not differ significantly (Fig. 6c, d). This is because at early time points the drug with the larger $V_d$ will show a lower degree of systemic effects sustained for a longer time period. However, it is the opposite for the drug with the lower $V_d$ such that just after dosing systemic effects will more pronounced but disappear faster. Overall, though, pulmonary selectivity after inhalation will be similar for the two drugs, whose half-lives differ only because of differences in their $V_d$ (Fig. 6c, d, assuming similar plasma protein binding). Figure 7 compares the differences between the effects of clearance and $V_d$ on pulmonary selectivity. Figures 7a and 7b show simulations where the half-life of a drug was modulated by changing either clearance or $V_d$. These simulations show that a drug with a long half-life because of a large $V_d$ is not necessarily a bad candidate for inhalation therapy, as long as the drug shows high systemic clearance.

The volume of distribution of a lipophilic drug is determined by its $f_u/f_{uT}$ ratio. This means that two glucocorticoids that differ in their plasma protein

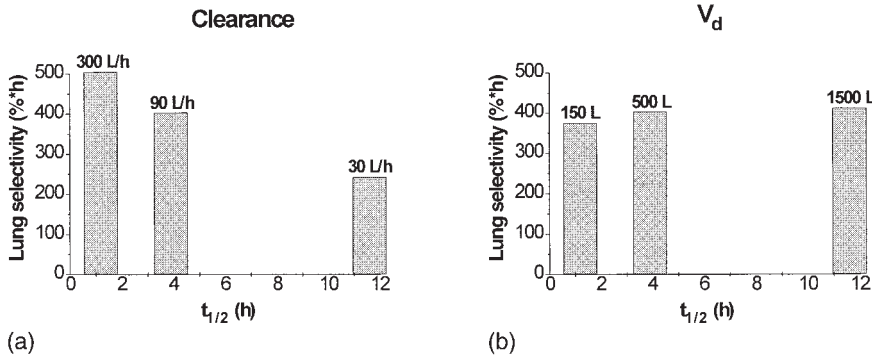



**Figure 7** Effect of clearance and volume of distribution on pulmonary selectivity. Clearance and volume of distribution was changed to result in the same half-lives (a: CL = 90 L/h; b: $V_d$ = 500 L).

binding can have the same volume of distribution if the $f_u/f_{uT}$ ratio stays the same. Considering that only free, pharmacologically active, not total drug concentrations are relevant for drug effects, it was of interest to test in the second series of simulations, again for the steady-state situation, how lung selectivity changes when $f_u$ and $f_{uT}$ change to the same degree ($f_u/f_{uT}$ and $V_d$ stays constant). Figure 8 shows such simulations for two hypothetical high extraction drugs that have the same $V_d$, but differ in their plasma and tissue protein binding. It is obvious that the drug with the more pronounced plasma protein and tissue binding shows reduced local, as well as, systemic effects when administered at the same dose (Fig. 8a vs. d). However, the differences in binding can be overcome by adjusting the dose (Fig. 8a vs. c, b vs. d), suggesting no pulmonary selectivity advantage is achieved for drugs differing in overall plasma and tissue binding. It is, however, important to realize that drugs with high plasma protein and tissue binding will show decreased pulmonary and systemic effects. Considering the lack of strong pulmonary surrogate markers in clinical studies, clinical studies for these higher binding drugs might suggest high "safety" because of the lower systemic activity on a μg basis.

Recently, the pronounced binding of fluticasone propionate to lung tissue has been suggested to be beneficial for pulmonary selectivity (37). The above simulations have shown that increased tissue binding, however, does not necessarily increase lung selectivity. This is true because only free drug levels are pharmacodynamically relevant and only a slow release from binding components, such as cell membranes, or the slow activation from intracellular "prodrug" pools (see below) will increase pulmonary residence time and, consequently, lung selectivity (6). Nonspecific tissue binding, however, is characterized by a high-capacity, low-affinity binding phenomenon, where generally dissociation is very fast under

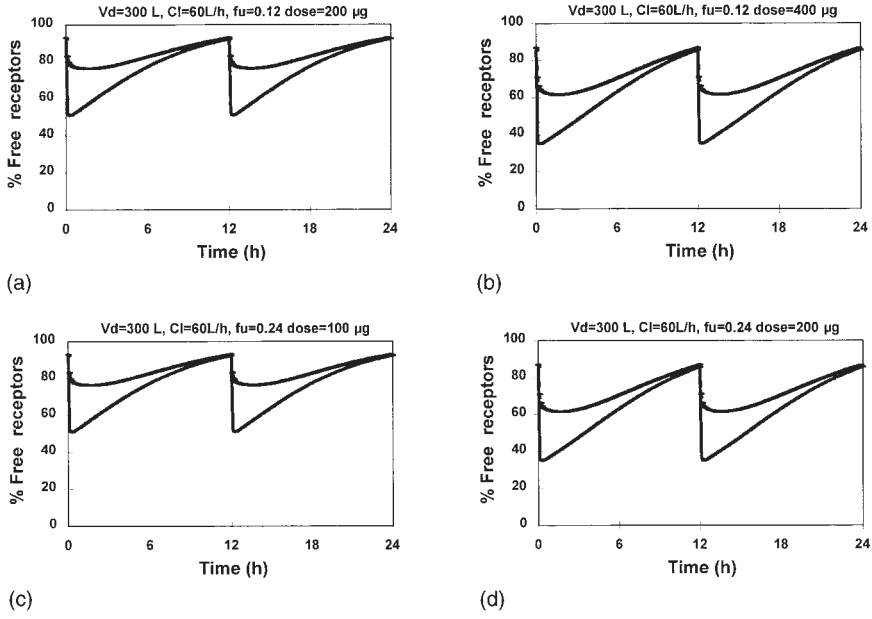



**Figure 8** Effect of plasma and tissue binding on pulmonary (lower line) and systemic (upper line) receptor occupancies. Simulations are shown for two hypothetical glucocorticoids given at certain doses twice a day at steady state. The drugs show the same clearance and volume of distribution, but differ in plasma and tissue binding ($f_u/f_{uT}$ is constant). Note that doses differ in situation a versus b, and c versus d.

sink conditions and should not improve lung targeting, as it does not increase the pulmonary residence time.

Receptor, plasma protein, and tissue binding seem to correlate roughly with the lipophilicity of the drug. Since an increase in tissue or plasma protein binding decreases the fraction of drug able to interact with the receptors, an increase in receptor binding because of an increase in lipophilicity might be buffered pharmacologically by a decrease in free drug levels. The activity at the site of action (and also in the systemic circulation), observed for more lipophilic drugs, might actually be lower than the difference in receptor-binding affinity would suggest. This will have an effect on the actual dose necessary for inhalation therapy but will not have any effect on the pulmonary selectivity.

Using the volume of distribution as a parameter that describes the extent of tissue distribution, we assumed that only passive diffusion processes are present. There is, however, increased evidence that active transport mechanisms are involved in the specific tissue distribution of glucocorticoids. Sergeev and coworkers

identified membrane-binding components that appear to be involved in the uptake of corticosteroids into thymocytes. Glucocorticoids have also been shown to be substrates for the *p*-glycoprotein transporter (38,39), as increased penetration of dexamethasone to the brain has been observed in mdr1A P-glycoprotein knockout mice (39,40). These results suggest that dexamethasone is being pumped out of the blood-brain barrier endothelial cells of wild-type mice.

It is likely that such efflux pumps are responsible for the significantly lower brain receptor occupancies after pulmonary and i.v. administration of the inhaled glucocorticoids triamcinolone acetonide, budesonide, and fluticasone propionate (41–43). A better understanding of the glucocorticoid transport mechanism might lead to improved lung selectivity by identifying glucocorticoids with optimized lung versus systemic tissue distributions.

### F. Inhaled Dose

The relationship between dose and pulmonary targeting is shown in Figure 9. Increasing the dose also increases the difference between pulmonary and systemic

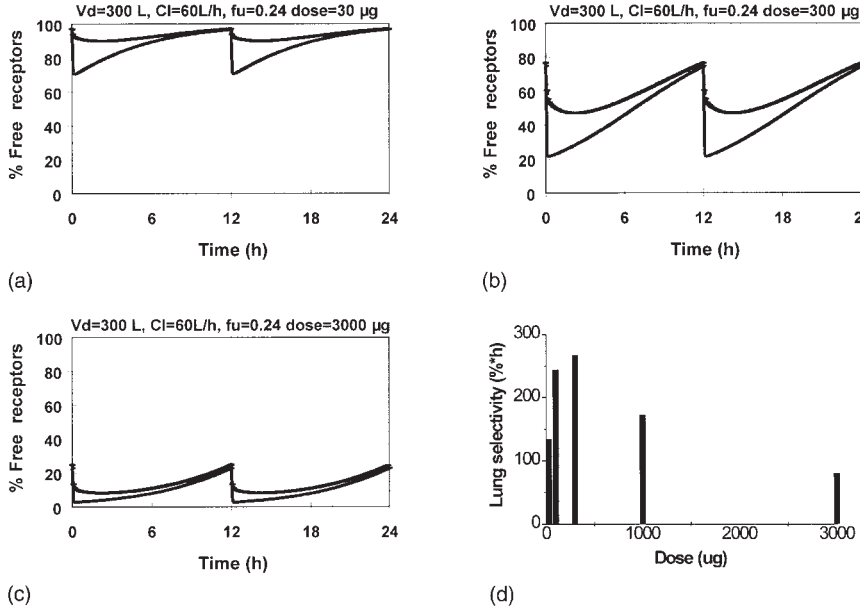



**Figure 9** Effect of dose on pulmonary selectivity. Pulmonary selectivities [areas between pulmonary (lower line) and systemic (upper line) receptor occupancies] observed in a–c are summarized in d. Additional doses are also shown in d.

receptor occupancies until a maximum is reached. Further increases in the dose will result in more pronounced receptor occupancies in both lung and systemic tissues, but pulmonary selectivity is lost. These simulations (6) suggest that there is an optimal dose that shows a most pronounced pulmonary selectivity. Thus, patients who need high doses of inhaled glucocorticoids to control their asthma might not benefit from inhalation therapy because of a lack of targeting. Support for these theoretical considerations has been shown in previously reported rat experiments by our group (44). Different doses of liposomal encapsulated triamcinolone acetonide were administered, and the receptor occupancies in the lung and liver were monitored for the assessment of pulmonary selectivity. These results strongly resembled those shown in Figure 9, supporting the hypothesis that pulmonary selectivity is lost at high doses. The PK/PD model was also used to probe how pulmonary selectivity is affected by dose and dosing frequency in multiple dosing regimens. Figure 10 depicts a pulmonary selectivity pattern when the same daily dose of 400 μg was given once (1 × 400 μg), twice (2 × 200 μg), and four times (4 × 100 μg). These simulations suggest that higher frequency dosing during

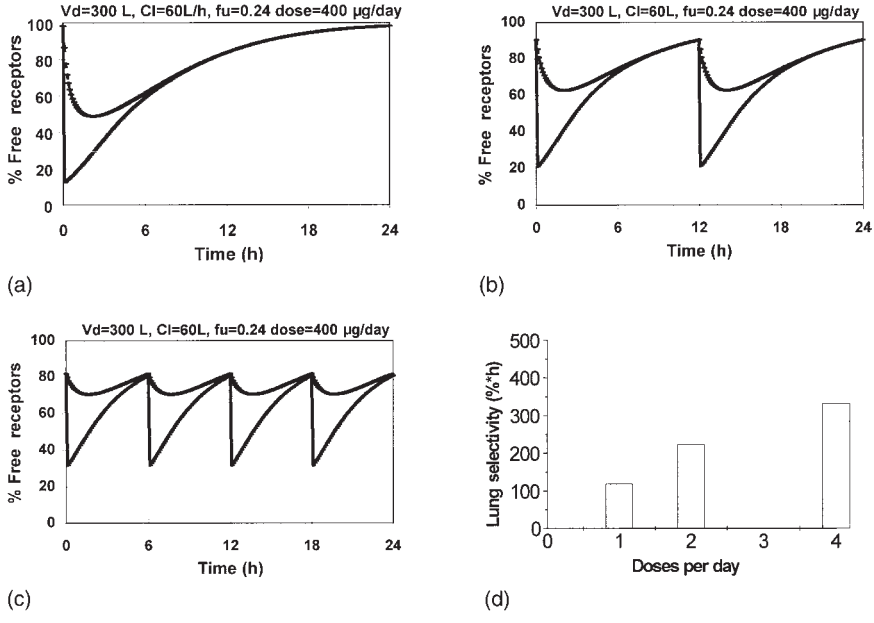



**Figure 10** Effect of the dosing regimen at steady state on pulmonary selectivity. The daily dose of 400 μg was given as one single dose (a), 200 μg b.i.d. (b), or as 100 μg q.i.d. (c). Pulmonary selectivities (areas between pulmonary (lower line) and systemic (upper line) receptor occupancies) observed in a–c are summarized in d.

the day will significantly increase pulmonary selectivity. These results are in close agreement with those of Toogood, who detected clinically an increase in anti-asthmatic efficacy when smaller doses were given more frequently (45). Since patient compliance is reduced with more frequent dosing regimens, an alternative strategy of improving pulmonary selectivity is the use of inhalation formulations with sustained release characteristics (see below).

### G. Pulmonary Release and Absorption

A glucocorticoid inhaled as a liquid suspension or dry powder aerosol has to (1) come in contact with the inside surface of the airways, (2) dissolve into the fluid lining (suspension and dry powders only), (3) diffuse into the cells, and then (4) interact with the glucocorticoid receptor. Using the integrated PK/PD model of pulmonary targeting, it was of interest to investigate what effect biopharmaceutical factors such as the dissolution/release rate might have on pulmonary selectivity. These simulations assume that the dissolved drug is readily absorbed into the airway cells and no other interaction to pulmonary tissue would modulate the pulmonary residence time. Figure 11 shows a simulation for deposition only into the upper part of the lung, where the mucociliary transporter is able to remove solids from the lung. These simulations indicate that glucocorticoids that dissolve immediately (or are immediately released from the formulation or are given as a solution) do not show any pulmonary selectivity. When the dissolution/release rate is slowed, pulmonary and systemic receptor occupancies dissociate/separate and pulmonary selectivity increases. If the dissolution rate is too slow, therapeutically effective concentrations are not maintained and pulmonary targeting disappears. Thus, an optimal release rate (e.g., dissolution rate or release rate from a carrier) exists for which an optimal pulmonary targeting is observed (6). Very fast pulmonary release, such as that observed for a glucocorticoid in solution, does not result in any pulmonary targeting because the drug is immediately absorbed from the lung into the systemic circulation (46,47). As a result, the free drug levels in both the systemic circulation and the lung are identical. Delivery systems with a prolonged pulmonary release rate result in free pulmonary drug levels that are higher over an extended period of time than those in the systemic circulation, leading to pronounced lung selectivity. If the dissolution/release rate is too slow, therapeutically effective concentrations are not achieved and the mucociliary transporter of the upper respiratory tract may remove the solid drug before it is able to dissolve into the lung and induce pulmonary effects. This portion of the pulmonary-deposited dose can be absorbed orally to induce systemic side effects (assuming a certain oral bioavailability). Thus, theoretically, an optimal pulmonary dissolution or release rate exists for the upper respiratory tract (6), which ensures a prolonged pulmonary residence time. It will be of interest to see how newer liquid-aerosol drug-delivery devices will perform due to the fact that glucocorticoids in solution are absorbed so rapidly from the alveolar region of the lung.

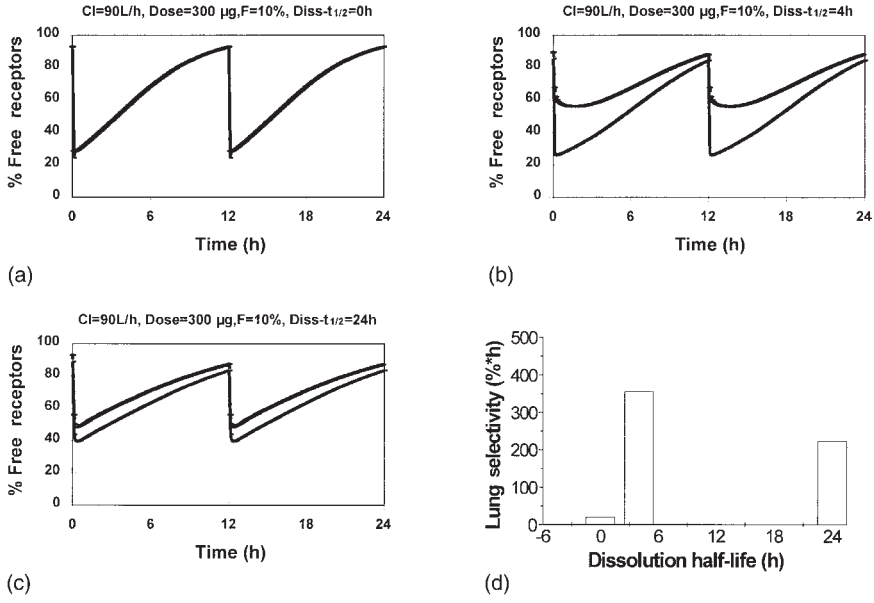



**Figure 11** Effect of the pulmonary dissolution rate on pulmonary selectivity. The dose of 300 µg was allowed to dissolve immediately (a), with a half-life of 4 hours (b), or with a half-life of 24 hours (c). Pulmonary selectivities [areas between pulmonary (lower line) and systemic (upper line) receptor occupancies] observed in a–c are summarized in d. The dose was given twice a day at steady state.

Noncompartmental analysis of inhaled glucocorticoids after intravenous injection and inhalation resulted in relatively long pulmonary residence times for fluticasone propionate [5 h (48)] and triamcinolone acetonide [2.9 h (21)], while that of budesonide was relatively short [0.75–1.55 h (49)]. Interestingly, these absorption times in humans correlated roughly with pulmonary selectivities determined in a rat model (utilizing lung without tracheobronchial sections) (41–43), suggesting the importance of the lung pharmacokinetics for lung selectivity.

Overall, the following mechanisms might be employed to prolong pulmonary residence time:

1. Use of drug particles with slow dissolution rate (e.g., drug lipophilicity or crystal structure)
2. Slow release from drug delivery systems (e.g., liposomes or microspheres/microcapsules)
3. Initiation of a cellular interaction resulting in prolonged residence times in the airways/lung (e.g., ester-formation or "capturing" in membrane structures)

To illustrate the effect of different formulations of the same drug, an animal model (rat) showed that various biopharmaceutical forms of triamcinolone acetonide (TA) exhibited drastically differing levels of pulmonary targeting when delivered intratracheally. Pulmonary and systemic receptor occupancies after intratracheal administration of a TA solution, a micronized TA dry powder, and a TA crystal suspension used for the treatment of arthritis, all at the same dose, were assessed. The comparison showed that the degree of pulmonary targeting (difference between pulmonary and systemic receptor occupancy) for TA increases from solutions to micronized powders to crystal suspension, which is in agreement with its anticipated dissolution behavior. Hill and coworkers demonstrated that BDP given as a dry powder showed much slower absorption profiles than beclomethasone dipropionate (BDP) given as MDI (29). This indicates that biopharmaceutical factors can affect the pulmonary fate of an inhaled drug. These examples indicate that the biopharmaceutical/formulation properties of inhaled glucocorticoids are important determinants of pulmonary selectivity (6).

Over the last 10 years, liposomal drug formulations have been investigated as an alternative way of controlling the pulmonary residence time of inhaled glucocorticoids (13,50). Different liposomal formulations of triamcinolone acetonide phosphate (TAP) showed that pulmonary targeting was directly related to the extended release rate of the formulation (44,50). Similarly, slow-release budesonide-palmitate liposomes were reported to increase selectivity in the peripheral region of the lung (13).

Alternative approaches for increasing pulmonary residence time, such as the use of novel excipient derived from oligomeric lactic acid (51), the use of nanofunctional drug coatings (52), or incorporation of drug into low-density microspheres (53,54), have been reported. As an example, ultra-thin coatings on budesonide powders resulted in a significant improvement of pulmonary selectivity (52). The implication of utilizing these various methods to improve pulmonary selectivity looks promising, but the therapeutic efficacy, as well as industrial scalability, remains to be seen.

Another mechanism for prolonging pulmonary residence time has been proposed, based on the fact that budesonide and other glucocorticoids form ester conjugates as depots intracellularly (55–57). These conjugates are unable to cross pulmonary membranes and are consequently trapped in the airways/lung as inactive "prodrugs." Sustained cleavage of these esters back to free drug by esterases provides a slow release of active species intracellularly, which will result in a prolongation of the pulmonary effects and potentially in an increase in pulmonary targeting (if a substantial fraction of the dose is retained by this mechanism). Although the potential benefits of the ester formation in the lung is intriguing, clinical studies need to demonstrate its therapeutic relevance.

Finally, it should be noted that the peripheral part of the lung lacks the mucociliary clearance mechanism. Applying the PK/PD model to drug deposition

deeper in the lung showed similar relationships for dose, dosing regimen, and pulmonary deposition to those seen for more central deposition. The only difference was that lung pulmonary selectivity increases with decreasing release rate of the drug.

## III. Conclusion

Identifying extrahepatic metabolic pathways with sufficient pulmonary stability (although a challenge after previous negative clinical studies with such drugs) and optimizing the pulmonary residence time of an inhaled glucocorticoid 1) via control of the intraluminal residence time (e.g., via control of the dissolution rate, use of liposomes or other drug delivery systems), 2) by exploiting rate-limiting interactions of the drug molecule with membranes or other cell structures, or 3) via optimizing formation of pulmonary depots through ester formations should be the main research areas for further improving pulmonary selectivity. More information is also necessary for assessing the regional differences in targeting observed for the tracheobronchial, central, and peripheral segments of the lung/airways and learning which segments are most important for controlling asthma of different severity. It is also of interest to study the critical effects of the mucociliary clearance in mild, moderate, and severe asthmatics.

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## Discussion

**Dr. Seale:** How do you measure percent receptor binding in your experiments?

**Dr. Hochhaus:** These studies use standard ex vivo receptor binding techniques, in which the rat is dosed intratracheally with unlabeled drug. Rats are killed after defined time points, tissues are removed, homogenized, unbound steroid is removed by charcoal, cytosol is prepared by ultracentrifugation, and the number of free receptors is determined by pulse-labeling with high concentration of radioactive triamcinolone acetonide.

**Dr. Brattsand:** These novel studies are based on lung tissue. Because the target tissue in asthma is the airways, we look forward to data on receptor occupancy at the airway level. For budesonide, prolonged tissue deposition by the esterification mechanism is seen much more at the airway than at the lung level.

**Dr. Schleimer:** You pointed out that at a certain dose lung selectivity would be lost when the circulating steroid levels become too high. John Toogood reported that selectivity was retained at doses of budesonide up to 2–3 mg. At what dose for commonly used steroids would you predict that pulmonary selectivity would be lost?

**Dr. Hochhaus:** These studies have been performed either by computer simulation or in rat experiments. It is very difficult to accurately predict from this work the dose in humans at which targeting is lost. It might well be that a dose of 2–3 mg budesonide will still be in the dose range which will induce targeting.

**Dr. Rohdewald:** Which dose should be given considering receptor occupancy? Is it possible to calculate the amount of drug which is needed for 100% occupancy of receptor sites?

**Dr. Hochhaus:** So far we are not fully able to predict in humans the dose which is necessary to achieve a certain receptor occupancy, mainly because we don't know exactly the degree of tissue binding observed for inhaled glucocorticoids in the human lung. This is necessary to calculate this key parameter.

**Prof. Dolovich:** What effect does the formulation have on the absorption rate of the drug in the lung? Specifically, are there differences in absorption between the solution HFA, suspension CFC, or HPA PMDI compared to powder formulation?

**Dr. Hochhaus:** The formulation is one of the key factors affecting pulmonary selectivity as it determines the pulmonary residence time of the glucocorticoid. We have shown in animal experiments that pulmonary selectivity depends on the physiocochemical state of a given glucocorticoid. We could

show for triamcinolone acetonide that pulmonary selectivity is lost for a triamcinolone acetonide solution while triamcinolone acetonide suspension showed distinct targeting because of the increased pulmonary residence time. It is therefore likely that there are differences in the pulmonary selectivity whether a drug is given as a solution in a MDI or a powder in a DPI. These differences need to be addressed.

# 13

# Reversible Glucocorticoid Esterification

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## I. Introduction

Glucocorticosteroids (GCSs) have a multitude of endocrinological effects and at higher doses also clinically important anti-inflammatory effects. Because GCS effects are primarily mediated via intracellular receptors, and because receptors are abundant in all cells, one fundamental problem has been to differentiate between wanted pharmacological effects and unwanted side effects. The primary pathway that industry has chosen to pursue to obtain maximum local anti-inflammatory effect in lung and minimum systemic effects (i.e., optimum airway selectivity) has been by pharmacokinetic means.

For an inhaled glucocorticosteroid to show an airway selectivity at any given moment, the free concentrations in the airway GCS receptor biophase must be higher than the corresponding concentration in the rest of the body, assuming similar receptor affinity and intrinsic response throughout the body. This will lead to higher receptor occupancy in the airway compartment than in the systemic compartments and thus give higher beneficial effects than unwanted effects. It has been suggested by PK/PD simulations (1) (see Chap.12) that the most important pharmacokinetic parameters for achieving high airway selectivity are the fraction of dose deposited in the airways, dissolution rate of drug in the airways, systemic ab-

sorption rate from the lungs, oral bioavailability, and systemic clearance. High systemic clearance and low oral bioavailability both contribute to decreased systemic effects, while a high fraction of dose deposited in lung, slow airway dissolution rates, and slow systemic absorption from the lungs contribute to both a prolonged effect duration and an increased airway selectivity. The systemic absorption rate for glucocorticosteroids is generally rapid (2,3) due to the fast movement across cell membranes and the high airway perfusion rate. Thus, one of the most obvious ways to enhance airway retention of an inhaled steroid is to decrease the release rate by having a slowly dissolving drug formulation.

Budesonide (BUD) has been shown to be a fast-dissolving steroid (4) with rapid absorption from the lung in both humans and animals, and was therefore not expected to show a prolonged duration of action. Contrary to what could be expected from early pharmacokinetic documentation, BUD is effective during once-daily treatment in patients with asthma, rhinitis, and inflammatory bowel disease (IBD) (5–7). As reviewed in Chapter 9, BUD has been shown to be retained in airway tissue to a greater extent than more lipophilic GCSs. The explanation for this tissue retention was subsequently shown to be the intracellularly formed and retained BUD fatty acid esters. These esters, which are produced in a variety of tissues in both animals and humans, are pharmacologically inactive and are rapidly formed intracellularly by enzymatic pathways. The BUD-esters are slowly hydrolyzed back to intact BUD by lipases. There is preclinical evidence that the intracellular ester depot increases the duration of the effect.

PK/PD simulations offer a unique way to study complex biological systems in a well-controlled manner, varying only one or a few basic parameters at a time, which is impossible in a real-life experiment. Experience has shown that compartmental models often give a good description of the pharmacokinetics and that a simple $E_{max}$ model can well describe receptor-mediated pharmacological effects.

Two issues are addressed in the present chapter: Can the BUD esterification mechanism theoretically improve lung selectivity, even though the fatty acid esters are formed both in the lung and in peripheral tissues? What are the pharmacokinetics of BUD and BUD fatty acid esters in selected rat tissues after administration by local and systemic routes?

## II. General Models for Lung Selectivity

A theoretical compartment model consisting of six compartments (Fig. 1) was used to study the impact of steroid ester formation (8). The model is an extension of previously presented models used to study lung selectivity (1). The model consists of two deposition compartments where steroid is deposited after inhalation—one for the lung-deposited fraction and one for the swallowed and gut-absorbed fraction of the drug. The lung deposition compartment has an additional outflow,

representing the mucociliary clearance of lung-deposited drug. Two compartments represent intact steroid in the body: one in lung and one in the system (i.e, body except lung). The steroid esters are represented by two compartments: one describing ester formed in the lung and one describing ester formed in rest of the body.

Steroid receptor occupancy was calculated in the compartments representing intact steroid in the lung and in the system by using a simple $E_{max}$ model:

$$Effect = \frac{E_{max} \cdot C_{steroid}}{EC_{50} + C_{steroid}} \quad (1)$$

The cumulative receptor occupancy during one dosing interval at steady state (after once-daily doses) was calculated as the area under the curve (AUC) for the 24-hour effect-time curve, both in the lung and in the rest of the body. Lung

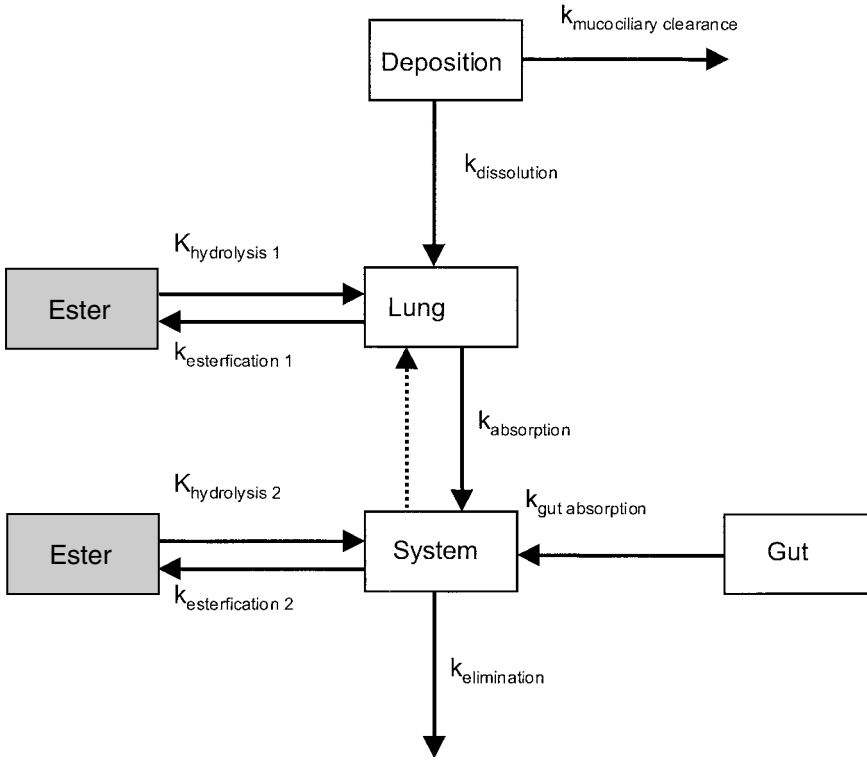



**Figure 1** Compartment model used to simulate the steroid concentrations in lung and system after inhalation. Dotted line indicates summation when calculating effect in the lung.

**Table 1** Parameters Used in Simulations (if Not Otherwise Specified)

| | | | |
|---|---|---|---|
| Dose (μg) | 100 | Oral bioavailability (%) | 10 |
| Lung volume (L) | 1.9 | Volume of distribution for unesterfied drug (L) | 150 |
| Lung deposition (%) | 30 | Clearance (L/h) | 60 |
| Lung dissolution $t_{1/2}$ (h) | 0.3 | Esterification $t_{1/2}$ (h) | 0.07 |
| Systemic absorption (h) from the lung $t_{1/2}$ | 0.07 | Ester hydrolysis $t_{1/2}$ (h) | 2 |
| Oral absorption $t_{1/2}$ (h) | 0.07 | $EC_{50}$ (ng/mL) | 0.24 |
| | | $E_{max}$ (%) | 100 |

selectivity was defined as the ratio between these two AUCs. When calculating the lung effect, the concentration in the lung was calculated as the sum of the concentration in the body and the concentration in the lung of drug dissolved, but not yet absorbed into the system, describing the fact that there is a fast redistribution of drug from the body to the lung. The theoretical steroid used in the simulations was assumed to have a high clearance (60 L/h), fast lung dissolution ($t_{1/2}$ = 18 min), fast systemic absorption ($t_{1/2}$ = 4 min), high lung deposition (30%), low oral bioavailability (10%), medium volume of distribution (150 L), and high receptor potency ($EC_{50}$ = 0.24 ng/ml, equal to 0.48 nM if assuming a molecular weight of 500 g/mol). If not stated otherwise, the parameters given in Table 1 were used in the simulations. The parameters were either taken from Ref. 1 (clearance, lung dissolution, systemic absorption, volume of distribution, and receptor potency) or based on experience with BUD given via Turbuhaler® (lung deposition and oral bioavailability). The lung selectivity obtained by the model including ester formation was compared to a model without ester formation, keeping all other parameters the same.

### A. Rate of Ester Formation and Hydrolysis

As illustrated in Figures 2–7, the rates of ester formation and hydrolysis may have a considerable effect on the lung selectivity of a GCS. If there is no ester formation, selectivity is low, only 1.1. Rapid formation of ester improves selectivity up to 1.8 at an ester formation half-life of 0.035 hour both in the lung and the system (Fig. 2) or only in the lung (Fig. 3). If ester formation rate were held constant in the lung ($t_{1/2}$ = 0.07 h), changes in the systemic formation rate would have only a minor influence on selectivity (Fig. 4). Thus, the very fact that ester is formed in the lung will influence the duration of the drug effect in the airways and hence selectivity, even though ester is also formed in the systemic compartment.

The opposite is true for ester hydrolysis: fast hydrolysis in the lung reduces selectivity (Figs. 5, 6), and changes in systemic hydrolysis rate at constant rate in the lung ($t_{1/2}$ = 2 h) has no effect (Fig. 7).

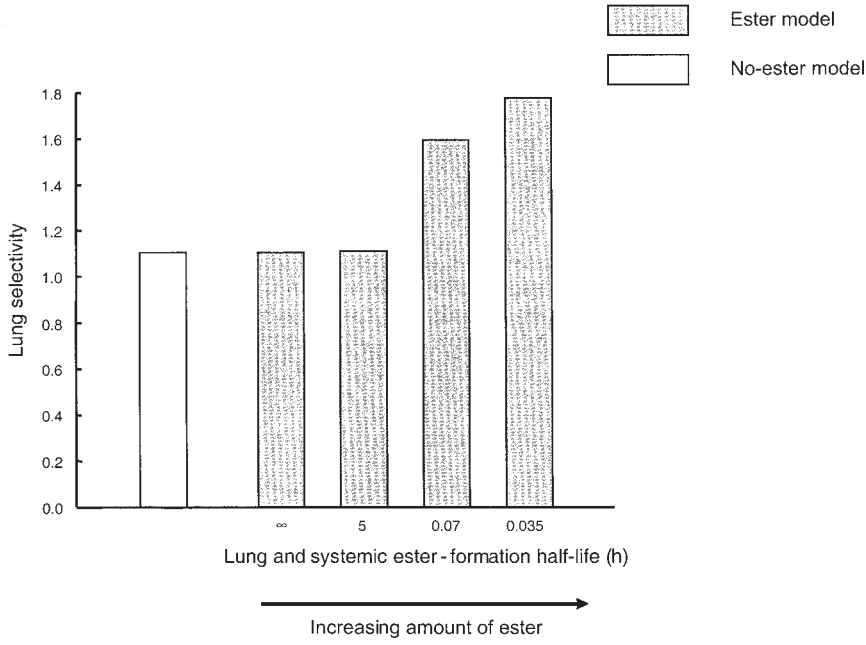



**Figure 2** Lung selectivity when esterification rate is changed in both lung and systemic compartments.

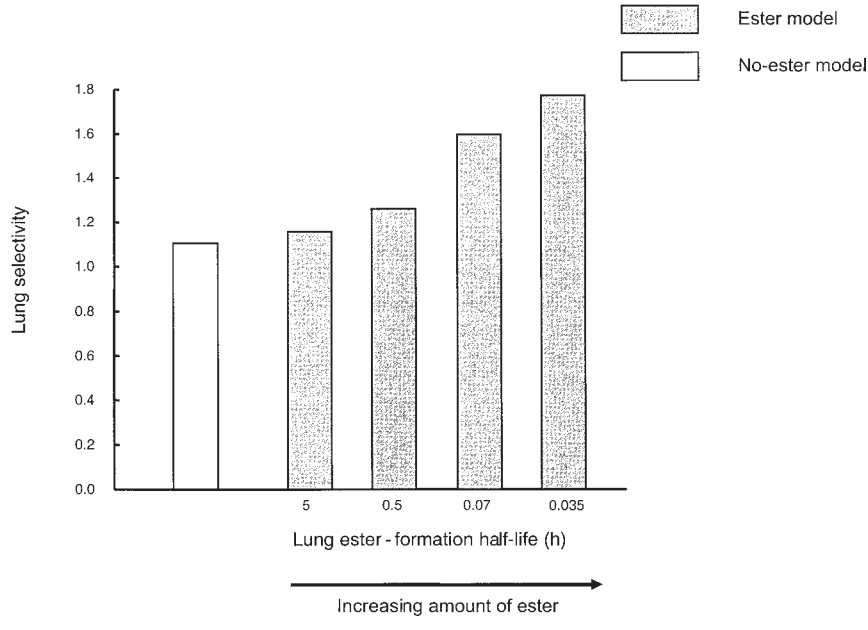



**Figure 3** Lung selectivity when esterification rate is changed in lung compartment.

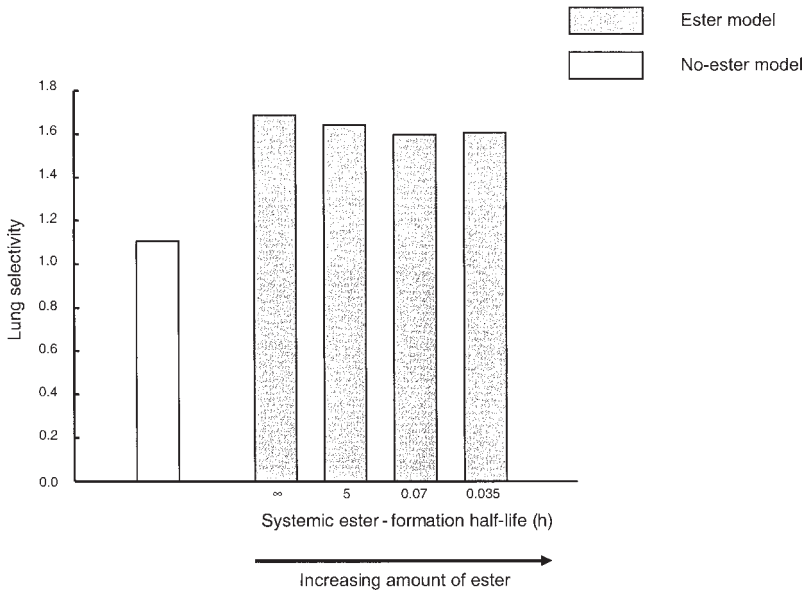



**Figure 4** Lung selectivity when esterification rate is changed in systemic compartment.

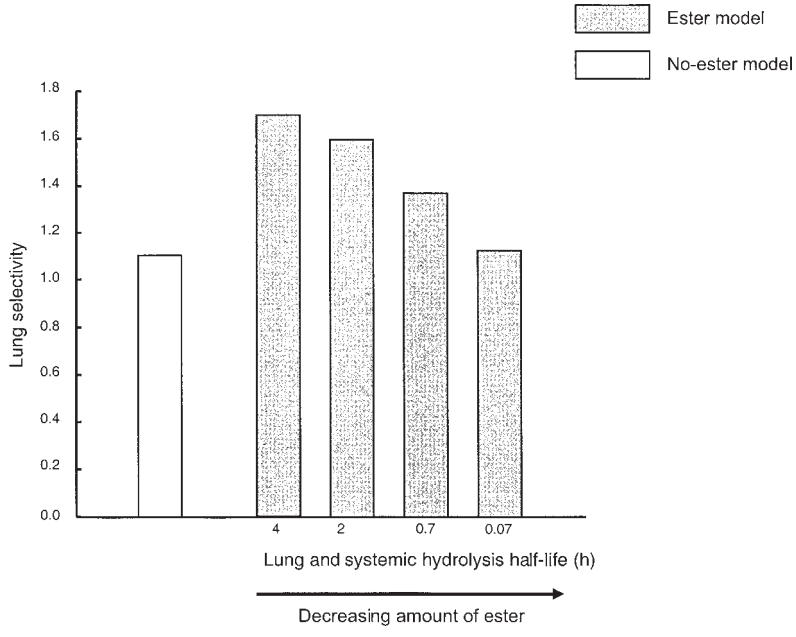



**Figure 5** Lung selectivity when hydrolysis rate is changed in both lung and systemic compartments.

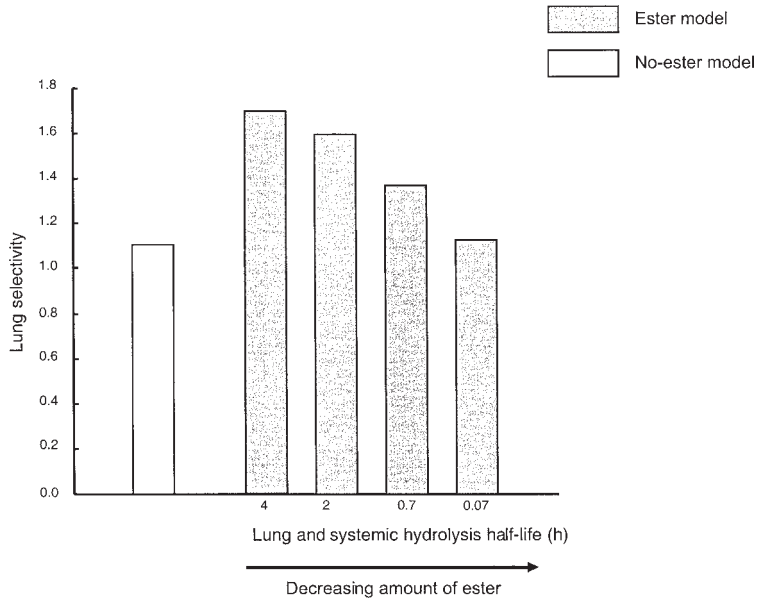



**Figure 6** Lung selectivity when hydrolysis rate is changed in lung compartment.

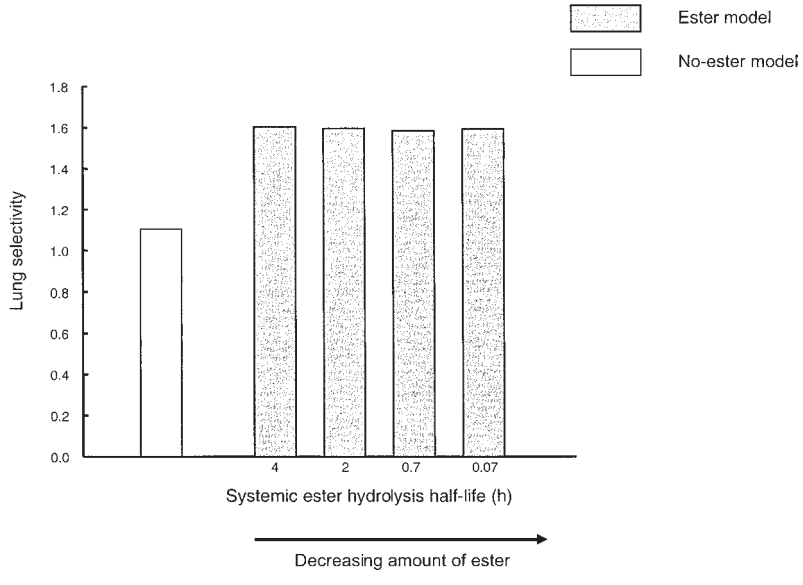



**Figure 7** Lung selectivity when hydrolysis rate is changed in systemic compartment.

**Table 2** Simulated Airway Selectivity (SEL) in Model with (nonshadowed rows) and Without (shadowed rows) Ester Formation

| Cl (L/h) | $t_{1/2}$diss (h) | $F_{lung}$ (%) | $F_{oral}$ (%) | V (L) | SEL |
|---|---|---|---|---|---|
| 10 ↓ 90 | | | | | 1.1 ↓ 2.0 |
| 10 ↓ 90 | | | | | 1.0[a] ↓ 1.2 |
| | | | | 30 ↓ 500 | 1.6 ↓ 1.6 |
| | | | | 30 ↓ 500 | 1.1[a] ↓ 1.1 |
| | 0.3 ↓ 8 | | | | 1.6 ↓ 2.0 |
| | 0.3 ↓ 8 | | | | 1.1[a] ↓ 1.8 |
| | | 5 ↓ 50 | | | 1.1 ↓ 1.9[b] |
| | | 5 ↓ 50 | | | 1.0[a] ↓ 1.3[b] |
| | | | 25 ↓ 5 | | 1.3[c] ↓ 2.1 |
| | | | 25 ↓ 5 | | 1.1[a,c] ↓ 1.2 |

[a] Model without ester formation.
[b] Lung deposited dose held constant at 30 µg.
[c] Total dose was 100 µg.
Cl = Clearance; V = volume of distribution for unesterified drug; $t_{1/2}$diss = dissolution half-life in lung; $F_{lung}$ = deposited fraction in lung; $F_{oral}$ = oral bioavailability. Parameters not given in the table were fixed at the values given in Table 1.

### B. Other Kinetic Parameters

As seen in Table 2, systemic clearance combined with ester formation in the lung increases selectivity up to 2.0 at 90 L/h, whereas less effect is seen without ester. Prolonged dissolution rate in the airways retards uptake, and selectivity is hereby improved, but moreso without than with ester formation. A high fraction deposited in the lung favors selectivity both with and without ester formation. Conversely, a high oral bioavailability is associated with a low selectivity. Volume of distribution of unesterified drug has no influence on selectivity.

### C. Discussion

The AUC for the concentration-time curve at steady state in the lung and central compartments in the compartmental system described does not change when the half- lives for ester formation and hydrolysis change. On the other hand, the AUC for the effect-time curve in the lung and central compartment do change (Table 3). Also, the AUC for the effect-time curve in the lung increases more than the AUC in the systemic compartment, leading to an increase in lung selectivity even when esters are present in both the lung and central compartments. As can be seen in Figure 8, the time profile of the effect changes to a more prolonged shape when ester formation is included, and thus the esterification leads to an increased effect duration as well as an increased lung selectivity.

Hence, the beneficial effects of the esterification mechanism are evident after repeated bolus doses. However, the advantage of esterification for selectivity will decrease with more frequent dosing. If the extreme of frequent dosing, a hypothetical continuous lung administration, is simulated, the effect in the lung and in the rest of the body in the presence of ester formation approaches the values obtained without ester formation (Fig. 9). Evidently, equilibrium is eventually reached where exactly the same effect will be reached with or without ester formation. For a rapidly absorbed drug (such as BUD), strict equilibrium will never be reached, and a beneficial effect of the esterification is therefore achieved.

To summarize, in the model described, fast formation and slow hydrolysis of esters improve airway selectivity. If this is combined with a high systemic clearance, selectivity increases further. Other factors that increase the time or amount

**Table 3** $AUC_{0-24h}$ at Steady State (after once-daily doses) for the Concentration-Time Curve and the Effect-Time Curve

| | Lung | | System | |
|---|---|---|---|---|
| | Without ester | With esters | Without ester | With esters |
| Concentration (AUC μg · h/L) | 8.3 | 8.3 | 2.3 | 2.3 |
| Effect (AUC % · h) | 460 | 1100 | 420 | 690 |

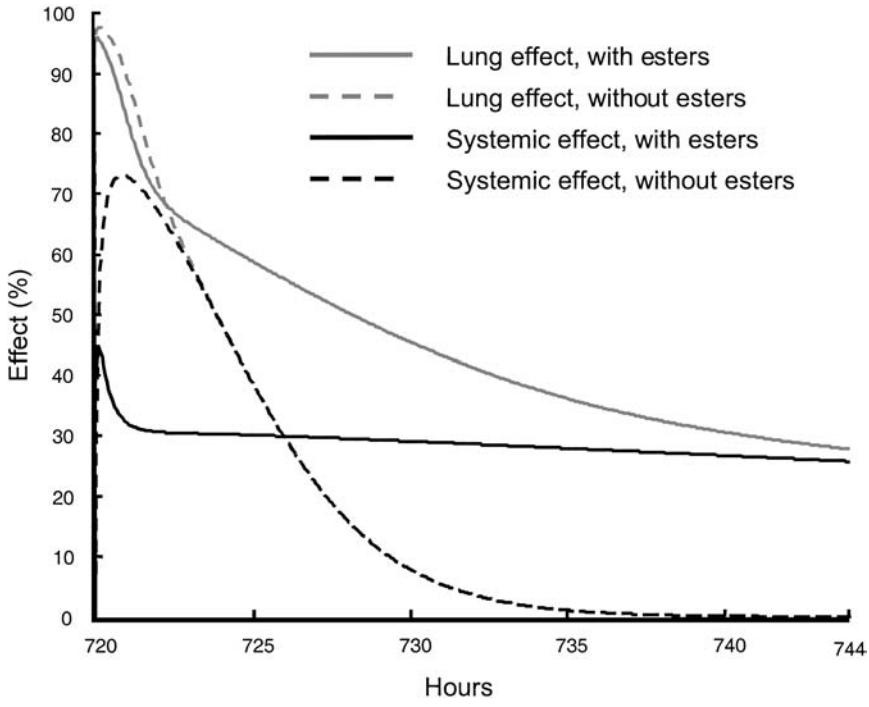



**Figure 8** Effect-time curves for one dose-interval at steady state. The graph shows the effect both in lung (light gray line) and in systemic compartment (dark lines) with (solid lines) and without (dotted lines) esters.

of drug in the lungs, such as a high lung deposition and slow dissolution in the airways, are also beneficial. Airway dissolution rate has less impact, however, for a drug that forms esters than for one that does not.

## III. Pharmacokinetic Modeling of BUD in the Rat

To characterize the pharmacokinetics of BUD and BUD fatty acid esters in tissue and in plasma, two animal studies were conducted (9). In one study, the pharmacokinetics of tritiated BUD and BUD-esters in lung, trachea, soleus muscle, and plasma were evaluated after intravenous administration. In another study, the pharmacokinetics of BUD and BUD-esters in lung, trachea, and plasma were evaluated after inhalation.

The resulting data were analyzed both by conventional noncompartmental analysis and by compartmental analysis. The compartment model used was a semi-

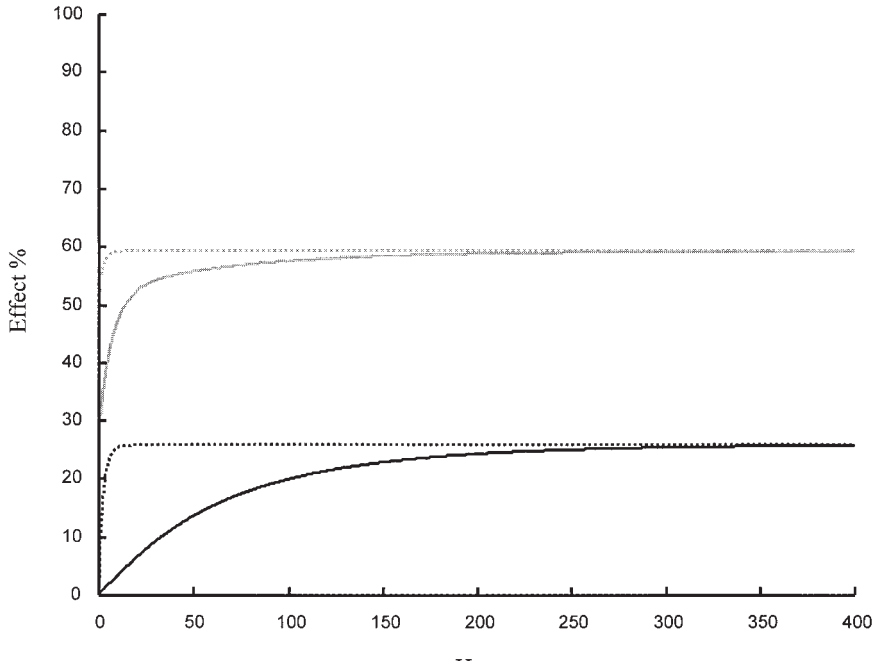



**Figure 9** Effect-time curves during a continuous lung input. Lung effect shown as light gray lines and systemic effect shown as dark lines. Model with esters shown as solid lines and model without esters shown as dotted lines.

physiological compartmental model, taking into account distribution between tissues as well as the enzymatic formation and hydrolysis of BUD-esters.

### A. Methods

#### *Intravenous Study*

A dose of 16 nmol/kg $^{3}$H-BUD dissolved in an aqueous ethanol solution was injected intravenously in the tail of male Sprague-Dawley rats. The animals were sacrificed by heart puncture after having been anesthetized with pentobarbital. Three rats at each time point were sacrificed at 5, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 8, 12, and 24 hours after drug administration.

Blood was collected from each rat, plasma separated by centrifugation and frozen at −70°C. Trachea, lung, and soleus muscle were removed from each rat, weighed, and frozen at −70°C.

BUD and BUD-esters were extracted from lung and soleus muscle by homogenization in ethanol followed by microwave extraction of the ethanolic

homogenate at 90°C and separation by centrifugation. For trachea there was no homogenization step, and the cut trachea was directly extracted by microwave extraction.

The tissue extracts were fractionated by HPLC. The BUD fraction and the fraction of combined BUD-esters were collected. Radioactivity was measured in a liquid scintillation counter. The plasma samples were pretreated by an automated solid phase extraction, followed by on-line liquid chromatography enrichment. The enriched samples were collected in scintillation tubes.

### *Inhalation Study*

A calculated dose of 220 nmol/kg micronized BUD powder was inhaled by nose exposure by male Sprague-Dawley rats during 10 minutes in a Batelle flow past inhalation chamber. The animals were sacrified by heart puncture after having been anesthetized with pentobarbital. Blood, trachea, and lung were sampled and treated as described above, except that no 5-minute sample was obtained. The lung and trachea extracts were fractionated using a separate LC system. BUD and BUD-esters in the fractions were subsequently quantitated with a different LC-MS-MS system.

BUD and BUD-esters in the plasma samples were analyzed by two different methods comprising solid phase extraction and APCI LC-MS-MS detection.

### *Modeling*

A semi-physiological compartment model (Fig. 10) was fitted to mean plasma and tissue concentrations of BUD and BUD-esters using the computer program NONMEM. The lung was described by three compartments representing solid BUD (deposition compartment), dissolved BUD, and BUD-ester; the trachea was described by four compartments representing solid BUD (deposition compartment), dissolved BUD, and BUD-ester (two compartments were needed to describe the nonlinear kinetics of BUD-ester in trachea); soleus muscle was described by two compartments representing BUD and BUD-esters; plasma was described by two compartments representing BUD and BUD-esters; the gut was described by one compartment; and one extra peripheral compartment accounted for BUD outside the characterized organs. The model was implemented as a set of differential equations with Michaelis-Menten functions to describe the rate of formation and rate of hydrolysis of BUD-esters and first-order rate constants to describe the rates of all remaining processes. The fraction of inhaled dose deposited in lung and trachea was fixed at 30% during the fitting procedure. The distribution between lung (94%) and trachea (6%), the oral bioavailability, and the half-life for gut absorption were all based on experimental data and were fixed during the fitting procedure (data on file). The volumes of the lung, trachea, muscle, and central compartment were fixed (lung = 1 mL, trachea = 0.5 mL, muscle = 200 mL, plasma compartment = 50 mL) (10).

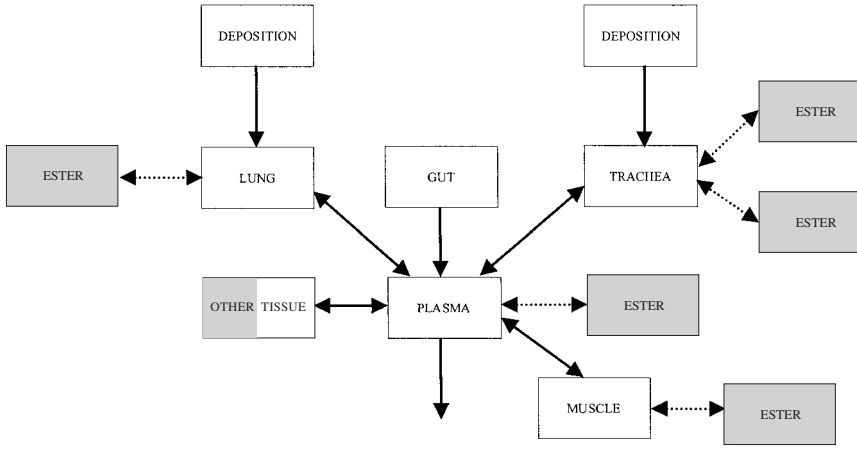



**Figure 10** Compartment model used to model the kinetics of BUD and BUD-esters in rat after inhalation and intravenous administration. Dotted arrows represent Michaelis-Menten kinetics and solid arrows represent first-order processes. Dark compartments represent BUD-ester compartments. (Redrawn from Ref. 9.)

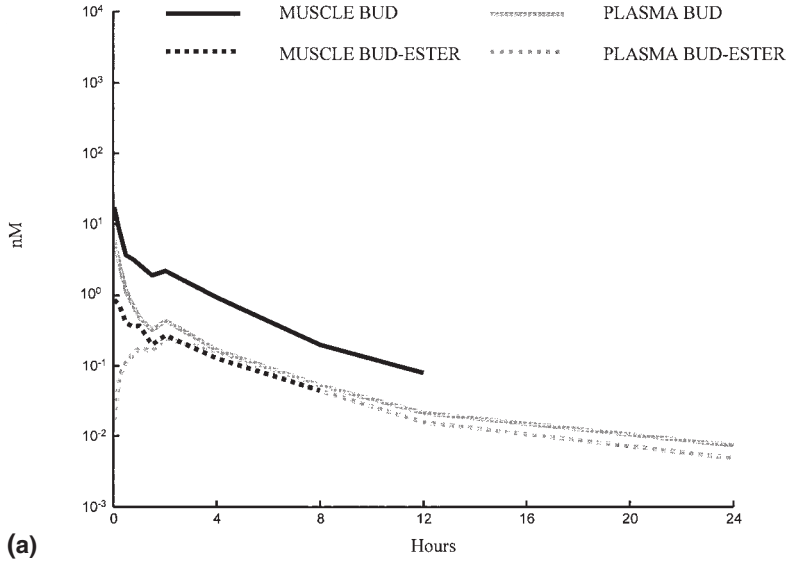
The $k_m$ for the Michaelis-Menten formation and hydrolysis processes, respectively, was assumed to be the same in all tissues except in the second BUD-ester compartment in trachea.

The $V_{max}$ for the Michaelis-Menten processes describing the formation and hydrolysis of BUD esters was unique for each tissue. The parameters were the same after both intravenous administration and inhalation, except in trachea and lung, where different $V_{max}$ values for the ester formation were used.

### B. Results

BUD concentrations were measurable in all tissues up to 24 hours except in plasma after inhalation (up to 12 h) and in muscle after i.v. (up to 12 h). BUD-ester concentrations were measurable in all tissues up to 24 hours except in muscle after i.v. (up to 8 h). The longest terminal disappearance half-life of BUD-esters was found in trachea and the shortest in muscle, about a 10-fold difference. The mean concentrations of BUD-ester were at later time points higher than the mean concentrations of BUD in trachea and lung, while they were lower in muscle and plasma (Fig. 11). AUC was much greater in the trachea than in other tissue (Fig. 12).

The compartmental model described the experimental concentrations of BUD and BUD-esters well in all tissues (Fig. 13) after both i.v. administration and inhalation. Parameters, obtained by fitting the model to the experimental data, are given in Table 4.



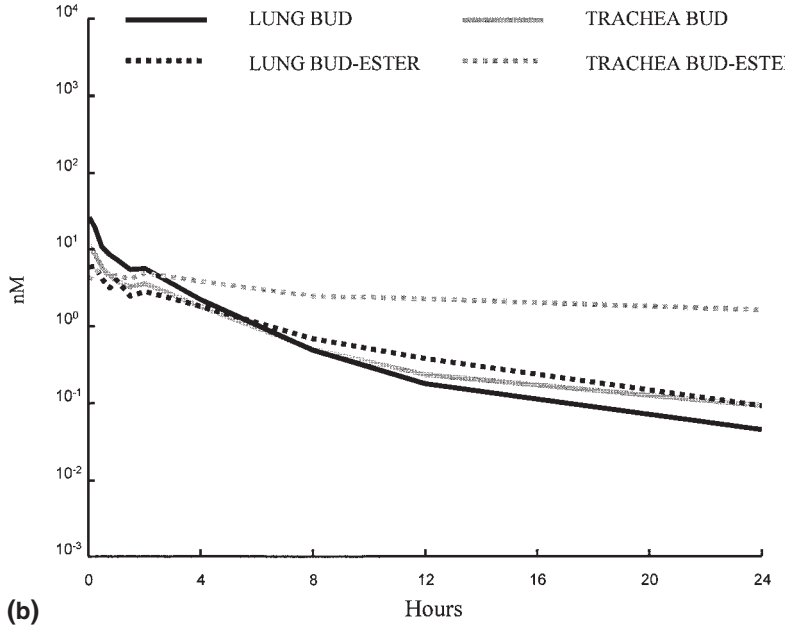
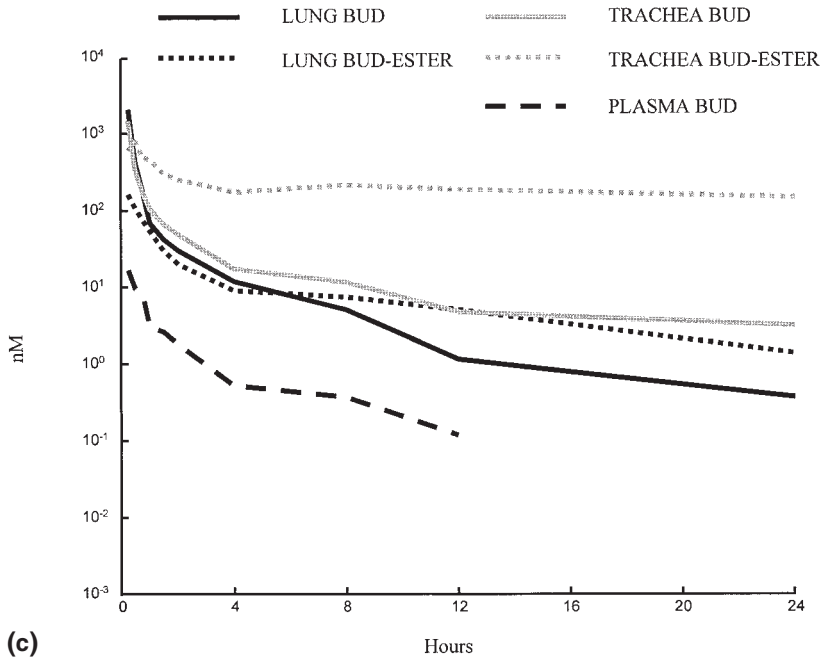




**Figure 11** (a) Intravenous administration (4 nmol BUD/rat): Experimental BUD and BUD-ester concentrations in rat soleus muscle and plasma. (b) Intravenous administration (4 nmol BUD/rat): experimental BUD and BUD-ester concentrations in rat lung and trachea. (c) Inhalation (55 nmol BUD/rat): experimental BUD and BUD-ester concentrations in rat. (Redrawn from Ref. 9.)



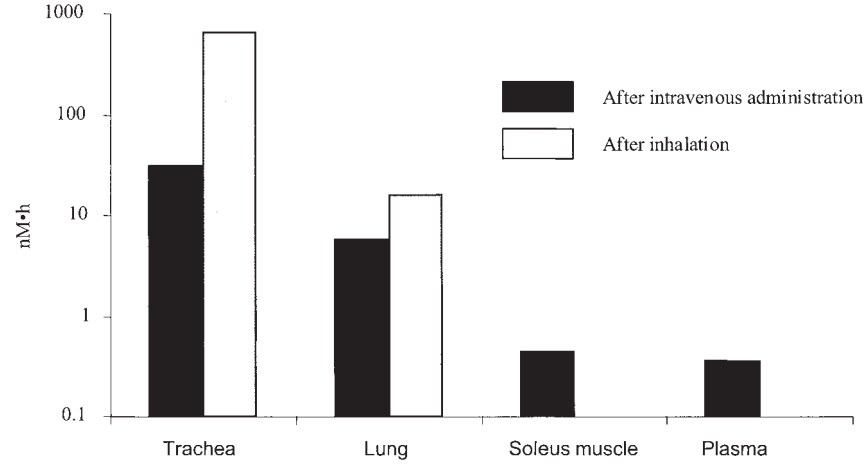




**Figure 12** $AUC_{0\text{-}\infty\,h}$ for BUD-esters in different tissues, normalized to $AUC_{0\text{-}\infty\,h}$ for BUD in plasma.

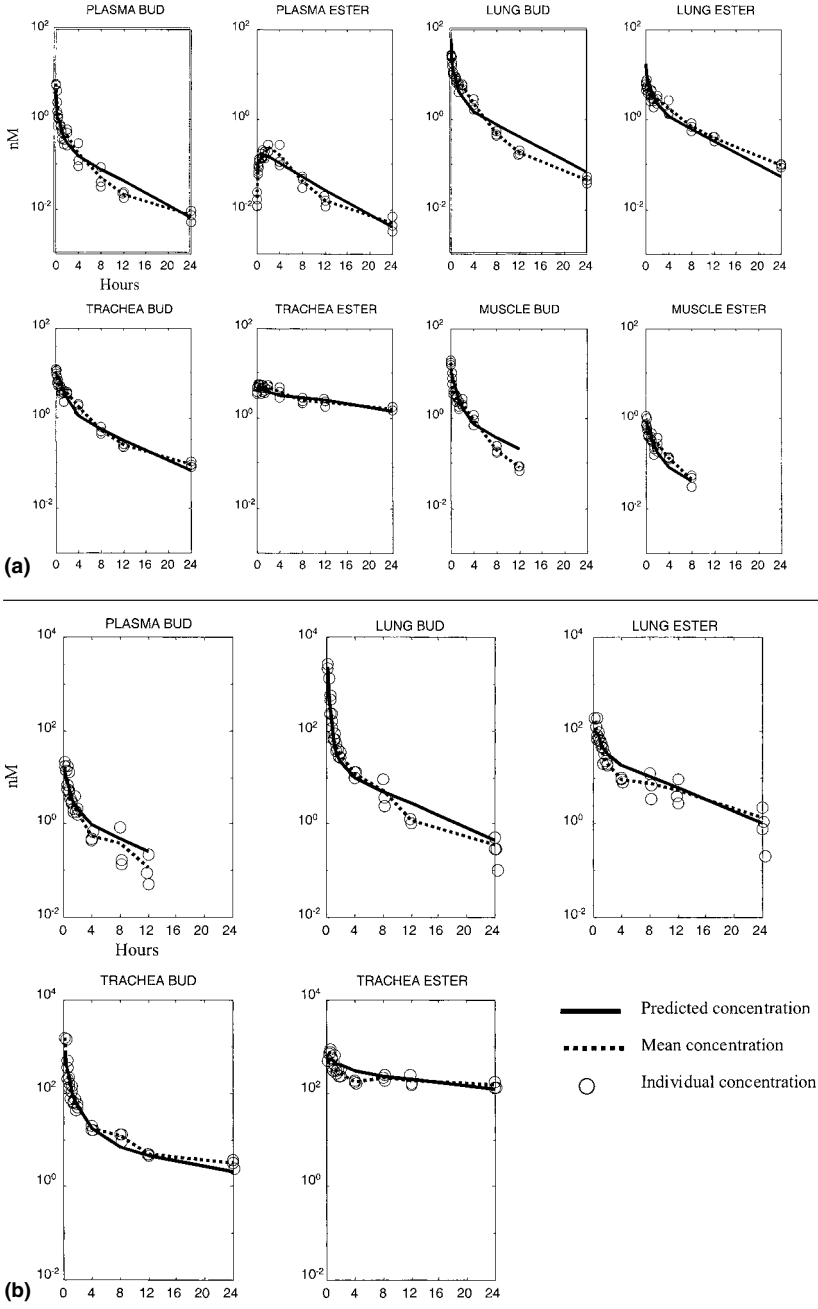



**Figure 13** (a) Model-predicted (solid lines) and experimental concentrations (means connected with dotted lines) after intravenous administration of 4 nmol BUD/rat. (b) Model-predicted (solid lines) and experimental concentrations (means connected with dotted lines) after inhalation of 55 nmol BUD/rat. (Redrawn from Ref. 9.)

**Table 4** Parameters of BUD and BUD-Esters Found by Noncompartmental Analysis and by Fitting the Multicompartmental Model to Experimental Rat Data

| | Ester terminal disappearance $t_{1/2}$(h) | Ester AUC[a] (nM*h) | BUD absorption[b] $K_m$ (min) | BUD reabsorption[c] $t_{1/2}$ (min) | Ester synthesis $V_{max}$ (nmol/h) | Ester hydrolysis $V_{max}$ (nmol/h) | Ester synthesis $K_m$ (nM) | Ester hydrolysis $K_m$ (nM) |
|---|---|---|---|---|---|---|---|---|
| Trachea | i.v. 20 | 31 | | | i.v. 26 | | | |
| | inh .18 | 662 | 14 | 216 | inh 220 | 328 | 30 | 230 |
| Trachea 2nd ester compartment | | | | | i.v. 0.26 inh 0.60 | 0.020 | 2600 | 710 |
| Lung | i.v. 5.6 | 6 | | | i.v. 97 | | | |
| | inh 6.6 | 16 | 2.6 | 13 | inh 280 | 951 | 30 | 230 |
| Muscle | i.v. 2.5 | 0.4 | 10 | 0.5 | 794 | $56 \cdot 10^3$ | 30 | 230 |
| Other tissue | | | 150 | 2.6 | | | 30 | 230 |
| Plasma | i.v. 5.4 | 0.4 | | | 0.201 | 4.4 | 30 | 230 |

[a] Normalized to BUD plasma AUC.
[b] To plasma.
[c] From plasma.

The dissolution of drug was rapid both in trachea and lung. Absorption and reabsorption between the tissues and plasma were rapid processes, except for reabsorption to trachea and absorption from the "other tissue" compartment to plasma. Based on the model fitting, we may conclude that all tissues except plasma and the second trachea compartment appear to have a high synthetic capacity for BUD-esters as judged from the estimated $V_{max}$ values. Hydrolysis was most effective in muscle.

### C. Discussion

The two presented animal kinetic studies show that BUD-esters are formed in a variety of tissues. It is further shown that the airways, especially trachea, possess a high capacity to form esters and that the esters are retained for a prolonged time.

The presented semi-physiological compartment model describes well experimental data from two different studies with two different administration routes. The model offers an explanation and parameterization of both the distribution of BUD and the tissue-specific formation and hydrolysis of BUD-esters after intravenous and inhalation administration in rat.

A possible explanation for the need for different $V_{max}$ for the formation of BUD-esters in lung and trachea after the i.v. versus the inhalation routes of administration might be an asymmetric cellular distribution of the enzymes responsible for the esterification of BUD, such that a higher enzyme concentration is found at the luminal than at the endothelial side of the airways. This could explain why more BUD-esters are formed after inhalation, compared to intravenous administration. The differences in $V_{max}$ among the different tissues are probably an indication of different amounts or activity of the enzymes responsible for the formation and hydrolysis of BUD-esters. BUD-esters in trachea seem to behave more nonlinearly than BUD-esters in the other tissues, and a sum of two compartments with different Michaelis-Menten kinetics (both $k_m$ and $V_{max}$) was needed to describe the kinetics of BUD-esters in trachea. Again, one can only speculate about the reason for this finding, but it might be a different distribution of the enzymes involved in the synthesis and hydrolysis of the BUD-esters. Further animal studies are needed to measure tissue-specific $V_{max}$ and $k_m$ values.

## IV. Concluding Remarks

This chapter has discussed the novel intracellular esterification mechanism that the glucocorticoid BUD possesses and has confirmed the presence of esters in the tissues analyzed in the present study (lung, trachea, plasma, and muscle). It has further been shown, on a theoretical basis, that the esterification mechanism might lead to *both* a prolonged effect duration and an increased lung selectivity of an inhaled steroid. A detailed description of the tissue and plasma kinetics of both

BUD and BUD-esters has been obtained through two rat studies using the intravenous and inhaled routes of administration. The experimental data obtained from the rat studies have been parameterized in a compartment model, and some fundamental insight in the behavior of BUD and BUD-esters has hereby been obtained.

The presented pharmacokinetic model may, apart from increasing the understanding of BUD and BUD-ester kinetics, be used to predict the concentration profiles of BUD and BUD-esters following other dosing regimens; the effect of coadministration of local esterification inhibitors on the duration of action and airway selectivity; and the effect of changing the dissolution profile of the BUD formulation.

In humans, further work is needed to model and fully characterize the pharmacokinetics of BUD and BUD-esters in a way similar to the above presented. Since it is impossible to obtain the same richness in experimental data in humans, the presented rat model could be used to guide the modeling efforts in humans. The rat model might also be used for interspecies scaling.

The esterification mechanism appears to offer a novel mode of improving the local pharmacokinetics of new chemical entities. In preclinical drug discovery projects, molecular structure could be optimized towards affinity to the enzymes responsible for esterification and hydrolysis and towards the affinity to the receptor concerned, thus offering a novel way of altering tissue retention and improving duration of action and local targeting.

## Acknowledgments

We thank Per Strandberg for performing the inhalation study, Hanna Falk Nilsson for performing the intravenous study, and Staffan Edsbäcker for valuable discussions and comments on the manuscript.

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## Discussion

**Dr. Schleimer:** It is interesting that there appear to be two compartments for the budesonide esters in the lung. Is this due to separate locations for the esterification step and the actual storage site in the lungs? Do you know the location of either the acyltransferase or the deacylase?

**Mr. Jendbro:** The need for two BUD-ester compartments, with first-order kinetics, in the lung in the presented preliminary model probably reflects the nonlinear behavior of the esterification process. These two ester compartments have been substituted with one compartment, utilizing nonlinear Michaelis-Menten kinetics instead of first-order kinetics, in the refined model presented in this chapter. The location of the enzymes involved in the esterification and hydrolysis processes are presently not known, but, interestingly, the rat model suggests that at least the tracheal acyltransferases are differentially exposed to budesonide entering from the airways relative to drug entering from the systemic circulation.

**Dr. Derendorf:** In some of your simulations, the terminal half-life in plasma is shorter than in tissue. Is this kinetically possible?

**Mr. Jendbro:** According to the present model, a longer half-life in airway tissues is possible, the reason being that strict equilibrium between tissues and plasma never is reached. First, different tissues have different capabilities to generate reversible depots of budesonide esters. Notably the esters are retained intracellularly within the respective tissue, and there is no indication of any redistribution of nonhydrolyzed budesonide esters in the body. Second, from a mass balance point of view, the airway tissues contain only a small fraction of drug in the body, and plasma kinetics of budesonide is governed by more rapid processes seen as a rather short half-life of budesonide. Of course, the vast majority of drug in plasma (and rapidly equilibrating tissues) is also distributed into airway tissues. So in these airway tissues there will be a mixture of budesonide coming from systemic circulation and from intracellular depots, where budesonide esters are slowly hydrolyzed to intact budesonide. Thus, the observed half-life in plasma will be shorter than that in tissue.

**Dr. Seale:** In your rat experiments, after delivering budesonide via the inhaled route, your graphs revealed some difficulty detecting esterified budesonide in muscle tissue, whereas it was detected in muscle following i.v. administration of budesonide. What is the explanation for this difficulty after inhaled administration?

**Mr. Jendbro:** This is correct. As tritium-labeled drug was used in the i.v. experiments and nonlabeled in the inhalation experiments, limits of quantitation

differed, and we were not able to systematically quantitate nonlabeled drug in muscle following inhalation. However, the scarce samples where quantitation was possible did not point towards any difference in muscle tissue in ester formation or hydrolysis depending on route of administration, which the model also predicts.

**Prof. Dolovich:** Can these models simulate clinical factors such as mucus layer, bronchoconstriction, and hyperreactivity and determine the effects on the selective absorption and dissociation site of inhaled steroids?

**Mr. Jendbro:** The general model presented treats the airways as one, homogeneous tissue, and thus no distinction can be made regarding local subcompartments. The effect and airway selectivity simulated in the first part of my presentation refers to glucocorticoid receptor occupancy. The rationale behind simulating receptor occupancy is that a majority of glucocorticosteroid-induced effects are receptor triggered. Of course, bronchoconstriction changing regional deposition can be built into the model taking mucociliary clearance in different regions into consideration, but this would make life much more complicated and does not address the basic question I tried to address—that of esterification and its effect on airway selectivity.

**Dr. Seale:** It would be hard to build bronchoconstriction into the rat model because the agents that would be used to induce bronchoconstriction may increase permeability (and hence absorption of drug).

**Dr. Schleimer:** I am wondering if you have had the chance to determine the rate of acylation in some of the tissues susceptible to side effects such as adrenal gland, skin, or bone?

**Mr. Jendbro:** Skin and bone were sampled during the study but have not been analyzed due to analytical difficulties.

**Dr. Brattsand:** The clinical efficacy of budesonide, FP, and mometasone furoate in rhinitis correlate largely with their nasal bioavailability, which in turn depends on their dissolution rate/water solubility.

**Dr. Derendorf:** Important for the efficacy is the unbound concentration at the site of action. This is governed by clearance and protein binding. Changes in volume of distribution will change the half-life, but not the average steady-state concentration.

# Part Five

## AIRWAY–LUNG SELECTIVITY OF CURRENT INHALED STEROIDS

# 14

# Airway Selectivity of Current Inhaled Corticosteroids in Properly Designed Studies

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## I. Methodology of Well-Designed Clinical Studies

Countless studies, involving either normal volunteers or asthmatic patients, have investigated some aspects of systemic activity, or efficacy of inhaled corticosteroids (ICS), but only a minority have been well designed. For obtaining useful clinical data about new drugs the most informative studies are those that (1) explore several doses from which a dose-response can be derived, (2) are placebo controlled and blinded, (3) allocate treatments by a random method, and (4) investigate both efficacy and toxicity. For ICS, it is difficult to design such studies, particularly for efficacy, as the time course for response extends over days and weeks. Some studies of systemic absorption of ICS in normal volunteers (1–3) and asthmatics (4,5) have incorporated some of these design features.

Crossover designs tend to be used in normal volunteers, whereas parallel group designs are more common in patient studies that tend to be conducted over a longer period of time than normal volunteer studies. Similarly, placebo controls are more often used in normal volunteer studies than for patient studies, as it may be unethical to offer placebo instead of ICS to patients with asthma, particularly if the duration of the treatment period is weeks or months. Most studies have investigated only one dose of drug, so there are very few studies that fulfill the ideal

criteria of a randomized, blinded, placebo-controlled multiple-dose trial. A recent meta-analysis of efficacy and safety of inhaled corticosteroids reviewed 14 trials, each of which involved a fixed dose of fluticasone compared to at least twice the dose of budesonide or beclomethasone (6).

### A. Quantitating Drug Delivery

Few studies pay much attention to this aspect of drug design, which is taken for granted if drugs are given orally or intravenously. The respirable fraction, which is highly dependent upon particle size (7), the characteristics of the delivery device, and the inspiratory effort of the subject will determine the extent of intrapulmonary deposition and thus the systemic availability of the inhaled drug. The use of large-volume spacer devices usually increases the respirable dose and decreases the amount of drug deposited in the oropharynx (8). Thus, spacer devices minimize the contribution of swallowed drug to systemic activity.

The contribution of absorption from the gut varies inversely to the extent of hepatic first-pass metabolism, which is high for fluticasone (99%), moderate for budesonide (90%), and less for beclomethasone (~70%). Thus, for budesonide 20–25% of systemic activity may come from gut absorption rather than direct absorption across the lung.

Drug-specific dry powder devices such as the Diskhaler and Turbuhaler have quite different characteristics, such as particle size and flow characteristics, resulting in considerable differences in intrapulmonary deposition (9).

All these variables mean that comparisons between inhaled corticosteroids, based on the nominal dose (ex-device), will necessarily be relatively crude estimates.

### B. Measuring Systemic Absorption

In the past plasma cortisol concentrations were the only measurements used to investigate systemic absorption of ICS. This is an indirect method because it depends upon a complex biological system comprising the hypothalamic-pituitary-adrenal (HPA) axis with its inherent variability. On the other hand, it does reflect the biological activity of the systemically absorbed drug, which provides more information than drug concentrations in isolation (10). The extent to which the systemic activity of inhaled corticosteroids on the HPA axis correlates with clinically important effects, such as easy bruising (11) and alterations in markers of bone metabolism (12), is uncertain. Further carefully planned long-term studies will be required to determine whether there are consistent correlations.

#### *Plasma Cortisols*

Morning plasma cortisols (usually taken between 8 and 10 a.m.) are extremely variable and therefore insensitive indices of suppression of HPA axis (10). A more sensitive measure is the 24-hour integrated plasma cortisol levels ($AUC_{24}$)

or urinary free cortisol excretion. $AUC_{24}$ cortisol, measurements which require repeated blood collections, are conducted in closely supervised laboratory studies. On the other hand, fractionated overnight and early morning urinary cortisol collections, corrected for creatinine excretion, are suitable for less intensively monitored clinical studies. This test is more sensitive than single morning cortisol levels, and it therefore is more reliable in detecting systemic activity of inhaled corticosteroids.

A third test sometimes carried out is the cosyntropin stimulation test in which this synthetic ACTH is injected to assess whether there is any impairment of adrenal cortisol reserve. It is not known whether this is any more sensitive in detecting HPA axis dysfunction than overnight urinary cortisols or 24-hour AUCs, although one study has suggested that this may be the case (12).

Studies that rely merely upon measuring plasma cortisol in single morning samples will underestimate the degree of systemic absorption of inhaled corticosteroids.

### *Direct Measurements of Plasma Concentrations*

With the development of sensitive assays it is now possible to measure the relatively low concentrations of corticosteroids that enter the systemic circulation following inhaled drug delivery (13,14). In general, there is rapid absorption following inhaled drug delivery so sampling times should be early after taking the dose. Studies in which drug concentrations have been measured have been carried out almost exclusively in normal volunteers.

Consideration should be given to whether the true characteristics of the systemic kinetics of the drug can be obtained from single-dose studies or steady-state studies. For drugs such as fluticasone with a terminal phase half-life of approximately 14 hours, single-dose studies may not reflect the situation following repeated dosing. Thus, with repeated twice-daily dosing of inhaled fluticasone, the average plasma concentration was approximately 1.7 times greater than after a single inhaled dose (14).

In summary, there are many factors, in addition to dose and drug, that may influence the clinical effect of inhaled corticosteroids. These factors should always be considered in papers describing comparative trials of inhaled corticosteroids to allow full interpretation of the study and comparison of the drugs. Some of the more important factors are:

Trials should be of a randomized, double-blind, and preferably placebo-controlled design. However, the use of different inhaler devices and the need to maintain asthma control may make such trials almost impossible, especially in patients with severe asthma.

Precise details of the devices and inhalation technique used to administer the corticosteroid are essential.

The unit dose delivered by the inhaler may influence delivered dose. The

amount of drug substance delivered to the lungs by five puffs of 50 μg of a corticosteroid from a pressurized metered dose inhaler (pMDI) may, for example, be greater than that delivered by one puff of 250 μg from an inhaler of the same type.

The time interval between actuation of the pMDI and inhalation should be standardized and specified when polycarbonate spacer devices are used as part of the delivery system. The cleaning and washing recommendations given to the trial participants should also be described, as the condition of the spacer device influences its electrostatic charge and thus its delivery characteristics.

Patients must receive full instruction in the correct use of all inhaler devices included in trials. Without this, results may be confounded by differences in efficacy or ease of use of the inhalers, especially if a familiar inhaler is compared with an unfamiliar inhaler.

Patient compliance with different forms of inhaled therapy may vary, so some measure of patient compliance should be included and documented.

Each of these factors could influence the measured effect of an inhaled corticosteroid by at least the same extent as doubling or halving the administered dose. The value of comparative studies of inhaled corticosteroids that ignore these factors in therefore doubtful. The optimal design of a comparative trial of corticosteroids is not yet established, but true differences between corticosteroids are most likely to be detected in well-designed trials that control for these factors.

## II. DO STUDIES IN NORMAL SUBJECTS ACCURATELY PREDICT DRUG BEHAVIOR IN ASTHMATIC PATIENTS?

Studies are frequently carried out on normal subjects as part of the clinical development program for new drugs. For inhaled drugs, there are theoretical reasons to expect that the intrapulmonary deposition and systemic bioavailability may differ between asthmatic subjects and normal subjects. First, the airways obstruction of asthma may limit the distribution of inhaled drug to peripheral sites from which systemic absorption can readily occur. Second, increased permeability of the airways, which is a feature of asthma, may enhance drug absorption (15). There are three types of studies that have compared systemic drug bioavailability in asthmatics and normal subjects.

### A. Studies That Compare a Given Drug in Normal and Asthmatic Subjects

Several studies have addressed this question. Lipworth and Clark (16) administered nebulized salbutamol (40 μg/kg) to three groups (each of 10 individuals),

comprising normal subjects, mild asthmatics, and patients with severe asthma. The concentration of salbutamol was measured in blood samples taken at 5, 10, 20, and 30 minutes after completion of the inhaled dose, and systemic responses were assessed by changes in finger tremor, heart rate, and serum potassium. For the patients with severe asthma ($FEV_1$ 49.2% of predicted normal), the salbutamol $C_{max}$ and $C_{av}$ were less than the values recorded in normal subjects and those with mild asthma. Furthermore, the increases in heart rate and finger tremor were less in the severe asthmatics than in the normal subjects and mild asthmatics. Serum potassium was not significantly different in any of the groups. Thus, both these pharmacokinetic and pharmacodynamic measurements showed that the systemic absorption of salbutamol was less in the patients with severe asthma. It is probable that the airways obstruction in the asthmatic subjects reduced peripheral distribution of the drug, thereby limiting its systemic absorption.

The findings in studies of intrapulmonary deposition of inhaled salbutamol are consistent with the results of pharmacokinetic and pharmacodynamic studies. A group of asthmatics (mean $FEV_1$ 1.45 L) were compared with normals after inhaling 200 μg of technetium-labeled salbutamol (17). While the intrapulmonary deposition, assessed by dual-headed gamma camera, did not differ between the two groups, the percentage of the intrapulmonary dose distributed to the periphery was 30.4% in the asthmatic subjects compared with 44% in the normal subjects ($p < 0.05$).

For sodium cromoglycate, urinary excretion can be used as an index of systemic bioavailability because less than 2% of drug is absorbed after oral administration and after intravenous administration the drug is excreted unmetabolized in the urine. In an early study (18) it was found that the urinary excretion of cromoglycate was reduced in patients with chronic bronchitis compared with normal controls, but it was not reduced in asthmatic subjects. In a more recent study it was found that the lung bioavailability of cromoglycate (administered via a Spinhaler) was reduced in asthmatics compared with normal subjects, reflected by reduced plasma $C_{max}$ and AUC, together with reduced urinary excretion of cromglycate (19).

After inhaling nedocromil 4 mg from a pMDI, the calculated bioavailability was 9.2% of the nominal dose in normal subjects and 5.7% in asthmatic subjects. The asthmatic subjects had a delayed $T_{max}$ and lower values for $C_{max}$ and AUC compared with the normal controls (20).

Comparisons between asthmatics and normal subjects can be made from two studies, each of which used the same drug doses and delivery devices for 7 days of treatment (3,5). In 20 normal subjects, the mean suppression of integrated AUC for plasma cortisol was 86% after fluticasone 200 μg (via Diskhaler) daily and 47% after budesonide 1600 μg (via Turbuhaler), whereas the values in 23 subjects with asthma (mean $FEV_1$ 80% predicted) were 34% and 16%, respectively. In a well-designed crossover study the pharmacokinetics of fluticasone were

determined in normal volunteers ($n = 13$) and asthmatic subjects ($n = 10$; mean $FEV_1$ 54% predicted) after the administration of i.v. and inhaled fluticasone 1000 μg (21). The systemic availability was significantly less in asthmatic subjects than in normals (10.1% vs. 21.4%; $p < 0.001$) and the suppression of cortisol was less in asthmatics. The systemic availability of fluticasone correlated with diffusing capacity but not $FEV_1$.

In contrast, a study of normal subjects and patients with mild asthma (morning PEF 67–113% predicted) reported no significant differences in plasma fluticasone AUC between asthmatics and normals after inhaling fluticasone 1000 μg (22). In another recent study, it was found that the systemic bioavailability of fluticasone 1500 μg (via Accuhaler) was less in a group of asthmatics (mean $FEV_1$ 60% predicted) than in 46 normal subjects, whereas the systemic bioavailability of budesonide (1600 μg via Turbuhaler) was not significantly different (23).

Thus, the evidence indicates that the systemic bioavailability of several classes of drugs (such as bronchodilators, cromoglycate, and inhaled corticosteroids) is reduced in asthmatic subjects compared with normal controls, presumably because the airways obstruction in asthmatic subjects limits the delivery of the drug to the major absorption sites in the periphery of the lung. The reason why reduced bioavailability in asthmatics should occur with fluticasone but not with budesonide may relate to differences in physicochemical properties of these steroids.

### B. Studies That Compare Different Drugs in Normal Subjects

Comparative studies of this kind have been confined to ICS and relevant studies to consider are those that have explored several doses of ICS. Three such studies (1–3) have shown the ratio of systemic activity (on a mg-for-mg basis) for fluticasone to budesonide ranged from 3.7 to 1.7. These studies have used one of the more sensitive indices of systemic activity, the integrated 24-hour plasma cortisol level. In the study with the lowest value (3), fluticasone and budesonide were administered via their dry powder devices (Diskhaler and Turbuhaler, respectively). Since Turbuhaler has a relatively high intrapulmonary deposition compared with other delivery devices (24), this study may have underestimated the systemic activity of fluticasone.

### C. Studies That Compare Different Drugs in Asthmatic Subjects

Several studies have compared different ICS in asthmatic subjects. Since most of these studies have used low doses, which did not affect plasma cortisol (25), they do not permit potency ratios to be calculated for the ICS. Only two published studies have compared repeated administration of fluticasone and budesonide, in doses sufficiently high to cause some cortisol suppression (4,5). These were double-blind, randomized crossover studies in which the drugs were taken twice daily for 4 and 7 days (Table 1). Measures of integrated cortisol secretion (10 h overnight

**Table 1** Comparative Multiple-Dose Studies

| Study (Ref.) | Design | Daily Dose, μg[a] (Device) | | Duration (days) | F:B potency ratio | |
|---|---|---|---|---|---|---|
| | | F | B | | $AUC_{0-24}$ | a.m. cortisol |
| Normals | | | | | | |
| Boorsma et al., 1996 (1) | Crossover placebo-controlled ($n = 21$) | 400, 750 2000 (MDI) | 400, 800 2000 (MDI) | 4 | 3.7 | 5.2 |
| Grahnén et al., 1997 (3) | Parallel group ($n = 20$) | 100, 200 500, 1000 (DH) | 100, 200 400, 800 (TBH) | 7 | 1.74 | 2.3 |
| Donnelly et al., 1997 (2) | Crossover placebo-controlled ($n = 28$) | 750, 1500 2000 (MDI) | 800, 1600 3200 (MDI) | 5 | 2.9 | 3.1 |
| Asthmatics | | | | | | |
| Clark and Lipworth, 1997 (4) | Double-blind crossover ($n = 12$) | 500, 1000 2000 (MDI) | 500, 1000 2000 (MDI) | 4 | >1[b] | 3.5 |
| Derom et al., 1999 (5) | Double-blind crossover ($n = 23$) | 400 2000 (DH) | 400 1600 (TBH) | 7 | >1[c] | >1 |

[a]Given in 2 divided doses.
[b]10 h overnight urinary cortisol.
[c]$AUC_{0-20}$ 0–20 h postdose.
$n$ = Number of subjects; AUC = area under plasma cortisol curve; MDI = metered dose inhaler; F = fluticasone; B = budesonide; DH = Diskhaler; TBH = Turbuhaler.

urinary cortisol and $AUC_{0\text{-}20}$h plasma cortisol) and morning plasma cortisol revealed that fluticasone had a greater systemic activity than budesonide. The ratios could not be quantitated by conventional criteria (e.g., $ED_{50}$) because neither drug in either study consistently produced greater than 50% inhibition. Log linear interpolation of 8 a.m. plasma cortisols revealed a F:B potency ratio of approximately 3:1 (4). Since dry powder devices (Diskhaler and Turbuhaler) were used in the Derom study (5), it may have underestimated the systemic activity of fluticasone, given relatively high intrapulmonary deposition of budesonide from the Turbuhaler at approximately 30% of dose (24) compared with a lower percent from Diskhalers (17).

In summary, it has been possible to accurately determine the potency ratios for systemic activity of ICS in normal subjects, using multiple doses to derive dose-response curves, and the estimated potency ratio for the newer ICS (fluticasone and budesonide, F & B) ranges of 2–3:1 (see Table 1). There are less accurate determinations of ICS potency ratios in asthmatic subjects because there is less systemic absorption, making quantitation of any effect imprecise. Nevertheless, the small number of studies that have used reliable methodology have indicated that fluticasone appears to be more potent than budesonide per μg nominal dose (26).

## III. Variability in Systemic Bioavailability Between Subjects

### A. Drug Absorption

Following inhalation the plasma pharmacokinetics of ICS vary considerably between individuals. In normal subjects, there were wide standard deviations in $C_{max}$,$T_{max}$, and AUC following inhalation (14) and intravenous (27) doses of fluticasone, suggesting that there are considerable differences between individuals' pharmacokinetics. Similar variability was observed with inhaled budesonide in normal subjects (28) and with fluticasone in asthmatic subjects (21). On the available evidence there is no reason to think that the variability between subjects is any greater in asthmatics than in normal subjects.

### B. Systemic Activity

Since there is considerable variability between individuals in drug absorption following inhaled administration of ICS, a similar and possibly greater variability might be expected in biological activity, which depends on the end result of plasma concentration, receptor sensitivity, and postreceptor translation. Measurements of AUC plasma cortisols have shown wide variation in the extent of inhibition for any chosen inhaled ICS among normal subjects (1,2,28). Although

studies in asthmatics have not been as comprehensive as in normal volunteers, the available data suggest that there are considerable differences in susceptibility to cortisol suppression between individual asthmatic subjects (4).

## IV. Comparative Clinical Studies

To draw comparisons between different inhaled doses of the same corticosteroid or between different corticosteroids, some general aspects of the dose-response relationships of inhaled corticosteroids need to be considered. For a given drug or inhaler, there will always be a dose below which no effect can be detected, no matter which investigative method is used. There will also be a dose range within which an effect is measurable for one or more outcomes. Within a certain dose range, there will be a linear (or log linear) relationship between the magnitude of the effect and the dose of drug. At a certain dose, the dose-response curve flattens out. Increasing the dose beyond that point is associated with only small changes in the outcome measurement. This means that the most important information that needs to be obtained to accurately compare the various drugs includes:

The lowest dose level at which an effect can be detected
The dose required to produce a certain effect (i.e., 50% of maximum), which is important for an accurate potency comparison
The slope of the dose-response curve, which is important for an accurate potency comparison and for assessment of the risk of overdosing; the steeper the slope, the higher the risk
The dose at which the dose-response curve starts to flatten out, which is important to assess the appropriateness of dosing in comparative trials

A number of dose-response studies (29–32) have provided useful information about different corticosteroids or inhalers. They all report marked improvements in the outcome variables that are most often measured in clinical asthma studies, that is, symptoms and lung function, at low daily doses (100–200 μg/day) of inhaled steroids in the type of patients—with mild or moderate asthma—that are most often studied in these clinical trials. The additional improvement achieved in these parameters by increasing the doses is small, often taking an additional fourfold increase in dose to produce further statistically significant effects. This means that, as low doses are clinically so effective, even very large, well-conducted studies usually fail to show any statistically significant or clinically relevant additional effect on symptoms and lung function between two adjacent and doubling doses on the dose-response curve (29,31,32). This has important implications when trying to draw conclusions from comparative trials of the clinical effect of different inhaled corticosteroids, when often halving or doubling doses are compared and no significant differences are found in effect between the test drugs.

Another factor that complicates the interpretation of clinical studies with inhaled corticosteroids is that differences in the measured response to treatment are influenced by the duration of treatment and monitoring in the study. This is because symptoms of asthma usually show a beneficial response within days, while maximal improvement in lung function may not occur for several weeks (33), and maximal improvement in airway hyperresponsiveness takes months to years of treatment with inhaled corticosteroids (34). Most dose-response studies of inhaled corticosteroids have been of short duration, so potential differences between the effects of different drugs or doses may not have become apparent for some outcome variables.

Though a substantial number of comparative studies have been performed, it has been difficult to draw firm conclusions about the comparative efficacy of different inhaled corticosteroids, for all of the reasons discussed above. However, these studies have suggested that budesonide administered by Turbuhaler and fluticasone administered by Diskhaler are approximately equipotent in efficacy, and these treatment modalities both seem to be more potent (on a nominal dose basis) than beclomethasone dipropionate (BDP) administered by pMDI or Rotahaler or budesonide administered by pMDI. Moreover, fluticasone by Diskhaler seems clinically more potent on a μg-for-μg basis than triamcinolone pMDI (35). Further well-designed comparisons are, however, required to confirm these suggestions.

## V. Systemic Unwanted Effects

The established inhaled corticosteroids have an excellent clinical safety profile, when used in doses needed by most patients to achieve symptom control, even with long-term use. However, concern exists about the long-term use of higher doses of inhaled corticosteroids leading to significant systemic unwanted effects, including adrenal suppression (36,37), bone demineralization (38), and, in children, impairment of growth (39,40). Therefore, it is important also to assess and compare the risk of systemic unwanted effects from various inhaled corticosteroids. This information is also required to accurately assess the therapeutic index.

Clinically relevant systemic unwanted effects should ideally be studied within the context of controlled, long-term clinical trials, which use clinically relevant doses in patients whose disease severities and ages are similar to the groups in which the drugs would normally be prescribed. Such studies require large numbers of patients and are difficult to conduct. As a substitute, the systemic effects of the various inhaled corticosteroids are often studied in short-term, crossover studies on healthy volunteers or patients with mild disease, who will tolerate treatment with placebo for a certain period. A recent study suggested that systemic effects are more likely in healthy volunteers than in asthmatic subjects (21). Therefore, the clinical relevance of findings from such studies for patients with moderate and severe asthma is not known.

### A. Hypothalamic-Pituitary-Adrenal Axis

Comparisons of the systemic effects of inhaled corticosteroids have mainly focused on the effects upon the hypothalamic-pituitary-adrenal (HPA) axis. Measures of HPA axis function provides the most sensitive and easily measured markers of systemic effects of inhaled corticosteroid therapy. Effects can be demonstrated with very short duration of dosing, and therefore this measurement is often used to compare and contrast different inhaled corticosteroid preparations. The clinical significance of small alterations in HPA axis function measured under controlled, artificial laboratory conditions is, however, doubtful. Some reduction in cortisol secretion merely reflects the normal functioning (feedback) of the HPA axis–control mechanisms in response to exogenous steroid rather than a clinically significant abnormality, and the total corticosteroid exposure of the body may remain within the physiological range. Significant laboratory findings are, therefore, not necessarily predictive of important clinical effects.

Despite these limitations, the systemic effect studies conducted so far do allow some conclusions:

All the currently available inhaled corticosteroids can produce suppressive effects on the HPA axis, and the effect is dose-dependent (1,5,41–43).

Overall, the systemic potency ratio of budesonide to fluticasone depends upon the inhaler devices compared and on whether the assessment is after single or repeated dosing. The systemic potency ratio between fluticasone pMDI and budesonide pMDI on a μg-for-μg basis has usually been around 3:1, i.e., three times as much budesonide is required to produce the same degree of systemic effect as fluticasone (1,44,45). For the DPIs this ratio seems to be around 1.5:1 in adults (5) and around 1:1 in children (46).

The risk of HPA axis effects with BDP pMDI is somewhat higher than that with budesonide pMDI (47), but there is inadequate information to calculate an accurate systemic effect ratio for BDP versus budesonide or fluticasone.

Higher doses of fluticasone demonstrated a twofold greater effect on the HPA axis when compared to higher doses of triamcinolone acetonide in adult asthmatics (48).

### B. Bone Density

Osteoporosis is an important complication of the use of ingested corticosteroids, particularly in high-risk patients, such as postmenopausal women (49). This occurs through an increase in bone resorption and a decrease in bone formation, and results in increased risk of fractures, especially hip and spine. Inhaled corticosteroids have been demonstrated to have effects on bone metabolism, although there is little evidence that, at the conventionally used doses, they cause osteoporosis, and no evidence that they cause increased risk of fractures.

The effects of inhaled glucocorticosteroids on bone metabolism have been demonstrated by measuring serum osteocalcin, which indicates changes in bone formation, and urinary hydroxyproline, measured after a 12-hour fast, which is increased with increased bone resorption. Pyridium cross-links in urine is another measure of bone resorption, which has the advantage over urinary hydroxyproline of not being dietary dependent; however, to date the effects of inhaled glucocorticosteroids on this measure of bone resorption have not been reported.

The effects of BDP and budesonide on serum osteocalcin and urinary hydroxyproline have been studied in adults. Both have been shown to influence serum osteocalcin levels in a dose-dependent manner (50), but only BDP increases urinary hydroxyproline excretion at doses up to 2000 μg/day. In children, doses of budesonide of less than 800 μg/day (51) and of fluticasone of 200 μg/day (52) have no effect on any biochemical marker of bone turnover.

Several studies have measured bone densitometry in adult asthmatics taking inhaled corticosteroids. In one study, adult patients were taking a mean dose of inhaled BDP 630 μg/day over 2 years (53), while in another a mean dose of BDP or budesonide of 980 μg was given for 3 years. In addition in the EUROSCOP trial, evaluating the efficacy of inhaled budesonide in chronic obstructive lung disease (COPD), older patients (mean age 52 years) were treated with inhaled budesonide 800 μg/day for 3 years (54). In none of these studies was there any evidence that these patients had increases in bone loss. Also, to date no studies have demonstrated that these biochemical markers of bone turnover are associated with increased risk of bone fracture.

### C. Posterior Subcapsular Cataracts

These occur more frequently in patients taking ingested corticosteroids, and this greatly complicates the issue of whether they occur with greater frequency in patients using inhaled glucocorticosteroids. Most studies in adults (55) and children (56) suggest that, once the confounding effect of ingested glucocorticosteroids is removed, there is no evidence that inhaled glucocorticosteroids increase the risk of developing posterior subcapsular cataracts. One recent study has, however, indicated that high inhaled doses of BDP is associated with a slightly greater risk of posterior subcapsular cataracts (57) in older patients. This study did not, however, stratify for the known risk of cataract formation associated with atopy (58).

## VI. Summary

The most rigorously designed studies of airway selectivity of the currently available ICS have been carried out in normal volunteers during short-term administration. These studies have shown that all of the ICS are absorbed via the lungs if sufficiently high doses are given. In general, the rank order of putative potency for anti-inflammatory activity is maintained with respect to systemic biological activ-

ity. The few well-designed studies in asthmatic subjects have indicated that the systemic bioavailability of ICS is less than in normal subjects, presumably because of the airways obstruction and the decreased surface area for drug absorption. Preliminary data suggest that there may be differences in systemic bioavailability between different ICS, possibly related to their physicochemical characteristics.

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## Discussion

**Dr. Brattsand:** My comment goes back to the difference in plasma cortisol diurnal variation between normals and patients with Cushing's disease, where the major difference is that these patients lack the afternoon and early night drop of plasma cortisol. Obviously the more prolonged trigger period mediates the adverse steroid actions seen in these patients. This suggests that such adverse actions are induced first when the receptor-triggering period surpasses a critical length, as a probable physiological adaptation to prolonged stress. Thus, the duration of a steroid peak in plasma may be at least as important as its initial height (see Chapter 14). Therefore, to minimize the adverse steroid actions after inhalation, the receptor triggering in the systemic compartment should ideally not last much longer than happens during normal diurnal cortisol variation.

**Dr. Derendorf:** Continuous administration of steroids will produce more cumulative effect than multiple dosing of the same dose.

**Dr. Seale:** Dr. Hochhaus, what assumptions are made about percent of receptor occupancy for efficacy in your computer modeling?

**Dr. Hochhaus:** We assume a direct relationship between receptor occupancy and efficacy; thus, maximum efficacy will be observed when 100% of receptors are occupied in the lung. Whether an asthmatic will feel a difference between a 50% or 70% receptor occupancy, I don't know.

**Dr. Rohdewald:** We not only have to look for percent of receptors occupied in the target tissue producing the wanted clinical effect, we should also know how many receptors have to be occupied at the peripheral sites to produce the unwanted effects. Presence of drug only, irrespective of the concentration, is not a sound reason for concerns.

**Dr. Schleimer:** The possibility that dividing doses of oral steroids, or using alternate daily doses, leads to maintained efficacy with reduced side effects has been thoroughly debated. I believe there are studies showing reduction of HPA suppression with alternate daily doses. It is my impression, however, that the cognoscenti believe that it is the cumulative dose that is most relevant with respect to osteoporosis.

**Dr. Derendorf:** It is a general concept of pharmacology that the unbound concentration at the response site is responsible for activity. One experimental way to measure unbound concentrations in the tissues is microdialysis.

**Dr. Hochhaus:** What if the diffusion into tissues is not homogeneous in all tissue because of transporters? We have shown the involvement of transporters in

the brain and distribution of glucocortcoids, which resulted in lower free glucocorticoid levels in the brain than in other tissues.

**Dr. Derendorf:** For FP, the amount of drug appearing in the blood mirrors the disappearance of the drug in the lungs, since there is little oral absorption.

**Dr. Pedersen:** Budesonide seems to be clinically more effective in the nose than other novel lipophilic steroids. Presumably this is due to budesonide being better absorbed because of its lower lipophilicity. To what extent does the same mechanism play a role in the airways? Does a lower fraction of the deposited drug become absorbed if the steroid is more lipophilic?

**Dr. Hochhaus:** I believe that it is not necessarily the lipophilicity affecting the uptake into the tissues, but the lipophilicity will affect the residence time in the lung or nose and consequently how fast the drug is leaving the lung. We have shown that there is an optimal release rate in the upper part of the lung and that there is a the need to design drugs that slow this release or dissolution pattern.

**Dr. Persson:** Topical airway steroids must dissolve in hydrophilic mucosal surface lipids before being absorbed across the lipophilic mucosal membranes. The importance of a good balance between hydrophilicity and lipophilicity with airway steroids may be seen especially in treatment of rhinitis where highly lipophilic nonabsorbed drugs in part may be lost through a runny nose (or through mucociliary clearance). The physical properties of budesonide leading to an efficient entry into mucosal tissues may explain why this drug is clinically more potent than highly lipophilic nasal steroids that are more potent than budesonide at the receptor level.

**Dr. O'Byrne:** Is the systemic absorption higher in mild vs. severe asthmatics?

**Dr. Seale:** There was no difference between healthy subjects and patients with mild asthma in cortisol suppression or plasma drug concentration following one week's treatment with FP via Diskhaler (Lofdahl et al. 1999). Taking these together with the Brutsche et al. data and Harrison et al. data from ERS 99, it appears that for the lipophilic steroids there is a difference in systemic exposure correlating with disease severity but that this difference does not appear to exist for budesonide. In addition, in the October issue of *Chest*, Weiner et al. showed a very clear correlation between lung function parameters and effect on HPA axis, so that the more normal the lung function, the greater the cortisol suppression. One factor contributing to these findings is the more central airway drug deposition seen in patients, which results in a greater mucociliary elimination, particularly for the slowly dissolving steroids.

**Dr. O'Byrne:** Perhaps we should stop doing these sorts of comparisons using adrenal output as the marker of systemic activity, as the results in normal subjects do not reflect what is happening in asthmatics and it has not been possible

to demonstrate meaningful differences when asthmatics are studied, even using excellent study designs.

**Dr. Busse:** There has been considerable interest in the genetic basis of drug responses to leukotriene metabolism. What information do we have about the genetic variants or genotypes in relationship to the response to corticosteroids?

**Dr. Seale:** I am not aware of any studies addressing this question.

**Dr. Hochhaus:** We have performed studies in human lung which showed that human lung differs in the $B_{max}$ and $K_d$ values of glucocorticoid receptor interaction and not only in the pharmacokinetic behavior. As an example, we measured 10-fold differences in the $K_d$ values in these samples. I would like to ask whether there have been sufficient studies performed that looked at pharmacokinetic and pharmacodynamic variability in patient populations?

**Dr. Busse:** There is no information available. We need to know about the different metabolic pathways for each steroid as well as racial/ethnic differences and polymorphisms. The disease process has been studied to some extent in terms of efficacy but not drug metabolism.

**Dr. Schleimer:** Dr. Hochhaus, is the variability that you have seen at the level of receptor number or receptor affinity or both?

**Dr. Hochhaus:** There have been differences in receptor number and affinity.

**Dr. Derendorf:** It is the pulmonary absorption that is decreased in asthmatics, not the gastrointestinal absorption. That may explain why the difference between normal subjects and asthmatics is most pronounced for fluticasone where there is no gastrointestinal absorption.

**Prof. Dolovich:** Deposition measurements have shown that intraindividual variability is greater than interindividual variability, which parallels clinical response data to some degree. Control of inhalation parameters for delivery of the test aerosolized drug/formulation should be part of any clinical trial protocol to try and reduce this variability.

**Dr. O'Byrne:** The reasons for the differences between normal subjects and asthmatics in systemic absorption must relate in some way to where the drug is going, possibly because of airway narrowing, mucus plugging, etc.

**Dr. Szefler:** Are you saying that we should no longer do studies of inhaled steroids in normal subjects?

**Dr. O'Byrne:** I think that the differences in the sensitivity of the adrenal suppression with IS between normal subjects and asthmatics makes it difficult to defend the use of normal subjects in comparing and contrasting the systemic effects of different IS, and then extrapolating these results to effects in asthmatics.

**Dr. Rand:** How might decreased adherence with ICS over time in clinical trials have influenced growth retardation results? Particularly, the marked differences between year 1 retardation and later years?

**Dr. O'Byrne:** This may potentially confound the results obtained. However, in the long-term studies conducted by Soren Pedersen, the clinical benefit of IS persisted, suggesting that adherence was reasonable, but the slowing of growth did not.

# 15

# Childhood Asthma and Growth

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## I. Introduction

Twenty years ago, the majority of children with asthma received only intermittent anti-asthma treatment in association with exacerbations of the disease. Evolution of treatment since that time has included the use of continuous treatment with theophylline, $\beta_2$-agonists, or sodium cromoglycate for children with moderate or severe, persistent asthma; some patients with very severe disease were also given daily or alternate-day prednisolone or inhaled corticosteroids. During the last decade, asthma therapy has included early introduction of anti-inflammatory medications, especially inhaled corticosteroids. This means that at many clinics inhaled corticosteroids are now given to patients with mild and moderate asthma severity, rather than reserving this therapy for the most severe cases. This change in treatment strategy, together with new knowledge about the pathophysiology of the disease, has provided useful information supporting a more aggressive introduction of inhaled corticosteroids into the treatment regimens of both children and adults. While this change in therapy seems justified both upon pathophysiological findings and efficacy data, it is often questioned whether it is also justified from a safety point of view. Thus, many pediatricians are still concerned about potential

adverse effects of long-term treatment with inhaled corticosteroids, particularly on growth.

The widespread use of inhaled corticosteroids for all disease severities has also made treatment with inhaled corticosteroids more complex. Thus it seems that conclusions obtained in trials in patients with a certain age and asthma severity may not always be valid for patients of a different age or disease severity. Clinical trials have repeatedly found that the vast majority of school children with mild asthma are very well controlled on a daily dose of 100–200 μg inhaled corticosteroid and that such therapy is as effective as or more effective than other antiasthma therapy in the majority of such patients (1). Several studies have also suggested that the systemic effects of a certain inhaled corticosteroid are more pronounced in patients with mild asthma than in patients with more severe disease (2–4). This means that the clinical relevance of the findings in studies using daily doses of inhaled corticosteroid ≥400 μg per day in patients with mild asthma should be questioned since such doses are rarely needed in patients with this disease severity. Clinical use of inhaled corticosteroids must be based upon the knowledge about beneficial and adverse effects obtained in controlled clinical dose-response trials in patients with a similar age and disease severity as the group in which the drug is going to be used.

The systemic effect potential of therapy with inhaled corticosteroids is still an issue of concern, and great efforts have been made to develop new inhaled steroids and inhalers with even less systemic activity for a given clinical effect. Although it seems that some inhaled steroids or inhalers have a higher clinical potency and/or a lower potential for systemic effects than others, there is still much debate about the long-term clinical importance of this since only few good, prospective, controlled trials have been designed to assess this. Until such trials exist, transferral of conclusions from one drug-inhaler combination to another drug-inhaler combination should be made with great caution.

In the following review, the influence of the asthma disease itself and the use of inhaled corticosteroids on growth will be discussed in some detail since this issue often causes great concern among prescribing pediatricians, patients, and their parents. Furthermore, some factors important for the assessment of the clinical relevance and general applicability of the findings of growth studies will also be briefly summarized.

## II. Definitions and Study Designs

The safety and occurrence of systemic effects of inhaled corticosteroids in children have been extensively studied over the past 20 years without a clear definition of the various terms used to describe these issues. Often no distinction is made between a *measurable systemic effect* and a *clinically relevant systemic side*

*effect*. This may lead to unwarranted and unnecessary fear among physicians and patients. Whether a systemic effect is measurable or not depends entirely upon the sensitivity of the method used for the measurement: when more sensitive methods are utilized, more systemic effects become measurable. Thus, for a given drug or inhaler, there will always be a dose below which no systemic effects can be detected no matter which method is used. As the dose increases, there will be a dose range within which systemic effects are measurable in one or more systemic effect models. However, more often than not, these measurable effects merely reflect small changes within the normal range of the normal biological feedback system, or they may be chance findings without clinical relevance (small changes in plasma cortisol levels frequently sampled under artificial laboratory conditions). With regard to growth there are several examples of detectable systemic effects the clinical relevance of that must be questioned: sodium cromoglycate treatment has been found to be associated with significant effects upon the excretion of growth hormone in the urine (5) and markers of bone metabolism (6). Treatment with inhaled $\beta_2$-agonists has also been found to adversely affect the secretion of growth hormone (7,8). Although statistically significant, these findings are probably not clinically relevant, though thorough clinical studies have not yet assessed this.

Another potential pitfall in studies of measurable systemic effects is that such studies are normally short-term or single-dose, standardized, crossover studies on healthy volunteers or patients with very mild disease, who will tolerate treatment with placebo for a certain period. The clinical relevance of findings from such studies to patients with more severe asthma is not known. Recent studies suggest that significant differences may exist between findings in patients and healthy volunteers (4), the systemic effects being markedly higher in healthy volunteers than patients. Similar differences may be seen between patients with mild and more severe disease (2,3).

Our knowledge about the dose level at which measurable systemic effects of inhaled corticosteroids are seen in short-term controlled laboratory studies in healthy volunteers or patients with mild disease is quite good. It varies with different steroid/inhaler combinations (9,10), and conclusions from one drug/inhaler combination cannot be extrapolated to other drug/inhaler combinations. Though the clinical relevance of the finding in such studies may be questioned, it can probably be assumed that doses of an inhaled corticosteroid not associated with any measurable systemic effects in sensitive laboratory test systems are also clinically safe.

Clinically relevant systemic side effects should be studied in controlled, long-term clinical trials, using clinically relevant doses in groups of patients with a disease severity and age similar to the groups in which the drugs would normally be prescribed. Such studies require large numbers of patients and are difficult to conduct.

### A. Delivery Device

A clinically very effective inhaler with high intrapulmonary deposition of drug will be expected to have a higher systemic effect than a clinically less effective inhaler. However, lower doses can be used if the inhaler is very effective, emphasizing the importance of dose titration. On the other hand, if the bioavailability after oral dosing is not zero, the contribution of the orally deposited drug to the systemic effect will be higher for an inhaler with a low intrapulmonary and high oral drug deposition than for an inhaler with a lower oral drug deposition and the same or higher intrapulmonary drug deposition. This may be clinically important since differences in inhaler characteristics may result in a threefold difference in therapeutic index for some inhaled corticosteroids (Fig. 1).

The clinical implication of this is that for inhaled corticosteroids with a high gastrointestinal availability of drug, the risk of unwanted systemic effects can be reduced without loss of efficacy by choosing an inhaler/drug combination with a high therapeutic index and tailoring the dose to the severity of the disease. Some recent controlled clinical trials on growth have not undertaken such measures (11–14). They found that longitudinal statural growth was retarded in children

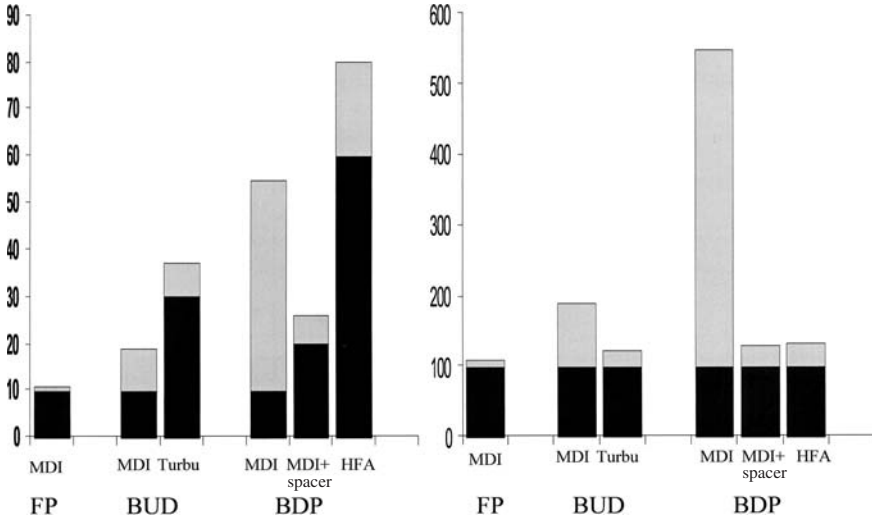


**Figure 1** (Left) Systemically available drug after inhalation of 100 μg of different corticosteroids from different inhalers. Black = Absorption from the lungs; gray = absorption from the gastrointestinal tract. (Right) Amount of drug that becomes systemically available if the patient inhales an amount of drug that would result in deposition of 30 μg of drug in the intrapulmonary airways. FP = Fluticasone propionate; BUD = budesonide; BDP = beclomethasone dipropionate; MDI = CFC metered dose inhaler; Turbu = Turbuhaler; HFA = Hydro Fluro Alkan.

treated with a fixed (no dose tailoring) daily dose of 400 μg beclomethasone dipropionate. Moreover, all used a pMDI or dry powder device for delivery. These devices deposit >85% of the dose in the oropharynx in children. This fraction of drug is extensively systemically absorbed due to a low first metabolism of beclomethasone dipropionate (15–17). Therefore, the therapeutic index of the treatment in these trials would have been better if a spacer device had been used for the delivery, since a spacer reduces oropharyngeal deposition of drug (15). Hence the clinical effect: systemic effect ratio would have been improved and the adverse effect upon growth probably reduced or even abolished if a spacer device and dose tailoring had been used (Fig. 1).

It is beyond the scope of this chapter to discuss in detail how the delivery characteristics of various inhalers influence the therapeutic index and the risk of clinically important systemic side effects. It is, however, important to realize that due to marked differences between the various products, conclusions from one inhaler/drug combination may not be transferable to other drug/inhaler combinations.

### B. Dosing Regimen

Once-daily dosing of inhaled corticosteroids is becoming more widely used in patients with mild disease severity. Although more studies are needed, preliminary evidence suggests that once-daily administration of budesonide may be associated with less effect on short-term lower leg growth rate and markers of collagen and bone turnover than the same budesonide dose divided into twice-daily dosing (18–20). Furthermore, little is known about the effect of once-daily dosing on the therapeutic index of the various inhaled corticosteroids.

### C. Pharmacodynamics

The clinical effects of inhaled corticosteroids are best evaluated in dose-response trials. A number of such studies have been performed in adults (10), but only a few have been done in children. The findings in such studies are important for our understanding of the clinically relevant doses of inhaled corticosteroid to use in growth trials in various patient groups. Therefore, the published dose-response trials conducted in children will be briefly presented.

#### *Budesonide*

A placebo-controlled study of 404 children with moderate asthma severity (21) assessed the dose-response relationships for lung function and other clinical outcomes with budesonide delivered through the multiple-dose dry powder inhaler, Turbuhaler in daily doses of 100, 200, and 400 μg. All doses produced statistically significant effects. The difference between placebo and low-dose budesonide was

much greater than the difference between low-dose and high-dose budesonide, the lowest dose producing a near maximum effect on most outcomes with little additional benefit being obtained by the increase in dose. Moreover, the differences in clinical effect between individual dose steps were not statistically significant.

A double-blind crossover study of 19 schoolchildren with moderate to severe asthma compared the effects of 100, 200, and 400 μg/day budesonide given by pMDI + large-volume plastic spacer (22). Morning PEF values and $FEV_1$ for all three doses were significantly better than for placebo. There was no dose-response effect in PEF values measured at home or diary recordings of symptoms and rescue $\beta_2$-agonists, indicating that the top of the dose-response curve had been reached at the lowest dose for these outcomes. However, a dose-response effect with significant differences between the effects of individual doses was seen in the $FEV_1$ values measured at the clinic. Moreover, the fall in $FEV_1$ or $FEF_{25-75\%}$ after exercise proved to be a sensitive marker of dose-response with significant differences detected between adjacent doses.

### *Fluticasone Propionate*

The effect of 100 and 200 μg/day fluticasone propionate Diskhaler® was compared with that of placebo among 169 asthmatic children (23). Both doses of fluticasone propionate led to significant improvements in PEF, lung functions, asthma symptom scores, and exacerbations over 6 and 12 weeks, but as in the other dose-response studies, no significant difference was seen between the effects of the two doses of fluticasone propionate.

Finally, a double-blind, dose-reduction trial in 216 children with moderate asthma found that the minimal effective daily dose of inhaled corticosteroid required to maintain optimal lung function and clinical control and prevent exercise induced asthma was 188 μg budesonide from Turbuhaler® and 180 μg fluticasone propionate from Diskhaler (24).

All these studies share some common features. They all demonstrate marked and rapid clinical improvements and changes in symptoms and lung function at very low daily doses (around 100 μg) of inhaled steroids in most children with mild, moderate, and even severe asthma (10,22,25,26). Additional improvement in these parameters with increasing doses is rather small, often taking an additional fourfold increase in dose to produce further significant effect. Low doses are clinically so effective that even very large, well-conducted studies normally fail to show any statistically significant or clinically relevant additional effect on symptoms and lung function when the dose is increased beyond 100 μg per day (10). In agreement with this, a large number of studies have found that the beneficial effects of low doses of inhaled steroid (200 μg/day) in children are normally more pronounced than for any other antiasthma drug to which they have been compared. (12–14,27–37).

## III. Growth

It is clear from efficacy studies in pediatric patients that 100–200 μg of inhaled steroid per day is more effective than any other treatment in the majority of patients and that these doses control the disease satisfactorily in the majority of patients with mild and moderate disease. Therefore, it is mainly relevant to assess the risk of clinically important systemic effects of such doses of inhaled steroids when comparing the clinical effect : side effect ratio with other drugs. When reviewing the literature about the safety of inhaled steroids in that dose range, it becomes clear that at present, no controlled studies have reported any clinically relevant systemic side effects with daily doses of 100–200 μg of orally inhaled corticosteroid (10,38–40).

Measurement of clinically important systemic effects of higher doses (>200 μg/day) of inhaled corticosteroids is mainly relevant for comparisons of different steroids or inhalers in order to define a therapeutic index. Knowledge obtained in such studies may also be of help in deciding whether a child whose asthma is not optimally controlled on a certain dose of inhaled corticosteroid should have this dose increased or additional therapy, such as a long-acting $\beta_2$-agonist. Findings in such studies are generally not relevant for decisions about choice of therapy between inhaled corticosteroids and other asthma drugs since doses of inhaled corticosteroids that are equi-effective with other asthma drugs are normally lower.

For inhaled steroids, the vast majority of safety data, including data on growth, has been obtained in school children with mild asthma who have not required the doses of inhaled corticosteroid under investigation to be optimally controlled. Furthermore, many studies have been conducted under conditions very different from the actual conditions occurring during day-to-day treatment. As mentioned earlier, conclusions from such studies should be applied with great caution to the day-to-day treatment of patients with mild or moderate asthma severity or treatment of patients with more severe disease, who may actually require the doses used in these studies to control their disease. Clinically relevant safety data should be obtained in clinical trials, which tailor the dose of inhaled steroid to the severity of the disease. Often the findings in such "dose-tailored" studies are different from the conclusions of studies carried out in the laboratory or from studies in which patients are overtreated due to a fixed (often high-dose) dosing regimen that does not allow for dose adjustments as indicated by the individual's clinical picture.

When the effects of steroids on growth in children are assessed, it is important to appreciate that growth may be divided into three distinct age-related components (41):

1. The rapid, rapidly decelerating, growth of the first 2–3 years of life. This phase is probably controlled by the same factors that are important for fetal growth, the main one being nutrition.

2. Childhood growth, which occurs from approximately, age 3–11 years. This phase mainly represents the contribution of the endocrine system, particularly growth hormone.
3. Pubertal growth, which depends largely on a combination of growth hormone and sex steroids.

Since the importance of the various factors that affect growth seems to differ between these three phases, studies should preferably be performed in all three age groups separately. Even though our knowledge about this area is still quite sparse, the importance of age for the growth-retarding effect of inhaled corticosteroids is supported by the findings in two recent studies, which found that the growth-retarding effect of an inhaled corticosteroid administered for one year was more marked in prepubertal than pubertal school-age children (12,42). This lends further support to the view that conclusions drawn from results obtained in one age group should be applied to other age groups with great caution.

### A. Influence of Disease

Most chronic diseases of childhood have been shown to adversely affect the normal growth pattern of a child. The most commonly observed influence of the asthmatic condition on growth is a reduction in growth rate, most often seen towards the end of the first decade of life (Fig. 2) (43–50). This reduced growth rate continues into the mid-teens and is associated with a delay in the onset of puberty. The prepubertal deceleration of growth velocity resembles growth retardation. However, the delay in pubertal growth is also associated with a delay in skeletal maturation so that the bone age of the child corresponds to the height. Ultimately, there is no decreased final height, although it is reached at a later than normal age (43–50). This difference in growth patterns seems to be unrelated to the use of inhaled corticosteroids, but it seems to be more pronounced in the children with the most severe asthma.

The deviant growth pattern seen in many children with asthma complicates the interpretation of results from cross-sectional studies comparing the heights of asthmatic children treated with inhaled corticosteroids with the heights of normal children or children with asthma who are not treated with inhaled corticosteroids. The results of Littlewood (51) illustrate this problem: a group of asthmatic children (mean age = 10.9 years) treated with inhaled beclomethasone had lower height standard deviations scores than a group of children never treated with inhaled corticosteroids (mean age = 6.6 years). The results of other studies (43–50) suggest that this difference might as well be due to differences in age as to differences in treatment between the two groups.

Recent studies suggest that a poorly controlled asthma may in itself adversely affect growth in populations of children who have never received inhaled corticosteroids. Thus, height standard deviation scores before treatment with in-

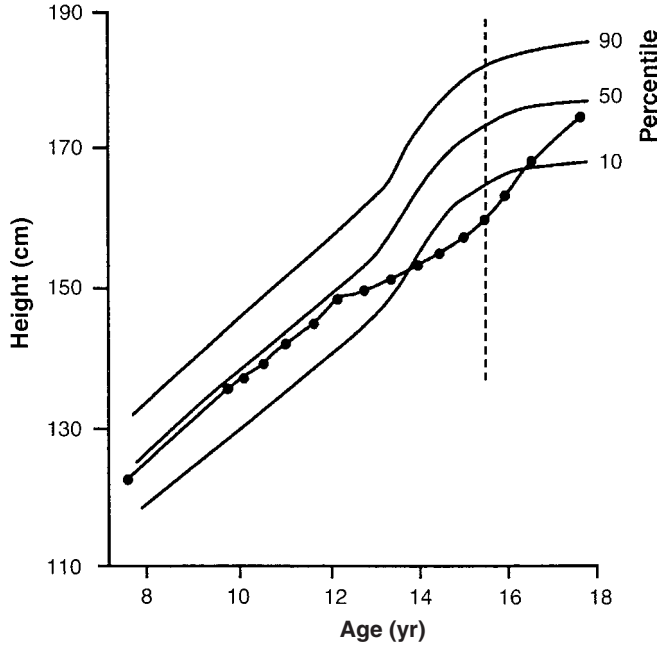


**Figure 2** The most commonly observed influence of the asthmatic condition on growth is a reduction in growth rate, which is most often seen toward the end of the first decade of life. The reduced growth rate continues into the midteens and is associated with a delay in the onset of puberty. This prepubertal deceleration of growth velocity resembles growth retardation. However, the delay in growth is also associated with a delay in skeletal maturation so that the bone age of the child corresponds to the height and, ultimately, there is no reduction in final height, which is reached at a later-than-normal age. This difference in growth pattern seems to be unrelated to the use of inhaled corticosteroids, but it seems to be more pronounced in children with the most severe asthma.

haled steroids were found to correlate significantly with lung functions (32,42) and degree of asthma control (52,53): the lower the lung function or poorer the asthma control, the lower the height standard deviation score. Furthermore, severe asthma was suggested to adversely affect expected final height in a retrospective study on a cohort of children with different degrees of asthma (54) and statural height in a large population based study on more than 3000 children with asthma (55). Exactly which mechanisms operate in poorly controlled asthma that can adversely affect growth is unclear. However, they may share some features with the factors operating in groups of children living under poor socioeconomic conditions, which have been shown to have an effect upon growth that is more pronounced than the effect of high-dose inhaled corticosteroids (55). These observations are

very important for our understanding of the various factors that should be considered and adjusted for when the general applicability of the findings of various controlled growth studies is assessed.

### B. Effects of Exogenous Steroids

When the effect of exogenous corticosteroids on growth is evaluated, the various studies can be arbitrarily but conveniently divided into:

*Growth marker studies*, which measure steroid-induced changes in various serum markers thought to reflect bone and collagen formation/degradation or growth

*Short-term studies*, which assess growth during periods of 6 months or less

*Intermediate-term studies*, which evaluate growth during periods longer than 6 months but do not assess final adult height

*Long-term studies*, which assess growth for many years and also include final adult height in relation to predicted adult height

This distinction is important to remember when the findings of a clinical trial are assessed.

Several studies have demonstrated poor correlations between short-term height velocity and annual height velocity (56–60) and between steroid-induced changes in short-term lower leg growth rate and statural growth during the subsequent year. One month lower leg length velocity explains virtually nothing of the variation in annual statural height velocity (59,60). In addition, the correlation between two consecutive annual height velocity values for normal prepubertal children is also very poor. A low gain in one year is not necessarily followed by a low gain the next year, and vice versa (59). The correlation between 1-, 2-, 3-, and 4-year values are only partially correlated with one another (59), and height velocity computed over a period of 3 or 4 years in childhood only explains 34% and 38%, respectively, of the variation in final height, respectively. Therefore, the clinically most important outcome measure of human growth is the final height in relation to expected final height, allowing for gender and mid–parental height differences. When the results from growth studies are evaluated, it is important to realize that a change in growth markers or an effect upon growth found in short- or intermediate-term studies is not necessarily equivalent to an effect upon long-term growth or final adult height.

### C. Markers of Bone Formation and Resorption

When the influence of various inhaled corticosteroids on growth is assessed, levels of various biological markers of bone and collagen formation and degradation or growth hormone concentrations and activity have been the most popular surrogate markers of statural growth studied.

Levels of all the markers of bone and collagen formation and resorption are usually measurable, as normal bone and collagen are in a constant state of turnover, maintaining a balance between resorption and formation. In simple terms, an elevation of all markers could occur when there is increased bone turnover without net loss or gain in bone mass, while a reduction of all markers, normally seen with low doses of oral steroids or high doses of inhaled corticosteroids, could signify a reduction in bone turnover with a constant bone mass. Therefore, it is probably most clinically relevant to consider the *net* effect of bone formation and bone resorption (61). If, for instance, formation and resorption decrease to the same extent, the changes may not be important since the net effect may be zero. An elevation of markers of bone resorption alone supposedly suggests net bone loss, whereas an elevation of markers of bone formation alone suggests net bone formation. However, no isolated marker can be considered a reliable guide to the extent of bone formation or resorption; moreover, the significance of some markers is not clear and the relevance of changes reported from short-term studies to long-term clinical outcomes remains to be demonstrated (62). However, evidence suggests that small but statistically significant changes in such markers are rarely of any clinical relevance.

### D. Inhaled Corticosteroids and Bone Markers

Reduced osteocalcin levels have been reported in children with asthma independent of whether or not the child received steroids (63), emphasizing that results from steroid-treated children with asthma should be compared with findings in children with asthma who are not receiving any exogenous steroids. Several studies have assessed markers of bone and collagen resorption and formation in such designs. They all found that only high daily doses of inhaled steroids (>400 μg budesonide) may have a detectable effect on some of these markers in children, suggesting a reduction in both bone formation and degradation at this dose. Daily doses of 400 μg or less of budesonide or fluticasone propionate had no effect in any of the studies (6,18,63–70). All studies were short term and involved patients with quite mild disease. In contrast, studies of children receiving low doses (2.5–5 mg) of oral prednisolone found significant reductions in serum levels of osteocalcin, PICP, and ICTP and in hydroxyproline excretion in the urine (67,71).

No adverse effects on markers of bone formation and degradation have been reported at standard pediatric doses of inhaled corticosteroids, whereas higher doses may cause significant changes, which suggest a reduced bone turnover rate. The importance of this finding has yet to be elucidated. Recent studies found no correlation between the levels of various markers and bone mineral density or between changes in levels of the various markers and changes in bone mineral density over 1 and 2 years (40).

### E. Growth Markers

During recent years the blood levels of various biochemical markers, such as growth hormone, somatomedin-1 (IGF-1), IGF-binding protein-3 (IGFBP-3), carboxyterminal propeptide of type-1 procollagen (PICP), and the amino terminal propeptide of type III procollagen (PIIINP), have been found to correlate to some extent with growth rate of lower leg growth velocity (72,73). However, the predictive value of drug-induced changes in the levels of these markers is not known. Thus, a recent controlled, prospective study found no correlation between the levels of various markers of bone and collagen formation and degradation or steroid-induced changes in these markers and growth during 1 and 2 years of budesonide treatment (40), indicating that the predictive value of steroid-induced changes in these markers is low and that assessment of changes is not clinically useful. This is in good agreement with the observation that growth markers have been found to correlate poorly with growth rate in healthy children (74) and the findings in a study that assessed the clinical usefulness of the various markers in children suspected of steroid-induced growth retardation and children with normal growth during treatment with inhaled corticosteroids. No clinically useful differences were found in growth hormone secretion, serum cortisol, ostecalcin, and IGF-1 levels or bone mineral density between the two groups (75).

Treatments with even low doses of prednisolone (2.5–5 mg/day) are associated with significant reductions in the levels of some of these markers, while daily doses of inhaled corticosteroids $\leq$400 μg are not (64,65,67,68,71). Furthermore, daily doses of beclomethasone and budesonide around 400 μg (range 200–1200 μg) do not adversely affect the output of urine growth hormone (76,77). The clinical relevance of findings of treatment-induced changes in growth markers remains unknown. Treatment with sodium cromoglycate has been found to be associated with significant effects upon the excretion of growth hormone in the urine (5) and markers of bone metabolism (6). Treatment with inhaled $\beta_2$-agonists has also been found to adversely affect the secretion of growth hormone (7,8,78). Although statistically significant, these findings are probably not clinically relevant, although thorough clinical studies have not yet assessed this.

Leptin is a recently discovered hormone that is believed to play an important role in regulation of body weight and perhaps also growth. Systemic steroids increase serum leptin levels, but daily doses of 800 μg budesonide from a pMDI with a spacer had no effect on this marker (79).

### F. Short-Term Studies

Knemometry measures changes in short-term linear growth of the lower leg within weeks. This may be a valuable adjunct/alternative to traditional growth studies since knemometry allows very controlled designs. However, the clinical implication of knemometry findings still needs further study since 1-month lower

leg growth velocity or steroid-induced changes in short-term in lower leg growth rate explains virtually nothing of the variation in annual statural height velocity (59,60). Furthermore, daily treatment with 2.5 or 5 mg prednisolone totally stopped lower leg growth (80,81). This indicates that knemometry is too sensitive and probably amplifies or exaggerates the growth-stunting effects of exogenous steroids. On the other hand, it also means that if an exogenous steroid has no adverse effect on lower leg growth in a properly performed knemometry study, it is most unlikely that such treatment will be associated with any growth suppression during long-term treatment. Thus, at present, no statural growth studies have found any adverse effects on statural growth of doses of inhaled corticosteroid, which in well-designed knemometry studies have been found not to adversely affect lower leg growth.

Several knemometry studies have evaluated the influence of inhaled budesonide delivered from a pMDI and a spacer (Nebuhaler) on short-term lower leg growth in school children (82–85). These studies, and a later meta-analysis, concluded that daily doses of budesonide ≤400 μg from pMDI and Nebuhaler did not adversely affect growth. In contrast, 800 μg budesonide per day from Nebuhaler or 400 μg/day from the dry powder inhaler Turbuhaler significantly reduced lower leg growth rate in the three studies evaluating these drug-inhaler combinations (82–84,86). Daily doses of 200 μg budesonide delivered from Turbuhaler or fluticasone propionate from Diskhaler did not adversely affect growth in the studies evaluating this dose (81,86,87). All children participating in these studies were mild asthmatics not requiring inhaled corticosteroids.

In preschool children, 200 μg budesonide per day from a Nebuhaler did not affect short-term lower leg linear growth in children aged 13–36 months (88), whereas 800 μg per day was associated with a significant reduction.

Beclomethasone dipropionate delivered from the dry powder inhaler Diskhaler has been assessed in two knemometry studies of different designs (89,90). In both studies, treatment with a daily dose of 400 μg was associated with significant growth suppression of lower leg growth. This growth suppressive effect of beclomethasone was significantly higher than that observed during treatment with 200 μg fluticasone propionate per day (89).

Budesonide from a Turbuhaler and fluticasone from a Diskhaler have been compared in a recent dose-response study (86). It was found that, μg for μg, the two drug-delivery combinations had similar effects. Doses of 200 μg/day did not adversely affect lower leg growth rate, whereas treatment with 400 μg/day was associated with a slight reduction in lower leg growth, which was significant for budesonide when compared to placebo but not when compared to fluticasone. No other inhaled corticosteroids have been compared with placebo in knemometry studies.

Short-term knemometry studies and long-term studies have found that treatment with oral steroids retards growth and induces changes in blood levels of

biochemical markers of growth (50,71,91–98), even at doses as low as 2.5 and 5 mg prednisolone per day (71,91,93,96). These effects have been consistently greater than the effects of 400–800 μg of inhaled corticosteroid.

### G. Statural Growth

It is well known that systemic steroids may adversely affect growth in children. The suppressive effect seems to depend upon the duration of treatment, dose, and frequency (94,95), and when treatment is stopped catch-up growth may occur (97,98). However, growth retardation caused by daily and alternate day administration of large doses of systemic corticosteroids for extended periods of time may be permanent (50,91).

Over the years the influence of inhaled corticosteroids on growth of asthmatic children has been studied extensively. There have been flaws in the designs of most studies. Several have been retrospective or uncontrolled. Others have been conducted under artificial conditions, which are very different from the day-to-day treatment situation. This makes it difficult for the clinician to draw firm, unequivocal conclusions about the clinical relevance of the findings. A brief summary of the findings and the conclusions is given below.

### H. Intermediate-Term Studies

A large number of intermediate-term studies have evaluated the effect of inhaled corticosteroids on statural growth (11,14,32,43,44,52,99–122). Until 1993, none included a control group. The vast majority have been in school-age children. Some have been historical follow-up studies, while others have been prospective, more or less controlled studies. A metered dose inhaler or a Diskhaler was used for the administration of beclomethasone and a Nebuhaler for budesonide administration. None of these studies, comprising more than 2000 children treated for mean periods of 1–13 years, found any adverse effect of the inhaled corticosteroid upon growth. In agreement with this, a meta-analysis of 21 studies representing 810 patients to some extent corroborated the findings in these studies. The analysis compared attained height with expected height of children with asthma treated with inhaled or oral steroids (123). Significant weak growth impairment was found in children receiving oral steroids, whereas children treated with inhaled steroids attained normal height. Furthermore, there was no statistical evidence of inhaled steroid therapy being associated with growth impairment either at higher doses or during extended therapy.

A recent follow-up of a cohort of 3347 children with asthma from general practices corroborated these findings, but in addition shed further light on the complexity of the growth process. The study found that the vast majority of children had normal growth rates. Only children receiving daily doses of inhaled cor-

ticosteroids of ≥400 μg showed growth impairment. However, this effect on growth was smaller than the effect of poor socioeconomic status or severe asthma (55). This study illustrates how important confounders may be in growth studies. Such confounders must be accounted for in the analysis of the data.

In contrast to these findings several studies of parallel group design conducted over the last few years have found significant growth retardation in children with mild asthma treated continuously for 9–12 months with a fixed daily dose of beclomethasone of ≥400 μg (11–14,39). This dose is markedly higher than the dose required to control mild asthma. Furthermore, a dry powder inhaler or a pMDI was used for the administration of beclomethasone. These devices deposit a large amount of drug in the oropharynx, which is extensively absorbed into the systemic circulation through the gastrointestinal tract, resulting in a subsequent increase in systemic effect. Therefore, the therapeutic index would have been better if a spacer device had been used for the delivery instead of a pMDI or a dry powder inhaler.

Tinkelman et al. (14) compared 400 μg beclomethasone with sustained-release theophylline in 195 asthmatic children 6–16 years of age. The observed mean growth rate for all children who had their first and last measurement greater than 100 days apart was 4.2 cm per year for the beclomethasone-treated children and 5.5 cm per year for the theophylline-treated children ($p < 0.005$). The growth-retarding effect was mainly observed in boys, and there was no significant difference between the two groups when the girls were studied separately.

Doull et al. (11) assessed the effect of daily doses of 400 μg beclomethasone on viral-induced wheezing episodes in 7- to 9- year-old children who, between episodes (around 5 per year), had no asthma symptoms requiring continuous inhaled corticosteroid treatment. Ninety-four children completed the study, the duration of which was 31 weeks. Growth rate during beclomethasone treatment was significantly lower than during placebo treatment, so that the placebo children grew on average 1 cm more during the 31 weeks of study. No catch-up growth was seen during the washout period.

Verberne et al. (12) found that the annual growth rate was significantly slower (4.7 cm/yr) in 35 children treated with 400 μg beclomethasone propionate per day than the growth rate in 32 children treated with salmeterol (6.1 cm/yr). A subgroup analysis revealed that the growth-retarding effect was only significant for prepubertal children. Pubertal children grew normally during beclomethasone treatment.

Similar findings were reported by Simons (13), who treated 241 children with mild asthma with either 400 μg beclomethasone dipropionate per day, placebo, or salmeterol for one year. Significant differences were observed in baseline height between the three groups at study entry. The annual growth rate was significantly lower in children treated with beclomethasone propionate (3.96 cm/yr) as compared with the growth rates in the other two groups (5.04 cm/yr) for placebo

and 5.40 cm/yr for salmeterol). The difference in growth rate between the three groups was due to a markedly lower growth rate in the beclomethasone-treated children during the first 3 months of the study. After this, the growth rate was similar in the three groups.

Somewhat different findings have been reported with budesonide and fluticasone propionate. A controlled, prospective study (32) measured growth in 216 children with asthma during long-term treatment with inhaled budesonide and 62 asthmatic children not treated with corticosteroids. The children were followed at 6-month intervals for 1–2 years without inhaled budesonide and then for 3–6 years on inhaled budesonide. During the period of budesonide therapy, the mean daily dose decreased from 710 to 430 μg. A pMDI or Turbuhaler was used for the administration of budesonide. No statistically significant differences were seen between the two groups in measured height or height SDS during run-in or the 3–6 years of treatment. Over the whole period the annual increase in height was 5.62 cm (controls) and 5.48 cm (budesonide). Because the dose of budesonide varied in each individual child during the treatment period, the influence of budesonide dose upon growth could not be accurately assessed. However, when high doses were used (>400 μg/day), both growth rate and lung functions were lower than during run-in and during treatment with 400 μg/day, indicating that either high doses or poor asthma control (or both) adversely affected growth. After 5 years of continuous treatment with a mean daily dose of 500 μg budesonide, statural height was still not significantly different between the two groups (124). A more recent analysis of these data confirmed the observation that growth rate was lower just before puberty (42). Moreover, an age-dependent effect was found on the growth-retarding effect of budesonide during the first year of treatment, the growth rate of young children (ages 5–10) being more reduced than the growth rate of pubertal children.

The prospective investigation of Merkus et al. (122) corroborated these findings. These investigators studied 40 asthmatic children who were randomized to treatment with 600 μg/day budesonide pMDI or placebo for 2 years. In addition, growth in these two groups was compared with the growth of 80 matched, healthy control subjects. The mean difference in growth rates between patients treated with placebo and their controls was −0.70cm/yr; that between children treated with budesonide and their controls was −0.44cm/yr. The observed mean (SEM) case-control difference between treatment groups was +0.27 (0.58) in favor of budesonide treatment. The authors concluded that asthmatic children (especially boys) have a prepubertal growth delay and that budesonide in a daily dose of 600 μg administered by a pMDI does not adversely affect growth over a 2-year period.

Finally, a recent prospective, double-blind, randomized, controlled study on 6 to 10-year-old children with mild asthma compared the growth rates of 46 children treated with budesonide Turbuhaler 100 μg b.i.d. and 45 children with ne-

docromil sodium with the growth of 45 healthy children during 9 months (40). No statistically significant differences were found in growth rates between the three groups. In contrast, growth rate was significantly reduced in the children in the budesonide group when they received treatment with 400 μg budesonide per day for a 3-month period. This study confirmed the safety of clinically effective low doses of budesonide in children with mild asthma (200 μg budesonide per day was clinically significantly more effective than treatment with nedocromil sodium). The double-blind period was followed by a one-year open label treatment with budesonide 400 μg per day for 3 months followed by 200 μg per day for 9 months. Even if this regimen was exactly similar to the regimen used during the first year of treatment the children grew significantly faster during the open phase than during the first year of treatment, suggesting that the growth-retarding effect of budesonide was reduced with continued treatment.

Two prospective, controlled studies assessed growth during fluticasone propionate treatment of children with mild asthma. Both found that fluticasone in daily doses of 100 and 200 μg did not adversely affect growth during one year's treatment (29,125).

Price et al. (29) measured growth during one year in 60 children treated with either 100 μg fluticasone per day or sodium cromoglycate 80 mg/day. No statistically significant differences were found between the two groups. The average growth rates in the two groups were 6.0 cm/yr (fluticasone) and 6.5 cm/yr (sodium cromoglycate), respectively.

Allen et al. (125) assessed the growth rate of 300 children treated with either placebo or 100 or 200 μg fluticasone per day for one year in a prospective, randomized, placebo-controlled trial. No statistically significant differences were found in growth rates between the three groups. The average growth rates were 6.15 cm/yr (placebo), 5.94 cm/yr (fluticasone 100), and 5.73 cm/yr (fluticasone 200), respectively. The authors concluded that prepubescent children treated with daily doses of 100–200 μg fluticasone propionate for one year grew at rates similar to placebo-treated children and at rates equal to expected growth velocity for age.

The effect of other inhaled corticosteroids upon growth has not been thoroughly assessed, although a retrospective study did not find any adverse effects upon growth during one-year treatment with triamcinolone (113).

### *Preschool Children*

The influence of inhaled corticosteroids on growth in preschool children is less well studied. Preschool children treated with 200–300 μg budesonide per day from a pMDI with a spacer were reported to grow normally during 3–5 years of continuous treatment (115). However, the conclusions of the study are weakened by the lack of a control group not receiving inhaled corticosteroids.

Skoner et al. (126,127) studied asthma patients recruited from three randomized, 12-week, double-blind, placebo-controlled studies with budesonide inhalation suspension (BIS). A total of 670 children were enrolled in three 52-week, randomized, open-label, controlled, parallel-group studies, comparing the growth rate during treatment with BIS and control treatment (which for some children included inhaled corticosteroids). BIS was initially administered at a dose of 0.5 mg once (studies A and C) or twice daily (study B), with attempts made at each clinical visit to gradually reduce the dose to the minimum effective dose. 223 subjects received control treatment and 447 budesonide inhalation suspension. Mean age at entry was around 5 years. Median total daily doses of BIS ranged from 0.5 to 1.0 mg. Changes in height SD scores differed significantly between the BIS and controls in study A, in which the controls did not receive inhaled corticosteroids, and there was a statistically significant decrease in growth velocity (−0.8cm/yr in the BIS-treated group compared with the controls). No statistically significant differences were observed between BIS and controls in changes in height SD scores or in growth velocities in studies B and C in which some of the control children also used inhaled corticosteroids.

### *Comparison Between the Effect of Inhaled Corticosteroids*

A study compared the annual growth rate during treatment with fluticasone propionate 400 μg/day with the growth rate in children receiving 400 μg beclomethasone propionate per day (128). Both groups were prepubertal. Growth rate was significantly higher during fluticasone treatment (4.99 cm/yr) than during beclomethasone treatment (4.09 cm/yr). This observation emphasizes that conclusions from studies with one inhaled corticosteroid should not be transferred to other inhaled corticosteroids. Each drug/inhaler combination must be studied separately.

The findings in these growth studies emphasize some important issues:

1. Important differences seem to exist between the growth retarding effects of various inhaled corticosteroids and inhalers (10).
2. Growth retardation may be seen with all inhaled corticosteroids when a sufficiently high dose is administered for long periods without any dose adjustment for disease severity.
3. Different age groups seem to differ in susceptibility to the growth-retarding effects of inhaled corticosteroids, children aged 4–10 years being more susceptible than pubertal children (12,42).

Furthermore, the findings in several of these studies suggest that the growth-retarding effect of an inhaled corticosteroid treatment may be more marked in the beginning of the treatment and in some way becomes attenuated with continued

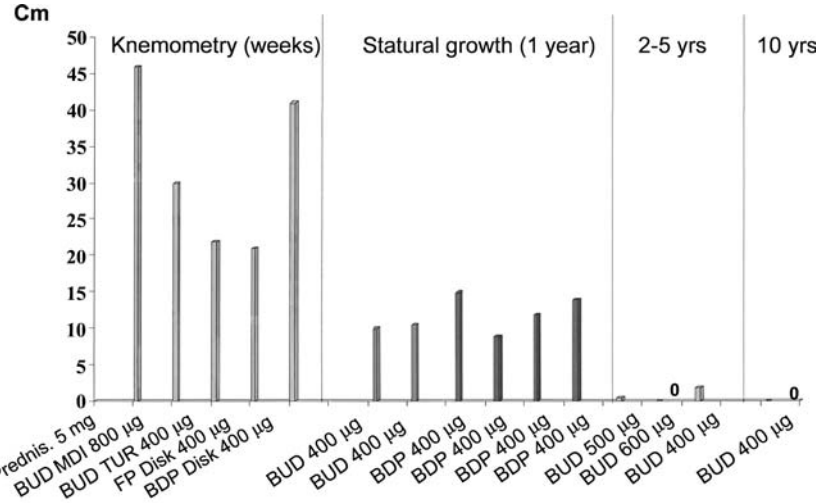


**Figure 3** Expected effect that a treatment would have on attained adult height if the growth retardation measured in each individual study persisted during 10 years of continued treatment. The data have been constructed upon the findings in randomized controlled trials published as full-length papers. Findings in short-term studies seem to suggest a much more marked effect upon attained adult height than studies of longer duration. These calculations should be assessed in light of the fact that all studies conducted so far (a total of six) have found that long-term treatment with inhaled corticosteroids has no adverse effect on attained adult height.

treatment (13,42,129,130). A time-dependent effect would also explain the marked steroid-induced reductions in lower leg growth rate observed in knemometry studies (80,82,83,89,131,132) compared with the smaller reduction in statural height in studies of longer duration. In an attempt to assess the possibility of a time-dependent, growth-retarding effect of inhaled corticosteroids, the data from various randomized controlled growth studies of different durations have been used to calculate the expected effect a treatment would have on attained adult height if the growth retardation measured in each individual study persisted during 10 years of continued treatment (Fig. 3). Although this analysis does not allow any firm conclusion about the possibility of a time-dependent effect upon growth, it does call for further studies to assess this question.

### I. Long-Term Studies and Studies on Final Adult Height

At present only few prospective long-term studies or studies on attained final adult height have been conducted.

Norjavaara (133) compared the recorded adult heights of all pregnant women ($n = 287.750$) born in Sweden between 1960 and 1974 with the heights of all pregnant women with asthma ($n = 2.738$) from the same period. Use of inhaled corticosteroids was very low in the study population since inhaled corticosteroids were reserved for the more severe cases during this period. It was found that the adult height of women with asthma was significantly lower than the adult height of women without a diagnosis of asthma and that patients who had been hospitalized with asthma early in life tended to be shorter than patients who had been hospitalized later in life. These data suggest that the asthma disease may in itself adversely affect adult height and that this effect may be more marked if the asthma is severe. This is in good agreement with the findings in other studies on children with asthma (42,54) and the observations in children with other chronic diseases (134,135) but in contrast to the findings in children with atopic dermatitis who, like children with asthma, may also have a prepubertal growth delay but apparently no adverse effect on adult height (136,137), probably because atopic dermatitis is a less severe disease.

Balfour-Lynn (43,44) followed 66 children for an average of 13.1 years. No difference was found in overall growth rate between children who received beclomethasone dipropionate and those who did not. Final heights were reported to be within the expected range in the children treated with beclomethasone dipropionate. No data on predicted height estimated from parental height were given (43,44). Similar results were reported in a retrospective study, which assessed the longitudinal growth of 97 children treated with beclomethasone dipropionate in daily doses of 300–800 μg for more than 8 years (138). Adult heights were within the expected range, but no predicted adult heights were given.

Other retrospective studies (54,139) have compared measured adult heights of the steroid treated children with their target adult heights. Silverstein found that adult height of patients with asthma was not significantly different from the adult height of nonasthmatic subjects; the overall difference, adjusted for mid–parental height, was −0.20 cm (95% C.I. from −0.27 to 1.64). Moreover, the adult height of asthmatic children treated with glucocorticoids was not significantly different from the adult height of patients with asthma not treated with glucocorticoids; the difference after adjusting for mid–parental height was −0.2 cm (95% C.I. from −0.1 to 0.6) (139).

Van Bever et al. (54) found that mean attained adult height was the same in subjects who took inhaled corticosteroids during childhood as compared to those who had never received this treatment. However, steroid-treated patients showed significantly lower values of adult height minus target height than in those who never took inhaled corticosteroids. Furthermore, patients who had ever been hospitalized for asthma showed a lower value for adult height minus target height than those who were never hospitalized, indicating that asthma severity negatively influenced attained adult height.

Larsson et al. (140) compared measured adult heights of 97 children treated with inhaled corticosteroids during childhood with the adult heights of 70 non–steroid-treated asthma patients and 136 healthy controls. Survey reported parental heights were used to calculate the target heights of the study population. It was found that inhaled corticosteroids did not adversely affect adult height; mean measured adult height/target height for the three groups were 2.6 cm (steroid-treated asthma), 2.6 cm (non–steroid-treated asthma), and 2.1 cm (healthy controls).

Recently, the findings of all these studies were confirmed in a controlled, prospective, long-term study in which asthmatic children were treated with inhaled budesonide for several years in doses tailored to disease severity (42). Sixty-two children who only received nonsteroid asthma treatment served as controls. One hundred and forty-two children attained adult height after a mean of 9.2 years of budesonide treatment at a mean daily dose of 412 μg (range 110–877 μg). Mean accumulated budesonide dose was 1.35 μg (range 0.41–3.99 μg). Eighteen of the controls and 51 healthy siblings were also followed until adult height had been attained. Mean differences between measured and target adult heights was +0.3 cm (95% C.I.: −0.6; +1.2) for budesonide patients, −0.13 cm (95% C.I.: −2.4; +2.1) for controls, and +0.9 cm (95% C.I.: −0.4; +2.2) for healthy siblings (Fig. 4). Height standard deviation scores (SDS) correlated positively with

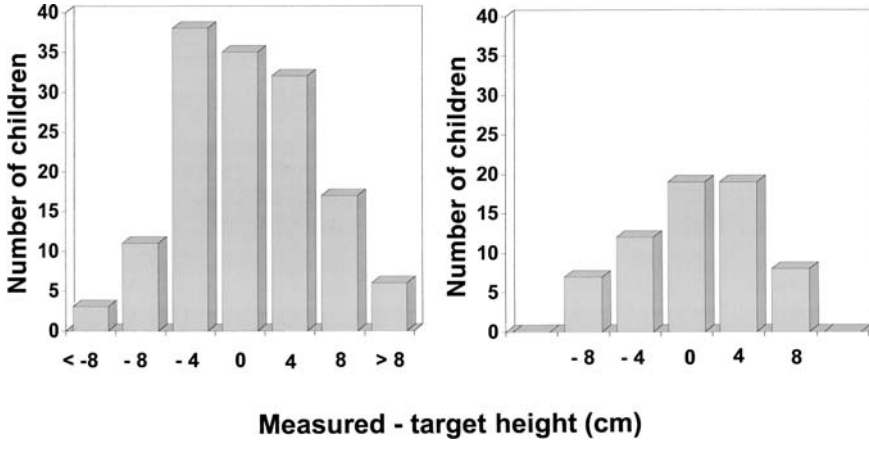


**Figure 4** Distribution of differences between measured adult height and target adult height in 142 children treated with inhaled budesonide for 3–13 years (left) and 47 healthy siblings +18 asthmatic children who had never received inhaled corticosteroids (right). Mean attained adult height in the budesonide group was 173.2 cm and mean target adult height was 172.9 cm.

percent predicted $FEV_1$ before asthma treatment, and the difference between measured and target adult height depended significantly upon height SDS before budesonide treatment. Growth rates were significantly reduced during the first years of budesonide treatment, but the reduction in annual growth rate did not persist and the changes in growth rate during this period showed no relation to the differences between measured and target adult height. Long-term treatment with inhaled budesonide did not adversely affect adult height, whereas poorly controlled asthma seemed to do so. Furthermore, changes in growth rate during the first year of budesonide treatment were not useful in predicting adult height.

Although prospective studies of several years' duration and retrospective analyses may always be criticized for being less controlled than prospective, short-term studies, some conclusions about the effect of inhaled corticosteroids on final height seem to be justified based upon the data from these studies, especially because a controlled, double-blind, randomized study of 15 years' duration is unlikely to be conducted in the foreseeable future.

The conclusions from the various studies addressing the question of attained adult height have been consistent in many areas:

- Children with asthma treated with inhaled steroids have consistently been found to attain normal final adult height (six studies).
- Uncontrolled or severe asthma seems to adversely affects growth and attained adult height (three studies).
- Corticosteroid-induced changes in growth rate during the first year of treatment do not predict adult height (the only long-term prospective study designed to answer that question).

The reason for the apparent discrepancy between the findings of some intermediate-term studies and the conclusions of final height studies is not clear. Studies with beclomethasone have found a growth retardation of 1.5 cm per year. An annual growth retardation of 1.5 cm, if persistent, would be expected to result in a cumulative mean reduction in measured adult height of 15 cm were the treatment given continuously for 10 years. Such marked effects would be difficult to miss in the day-to-day clinic or in long-term prospective studies. Some possible explanations for the discrepancy are as follows.

- The correlation between two consecutive annual height velocity values for normal prepubertal children is poor. A low gain in one year is not necessarily followed by a low gain the next year, and vice versa (59). The correlation between 1-, 2-, 3-, and 4-year growth velocities are only partially correlated with one another (59), and growth rate computed over a period of 3 or 4 years in childhood only explains 34% and 38% of the variation in final height, respectively.
- It seems as if the growth-retarding effect of exogenous steroids is most pronounced during the first year of treatment (13,42,129,130). Therefore,

conclusions from rather short-term studies of one year should be extrapolated to the long-term situation with caution.

Steroids also seem to retard bone maturation. If this occurs to the same extent as the retardation of growth, then final height is not expected to be adversely affected (since bone age will correspond to height age). Such children will grow for a longer period than their peers and eventually attain normal final height.

The results obtained in prepubertal schoolchildren may not be valid in other age groups because prepubertal children may be more sensitive to the growth-retarding effect of inhaled corticosteroids (12,42). If the pubertal growth spurt is not adversely affected, the effect on final height may be rather small.

In a real-life situation, children with mild asthma are not treated continuously with a rather high, fixed dose of inhaled corticosteroid: the dose is adjusted to the severity of the disease. Only children with moderate or severe asthma would require continuous treatment with daily doses of 400 μg or more. The systemic effects in these children may be lower than in children with mild disease (2–4). Many clinics would use spacers for inhaled corticosteroids with low first-pass metabolism. Compliance may be lower during long-term real-life treatment than during a controlled trial with several visits to the clinic. More prospective studies are needed to assess the relative importance of these possibilities.

### J. Problems in Growth Assessment—Individual Sensitivity to Steroids?

Case reports sometimes suggest that growth inhibition in individual children may be seen (141). When such reports are evaluated, it must be remembered that growth is a very complex process that may be affected by a host of factors, including disease severity, psychological factors, growth factors, receptor affinity, nutrition, body composition, age, puberty, genetic factors, and treatment. Children show spontaneous fluctuations in growth velocity often with seasonal variations—most children growing faster in the summer than in the winter (56). More recently it has also become clear that in some children fluctuations are not purely seasonal, but cycles of growth may span 2 or more years (Fig. 5) (57). This leads to a very poor correlation between the growth velocity in one year and that in the next year (58). These variations, in combination with a standard error of the height measurement, which for trained observers is around 0.2–0.3 cm, and the abnormal growth pattern seen in many asthmatic children unrelated to the use of inhaled steroids, mean that case reports of apparently reduced growth in association with an asthma treatment should be interpreted with caution. No firm conclusions about cause-and-effect relationships should be made without the support of further controlled studies.

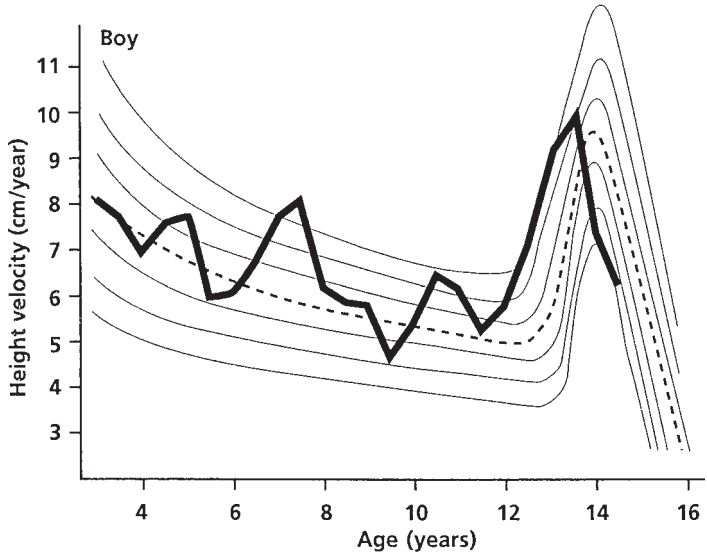


**Figure 5** Individual growth-velocity curve in a normal child. Marked variations are seen in annual growth rate. If a steroid treatment had been started at age 7 and stopped at age 9.5, it would probably have been concluded that the treatment caused growth stunting, particularly because an "apparent catch-up growth" is seen from age 9.5 to 10.5.

### K. Conclusions

In general, the literature reporting the effects of inhaled corticosteroids on growth has been reassuring, considering that the majority of children with mild and moderate asthma only require low doses of inhaled corticosteroids around 100–200 μg/day to achieve optimal control. Such doses do not adversely affect growth. Furthermore, final height does not appear to be adversely affected by long-term treatment with somewhat higher doses of inhaled corticosteroids even if such doses may retard growth rate in short-term trials. In contrast, the growth-retarding effect caused by uncontrolled asthma may be persistent, so uncontrolled asthma may also adversely affect final adult height.

Since the occurrence of measurable systemic effects and risk of clinical side effects increases with dose, the lowest dose of inhaled corticosteroid able to control the disease should always be used. Furthermore, inhaler-steroid combinations with a high clinical efficacy : systemic effect ratio should be used.

The possibility of increased sensitivity to the growth-retarding effect of inhaled steroids by susceptible individuals is complex and requires further study. Some data suggest that an increased sensitivity exists within a population at a cer-

tain unknown frequency. However, normal fluctuations in growth rate and deviant growth pattern of asthmatic patients not receiving inhaled steroids complicate the interpretation of individual growth data.

Finally, although results from short-term studies reflect systemic effects of inhaled corticosteroids, they do not predict intermediate- or long-term growth. Similarly, results from intermediate-term studies are poor predictors of attained adult height.

## IV. Summary

Inhaled steroids have been used for the treatment of asthma in children for more than 20 years. During this time, a substantial number of studies have been performed evaluating the safety and efficacy of this therapy. Generally, the results have been reassuring. Inhaled corticosteroids have a marked effect on both immediate- and long-term aims of asthma therapy. In patients with mild and moderate asthma, low daily doses of around 100–200 μg/day of inhaled steroid produce a clinical effect that, in most trials, is better than the effect of any other treatment to which it has been compared. No adverse effects on growth have been associated with treatment in this dose range, and idiosyncratic adverse reactions are rare. Higher doses of inhaled corticosteroids may reduce growth rate during the first years of treatment. However, even if this is the case, attained adult height is not adversely affected, even if such doses are used for several years.

Since the occurrence of measurable systemic effects and risk of clinical side effects increases with dose, the lowest dose that controls the disease should always be used. Furthermore, inhaler-steroid combinations with a high clinical efficacy : systemic effect ratio should be used. If a child is not sufficiently controlled on a low dose of inhaled steroid, it might be better to add another drug to the low-dose inhaled steroid treatment rather than to increase the steroid dose. Further studies are needed to assess at which dose this should be done for individual steroid/inhaler combinations.

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## Discussion

**Dr. Boulet:** You showed in previous studies the powerful effect of oral steroids on growth suppression. Have any studies been done looking at the benefit of reducing the need for oral steroids for exacerbations from inhaled steroid treatment in the more severe child asthmatic population in regard to growth?

**Dr. Pedersen:** Not to my knowledge.

**Dr. Schleimer:** Do oral steroids show the same pattern—early suppression, with delayed puberty and "catch-up," or is there a qualitative difference, with a reduction of ultimate statural height with oral steroids?

**Dr. Pedersen:** This has not been so extensively studied. However, it seems as if the same pattern is seen when low doses of oral steroids are used. However at higher doses (>5 mg/day), adult height may also be adversely affected.

**Dr. Edsbäcker:** Added to your convincing final height data following budesonide treatment, there have been two relatively recent Swedish compilations of final height data, one from the Swedish birth register involving almost 290,000 mothers born from 1960 to 1974, where 2,700 had been hospitalized for asthma; one group at ages 0–8, one group at 9–15. Final height in both of these groups of asthmatics did not differ from the control mothers. Also, the Swedish conscript registry data involving 164,000 healthy controls and 8,500 asthmatics showed that asthma per se is associated with a reduced final height by 0.7 cm, but that this was unrelated to steroid therapy.

**Dr. Pedersen:** Interesting. Our data also show that uncontrolled asthma may adversely affect growth and adult height.

**Prof. Dolovich:** Have you expressed the dose of ICS given as the microgram quantities contained in the drug available at the mouth from the delivery system, that is, as the emitted dose and also as the fine particle component of the emitted dose? This might correct for differences in growth suppression noted between inhalers.

**Dr. Pedersen:** We use the actual prescribed doses. It seems that the systemically available dose of an inhaled corticosteroid varies with age, being lower in young children (when a corticosteroid with high first-pass metabolism is used). Therefore, the plasma levels are quite similar over a wide age range when the same dose is inhaled.

**Dr. Derendorf:** Do you think that we are likely to see an effect on growth if there is no effect on serum cortisol?

**Dr. Pedersen:** Some believe that this may be the case. I am not convinced that this may be the case. More data are needed.

**Dr. Denburg:** What is the biological explanation for the growth pattern observed? Has this phenomenon been studied in vitro? Is there a relationship between growth suppression and efficacy of therapy?

**Dr. Pedersen:** We don't know, but it does not seem to be the case. The biological explanation is not known. However, the phenomenon is also seen in other chronic diseases. It is as if the body in some way adjusts to maintain the situation before the exogenous influence was there.

# 16

# Evaluation and Comparison of Inhaled Steroids

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## I. Introduction

Asthma is a chronic inflammatory disorder of the airways (1,2). Airway inflammation contributes to airway hyperresponsiveness, airflow limitation, respiratory symptoms, and disease chronicity. Considering asthma as an inflammatory disorder has implications for its diagnosis, prevention, and management. Recently, asthma has also been recognized as a cause of rapid loss of pulmonary function over time in some patients (3–6). The loss in pulmonary function may be incompletely reversible or irreversible (7). Thus, the consequences of poorly controlled asthma can include not only clinical symptoms and variability in pulmonary function, but also progressively worsening airflow obstruction (Table 1).

Numerous studies have clearly demonstrated that inhaled glucocorticoids alleviate clinical symptoms, improve pulmonary function, and reduce airway inflammation; there is some evidence that they alter disease progression (8–10). Current guidelines identify inhaled glucocorticoids as the "preferred" long-term controller asthma medication, especially for moderate and severe persistent asthma (1,2). Recent observations suggest that the response to inhaled glucocorticoids is highly dependent on the time of intervention: the earlier they are used,

**Table 1** Asthma Control Indicators

| |
|---|
| *Clinical measures* |
| Mortality |
| Hospitalizations |
| Acute exacerbations: |
| Emergency department visits |
| Courses of prednisone or high-dose inhaled steroids |
| Nocturnal symptoms |
| Breakthrough symptoms |
| β-Agonist use for symptom relief |
| Wheezing or symptomatic (including chest tightness, cough, shortness of breath) episodes affecting activity |
| *Pulmonary measures* |
| Peak expiratory flow |
| Pulmonary function—spirometry |
| $FEV_1$ % predicted |
| $FEV_1$/FVC or RV/TLC |
| Total lung volume |
| Bronchial hyperresponsiveness to exercise, methacholine, or histamine |
| *Markers of inflammation* |
| Bronchoalveolar lavage—cytology and mediators |
| Induced sputum—cytology, especially eosinophils, or mediators |
| Blood—total eosinophils, activated eosinophils |
| Plasma—eosinophilic cationic protein |
| Exhaled nitric oxide |
| *Progression* |
| Increasing medication requirements |
| Decline in pulmonary function |
| Increasing airways hyperresponsiveness |
| Serial biopsy—limited application at the present time |
| Cytology— eosinophils, mast cells, lymphocytes, and neutrophils |
| Features of airway remodeling—collagen, elastin, tenascin tissue deposition |

the more efficacious they are (7,11–13). Inhaled glucocorticoids are effective only as long as the medication is continued, with loss of beneficial effect occurring as early as days or weeks after the medication is discontinued (11). This has implications for the importance of continuing therapy for effective asthma control. Guidelines recommend doses for inhaled glucocorticoids based on severity of disease (Table 2). In general, the more severe the asthma, the higher the dose recommended (1,2). A body of literature has now been assembled reporting effects of inhaled glucocorticoids on growth velocity, cataracts, ocular hypertension, and bone loss in susceptible patient populations (14). An obvious question is whether

there are differences among the various inhaled steroids and delivery systems that could indicate a "preferred" product and delivery device for inhaled steroid therapy.

## II. Why Compare Inhaled Steroids?

Following the introduction of inhaled steroid therapy in the early 1970s, questions were raised regarding the dose dependency of their effects and about methods to limit the potential for adverse effects. At that time, the standard dose of an inhaled steroid, specifically beclomethasone dipropionate, was 400 μg per day and rarely exceeded 1000 μg per day. In a series of elegant studies, Toogood and associates demonstrated the dose-response relationship of beclomethasone dipropionate on an array of clinical and pulmonary function parameters (15). Using the asthma attack frequency as a primary outcome measure, they demonstrated that in oral steroid–dependent asthmatics, 200 μg of BDP per day reduced the frequency of attacks by 12%. Increasing the dose to 1600 μg per day would reduce the attack rate by 22%. The reduction in attack frequency came at the cost of a dose-related increase in cortisol suppression. Figure 1 summarizes the log dose-response relationships for clinical and pulmonary function parameters for beclomethasone dipropionate. A correlation analysis of the dose-response study failed to identify clinical parameters that would predict a steroid response, but it did identify fixed obstructive lung disease to predict a poor response (16).

This group of investigators then turned their attention to evaluating the dose-response relationship of another inhaled steroid, budesonide, on asthma control, pulmonary function, and laboratory measures in comparison to oral prednisone in prednisone-dependent and nondependent asthma patients (17). This model consisted of a 2-week placebo period followed by graduated increases in the dose of the steroid medication. Each of the three doses was administered for a period of 2 weeks, and clinical, pulmonary function, and laboratory measures were obtained after the second week of treatment. The first dosing series of the steroid medication was followed by a 4-week washout period. Then the same sequence was started with the alternate steroid medication.

Dose-response relationships were demonstrated for both medications. Using this model, equivalencies were established for various efficacy and systemic effect measures. The investigators concluded that the dose required to eliminate recurrently disabling asthma relapses was about 2.0 mg of budesonide per day or $>$40 mg of prednisone per day. They also concluded that the doses causing similar systemic effects based on the measures of 8 a.m. serum cortisol and blood eosinophil counts that were equivalent to $\geq$15 mg of prednisone per day were budesonide doses $\geq$1.84mg/day/70 kg adult or 26.3 μg/kg/day. This dose of prednisone is known to be associated with steroid-induced adverse effects, such as osteoporosis. Of great interest was their observation that the level of systemic

**Table 2** Dosage Guidelines Presented in the National Asthma Education Program Expert Panel Report II: Estimated Comparative Daily Dosages

| Glucocorticoid | Low dose | Medium dose | High dose |
|---|---|---|---|
| Adults | | | |
| Beclomethasone dipropionate | 168–504 μg | 540–840 μg | >840 μg |
| 42 μg/puff | (4–12 puffs—42 μg) | (12–20 puffs—84 μg) | (>20 puffs—42 μg) |
| 84 μg/puff | (2–6 puffs—84 μg) | (6–10 puffs—84 μg) | (>10 puffs—84 μg) |
| Budesonide Turbuhaler | 200–400 μg | 400–600 μg | >600 μg |
| 200 μg/puff | (2–4 puffs) | (4–8 puffs) | (>8 puffs) |
| Flunisolide | 500–1000 μg | 1000–2000 μg | >2000 μg |
| 250 μg/dose | (2–4 puffs) | (4–8 puffs) | (>8 puffs) |
| Fluticasone propionate | 88–264 μg | 264–660 μg | >660 μg |
| MDI: 44, 110, 220 μg/puff | (2–6 puffs—44 μg) or (2 puffs—110 μg) | (2–6 puffs—110 μg) | (>6 inh—100 μg) (>3 puffs—220 μg) |
| DPI: 50, 100, 250 μg/dose | (2–6 inhalations—50 μg) | (3–6 inh—100 μg) | (>6 inh—100 μg) or (>2 inhalations—250 μg) |
| Triamcinolone acetonide 100 μg/puff | 400–1000 μg (4–10 puffs) | 1000–2000 μg (10–20 puffs) | >2000 μg (>20 puffs) |

| | | | |
|---|---|---|---|
| Children | | | |
| Beclomethasone dipropionate | 84–336 μg | 336–672 μg | >672 μg |
| 42 μg/puff | (2–8 puffs—42 μg) | (8–16 puffs—42 μg) | (>16 puffs—42 μg) |
| 84 μg/puff | (1–4 puffs—84 μg) | (4–8 puffs—84 μg) | (>8 puffs—84 μg) |
| Budesonide Turbuhaler | 100–200 μg | 200–400 μg | >400 μg |
| 200 μg/dose | | (1–2 inhalations—200 μg) | (>2 inhalations—200 μg) |
| Flunisolide | 500–750 μg | 1000–1250 μg | >1250 μg |
| 250 μg/dose | (2–3 puffs) | (4–5 puffs) | (>5 puffs) |
| Fluticasone propionate | 88–176 μg | 176–440 μg | >440 μg |
| MDI: 44, 110, | (2–4 puffs—44 μg) | (4–10 puffs—44 μg) or | (>4 puffs—110 μg) |
| 220 μg/puff | | (2–4 puffs—110 μg) | (>2 puffs—220 μg) |
| DPI: 50, 100, | (2–4 inhalations—50 μg) | (2–4 inhalations—100 μg) | (>4 inhalations—100 μg) |
| 250 μg/dose | | | (>2 puffs—250 μg) |
| Triamcinolone | 400–800 μg | 800–1200 μg | >1200 μg |
| acetonide 100 μg/puff | (4–8 puffs) | (8–12 puffs) | (>12 puffs) |

*Source*: National Asthma Education and Prevention Program Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma. National Institutes of Health, National Heart, Lung, and Blood Institute, Publ. No. 97-4051, 1997.

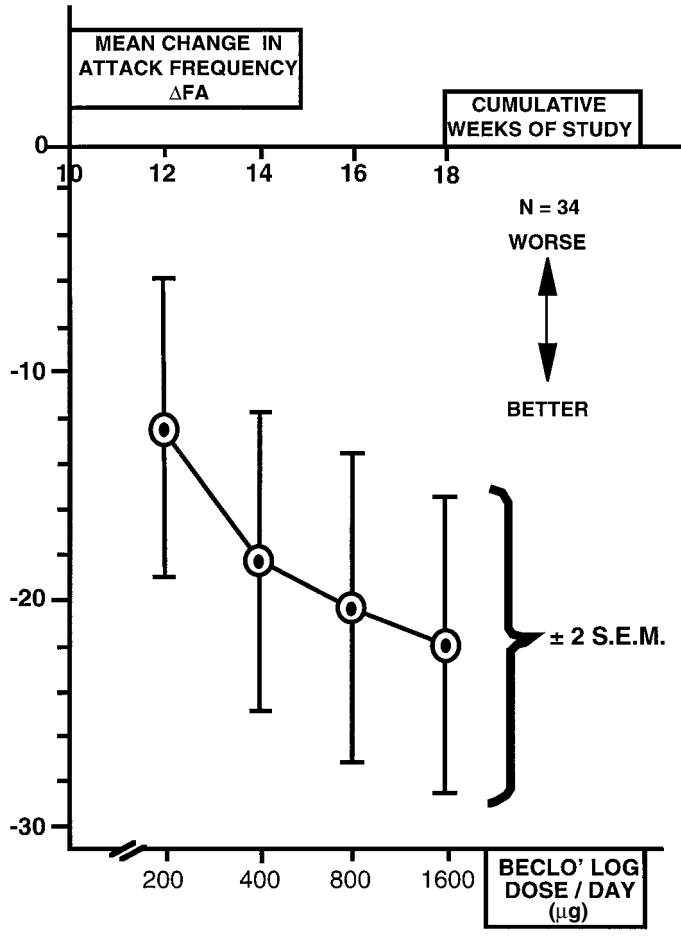


**Figure 1** The group mean change in asthma attack frequency per 2 weeks during beclomethasone aerosol (BA) treatment is plotted against log dose BA. Targets indicate values significantly improved compared with the pre-BA "baseline" values ($p < 0.05$ or better). The I bars illustrate the variability among these patients in their response to the drug at each dose tested. (From Ref. 16.)

glucocorticoid activity was much lower than that produced by the dose of prednisone needed to achieve an equivalent level of antiasthmatic response. They therefore concluded that the use of high-dose inhaled budesonide is reasonable in patients with severe asthma who would ordinarily require continued prednisone therapy.

The first attempt to compare one inhaled steroid to another was reported by McCubbin et al. (18). These investigators measured the effects of treatment on the immediate response to an allergen challenge in sensitized patients and on 24-hour urinary free cortisol and constructed a three-point dose-response curve for beclomethasone dipropionate (50 μg/inhalation), triamcinolone acetonide (100 μg/inhalation), and flunisolide (250 μg/inhalation). They studied 25 subjects in a randomized, parallel, single-blind study. Dosing began at the lowest dose (one inhalation four times per day for flunisolide and two inhalations four times per day for the others) and continued for one week and then was doubled sequentially until the third dose was completed. The investigators derived relative potencies for the three study preparations and concluded that they were approximately equivalent for both topical and systemic effects when the dose was expressed in micrograms. This model was proposed as a method to compare relative topical and systemic effects, but it has not been widely applied, possibly because their efficacy endpoint, partial suppression of the immediate response to an allergen challenge, is not regarded as clinically meaningful.

With growth in information about the efficacy of inhaled steroids and the recognition of chronic inflammation as a component of asthma, inhaled steroids rose to the forefront of asthma care in the early 1990s. Expert panels concluded that because inhaled steroids improved asthma control, reduced hospitalizations, increased pulmonary function, and reduced airway inflammation, they should be the cornerstone in the management of persistent asthma, especially moderate and severe forms. Consequently, a series of steroids characterized by increasing topical potency and suitability for aerosol administration were developed. As of June 2001, five inhaled steroids were available in the United States for asthma therapy: beclomethasone dipropionate, triamcinolone acetonide, flunisolide, budesonide, and fluticasone propionate. It is likely that a sixth inhaled steroid, mometasone, will be available soon. In addition, the recognition of the ozone-depleting properties of the chlorofluorocarbons (CFC) prompted the move to an alternate propellant, such as a hydrofluoroalkane, or a dry powder, breath-actuated delivery system. The simple change to these alternate delivery systems can affect particle size, however, and possibly influence the proportion of the actuated dose delivered to the lung (19,20).

Until recently, expert panels considered the inhaled steroids equivalent on a μg-per-μg basis (1). With the introduction of fluticasone propionate, it was recognized that its higher potency resulted in significantly greater cortisol suppression at doses exceeding 1500 μg than had been previously recognized with the other inhaled steroids (Fig. 2) (21). It should be noted that both steroids were evaluated with pressurized metered dose delivery (pMDI) systems. Observations related to differences in cortisol suppression with comparable doses of inhaled steroids (21) prompted the NHLBI Expert Panel to evaluate the relative potencies of the various inhaled steroids (2,22). Based on receptor-binding properties and limited

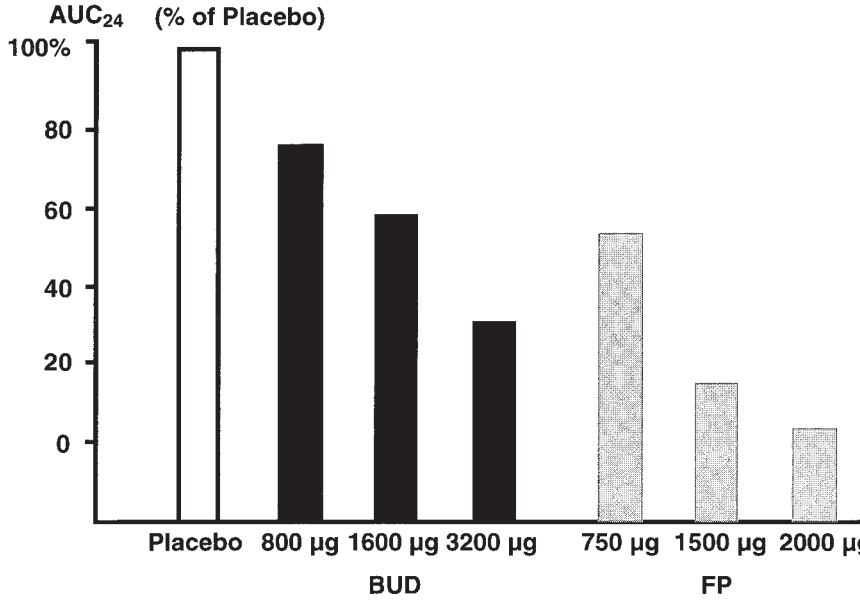


**Figure 2** $AUC_{24h}$ values for each treatment expressed as a percentage relative to $AUC_{24h}$ (placebo). (From Ref. 21.)

comparison studies of beneficial and systemic effects, the NHLBI Expert Panel developed a potency scale for the various inhaled steroids along with dosage guidelines for older children and adults as well as young children (Table 2).

Potency was defined by assay systems, such as the skin blanching technique, and by glucocorticoid receptor-binding studies that have not been verified as standards for asthma clinical response. Even to estimate the true potency of a medication requires a miminum of three carefully selected doses, and five doses are necessary to describe the dose-response curve from absence of effect through the dose-related increases in effect to plateau effect (Fig. 3). Defining a dose-response curve for a clinical endpoint thus appears cumbersome and difficult. Unfortunately, no long-term, placebo-controlled, randomized, prospective studies have been done to substantiate the inferences drawn from short-term studies of safety of inhaled steroids.

The amount of published information on dose-response for safety and efficacy of various available inhaled steroids differs markedly; some have been studied extensively, and others have been studied hardly at all. In general, doses under 400 µg per day seem unlikely to cause clinically significant systemic effects. Interestingly, several studies have indicated that small changes in growth velocity can be observed with doses as low as 400 µg per day administered to children for

periods of one year or less (23–27). Very little information is available for any inhaled glucocorticoid at doses exceeding 2000 μg per day. One marker, cortisol suppression, is very sensitive and shows a dose-related effect. Cortisol suppression has been noted with relatively low doses of some inhaled steroids and is noted with all available inhaled glucocorticoids given in doses of 1500 μg per day (28). What is not clear is how the endpoint measured, cortisol suppression, is related to the risks of adrenal withdrawal syndrome or with clinically significant systemic adverse effects. Until recently, systemic effects, such as significant reduction in bone density (associated with a risk of fracture), cataracts, hypertension, and growth impairment, were thought not to occur with conventional doses of inhaled glucocorticoids. However, several studies, although retrospective and uncontrolled, have raised concern about the effects of long-term use of inhaled glucocorticoids, especially high doses, on glaucoma and cataracts (29–32). It is not clear whether individual steroids can vary in their effect on the different target organs.

At a meeting of the Pulmonary and Allergy Drug Advisory Panel, the U.S. Food and Drug Administration (FDA) raised concerns about the effect of inhaled steroids on growth velocity in children. They also concluded that more informa-

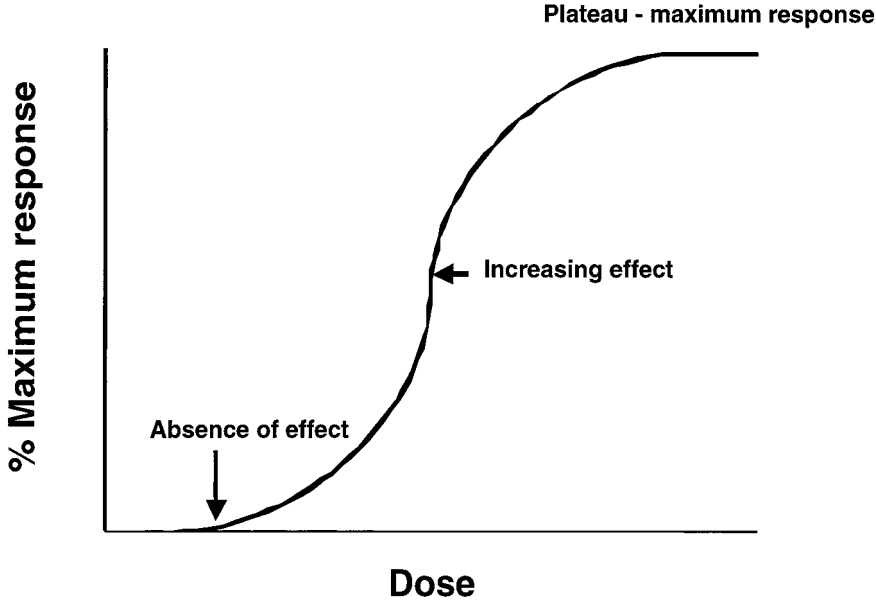


**Figure 3** Simulated dose-response curve showing minimal effect, increasing effect with dose, and plateau or maximum effect.

tion is needed about safe dosage schedules in children less than 5 years of age for all asthma medications, especially inhaled steroids, now that early recognition, early intervention, and long-term use are advocated (33). Dose-response studies are available defining the degree of cortisol suppression for a few inhaled steroids (14,18,21,28,34). Similar studies are needed to define dose-response relationships for efficacy. Excellent summaries have defined the adverse effects of inhaled glucocorticoids (14,35). In general, more information is needed for the safe use of these medications in patients at potential risk for adverse glucocorticoid effects (elderly, postmenopausal women, young children) for all inhaled glucocorticoids at various dosage ranges, especially high-dose inhaled steroids used for moderate to severe asthma (36,37).

The recent attention to risk for adverse effects has prompted a movement to limit doses to the low to medium range. Of interest, a recent study by Sont et al. (37) evaluated the level of asthma control resulting from inhaled steroid dose adjustments using airway hyperresponsiveness as an additional guide to long-term treatment. This study used the approach of adjusting inhaled steroid dosage according to levels of clinical symptoms, bronchodilator use, peak expiratory flow variability, and $FEV_1$ in one group (reference group) and according to the same criteria plus the addition of another, airway responsiveness, in another (AHR strategy group) (Table 3). Based on the score determined at 3-month interval evaluations, the dose could be adjusted to no inhaled steroid or low-, medium-, or high-dose inhaled steroid.

After a 2-year follow-up, the investigators made several interesting observations. First, they observed greater improvements in asthma control and pulmonary function with the AHR strategy. Second, biopsy samples obtained before and after 2 years of treatment showed significant reductions in reticular layer thickness and reduced eosinophil inflitration only in the AHR strategy group. Finally, they observed that a higher proportion of patients in the AHR strategy group required high and moderate-dose inhaled steroid therapy (Fig. 4). These observations suggest that patients with persistent asthma would receive better control and better resolution of inflammation, and the consequences of chronic inflammation, such as airway remodeling, if treatment were based on periodic measures of airway hyperresponsiveness. However, this strategy would result in treatment with higher doses of inhaled steroids, at least temporarily. On a cautionary note, clinical experience and available literature suggest that aggressive therapy rarely normalizes AHR, usually changing the provocative dose of methacholine and histamine by no more than two fold. Therefore, attempting to normalize AHR could result in protracted therapy and incur risk for adverse effect with little gain in response. Therefore, the application of measures of AHR requires careful consideration. Perhaps other measures, such as sputum eosinophils, or an alternative measure of ongoing inflammation could be included as indicators for the adjustment of inhaled steroid therapy.

**Table 3** Severity Classes of Symptoms, Bronchodilator Usage, Diurnal Variability in PEF, $FEV_1$, and Airway Hyperresponsiveness with Corresponding Treatment Steps

| Treatment step | Both strategies | | | | AHR strategy only, hyperresponsiveness: methacholine $PC_{20}$ (mg/mL) |
|---|---|---|---|---|---|
| | Symptoms >3 d/2 week | Bronchodilator use | PEF variability | $FEV_1$ | |
| 4 | Disturbed sleep/early wakeup/limited physical activities | >4 hourly | >50 | <50 | <0.25 |
| 3 | Nighttime symptoms/early wakeup/affect activities | >6 hourly | 30–50 | 50–60 | 0.25–1.0 |
| 2 | Mild nighttime/morning symptoms/may affect activities | 1–4 x/d | 20–30 | 60–70 | 1.0–4.0 |
| 1 | <3 d/2 wk | <daily | <20 | >70 | >4.0 |

*Source*: Ref. 37.

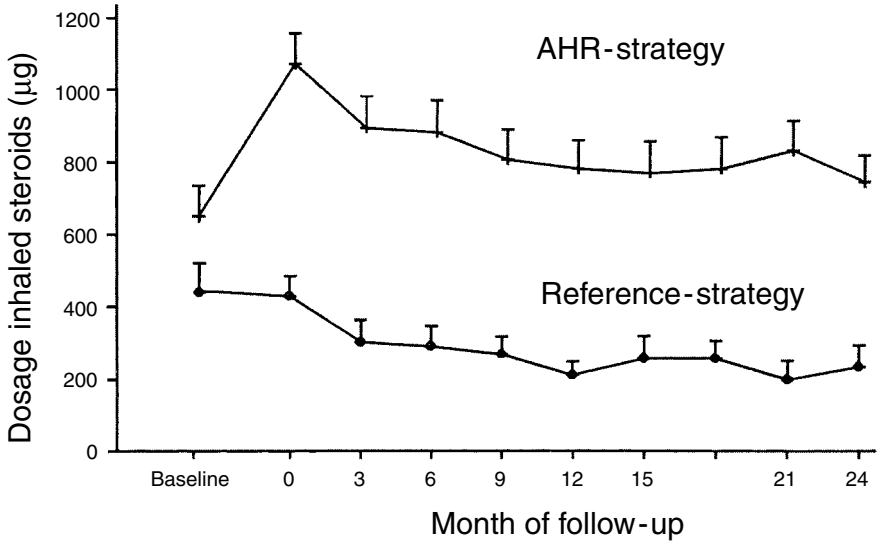


**Figure 4** Actual daily doses of inhaled steroids (µg; mean ± SEM) according to the AHR strategy and the reference strategy. The median difference in treatment with inhaled steroids was ±400 µg during the 2-year follow-up. Treatment requirement decreased with both strategies. However, the decrease with the AHR strategy was somewhat greater than with the reference strategy. (From Ref. 37.)

## III. Where Are We Now?

Currently, persistent asthma is considered a disease of chronic inflammation. Several studies have suggested that this chronic inflammation can contribute to declining pulmonary function over time and possibly to altered lung growth in children (3–6). This has prompted a movement for earlier intervention and prolonged therapy to alter the course of the disease (7,11–13). In addition, if the observations by Sont et al. (37) are applied, it could mean not only earlier use, but also higher-dose therapy for extended periods of time.

This necessitates the careful evaluation of the topical to systemic effect relationship of the available inhaled steroids and delivery systems. FDA has recommended the evaluation of surrogate markers to measure the effect of medications, especially in determining bioequivalence. The identification of surrogate markers is an attempt to focus the evaluation, but the marker must be clinically meaningful and reflect a desired therapeutic outcome. In addition, a "gold standard" inhaled steroid and delivery system would simplify comparisons.

In the absence of a gold standard, most clinical studies have addressed questions of comparative effects by testing one or two doses of, at most, two steroids.

The available studies are limited by problems of inconsistent methods of delivery, failure to monitor adherence with treatment, and narrow spectrum of measures of efficacy—usually consisting of clinical symptoms, use of rescue therapy along with peak flow and periodic spirometric measures—over a relatively short period of treatment, usually 3 months. There is a critical need for standardization of these testing procedures (2,14,22,38–40).

A marker for asthma control should predict the clinical consequences of poor outcomes. Some possible candidates are summarized in Table 1 and include pulmonary function changes, measures of inflammation, and measures of disease progression. Other potential markers are listed in Table 4. In evaluating the effects

**Table 4** Candidate Surrogate Markers for the Assessment of Response to Asthma Therapy

| |
|---|
| Disease parameters |
| Asthma control |
| Symptom-free days—number of days there is no disruption in activity or need for additional medical attention or therapy |
| Need for rescue therapy, e.g., as needed $\beta_2$-adrenergic agonists |
| Treatment failure, e.g., time to a first significant asthma exacerbation that requires systemic corticosteroid therapy |
| Progression of severity, e.g., the advancement to a next level of treatment |
| Pulmonary function |
| $FEV_1$—requires maximal effort of a forced expiration for a reliable measurement |
| $FEV_1$/FVC—requires maximal effort of a forced expiration for a reliable measurement |
| Peak expiratory flow—facilitates the collection of daily records in the home setting |
| Resistance—requires the use of forced oscillometry and may be useful in young children |
| Airways hyperresponsiveness—requires patient cooperation and a standardized procedure |
| Adverse effects of treatment |
| Inhaled glucocorticoids |
| Cortisol suppression—serial plasma collections over a 12- or 24-hour period |
| Linear growth |
| Stadiometry—most informative measure if performed with a reliable instrument and trained personnel |
| Knemometry—short-term measure that may be a useful bioassay procedure to detect a systemic effect, but long-term predictive value is poorly defined |
| Osteopenia |
| Bone densitometry—most reliable indicator to obtain measures that are predictive of clinical risk for fracture; generally requires long-term treatment to detect an effect |
| Bone markers—easily obtained objective measure of effect but not predictive of long effects; some bone markers reflect bone deposition and others resorption |

of an asthma medication such as inhaled steroids, one also has to identify a marker for adverse effects of clinical concern, such as growth suppression, cataracts, and osteoporosis (Table 4). There is no single short-term surrogate marker for either the best asthma outcome (e.g., control of disease progression) or for the most significant adverse effects (growth retardation, osteoporosis). Evaluating available markers for asthma progression, such as a decline in $FEV_1$, and for adverse effects on linear growth and bone development takes a long period of time (i.e., years) to measure changes of statistical and clinical significance.

## IV. What Would We Like to Know in Order to Advance Inhaled Glucocorticoid Therapy?

The previous discussion raises important questions related to patient care about the many inhaled glucocorticoids and delivery systems available. Can we define a minimally effective dose for the available inhaled glucocorticoids? Is this dose age-specific? Does it change with the use of different delivery systems? Are there maximum safe doses of the various inhaled glucocorticoids? For patients who do require high-dose inhaled steroid therapy, how can the risk for adverse effects be minimized? Is there a single objective marker that can be used to define the "best clinical outcome"? What is the best way to monitor disease progression?

## V. How Do We Get the Necessary Information?

To define a "preferred" inhaled steroid and delivery system, it is necessary to conduct carefully designed clinical trials evaluating dose-response relationships for selected efficacy and systemic effect parameters. It is also important to define a "standard" for this class of medications for study comparisons in order to limit the complexity and interpretation of future comparative studies. To identify minimally effective and maximally safe doses, it is additionally important to examine various categories of disease severity since maximal achievable response may differ based on level of severity (41). It is also important to identify meaningful surrogate markers of long-term outcomes. This measure should attempt to incorporate an analysis of the progressive nature of the disease to evaluate surrogate markers in projecting efficacy or unwanted systemic effects. To evaluate the risk of adverse effects, it is also important to evaluate all patient populations, including young children, women, and ethnic minorities.

## VI. The Approach of the National Heart, Lung and Blood Institute's Asthma Clinical Research Network

Five inhaled steroids are currently available in the United States for asthma therapy and possibly two more will be introduced by the time of publication.

Budesonide is the only nonhalogenated inhaled steroid. Budesonide and fluticasone are the only inhaled steroids available in a dry powder breath-actuated delivery system. Recently, a hydrofluoroalkane-based propellant for a pressurized metered dose inhaler was approved. There is also the possibility that additional new inhaled steroids will be approved (e.g., mometasone) that reportedly have less systemic absorption than presently available inhaled steroids, or new drugs with effects similar to steroids, e.g., blockers of transcription factors, such as NF-κB, AP-1, or NF-AT (40,42).

Although available guidelines describe inhaled steroids as the preferred long-term control medication for persistent asthma, especially disease of moderate or greater severity, expert panels have not had sufficient information to identify a preferred inhaled steroid (2,22,38). The ideal inhaled steroid and delivery system would have little or no systemic effect with a high level of topical activity in the lung (Fig. 5); in other words, they would have a high therapeutic index. For example, in Figure 5, treatment A is more potent than treatment B because lower doses achieve comparable effect. In addition, treatment A is more efficacious than treatment B because its maximal effect is greater.

To address these questions, it is important to identify reliable measures that could be obtained in short-term studies that bear significant clinical relevance. One approach is to define a measure of clinical benefit, such as an increase in $FEV_1$, and then assess comparative levels of systemic effect, such as cortisol suppression: an alternative approach is to identify a level of systemic effect and then examine comparative levels of beneficial effect.

The NHLBI's Asthma Clinical Research Network designated the comparison of inhaled steroids a significant therapeutic issue meriting a concentrated effort and sought to develop techniques of comparison that are reliable and easily

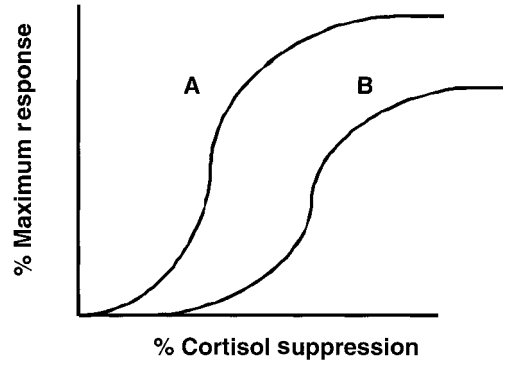


**Figure 5** Simulated dose-response curves depicting a comparison of two inhaled steroids. Inhaled steroid A is not only more potent, showing a similar effect to inhaled steroid B at lower doses, but it also has a greater maximal effect at high doses.

applied to a clinical research setting (38). The approach taken has been to obtain dose-response measures of systemic effect for different inhaled steroids and then to evaluate the relative pulmonary effects of treatment, with doses providing equivalent systemic effects.

### A. Markers of Systemic Effect

The most significant potential adverse effects of inhaled steroids include growth suppression, reduction in bone density, and cataracts. Unfortunately, measuring these effects requires large numbers of patients and long study periods. In lieu of reliable indicators for these significant clinical effects, attention has turned to measures of cortisol suppression as an indicator of systemic effect.

Although cortisol suppression cannot be directly extrapolated to a concerning clinical toxicity, it is an easily obtained and quantifiable measure of systemic availability (14). There are special considerations for the procedures used to measure cortisol suppression. In the past, cortisol suppression was assessed by measuring the adrenocortical response to stimulation with ACTH. This test was originally designed to measure dysfunction of the adrenal gland. It became apparent that the dose of ACTH used was supra-physiological and not sufficiently sensitive to detect small levels of adrenal suppression. Attempts at developing a more sensitive test, by using a low dose of ACTH, have not been sufficiently reliable to use in clinical studies (43).

Other methods for measuring cortisol suppression utilized either urinary or serial plasma cortisol measurements. Urinary cortisol measurements are usually obtained over 24- or 12-hour time periods. Although this test can be sufficiently reliable, it is highly dependent on the subject's adherence to the urine collection schedule. Serial plasma collections can provide reliable measures of cortisol suppression, but they require a specialized study unit to assure reliable and safe collection techniques. Blood samples are usually collected every hour over 24 hours or, alternatively, over a 12-hour nighttime period. It is important to carefully regiment the collection procedure to assure reproducible results. For the ACRN studies, several measures of cortisol suppression will be tested. These include 12-hour serial plasma cortisol and 24-hour urine cortisol measurements.

### B. Markers of Beneficial Effect

Several markers of clinical benefit have been used in studies including pulmonary function measures, determination of as needed β-agonist therapy for symptoms, evaluation of airway hyperresposiveness (histamine, methacholine, exercise, adenosine), and markers of inflammation. In general, measures of pulmonary function such as peak flow and $FEV_1$ improve quite rapidly and with low doses of inhaled steroids (22,41,44). Although useful for comparisons of effect, these pulmonary function measures often fail to demonstrate a dose-response relationship

in clinical studies (41,45). One proposed marker of inflammation, exhaled nitric oxide, has shown a dose-response relationship to inhaled steroid dosing, but its relevance to the measurement of chronic inflammation is still controversial (46,47). To date, a careful evaluation of sputum cytology, e.g., sputum eosinophils, in relation to inhaled steroid dose has not been conducted. If this measure is used, it will necessitate the enrollment of subjects with a specified number of sputum eosinophils in order to measure a response.

Attempts to identify a dose-response relationship of steroid effect using a measure of airway responsiveness, such as the provocative dose of histamine or methacholine, have been disappointing. The changes in airway responsiveness are usually limited to one doubling dose and are insufficiently broad to define a dose-response relationship. In addition, this measure is time dependent, improving with treatment even after a year of continuous therapy. One measure of airway responsiveness that shows promise as a discriminator of a dose-response effect is exercise challenge. Exercise challenge is attractive since this is a clinically meaningful measure of an asthma outcome that is relevant to real-life situations. A study conducted by Pedersen and Hansen in severe asthmatic children reported a dose-response relationship for inhaled budesonide using exercise challenge (48) (Fig. 6). This is the only study to date using this measure as an indicator of inhaled steroid response, and it needs to be replicated.

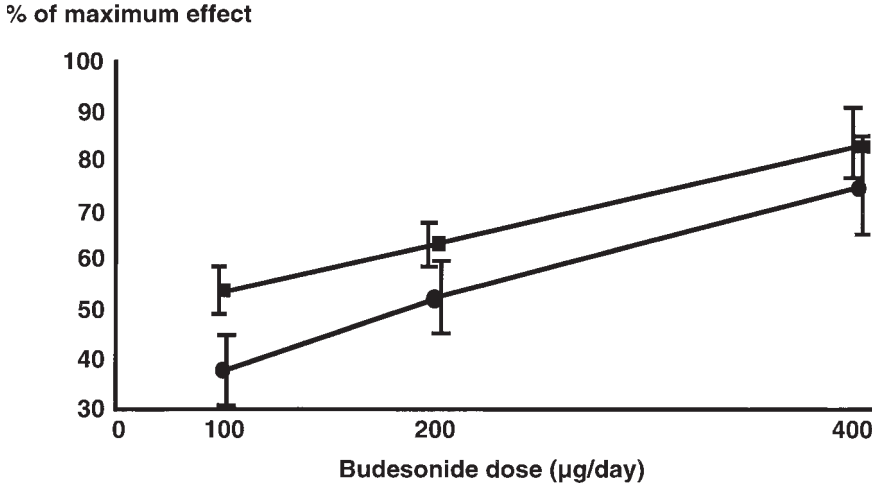


**Figure 6** Percent of maximum achievable protection (mean and 95% confidence limits) against fall in $FEV_1$ (■) and FEF 25–75 (●) after exercise in 19 children treated with 100, 200, and 400 µg of budesonide per day in randomized sequence. Each treatment period was 4 weeks. (From Ref. 48.)

The Asthma Clinical Research Network (ACRN) is incorporating a battery of markers of pulmonary and clinical response, as well as proposed markers of inflammation in its comparisons of inhaled steroids. These will be summarized in the following project description.

### C. The ACRN Study Design

In order to evaluate the comparative systemic and beneficial effect of inhaled steroids, the ACRN developed a series of studies that are now in progress.

#### *Dose of Inhaled Corticosteroid with Equi-systemic Effect (DICE)*

This series of studies was designed to quantify the level of cortisol suppression for increasing doses of selected inhaled steroids. The study design, summarized in Figure 7, is being conducted in two phases:

Phase I (pilot study)—evaluation of two inhaled steroids to verify study procedures

Phase II—expanded study with five inhaled steroids incorporating defined markers of systemic effect

The purpose is to develop an experimental paradigm to characterize inhaled steroids in terms of systemic effect (cortisol suppression) so that doses that produce an equi-systemic effect can be used in the second series of studies to measure comparative efficacy. The designated levels of cortisol suppression to be defined include 10% (CS10), 20% (CS20), 30% (CS 30), and 40% (CS40) cortisol suppression of overnight cortisol secretion.

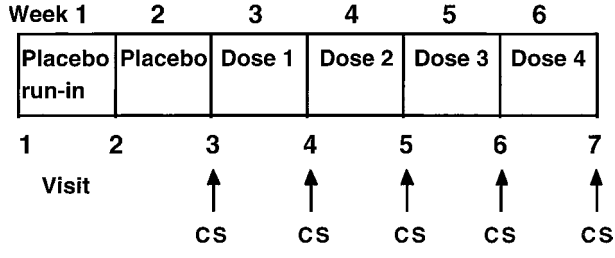


**Figure 7** DICE Phase I and II study design—outline of core design indicating a total of 7 weeks that incorporates a four-dose incremental procedure for the selected inhaled steroid. The subject receives each dose level for one week. Before starting inhaled steroid and after each incremental dose, cortisol suppression (CS) is measured via 12-hour serial one-hour plasma cortisol measurements and a 24-hour urine collection of cortisol measurement.

**Table 5** Inhaled Steroid Dosing Schedule for DICE Phase I Study

| Dose | Beclomethasone dipropionate, μg (84 μg/inhalation) | Fluticasone propionate, μg (44 μg/inhalation) |
|---|---|---|
| 1 inhalation twice daily | 168 | 88 |
| 2 inhalations twice daily | 336 | 176 |
| 4 inhalations twice daily | 672 | 352 |
| 8 inhalations twice daily | 1344 | 704 |

The study population includes subjects with mild to moderate asthma, 18–45 years of age. Asthma criteria include $FEV_1$ 65–90% predicted, 12% reversibility with inhaled albuterol, and $PC_{20}$ methacholine ≤8 mg/mL. In addition, subjects must have a baseline morning plasma cortisol ≥5 μg/dL, take no hormonal therapy, and be neither morbidly obese nor cachectic. Specific exclusion criteria were developed for steroid exposure to minimize the possibility of altered cortisol response. These criteria include no oral or injectable steroids within 2 years, no orally inhaled or nasal steroids within one year, and no topical steroids applied to the skin within one year, with the exception of over-the-counter topical steroids, and even these cannot have been administered within 2 months of study participation.

In the Phase I DICE studies, three inhaled steroid treatment arms were evaluated: fluticasone propionate (CFC propellant) administered via an OptiChamber, beclomethasone dipropionate (CFC) without chamber, and beclomethasone dipropionate (CFC) with Opti-Chamber. The study doses for the two steroids are summarized in Table 5. There were 20 subjects in each treatment arm.

After receiving placebo and each study dose for one week, the subject reported to the study center at 5:30 p.m. for overnight testing. The subject remained in the study unit until 8 a.m. the following day. During that time, urine collection for cortisol were collected from 6 to 11 p.m. and from 11 p.m. until 7 a.m. Hourly plasma cortisol collection samples were obtained from 10 p.m. to 7 a.m. In addition, in the Phase I studies, low-dose ACTH stimulation tests were conducted at 7 a.m. The dose of ACTH was 0.5 μg/1.73 $m^2$, and blood samples for cortisol measurement were obtained prior to ACTH administration and 20 minutes and 30 minutes after the dose. In addition, bone remodeling markers, *N*-telopeptide in pooled urine, and osteocalcin in blood were measured.

Recognizing that inhalation technique can greatly influence deposition of inhaled corticosteroids, consistency in inhalation technique was standardized using the Technique Assessor TM (Aradigm) controlling for inspiratory flow, volume inhaled after actuation, and breath-hold time.

In the DICE Phase I study, considerable information was obtained, including the following:

1. Neither the 6–11 p.m. urinary cortisol, the 11 p.m. to 7 a.m. urinary cortisol, nor the combination showed a dose-response effect on cortisol suppression.
2. Morning low-dose ACTH stimulation testing was not a useful measurement.
3. The overnight AUC plasma cortisol concentration is a sensitive test for adrenal function.
4. Urine *n*-telopeptide did not show a dose-response relationship.
5. Plasma osteocalcin did show a trend towards a dose-response relationship.
6. The application of a spacer did not have a significant effect on altering cortisol suppression with equivalent doses of beclomethasone dipropionate in the absence of a spacer.

A full protocol evaluating six inhaled corticosteroid preparations was developed and is now in progress based on experience gained from the DICE Phase I study. The DICE Phase II protocol, adjusted based on the DICE Phase I experience, includes.

1. Hourly plasma cortisol concentrations collected for 12 hours, from 8 p.m. until 8 a.m. the following morning. This will serve as the primary basis for comparison of systemic effect.
2. The incorporation of a 24-hour urine collection with two timed aliquots of urine collection, 8 a.m. to 8 p.m., and then 8 p.m. to 8 a.m. the following morning. The two individual 12-hour increments will be analyzed and also combined for a total 24-hour collection.
3. Steroid exclusion criteria modified to allow use of oral or injectable steroids between 1 and 2 years, and inhaled or nasal steroids between 6 months and 1 year, if results of the low-dose ACTH stimulation test shows normal responsiveness.

### *Measuring Inhaled Corticosteroid Efficacy (MICE)*

Definition of the magnitude of cortisol suppression for a range of doses of selected inhaled steroids will enable identification of doses that result in equivalent cortisol suppression and that can then be compared in a study of their relative beneficial effects on an array of indicators. This set of ACRN studies is also being conducted in series.

MICE Phase I—Comparison of two inhaled glucocorticoids, specifically beclomethasone dipropionate and fluticasone propionate. The selected

doses for comparison in MICE I are based on DICE Phase I experience and determination of inhaled steroid doses that result in cortisol suppression less than 5%, 20–30%, and 40–60%.

MICE Phase II—Expanded study with additional inhaled steroids of clinical interest. The inhaled steroids and corresponding delivery systems will be selected based on their measured levels of cortisol suppression in the DICE Phase II study and the clinical measures of efficacy with greatest reliability as determined from MICE Phase I experience.

The design features for MICE Phase I includes an initial placebo run-in phase, followed by a sequential series of increasing doses of inhaled steroids. The study design is summarized in Figure 8. The study doses selected include inhaled steroid doses that produce minimal cortisol suppression (<5%), 20–30% cortisol suppression, and 40–60% cortisol suppression. After the three 6-week dosing schedules of the inhaled steroid under evaluation are complete, the subject receives a 3-week course of high-dose inhaled fluticasone propionate to define the maximal effect for the study measures. Following administration of each study

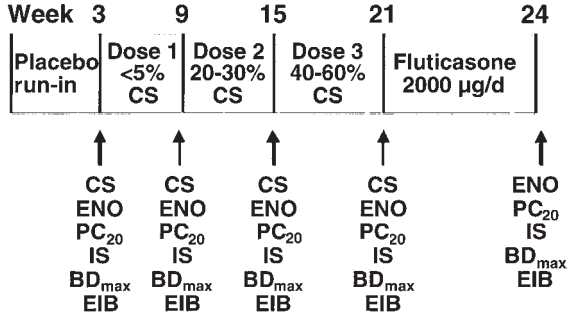


**Figure 8** MICE Phase I study design—outline of core design indicating a total of 24 weeks that incorporates a three-dose incremental procedure for each of the two inhaled steroids studied. A subject would receive either beclomethasone dipropionate or fluticasone propionate. Each dose level is administered for 6 weeks. After the three steroid dose levels, the subject receives fluticasone propionate at 2000 µg per day for 3 weeks. Before starting inhaled steroid treatment and after each incremental dose, cortisol suppression is measured via 12-hour serial one-hour plasma cortisol measurements and a 24-hour urine collection of cortisol measurement. Efficacy parameters are measured before starting inhaled steroid and after each dose increment of the inhaled steroid studied and also after the high-dose fluticasone propionate treatment. CS = Cortisol suppression; ENO = exhaled nitric oxide; IS = induced sputum; $PC_{20}$ = methacholine challenge; $BD_{max}$ = spirometry before and with maximum reversibility with bronchodilator; EIB = exercise challenge.

dose of inhaled steroid for 6 weeks, cortisol suppression is measured via overnight plasma cortisol (collected in 1-hour intervals from 8 p.m. to 8 a.m.) and urinary cortisol (two 12-hour increments and a combined 24-hour measurement).

The study population includes patients with $FEV_1$ 55–85%; reversible airflow obstruction ($\geq$12% or >200 mL improvement in $FEV_1$ following two to four inhalations of albuterol by metered dose inhaler and a $PC_{20}$ to methacholine $\leq$8 mg/mL); exercise-induced bronchospasm, as defined as a fall of $\geq$12% following exercise challenge; and baseline morning plasma cortisol concentration $\geq$5 μg/dL. Similar steroid exclusion criteria are applied as described for the DICE Phase II protocol.

As described in Figure 8, measures of efficacy include $FEV_1$ pre- and postbronchodilator, $PC_{20}$ for methacholine, exercise-induced bronchospasm, peak flow measured twice daily, asthma control assessment, induced sputum cytology, and exhaled nitric oxide. The study will be analyzed for the various measures of efficacy versus incremental increase in dose (cortisol suppression) facilitating a comparison of two inhaled steroids, inhaled beclomethasone dipropionate and fluticasone propionate, both administered with a pressurized metered dose inhaled and Opti-Chamber delivery device. Comparisons of efficacy can then be made analogous to Figure 1 provided by Toogood and colleagues (15–17). A time-response analysis can also be conducted to evaluate the comparative onset of effect (daily peak expiratory flow measures) for the two inhaled steroids. Theoretically, higher-potency inhaled steroids should result in a more rapid onset of effect. Based on the results of this study, the most reliable and cost-efficient measures of efficacy can be selected for the MICE Phase II study, which will use a wider array of inhaled steroids, and also increase convenience for study subjects. As technology improves and the degree of cortisol suppression is lowered, the procedure can be reversed to identify comparative efficacy and then evaluate corresponding systemic effect.

## VII. What Is the Best Way to Select Inhaled Glucocorticoids?

### A. Selecting an Inhaled Glucocorticoid

The present health care system, based on principles of cost management, encourages physicians to utilize the lowest-cost preparation. Unfortunately, this makes it difficult for the clinician to select the "best" preparation for the patient and does not reward advances in drug development. However, the emphasis in health care is gradually shifting to quality control. This direction challenges clinical research to define reliable markers of quality. Five inhaled glucocorticoids are now available in the United States with varying potencies, strengths, and delivery systems. Selection is now based on patient preference, taste, number of actuations per dose,

frequency of dose, and cost to the patient or health care provider. For the most part, comparisons have been limited to study of relative effects on cortisol suppression with limited analysis of relative efficacy. Fluorocarbon propellants are gradually being removed necessitating reformulation of all pressurized metered dose inhaler preparations. Market uptake for HFA-albuterol, however, has been slow possibly due to the associated higher cost or apathy about the issue of environmental protection. Dry powder, breath-actuated delivery systems will continue to be introduced. This change in delivery system formulation will require careful attention to patient education, since the technique of administration is different from the pressurized metered dose inhaler with or without a spacer. Recent studies suggest that the new delivery systems improve lung delivery (19,20), raising questions about the dose needed to minimize the amount of drug absorbed.

### B. Selecting the Dose of an Inhaled Glucocorticoid

Medication adjustments for asthma control will continue to be based on symptom reports by patients, monitoring use of rescue therapy, and pulmonary function when applied. There is inadequate information regarding the dose-response relationship of inhaled glucocorticoids for clinical effects and systemic effects (38). The dose-response relationship for efficacy parameters depends on the parameter measured. For example, reduction in symptoms and improvement in $FEV_1$ may be affected by relatively low doses, while subtle features, such as airway hyperresponsiveness and airway normalization may require higher doses over a prolonged period of time (15,37,48). Response is also related to duration of treatment. After approximately 2 months of treatment, low doses of inhaled steroids have effects similar to a medium dose administered for the same duration of treatment (36). Patients with severe persistent asthma may have persistent inflammation even in the presence of medium to high dose plus systemic glucocorticoids (49,50). Therefore, it will be useful to incorporate convenient and reliable markers of inflammation, such as exhaled nitric oxide, sputum cytology, and airway hyperresponsiveness, as they become readily available for clinical care.

### C. Minimizing the Risk of Long-Term Inhaled Glucocorticoid Therapy

There is no clinical definition of a maximally safe dose and minimally effective dose. Until this information is available, clinicians should monitor efficacy and potential adverse effects. Growth measurements in children are easily obtained, but other measures incur additional costs for laboratory testing, i.e., bone densitometry, tonometry, and slit lamp examinations. Patients at risk should be monitored for specific adverse effects, e.g., growth in children, bone density in postmenopausal women, and periodic ocular examinations in the elderly, especially in patients receiving high-dose inhaled steroid for prolonged periods of times, e.g.,

longer than one year. One should also be prepared in cases of significant physiological stress, e.g., severe asthma exacerbations, accidents, or surgery, where adrenal insufficiency could be a risk if cortisol response is impaired.

At the present time, based on concerns of risk of long-term, high-dose inhaled steroids, clinicians frequently elect to utilize low- to medium-dose inhaled steroids and supplement with nonsteroid long-term control medications, such as long-acting $\beta_2$-adrenergic agonists (salmeterol, formoterol), leukotriene modifiers (zileuton, zafirlukast, montelukast), or theophylline. Although benefits are derived in pulmonary function, additional information is needed to determine whether these supplementary therapies are more effective than high-dose inhaled steroid therapy in altering the course of the disease.

## VIII. Prospectus

Inhaled glucocorticoids will remain the cornerstone of managing persistent asthma for the near future. The leukotriene antagonist class of medications is considered an alternative form of first-line therapy or additive therapy in the management of mild persistent asthma. This is largely based on ease of administration. Additional studies are needed to determine if the leukotriene antagonists and other nonsteroid long-term control medications are equally effective in controlling disease progression. While concern remains regarding the risk of long-term adverse effects of inhaled steroids, especially at high doses over prolonged periods of time, new inhaled steroids and new delivery systems are being developed with less systemic absorption.

New insights regarding mechanisms of glucocorticoid effect, for example, reduction of peripheral inflammation, could lead to enhanced lung delivery or new drugs that maximize the desired cellular effect of steroids and minimize the risk for undesirable systemic effects. New insights into asthma management, for example, further understanding of the concept of "airway remodeling" and its clinical implications, could lead to identification of better methods to monitor airway inflammation, for example, airway hyperresponsiveness, induced sputum, and exhaled nitric oxide, to guide therapy. In the future, our understanding should further reduce the morbidity and mortality associated with asthma.

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## Discussion

**Dr. O'Byrne:** The term "adrenal suppression" may not be the best one to use for what you are measuring. The term "cortisol replacement" may be better as you are replacing endogenous with exogenous steroid. There is no evidence of true adrenal suppression with inhaled steroids.

**Dr. Szefler:** That is a good point, but the commonly used term has been cortisol suppression. This reflects a reduction in the plasma or urine cortisol measurements.

**Dr. Derendorf:** What was the rationale for measuring cortisol from 10 p.m. to 7 a.m. and not over 24 hours? You may miss the morning peak in some of your subjects.

**Dr. Szefler:** We chose a fixed time interval that would be reproducible and convenient for study subjects.

**Dr. Rohdewald:** Did you include in your study the measurement of frequency of use of $\beta_2$-antagonists?

**Dr. Szefler:** Yes, we have collected data on this parameter.

**Dr. Brattsand:** Do you expect that a selected steroid will be the best under all conditions (mild–severe, q.i.d.–q.d., etc.)? One steroid may be the best in severe asthma due to a need for some systemic efficacy (see Dr. Denburg's presentation), while another is better in mild asthma when given once daily. There may well be different threshold concentrations and trigger times for achieving the various antiasthmatic actions. In mild asthma—where the main emphasis is safety—a preferred glucocorticoid may not need to reach all efficacy levels.

**Dr. Szefler:** You raise some good points, and this will have to be considered in the interpretation of the data and subsequent application to clinical practice.

**Dr. Schleimer:** I am concerned that even the lower doses to be used ($<10\%$ cortisol suppression) may still be high on the efficacy dose response (near the "plateau"), weakening the ability to detect efficacy differences.

**Dr. Szefler:** You are correct, and that will have to be considered in the analysis. The results will likely be parameter specific.

**Dr. O'Byrne:** I would suggest that you also include an exercise test in the middle of the treatment period. That might increase the sensitivity.

**Dr. Busse:** As to the development of study design, you are pushing the probability of efficacy to a precision that may exceed the precision allowed by the disease itself. It would seem more appropriate to determine the effect of steroids

on regulation of the disease, rather than on different preparations of corticosteroids. One can then extrapolate these observations to studies of other steroids.

**Dr. Hargreave:** Is the design cumulative? The response is going to be determined by the presence and severity of eosinophilic inflammation. My suggestions are to select subjects with sputum eosinophilia.

**Dr. Szefler:** We did not make an attempt to screen subjects by sputum eosinophils, but this will certainly be looked at carefully as a response parameter.

**Dr. Rohdewald:** Will patients record PEF on a daily basis in a diary? That would provide a profile with many points indicating improvement of lung function.

**Dr. Szefler:** Patients in both our efficacy and systemic effect studies collect twice-daily pulmonary function measures. This will be analyzed as an outcome variable and will help us determine onset of effect of each dose.

**Dr. Georas:** You mentioned that 50% inhibition of cortisol secretion is the level at which you get concerned with inhaled glucocorticoids. I'm wondering how you came up with this number.

**Dr. Szefler:** That is just my own empirical assessment. There have not been studies that have closely looked at the relationship of the magnitude of cortisol suppression to other systemic effects, such as growth.

**Dr. Hargreave:** Why do you select 6 weeks? In fact, the steroid acts more quickly. The longer the duration of use, the more likely the outcome is to be influenced by external influences such as infection and allergen. What are the questions that you are asking in these studies? Can you identify a dose response? Which outcomes are the best ones to choose? I like to look at inflammatory parameters (e.g., sputum eosinophils, blood eosinophils, and exhaled NO).

**Dr. Szefler:** We chose 6 weeks to allow a sufficient period of time to reach near-plateau effect with each dose for pulmonary function, i.e., spirometer measures, The time relationship could very well be different for other measures, such as exercise-induced bronchospasm.

# Part Six

## IN VIVO RESEARCH ON AIRWAY–LUNG SELECTIVITY

# 17

# The Role of Direct Assessment of Airway Inflammation in Evaluating Inhaled Glucocorticosteroid Efficacy and in Managing the Asthmatic Patient

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## I. Introduction

In this chapter I will review the evidence that inhaled glucocorticosteroid (GCS) therapy can reverse specific aspects of airway inflammation observed in asthma. The focus here will be on cellular aspects of inflammation, as airway remodeling will be covered in Chapter 25.

Initially, I will review studies in which direct measurements of airway inflammation have been made, either pre- and postinhaled GCS therapy or in a placebo-controlled study. To date, direct measures of airway inflammatory status include bronchial biopsy, bronchoalveolar lavage or wash, and induced sputum; all of these methods have been used to evaluate the efficacy of inhaled GCS treatment. Following this, I will address the question of whether directly measuring airway inflammatory status should be routinely performed in order to tailor the therapy for specific patients. As invasive procedures would not be practical for this purpose, this section will focus on the potential role of induced sputum.

## II. Direct Measurement of Airway Inflammation to Assess the Efficacy of Inhaled Glucocorticosteroids

### A. Bronchial Biopsy

While evaluation of asthmatic lung tissue can be made postmortem (1), it is only relatively recently that endobronchial biopsies have been used for the analysis of tissue from the proximal airways of living asthmatics (2). This technique has made it possible to evaluate the effects of various treatments on inflammatory indices in this compartment.

Several investigators have observed beneficial effects of treating asthmatics with inhaled GCS on biopsy markers of airway inflammation. Jeffery et al. (3) compared indices of inflammation from biopsies of healthy and asthmatic subjects and also observed the effects of a brief period of inhaled GCS therapy on these indices (Fig. 1). In that study, asthmatics, having been managed with inhaled GCS for at least 6 months, had increased percentage of eosinophils infiltrating the bronchial mucosa compared to normals. Furthermore, mild asthmatics, managed with only inhaled bronchodilators, had an increased total number of inflammatory cells infiltrating the bronchial mucosa and a significantly greater percentage of both eosinophils and mast cells compared to normals. However, the eosinophil and mast cell percentages in this mild asthmatic group returned to normal after a 4-week treatment with inhaled budesonide (200 μg bid), but not with inhaled terbutaline (500 μg qid).

Laitinen et al. (4) treated seven mild steroid naive asthmatics with inhaled budesonide (600 g bid) for 3 months and observed decreased airway responsiveness and improved spirometry. Biopsies taken before and after treatment indicated significant reductions of total numbers of inflammatory cells infiltrating both the epithelium and lamina propria. Budesonide treatment was also associated with a decrease in eosinophil and lymphocytes in the epithelial layer and in mast cells, lymphocytes, and plasma cells in the lamina propria. Following treatment, eosinophils were detected in the epithelial layer of only one of these mild asthmatic subjects. Treatment with inhaled terbutaline of another group of similar subjects was not associated with improvement in epithelial inflammatory indices, although there was a significant reduction in total inflammatory cells infiltrating the lamina propria.

Djukanovic et al. (5) evaluated biopsies from asthmatic subjects prior to and following the commencement of inhaled GCS therapy (beclomethasone 2000 μg/day for 2 weeks, 1000 μg/day for 4 weeks) that was deemed necessary for optimal management of their asthma. Although this was an uncontrolled study, it is worth noting that the clinical improvement (decreased symptoms and airway responsiveness and improved spirometry) was associated with reductions in eosinophils and mast cells in the epithelial and submucosal layer and decreased submucosal lymphocytes.

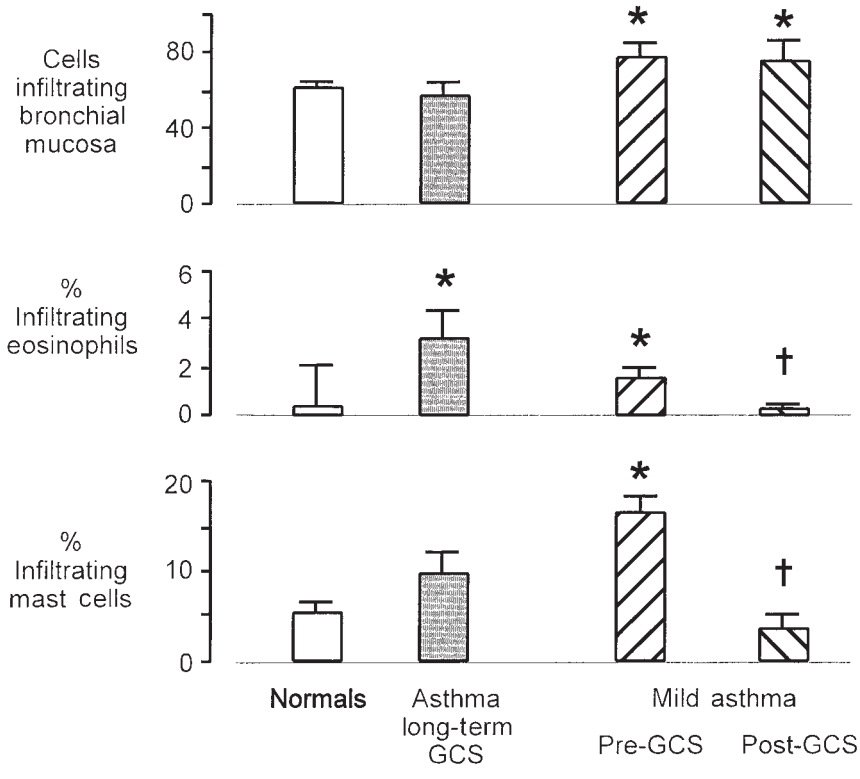


**Figure 1** Bronchial biopsy evaluation of inflammatory cells in the airways of normals, asthmatics receiving ongoing inhaled GCS treatment, and mild asthmatics before and after 4-week treatment with inhaled budesonide (200 μg) b.i.d. * = Different from normals ($p < 0.05$). † = Different than pre-GCS ($p < 0.05$).

Trigg et al. (6) randomized mild asthmatic subjects to receive either inhaled placebo or beclomethasone (500 μg bid) for 4 months. There were significant improvements in $FEV_1$ in both groups and a trend towards improvement in airway responsiveness in the steroid-treated subjects only. In the steroid-treated but not the placebo group there was a significant reduction in the numbers of mast cells and eosinophils in the bronchial mucosa.

Using a randomized, placebo-controlled parallel group design, Olivieri et al. (7) observed the effects of 6-week treatment with inhaled fluticasone propionate (FP), 250 μg bid, in mild asthmatic subjects. Treatment with FP was associated with decreased airway responsiveness and also with an 83% and 69% reductions in the number of eosinophils and mast cells, respectively, in the lamina propria. Placebo treatment was not associated with improvement of any of these outcomes.

Most recently, Lim et al. (8) compared inhaled budesonide (800 μg bid) and placebo for 4 weeks each in a randomized double blind crossover trial in mild athmatic subjects. When compared to placebo, budesonide treatment was associated with an improvement in morning and evening peak expiratory flow and a reduction in airway responsiveness. Comparison of bronchial biopsies between treatment arms indicated a 56% reduction in the number of eosinophils and 48% reduction in the number of macrophages in the submucosa in the steroid-treated group.

Clearly the results of these studies indicate that treatment of mild asthmatics with inhaled corticosteroids can lead to significant reductions in the numbers of eosinophils and also, possibly, the numbers of mast cells, lymphocytes, and macrophages in compartments of the airway wall. Encouragingly, the results of Jeffrey et al. (3) suggest that inflammatory cell numbers may be reduced to normal levels in these mild subjects. However, in that same study patients receiving long-term treatment with inhaled GCS had greater than normal eosinophil numbers in the bronchial mucosa. Possibly, these findings indicate that inhaled GCS cannot normalize eosinophilia in more severe asthmatics. It is also possible that patient compliance—especially given that treatment in this group was not part of the study design—was low and that the treatment effect was suboptimal. Answering the question of whether inhaled GCS can fully reverse specific aspects of airway inflammation in severe asthma will be difficult to determine using biosy studies, as the risk of bronchoscopy may not be justified in this population.

### B. Bronchoalveolar Lavage

In addition to obtaining biopsies for the evaluation of airway inflammation, fiberoptic bronchoscopy has also allowed researchers to perform bronchoalveolar lavage (BAL) and bronchial washing (BW) for this same purpose. Usually, BW involves infusing a small volume (approximately 20 mL) through the bronchoscope and immediately aspirating fluid back into a collection chamber, while BAL involves the infusion of a larger volume (often $2 \times 60$ mL), which is also aspirated into a collection chamber. Although it is not entirely clear which compartment is sampled using each technique, it is presumed that BW samples proximal airways while BAL samples the entire airway.

Ädelroth et al. compared inflammatory indices in both BAL and BW between normal and asthmatic research subjects and also have observed the effects on these indices of treatment with inhaled GCS (9). Eosinophil percentages were significantly increased in mild and steroid dependent asthmatics compared to normals. Unlike the results observed in bronchial biopsy studies, however, there was no significant reduction in eosinophil percentage in either BAL or BW in the mild asthmatic subjects following a 4-week treatment period with inhaled budesonide (200 μg bid) (Table 1).

**Table 1** Effects of Treatment with Inhaled GCS on Eosinophil Percentages in Bronchoalveolar Lavage and Bronchial Washing

| Study (Ref.) | Treatment | Outcome | Control | Treated | *p*-value |
|---|---|---|---|---|---|
| Adelroth et al. (9) | Budesonide 4 wks (200 μg b.i.d.) | BAL eosinophils (%) | 1.8 | 2.6 | >0.05 |
| Adelroth et al. (9) | Budesonide 4 wks (200 μg b.i.d.) | BW eosinophils (%) | 3.2 | 2.6 | >0.05 |
| Olivieri et al. (7) | Fluticasone 6 wks (250 μg b.i.d.) | BAL eosinophils (%) | 1.01 | 0.81 | >0.05 |
| Trigg et al. (6) | Beclomethasone 4 mo (500 μg b.i.d.) | BAL eosinophils (%) | ~1.7 | ~0.4 | >0.05 |
| Lim et al. (8) | Budesonide 4 wks (800 μg b.i.d.) | BAL eosinophils (%) | 1.76 | 0.81 | >0.05 |

Three other groups have also observed that inhaled GCS treatment does not affect inflammatory indices in BAL from mild asthmatic subjects (6,7). Olivieri et al. (7) observed that the cellular measurements in BAL before and after 6-week treatment with inhaled fluticasone propionate (FP), 250 μg bid, were not different, while there were no differences between the effects of 4-week treatment with inhaled budesonide (800 μg bid) or placebo (Table 1). Trigg et al. (6) found no significant changes in BAL following 4-month treatment with beclomethasone, 500 μg bid.

It is worth noting that in all four of these studies, where BAL or BW eosinophilia was not affected by inhaled GCS, indices of airway eosinophilia as assessed by bronchial biopsy were reduced in association with treatment. It has therefore been proposed that BAL may not be a sensitive tool for monitoring airway eosinophilia (8). However, it is possible that in more severe subjects, where there could be expected to be a greater percent of eosinophils in BAL or BW, a significant treatment effect would be observed. This likelihood is supported by observing the data of Trigg et al. (6) in which subjects with high baseline BAL eosinophilia appeared to improve following steroid treatment. Another possibility that may explain the lack of treatment effect of inhaled GCS on BAL eosinophils is that this outcome measurement reflects airways that are more distal than those sampled using bronchial biopsy and therefore not receiving optimal treatment from the delivery devices used in these studies. This possibility would need to be tested by repeating these studies using delivery devices with smaller particle sizes and therefore distributing to smaller airways. If this proved to be case, then one might argue that rather than being an insensitive tool for monitoring anti-inflammatory effects of inhaled GCS, BAL or BW may provide important information not available from bronchial biopsy.

### C. Induced Sputum

Induced sputum has been used since 1986 to provide a noninvasive means of assessing the extent of cellular and fluid-phase disturbances in the airway (10–12). Measurements made using this technique have proved reproducible and responsive (13), indicating that it may be a valuable method for assessing the anti-inflammatory effects of inhaled GCS.

Van Rensen et al. (14) compared the effect on mild asthmatic patients of 4-week treatment with inhaled fluticasone propionate (500 μg b.i.d.) or placebo on sputum eosinophilia in a double-blind, randomized, parallel group design. In this study, GCS but not placebo was associated with a two doubling concentration increase in $PC_{20}$. The percentage of eosinophils in induced sputum decreased significantly after 2 weeks of GCS but not placebo treatment (Fig. 2). This treatment effect was maintained over the 4-week treatment period and was lost after a 2-week washout period (Fig. 2).

Using a double-blind, randomized, crossover design, Lim et al. (8) compared the effects of 4-week treatment with placebo or inhaled budesonide (800 μg bid) on percent eosinophils in induced sputum. These authors also observed a significant reduction (pretreatment: 4.9%; posttreatment: 1.38%) in percent eosinophils after GCS treatment. In this study there was an associated reduction in eosinophils in bronchial biopsy tissue (see above).

Thus, it is clear that analysis of eosinophilia in induced sputum from asthmatic patients is sensitive to inhaled GSC treatment. While the study by Lim et al.

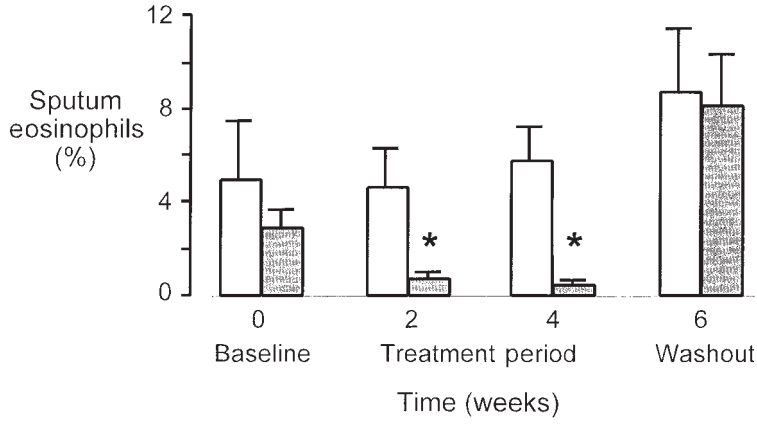


**Figure 2** Eosinophils expressed as a percentage of total cells recovered from sputum induced from asthmatic subjects before, during, and 2 weeks after a 4-week period of treatment with either placebo or fluticasone propionate (500 μg). * = Different than in placebo-treated group.

(8) demonstrated an associated decrease in eosinophils in bronchial biopsy, it is not clear whether information from these two tests can be considered as equivalent, or whether there are subtle differences in the value of these two tests.

### D. Conclusion

Results from several studies are in agreement that inhaled GCS can reduce the extent of several cellular aspects of asthmatic inflammation. This information has played a large role in the increased use of inhaled steroids in several countries and the move to introduce this class of drugs early in the natural course of the disease in the belief that this will perhaps reduce the ultimate severity of the disease. However, the information from studies of this type is not particularly useful for the physician who needs to decide on the appropriate dose of inhaled GCS for each patient or to determine whether this treatment is achieving the desired anti-inflammatory effect. Achieving these practically useful aims would require tests that can monitor the inflammatory state and provide information that can be incorporated into management decisions, much as is currently the case for symptom and pulmonary function tests. This issue will be addressed in the following section.

## III. Measurement of Airway Inflammation as a Guide for Treatment Decisions

It has been suggested by the Global Initiative on Asthma (GINA) that evaluation of airway inflammation should be incorporated into the management of the asthmatic patient. To date there are no studies in which the value of direct measurement of airway inflammation for this purpose has been assessed. However, Sont and colleagues have provided evidence that incorporating regular measurement of airway responsiveness into a treatment decision algorithm can result in improved asthma-related outcomes (15). Specifically, patients in whom the dose of inhaled GCS was determined by airway responsiveness as well as conventional markers of disease severity (symptoms, bronchodilator use, and spirometry) fared better in terms of exacerbation rate and improvements in lung function and airway remodeling than did patients who were assessed using only conventional markers. While these results provide encouragement that individualized treatment based on direct assessment of each patient can decrease morbidity, it is not clear whether measurement of nonspecific airway responsiveness is the most appropriate test for this purpose. While there are many studies demonstrating a significant relationship between the magnitude of airway hyperresponsiveness (AHR) and the extent of eosinophilic airway inflammation in biopsy, BAL, or sputum (e.g., 16–19), there are as many that fail to observe such a relationship (e.g., 9,20–22). The most likely explanation for these results is that a component of airway hyperrespon-

siveness in asthma is related to eosinophilic inflammation (either directly or indirectly), but the fact that there are many other contributing factors (Fig. 3) can confound this relationship when a heterogeneous sample of asthmatics is studied. Thus, the multifactorial basis of airway hyperresponsiveness means that the results of the study performed by Sont et al. (15) should be viewed with some caution. If, as the authors propose, GCS dose was influenced by the degree of airway hyperresponsiveness, subjects with severe AHR due to factors that are not responsive to GCS treatment would still receive the highest treatment dose. While this study demonstrated that, on the whole, this approach can lead to a group benefit, it is likely that more specific information about the nature of the inflammation will result in treatment strategies that are appropriate for the individual patient. Currently, the only validated methods to describe the nature of airway inflammation are the direct measurement techniques described above. Of these, only induced sputum is noninvasive and potentially useful as a tool for directing therapy on an individual patient basis.

### A. Sputum as a Predictor of Which Patients Will Improve with GCS Treatment

For sputum to be useful at guiding inhaled GCS therapy, it must provide information that will predict whether a specific patient will benefit from added inhaled GCS. Pavord et al. observed the effects of inhaled GCS therapy in two groups of asthmatics, one with sputum eosinophilia $<3\%$ and one with eosinophilia $\geq 3\%$ (23). These authors observed that following a 2-month period of treatment with inhaled budesonide (400 μg bid) there was greater improvement in spirometry, symptoms, and airway responsiveness in the group with higher baseline eosinophilia and that there was a significant relationship between the magnitudes of baseline eosinophilia and the improvement in airway responsiveness observed with GCS therapy (24). These results, suggesting that a significant level of sputum eosinophilia is a predictor for a favorable response to GCS therapy in asthma, are supported by observations that baseline sputum eosinophilia also predicts a beneficial effect of oral GCS on symptoms and spirometry in patients with COPD (25) and chronic cough (26). While these studies suggest that sputum can be used to predict steroid responsiveness, clearly there is a need for well-controlled trials in asthmatic patients to describe this relationship more precisely.

### B. Sputum as a Tool for Identifying Noneosinophilic Asthma Exacerbations

Induced sputum can be used to identify symptomatic asthmatic patients who do not have greater than normal levels of sputum eosinophila. Turner et al. examined the sputum from 34 consecutive patients presenting with an ongoing exacerbation

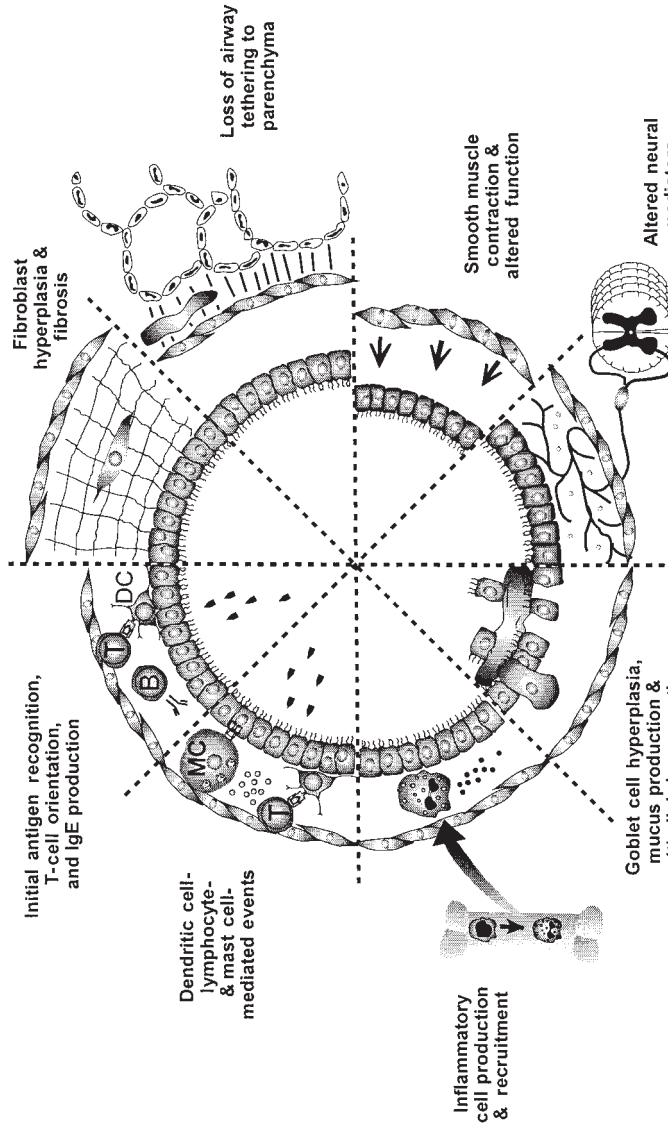


**Figure 3** Several of the many possible contributors to airway hyperresponsiveness. T, T lymphocyte; MC, mast cell; B, B lymphocyte; DC, dendritic cell.

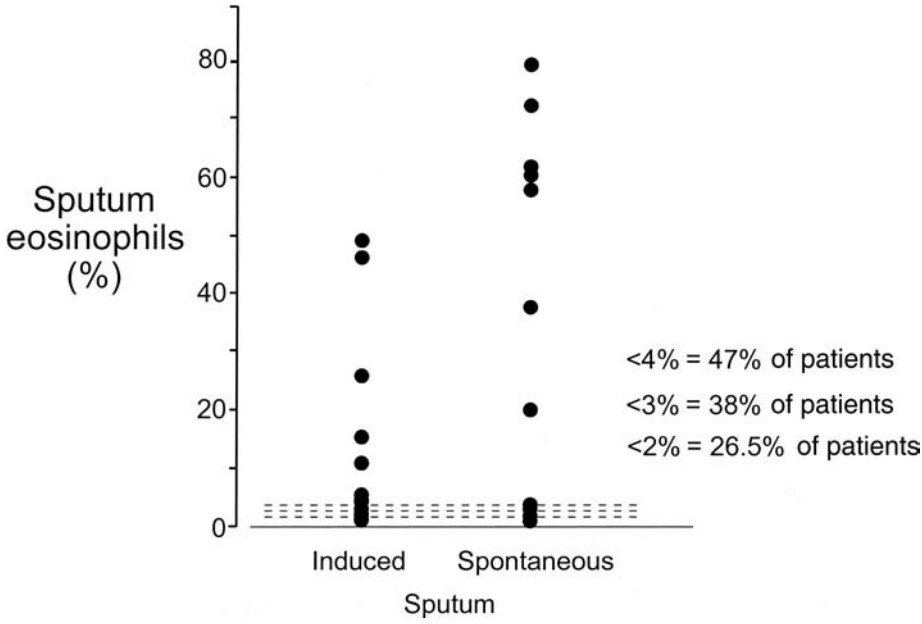


**Figure 4** Eosinophils, expressed as a percentage of total cells in spontaneous or induced sputum from 34 patients suffering from an exacerbation of asthma. Legend indicates percentage of patients with eosinophils less than the indicated level. (Modified from Ref. 27.)

of their asthma (Fig. 4) (27). In this study it was reported that 47% of the patients had a sputum eosinophilia of less than 4%, which at the time was considered to be the upper limit of normal. However, a more recent analysis of sputum from healthy individuals has shown that the upper limit of normal sputum eosinophilia is 2.2% (mean + 2 SD) (28). When these stricter criteria are applied to the data of Turner et al. (27), one still observes that greater than 25% of the patients with a current asthma exacerbation had sputum eosinophil levels in the normal range. Fahy et al. have also evaluated induced sputum from 18 patients with an ongoing exacerbation of asthma and found that 50% of these had less than 2% eosinophils (29). Futhermore, in the study by Fahy et al. it was observed that in patients with an associated viral respiratory tract infection, there was a more prominent neutrophilia in the sputum when compared to patients without an associated infection (85% vs. 57%). In this comparison it was also noted that the eosinophils were also lower in the group with a viral infection (5% vs. 31%), although this difference was not statistically significant.

Thus, the information from these studies suggests that induced sputum could be used in the evaluation of patients experiencing an exacerbation of their

asthma. While more information is required, it is conceivable that only those patients with a significant level of sputum eosinophilia should be managed with initiation of or increased GCS therapy.

### C. Sputum as a Tool for Predicting Asthma Exacerbations

Another potential use for induced sputum is to determine the optimal dose of inhaled GCS therapy in the management of a specific patient, the rationale being that analysis of sputum could identify the lowest GCS dose that will maintain an acceptably low level of specific airway inflammation. Recently, Jatakanon et al. measured inflammatory markers in induced sputum before and during a period of inhaled GCS reduction (30). In this study all patients were receiving at least 800 μg inhaled beclomethasone dipropionate (or equivalent) per day prior to the study and then switched to 200 μg budesonide daily for an 8-week period. It was observed that in subjects who experienced an exacerbation of their asthma over the course of the study, there was a significantly higher baseline sputum eosinophilia than in those who did not (medians: 13.6 vs. 0.2). Furthermore, increase in sputum eosinophilia during the course of the study was a better predictor of an exacerbation than was exhaled nitrous oxide.

Based on these results, it appears feasible that measuring the degree of eosinophilia in induced sputum could be used as a guide for whether a reduction in the dose of inhaled GCS is appropriate or likely to precipitate an exacerbation.

Based on observations by Jatakanon et al.(30) of the individual patient responses during the period of reduced inhaled GCS, it appears that sputum eosinophilia increased approximately 2 weeks prior to the symptomatic exacerbation, although this was not formally addressed. While it would be desirable to receive such warning prior to an exacerbation, clearly it is not practical to monitor patients sputum every 2 weeks.

### D. Does Sputum Provide Information That Is Not Available Clinically?

The above studies indicate that information obtained from induced sputum about the nature of airway inflammation could be useful in the management of the asthmatic patient. For this approach to be put into practice, however, it would need to be demonstrated that the information obtained from induced sputum was superior to the assessment of inflammatory status that is currently made based on routinely performed tests. Paramasweran et al. addressed this concern by comparing information obtained from induced sputum with a physicians assessment of patient's inflammatory status (31). In this study, respiratory physicians were asked to assess a total of 76 asthmatic patients based on an office visit, spirometry, response to prior treatment, and a macroscopic description of the patient's sputum. Physicians then assessed the patients as being controlled or uncontrolled, predicted the results

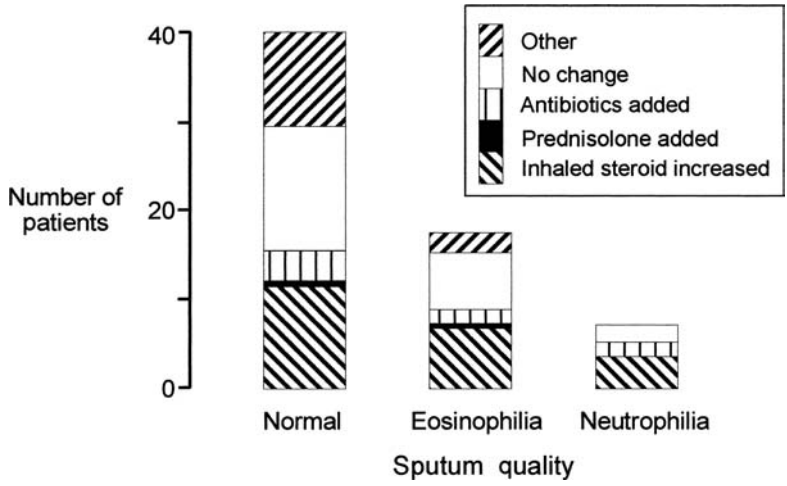


**Figure 5** Treatment plan based on clinical presentation in patients with normal, eosinophilic, or neutrophilic induced sputum. Note that there was a decision to increase GCS in less than 50% of the patients with an abnormally high eosinophilia, but that there was a decision to increase GCS in almost 50% of patients with either normal or neutrophilic inflammation. "Other" treatment decisions included adding other treatment, decreasing GCS, removal from work. (Adapted from Ref. 31.)

of the sputum analysis (normal, eosinophilic, neutrophilic, etc.), and offered a treatment plan. The study demonstrated that there was agreement between the physician's prediction of the quality of the sputum with the observed quality only 45% of the time. There was abnormal sputum findings in 22% of the patients whom the physicians described as being controlled. There were normal sputum findings in 38% of the patients whom the physicians described as being uncontrolled. The most striking finding from the study was the lack of agreement between the physicians' treatment plan and the quality of the induced sputum (Fig. 5). If, based on studies reviewed above, one assumes that eosinophilic inflammation is a predictor of responsiveness to inhaled GCS therapy, then it is clear that relying on physician impression will result in both unnecessary GCS treatment in some cases and failure to give appropriate GCS in others.

## IV. Summary

The purpose of this review was to summarize some of the evidence suggesting that there is a role for monitoring patients' inflammatory status as a means for deciding on appropriate treatment plans. The only noninvasive method of directly and qualitatively assessing airway inflammation currently available is induced

sputum. While there is much evidence to support that induced sputum may be useful in directing therapy, there is a need for trials that will demonstrate that this approach can result in decreased morbidity in asthmatic patients in a cost-effective manner.

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## Discussion

**Dr. Busse:** Can you comment on the possibility that sputum and lavage eosinophils represent cells from a different location? Furthermore, lavage is usually performed with large volumes of fluid and may represent airway and tissue cells. Can you comment on the possibility that eosinophils are more likely to be associated with exacerbations than chronic persistent asthma?

**Drs. Hargreave/Inman:** We certainly agree with the idea that sputum and lavage are sampling cells from different compartments. This is supported by the frequent observations that sputum eosinophilia is highly sensitive to corticosteroid treatment, while to date, BAL eosinophilia seems to be insensitive. As to whether sputum eosinophils are more likely to be associated with exacerbations than with chronic persistent asthma, this is likely a question of degree. For example, abnormally elevated sputum eosinophil levels can be observed in very mild asthmatics, including those who are essentially asymptomatic until challenged with allergen (Gauvreau et al., AJRCCM 1997; 156:1738–1745). However, it is well established that the degree of sputum eosinophilia will increase in association with an exacerbation. Thus, it would not be appropriate to say that a greater than normal level of sputum eosinophils is indicative of an exacerbation, while it might be appropriate to conclude this following a documented increase in sputum eosinophils in a patient. This is not to say that the simple observation of a greater than normal eosinophil level might indicate that asthma is not optimally controlled.

**Dr. Busse:** Could you comment on the feasibility of sputum samples to measure chemokines and cytokines?

**Drs. Hargreave/Inman:** Several groups are measuring levels of cytokines and chemokines in sputum supernatants (e.g., Kelly et al., JACI 2000; 105:1162–1168). However, as was observed using spiking experiments in this study, recovery of cytokines is not complete and thus, interpretation of measurements should be made with caution. Recovery may in some examples be improved by the addition of protease inhibitors during processing. It is likely that different optimal processing techniques will be found for different mediators.

**Dr. Persson:** The appearance of plasma proteins in sputum is airway inflammation. It reflects the airways plasma exudation process that involves passage of almost nonsieved plasma proteins across microvascular-epithelial barriers. Differing from the cell traffic, plasma exudation indices in airway lumen closely reflects the intensity and the time course of subepithelial inflammatory extravasation events. Interestingly, sputum analyses during the last 50 years show steroid-induced inhibition of plasma exudation along with improvement of

asthma. So, my question is whether you have determined plasma proteins in your work; if so, what did you observe?

**Drs. Hargreave/Inman:** We have focused on the measurement of fibrinogen as a marker of plasma exudation. This marker seems to be responsive to a number of inflammatory conditions in the airway, including severe asthma exacerbation (Pizzichini, AJRCCM 1997; 155:1501–1508) and to a greater extent in viral infection of the lower respiratory tract (Pizzichini, AJRCCM 1998; 158:1178–1184).

**Dr. Persson:** You demonstrated nicely an increase in sputum eosinophils occurring prior to exacerbation of asthma. Since airway luminal entry of eosinophils may be a major elimination route for these cells, an increase in sputum eosinophilia can be seen secondary to increases in airway tissue eosinophil numbers and particularly during resolution of asthmatic eosinophilic inflammation. How do you interpret the luminal entry of eosinophils in your study, as a pro-inflammatory event or as a sign of preceding mucosal tissue processes?

**Drs. Hargreave/Inman:** We agree that eosinophils observed in sputum may well reflect cells that are being cleared from the airway tissue. Certainly animal studies have demonstrated that appearance of eosinophils in the lumen lags behind their appearance in tissue. However, this does not necessarily imply that elevated sputum eosinophilia is indicative of recovery from a preceding process in the tissue. For example, following an allergen challenge, there is an increase in sputum eosinophils as early as 7 hours after challenge (Gauvreau et al., AJRCCM 1997; 156:1738–1745), while there is still an elevation in tissue eosinophils 24 hours after allergen (Woolley et al., AJRCCM 1995; 151:1915–1924), suggesting that elevations in sputum eosinophils may be observed during, rather than following active mucosal processes.

**Dr. Denburg:** What compartment/layer does the sputum represent—epithelial or another level?

**Drs. Hargreave/Inman:** This is obviously a very difficult question, and one to which we do not know the answer. One approach would be to measure the relationship between sputum eosinophilia and a quantification of the degree of eosinophilia in different tissue compartments and airway levels. However, generally the relationship between sputum and tissue eosinophilia has not been good (Maestrelli et al., AJRCCM 1995; 152:1926; Fahy et al., AJRCCM 1995; 152:53–58). Possibly this indicates that sputum is reflecting compartments other than those sampled through the bronchoscope. It is also likely, however, that the variability in both measurements precludes strong correlations.

**Dr. Busse:** There are preliminary data that airway eosinophils may not come only from the circulation. Some investigators have data to suggest that airway

eosinophils may migrate from the airway to lung tissues. This suggests that airway cells may be a dynamic collection of cells with the ability to move in and out of the airways.

**Dr. Persson:** Quantitatively, the number of cells that may reenter are exceedingly few compared to the number of eosinophils that are actually eliminated by entering the lumen and dying there or being cleared in the sputum alive.

**Dr. Schleimer:** Eosinophils may in some sense simply be a marker for $TH_2$ cells. Have you had a chance to look for mRNA for T-cell–specific genes in sputum?

**Drs. Hargreave/Inman:** Groups are starting to measure mRNA in sputum using in situ hybridization techniques. Encouraging results have been reported (Olivenstein et al., JACI 1999; 103:238–245) indicating that cells expressing message for $TH_2$ cytokines (IL-4 and IL-5) are increased in sputum from asthmatics compared to normal controls. Not surprisingly, and in agreement with your view, this increase in $TH_2$ activity was associated with elevated eosinophil numbers.

**Dr. Busse:** Although eosinophils appear in the airway 2–6 hours after antigen challenge, IL-5 is usually detected later. This raises the possibility that IL-5 may be more important to maintain eosinophil viability and in the airway rather than attracting them to the airway—a job for RANTES and eotaxin.

**Drs. Hargreave/Inman:** I completely agree with this view. In fact, we have evidence in a mouse model of allergen challenge, suggesting that local airway production of IL-5 is not needed for eosinophil recruitment, but that IL-5 is required at a systemic level in order to produce the increase in circulating eosinophils (Wang et al., J Clin Invest 1998; 102:1132–1141). These results support your idea that early increases in airway eosinophils are mediated through the actions of chemokines (RANTES and eotaxin), together with upregulation of adhesion molecules, acting on an already existing pool of circulating eosinophils. Prolonged recruitment however seems to require the added effects of IL-5 in stimulating eosinopoiesis.

**Dr. Rand:** What do we know about the effect of cycling on and off ICS (real life clinical practice) on inflammatory markers?

**Drs. Hargreave/Inman:** While to our knowledge the effects of cycling on and off steroids have not been studied using induced sputum, the effects of steroid reduction have been documented. In one of these studies (Gershman et al., ERJ 2000; 15:11–18), patients were placed on low (100 μg/day) or high doses (1000 μg/day) of inhaled fluticasone for 42 days and then switched to placebo. Not surprisingly, there was a rapid reduction in eosinophils following initiation

of treatment (this was sustained in the high-dose group only). Also not surprisingly, sputum eosinophils increased following removal of treatment, but this worsening appeared to be slower in the high-dose group, requiring 2–3 weeks to return to baseline levels. Thus, although this is an area that needs further study, it appears that short periods off steroids can be tolerated before eosinophilia (and presumably, as Dr. Schleimer suggests, $TH_2$ activity) increases to pretreatment levels.

# 18

# Use of Exhaled Nitric Oxide as Readout for Inhaled Corticosteroid Efficacy

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## I. Introduction

Optimal and noninvasive monitoring of the effect of anti-inflammatory drugs in asthma is important, as asthmatic patients may require life-long, individually tailored treatment with corticosteroids. The lack of success of the traditional parameters—lung function, airway reactivity, and symptoms—in monitoring the effect of corticosteroids, especially in dose-related studies, seems to be related to their relative insensitivity and slow responsiveness.

It has been suggested that it may take several weeks for inhaled corticosteroids to become effective. Recently, a variety of noninvasive approaches such as exhaled breath analysis (exhaled gases and condensate) and induced sputum have been developed, which are changing our understanding about the speed of action of corticosteroids and their effect in asthma. However, sputum induction (1) cannot be used for day-to-day monitoring, as it provokes transient neutrophilia (2,3), and the use of exhaled condensate is still in the early stage of development.

Exhaled nitric oxide (NO) as a marker of inflammation is comparable to invasive measurements of inflammation, such as bronchial biopsies and bronchoalveolar lavage (4,5) and induced sputum in asthma (6–8). It is not influenced

by other anti-asthma drugs, such as albuterol or salmeterol (9–11). The inflammatory origin (5,12–15) of elevated levels of exhaled NO in asthma (14,16), its responsiveness to suppression by corticosteroids (17,18), and accumulating evidence of its association with asthma severity (19–21) makes exhaled NO an effective and practical marker to monitor the effect of corticosteroid treatment in asthma. Dose-dependent reduction in exhaled NO has also been reported in mild asthmatics treated with low doses of budesonide (BUD) (22).

## II. General Principles of Exhaled Nitric Oxide Measurements

### A. Technical Factors Affecting Exhaled NO Measurements

Most of the measurements of exhaled NO have been made by a variety of commercially available chemiluminescence analyzers and are based on the photochemical reaction between NO and ozone generated in the analyzer. The specificity of exhaled NO measurements by chemiluminescence has been confirmed using gas chromatography–mass spectometry (23).

There are two main approaches to measurement of exhaled NO: on-line, during a single, flow-controlled exhalation against a resistance, and off-line, using similar controlled exhalation during a single exhalation into reservoir. There are a few technical factors that should be considered when exhaled NO measurements are used to monitor asthma treatment (Table 1).

The European Respiratory Society Guidelines on exhaled and nasal NO measurements were established in 1997 (24). Recommendations for standardized procedures for the on-line and off-line measurement of exhaled and nasal NO in adults and children have been published (25). The standardization of techniques makes it possible to compare the results of clinical trials from different centers.

### B. Conditions and Habits Affecting Exhaled NO Measurements

Conditions that may affect NO concentrations in exhaled air should be avoided or recorded and used for the interpretation of the data (Table 2).

**Table 1** Technical Factors Affecting Exhaled NO Measurement

| Increased NO | Decreased NO |
|---|---|
| Low exhalation or sampling flow rate | High exhalation or sampling flow rate |
| Breath holding | Spirometric maneuvers (transiently) |

**Table 2** Physiological, Pathophysiological Conditions, and Habits Affecting Exhaled NO Measurement

| Increased NO | Decreased NO |
|---|---|
| Allergen and/or pollen exposure | Menstruation |
| Air pollution | Smoking |
| Occupational exposure (ozone) | Acute alcohol ingestion |
| Arginine ingestion, nitrite/nitrate-enriched food | Mouth washing |
| Asthma | |
| Unstable/severe COPD | Nonasthmatic chronic cough |
| Allergic rhinitis | Pulmonary hypertension |
| Upper respiratory tract infection | Kartagener's syndrome |
| Influenza vaccination | Primary cilia dyskinesia |
| LPS administration | Cystic fibrosis |
| Bronchiectasis | |
| Ulcerative colitis | |
| Tuberculosis | |
| Lung cancer | |
| Active pulmonary sacroidosis | |

## III. Origin of Nitric Oxide in Exhaled Air

The correct use of exhaled NO as a readout for inhaled steroid (IS) efficacy depends on the understanding of its origin in exhaled air. Exhaled NO has multiple origins, as nitric oxide synthase (NOS) is found in several cell types in the respiratory tract, e.g., epithelial and vascular endothelial cells, macrophages, eosinophils, and neurons (26), and cannot be a marker of inflammatory response from a specific cell type. In fact, in most studies in normal subjects free of inflammation (27–29), cNOS rather than iNOS was found to be predominant in the airway wall.

Predominantly bronchial epithelial iNOS production has been shown to be the major source of exhaled NO in conditions in which airway inflammation is present, and this has been confirmed by direct measurements during bronshoscopy (4,30) or in tracheotomized patients (31), as well as indirectly by a remarkable exhalation flow-dependence of exhaled NO and accumulation of NO during a breathhold (4,32). The inflammatory origin of NOS induction (12,13,15) and related increase in exhaled NO in asthma (14,16) has been supported by high NO in exhaled air and increased nitrotyrosine and iNOS in bronchial epithelium of asthmatics (5) and lung transplant recipients (33). Exhaled NO levels were highly dependent upon the intensity and extent of expression of iNOS in bronchial epithelium and BAL neutrophilia, but not in the subepithelial area. This confirms that

exhaled NO may be not only a valid noninvasive measure of airway inflammation, but also a marker of the development of airway remodeling.

The profound effect of a nebulized (34–36) versus a comparable intravenous dose (37) of NOS inhibitors on exhaled NO strongly indicates that respiratory epithelium, but not vascular endothelial cells, is the major source of NO in exhaled air and explains its particular sensitivity to inhaled corticosteroids.

## IV. Clinical Relevance of Exhaled Nitric Oxide in Asthma

### A. Use of Exhaled NO as Readout for Corticosteroids Efficacy

*Oral Corticosteroids*

The overall effect of steroids on exhaled NO depends on the prevalence and the degree of iNOS activation and, therefore, has no effect in normal subjects, and is more effective in patients with more severe disease.

Oral prednisolone (30 mg daily for 3 days) reduces the elevated exhaled NO in asthmatic patients, whereas it has no effect on exhaled NO in normal subjects (34). Oral dexamethasone (4 mg/day for 2 days) similarly has no effect on exhaled NO or serum concentrations of interferon-$\gamma$ and interleukin-1$\beta$ in normal subjects (38). This is presumably because iNOS is the major source of increased exhaled NO in asthma, whereas the major source of exhaled NO in normal subjects is the constitutive NOS, which is not suppressed by corticosteroids.

The same dose of prednisolone (30 mg/day), however, given to mild asthmatics (34) produced a significant but moderate (22%) reduction in exhaled NO

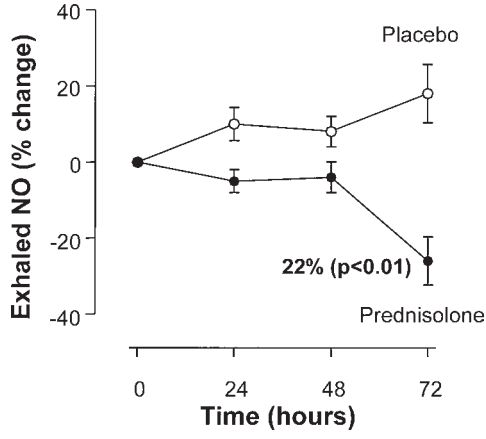


**Figure 1** Effect of oral prednisolone (30 mg/day) on exhaled nitric oxide (NO) in mild asthmatic patients (–●–) and normal subjects (–○–).

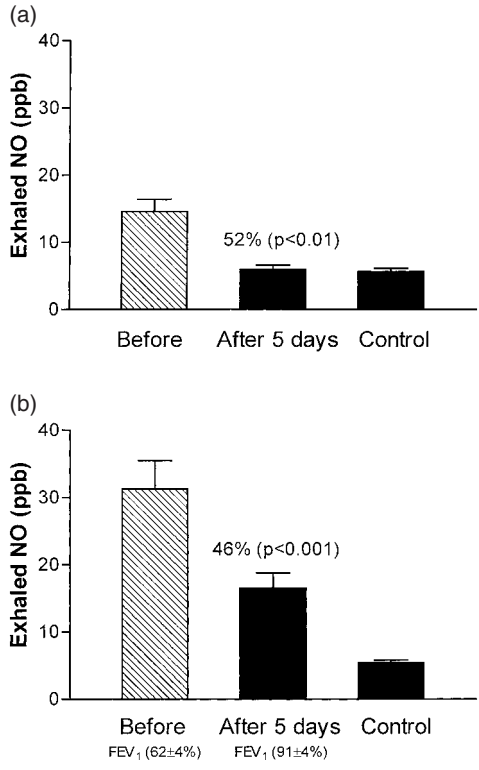


**Figure 2** The effect of oral prednisolone on exhaled NO in children with moderate-to-severe asthma.

within 72 hours (Fig. 1), whereas a cumulative dose of methylprednisolone (180–500 mg) caused 36% reduction within 50 hours in the majority of severe patients with acute asthma (39). A large dose (1 mg/kg/day for 5 days) of oral prednisolone normalized exhaled NO in infants and young children with wheezing exacerbations (Fig. 2a) (40), whereas the same dose in more severe asthmatic children only shifted their exhaled NO down to the levels of mild-to-moderate asthma in spite of the improvement in lung function (Fig. 2b) (10).

Recently it has been shown that NO levels correlated with the percentage improvement in $FEV_1$ from baseline to the poststeroid (30 mg prednisolone/day for 14 days) postbronchodilator value, with an NO level of $>10$ ppb at baseline having a positive predictive value of 83% for an improvement in $FEV_1$ of $\geq 15\%$ (41), and therefore may be useful in predicting the response to a trial of oral steroid in asthma.

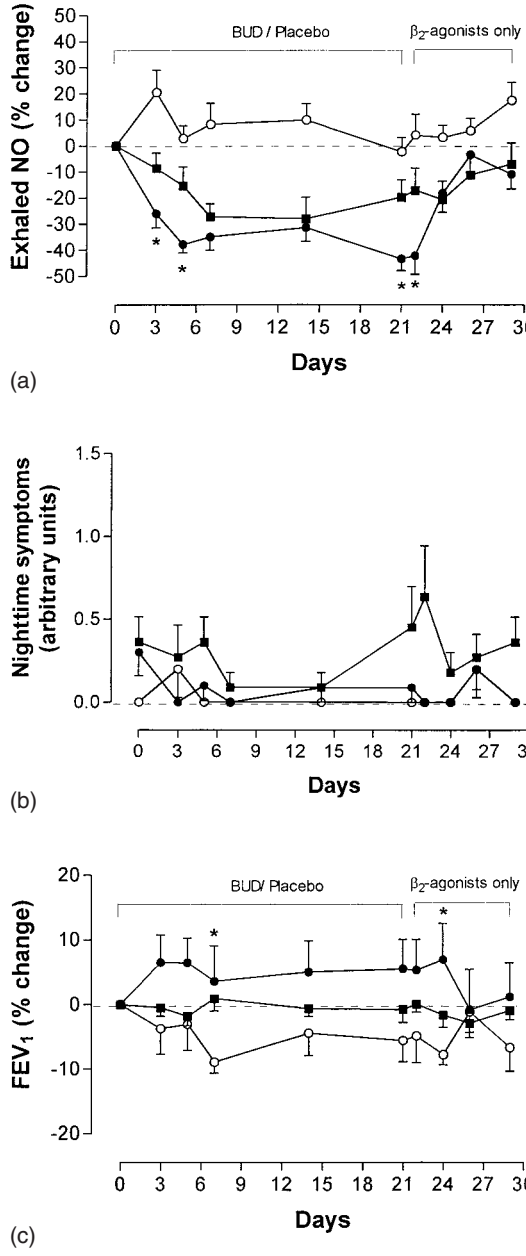


**Figure 3** (a) The effect of inhaled budesonide (BUD) on exhaled nitric oxide (NO) in mild asthmatic patients. Mean values ± SEM in patients treated with 400 μg BUD (–●–)

### *Inhaled Corticosteroids*

Exhaled NO has been used successfully to monitor anti-inflammatory treatment with inhaled corticosteroids in asthma (14,18,42–44), as it is an extremely sensitive and rapid marker of the effect of steroid treatment (Table 3). A significant reduction in exhaled NO levels was observed 6 hours after a single high dose (8 mg) of BUD (Pulmicort Respules®) in symptomatic moderate asthma (45). Dose-dependent changes in NO were reported during 3-week treatment with 100/400 μg BUD in mild asthma (22).

Recently we have shown for the first time that the onset of action of inhaled BUD on exhaled NO and the time to reach the maximal reduction were dose-dependent. The higher dose of BUD rapidly reduced NO (within 3 days) and prevented nighttime asthma symptoms in all patients (Fig. 3a,b). This was also associated with amelioration in $FEV_1$ (Fig. 3c). The onset of action of 100 μg BUD was slower and its effect on NO was less, and this was related to a slower improvement in nighttime symptoms. The difference between the effect of 100 μg versus 400 μg BUD on NO was apparent within 3 days and was maximal after 5 and 21 days. NO was further reduced during the third week of treatment with 400 μg BUD, in contrast to a minor NO increase in the 100 μg BUD group, which coincided with the return of asthma symptoms and increased use of $\beta_2$-agonists.

The time scale of the effect of steroids on exhaled NO was within the scope of the time needed for inhibition of iNOS activity (24 hours) (46), or nuclear transcription factor κB (30 min) after application of corticosteroids (47). However, the main effect of corticosteroids on iNOS is probably via inhibition of inflammatory cytokines, such as TNF-α, or IL-1β, which induced iNOS, or by inhibition of inflammatory cells such as eosinophils, which express iNOS (48).

The onset of action of steroids on exhaled NO depends not only on their dose, but also on asthma severity and, perhaps, on formulation and route of their administration. Thus, oral prednisolone given to mild asthmatics (34) produced a significant but moderate (22%) reduction in exhaled NO, whereas cumulative dose of methylprednisolone (180–500 mg) caused faster and more profound (36%) reduction within 50 hours in the majority of severe patients with acute asthma (39). The lack of changes in NO 3 and 6 hours after the first dose of 100/400 μg BUD in our study may suggest that the dose was too low and the patients had only mild asthma.

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or 100 μg BUD (–■–) or placebo (–○–). Level of significance of difference between 400 μg BUD and 100 μg BUD: $*p < 0.05$. (b) The effect of inhaled 400 μg, or 100 μg BUD, or placebo on nighttime asthma symptoms in patients with mild asthma. (c) The effect of inhaled 400 μg, or 100 μg BUD, or placebo on $FEV_1$ in patients with mild asthma. Level of significance of difference from placebo: $*p < 0.05$.

**Table 3** Effect of Corticosteroids on Exhaled NO

| Drug class | Effect (from baseline) | Onset (reported) | Duration (reported) | Recovery (reported) | Ref. |
|---|---|---|---|---|---|
| Corticosteroids | | | | | |
| *1600 μg/day BUD (Mil A) | ↓ 30% | | 7 days | | 18 |
| | ↓ 34% | | 14 days | | |
| | ↓ 41% | | 21 days | | |
| *BUD 1600 μg/day (Mil A) | ↓ 54% | | 28 days | | 42 |
| *BUD 100 μg/day (Mil A) | ↓ 29% | | 28 days | | 22 |
| BUD 400 μg/day | ↓ 50% | | 28 days | | |
| *Pred 30 mg/day, 3 days (Mil A) | ↓ 22% | 72 h | | | 34 |
| Pred + IS (Sev A) | ↓ 40% | 48 h | | | 39 |
| Pred 1 mg/kg, 5 days (Sev A) | ↓ 46% | | 5 days | | 10 |
| Pred 1 mg/kg, 5 days (Mod A) | ↓ 52% | | 5 days | | 40 |
| Pred + IS, 5 days (Sev A) | ↓ 65% | | 5 days | | 21 |
| IS (Mil A) | ↑ 9 ppb | | | 4 days | 81 |
| IS (Mod A) | ↑ 24 ppb | | | 15 days | |
| BUD 8 mg nebulized (Mod A) | ↓ 31% | 6 h | | | 45 |
| BDP 1 mg/day (Mil A) | ↓ 28% | | 7 days | | 82 |
| | ↑ 12% | | | 7 days | |
| *BUD 100 μg/day (Mil A) | ↓ 15% | | 5 days | | Kharitonov et |
| | ↓ 27% | | 7 days | | al. (ATS 2000) |
| | ↓ 20% | | 21 days | | |
| | ↓ 7% | | | 7 days | |
| BUD 400 μg/day | ↓ 26% | | 3 days | | |
| | ↓ 38% | | 5 days | | |
| | ↓ 35% | | 7 days | | |
| | ↓ 44% | | 21 days | | |
| | ↓ 3% | | | 5 days | |

BUD: Budesonide; Pred: prednisolone; IS: inhaled steroids; ↓: decrease; ↑: increase; *: placebo-controlled randomized trial; Mil A: mild asthma; Mod A: moderate asthma; Sev A: severe asthma; ppb: parts per billion (volume by volume).

An important issue that remains to be resolved is what level of exhaled NO needs to be achieved during the treatment. Exhaled NO levels in mild asthma are substantially reduced, but not normalized after a course of different doses (100–1600 μg) of inhaled steroids (22,34,44). However, the larger dose (1 mg/kg/day for 5 days) of oral prednisolone normalized exhaled NO in infants and young children with wheezing exacerbations (40), while more severe asthmatic children had exhaled NO levels reduced to the levels in mild-to-moderate asthma (10).

Affinity for the glucocorticoid receptor (GR) is perhaps another factor influ-

encing the effect of steroids on NO. Fluticasone propionate (FP), for example, has a threefold higher GR affinity than BUD, as it is more lipophilic than BUD, and its half-life of active steroid-receptor complexes is longer (>10 h vs. 5 h). Therefore, the rate of association of FP with the receptor is faster, and the rate of dissociation is slower than BUD (49). The high affinity of FP might be the reason for a profound and stable NO reduction by 76% after 2 first weeks and 77% after 4 weeks of treatment reported with 1000 μg/day FP (44). Indeed, NO levels were not fully recovered (83% of the baseline) 2 weeks after stopping of treatment. High-dose BUD (1600 μg/day), however, reduced exhaled NO by only 48% at the end of the third week, with further reduction to 54% after 4 weeks of treatment (18,22,42). The levels of NO were not fully recovered 2 weeks after FP was stopped, while NO levels in our study returned to the almost pretreatment values within 3–5 days regardless of the dose of BUD.

Speed, magnitude, and duration of changes in exhaled NO caused by steroids may be useful not only to monitor therapeutic efficacy of steroids, but also to assess their side effects, which are difficult to measure if conventional methods are applied. Thus, despite the greater cortisol suppression caused by FP, there were no differences between the effect of FP or BUD on $FEV_1$ or blood eosinophils (50).

### *Inhaled and Oral Corticosteroids*

The mechanisms of airway inflammation in asthmatic patients who respond well to corticosteroids could be different from those patients with severe persistent asthma who remain symptomatic despite corticosteroid treatment. With exacerbations, the number of eosinophils capable of expressing iNOS and producing NO (48,51) or prevalence of neutrophilic inflammation and oxidative stress (52) may increase, and this may also explain a further elevation of exhaled NO in these patients despite high-dose inhaled and/or oral steroid treatment (19,52). Thus, over half of children with very severe asthma had raised NO levels, indicating persisting airway inflammation and oxidative stress despite maximal doses of corticosteroids (53).

The rationale to quantify endogenous NO formation as the sum of its N-oxides is that nitrite has a relatively short half-life (110 s) (54) and may be further oxidized to nitrate ($NO_3^-$) by hydroxyl radical, hypochlorous acid, or various heme proteins (55). Therefore, it is difficult to distinguish whether the nitrite aqueous solution is derived from NO synthesis, peroxynitrite, or S-nitrosothiols. The significance of these various oxidative processes depends on local levels of $NO_2^-$ and $O_2^-$ formation. Thus, autoxidation of NO with $O_2$ is of a particular importance in asthma where NO production is elevated, and increased levels of nitrotyrosine have been correlated with elevated levels of oxidants (56). The presence of nitrotyrosine in the airways of patients who died of status asthmaticus supports the concept of widespread airway and parenchymal inflammation in asthma (57).

Corticosteroids reduce the formation of reactive oxygen species and nitrotyrosine in bronchial biopsies (5) and BAL (58) in asthma or formation of $NO_2^-$/$NO_3^-$ in nasal lavage and nitrotyrosine in nasal mucosa in allergic rhinitis (59).

However, considering the importance of oxidative stress in severe persistent asthma, a combination of corticosteroids with antioxidants and/or NOS inhibitors may be considered in these patients.

### B. Effect of Other Treatment on Exhaled NO

#### *Inhaled $\beta_2$-Agonists*

The short-acting $\beta_2$-agonist salbutamol (5 mg via nebulizer or 400 μg by metered-dose inhaler) has no acute effect on exhaled NO (9,10,60). Similarly, 1 week of treatment with a long-acting inhaled $\beta_2$-agonist, salmeterol, did not reduced NO in adults or children (9–11). This is entirely consistent with the fact that inhaled $\beta_2$-agonists do not have any effect on chronic inflammation in asthma and validates the use of exhaled NO to measure inflammation independently of airway caliber.

#### *Leukotriene Antagonists*

A leukotriene synthase inhibitor (pranlukast) inhibits the rise in exhaled NO when the dose of inhaled corticosteroids is reduced (61). Rapid reduction of exhaled NO has been recently reported within 2 days of starting montelukast, leukotriene receptor antagonist, in children with asthma (Table 4). The mechanism of this moderate 15% (62) or 30% (63) reduction is not clear, but it may reflect an inhibitory effect on inflammatory cytokines and, therefore, a reduced impact on iNOS. These data may also suggest an anti-inflammatory role for leukotriene $D_4$ receptor antagonism in the treatment of children with mild to moderate asthma.

#### *iNOS Inhibitors, Prostaglandins, and Other Drugs*

The use of NO modulators, e.g., iNOS inhibitors, or prostaglandin $PGE_2$, is presently at the stage of clinical research. Potentially, NO modulators may be important in management of severe asthma in which a combination of airway inflammation and oxidative stress together with an inherited or acquired resistance to steroids makes their treatment difficult.

Endogenous NO may play an important role in persistent airway inflammation and hyperresponsiveness, and treatment with aminoguanidine, a specific iNOS inhibitor, which has direct scavenging activities against $H_2O_2$, hypochlorous acid, and peroxynitrite (64) may be beneficial. Both aminoguanidine and $N^G$-nitro-L-arginine methyl ester (L-NAME) can be safely given and have been known to cause a significant reduction in exhaled NO in asthmatic patients (34,35) (Table 5). More long-term treatment will be required to demonstrate whether NO contributes to the persistence of asthmatic inflammation.

**Table 4** Effect of Nonsteroidal Drugs on Exhaled NO

| Drug class | Effect (from baseline) | Onset (reported) | Duration (reported) | Recovery (reported) | Ref. |
|---|---|---|---|---|---|
| Nonsteroidal anti-inflammatory drugs | ↓ | | | | |
| *Ibuprofen 2.4 g, (N after i.v. endotoxin) | | | | | 83 |
| $\beta_2$-Agonists | | | | | |
| *Terbutaline (Mil A) | No effect | | | | 18 |
| *Salbutamol, *salmeterol (Mil A) | No effect | | | | 11, 84, 85 |
| Interleukin inhibitors | | | | | |
| *IL-4 receptor* (Mod A) (Neb) | ↓ 8 ppb | 4 days | 15 days | | 81 |
| Leukotriene antagonists | | | | | |
| *Montelukast (Mil A) | ↓ 15% | | 2 days | | 62 |
| *Montelukast (Mil A) | ↓ 20% | | 14 days | | |
| *Montelukast (Mil A) | ↓ 30% | | 28 days | | 63 |
| After end of treatment | ↑ 19% | | | 14 days | |
| Pranlukast (Mil A) | ↓ | | 4 wk | | 61 |

↓: decrease; ↑: increase; *: placebo-controlled randomized trial; Neb: nebulized; i.v.: intravenous; N: normal subjects; Mil A: mild asthma; Mod A: moderate asthma; ppb: parts per billion (volume by volume).

**Table 5** Effect of NOS Inhibitors on Exhaled NO

| Drug class | Effect (from baseline) | Onset (reported) | Duration (reported) | Recovery (reported) | Ref. |
|---|---|---|---|---|---|
| NOS inhibitors | | | | | |
| *L-NMMA (N) | ↓ 44% | 15–45 min | 4 h | | 34 |
| *L-NMMA (Mil A) | ↓ 40% | | | | |
| *L-NAME* (N) | ↓ 53% | 15–45 min | 4–6 h | | 35 |
| *Aminoguanidine (Mil A) | ↓ 67% | | | | |
| *Aminoguanidine (N) | ↓ 28% | 15–45 min | 4–6 h | | |
| *Aminoguanidine (Mil A) | ↓ 53% | | | | |
| *L-NAME (Mil A) | ↓ 55% | 30 min | 2 h | | 36 |
| L-NMMA (N) (i.v.) | ↓ 10% | 30 min | | | 37 |
| L-NAME (N) (Neb) | ↓ 37% | 10 min | | | |

L-NMMA: $N^G$-monomethyl-L-arginine; L-NAME: $N^G$-nitro-l-arginine methyl ester; ↓: decrease; ↑: increase; *: placebo-controlled randomized trial; Neb: nebulized; i.v.: intravenous; N: normal subjects; Mil A: mild asthma.

Prostaglandins $E_2$ and $F_{2\alpha}$ decrease exhaled NO in normal and asthmatic subjects irrespective of changes in airway caliber (65). This effect occurs rapidly and is presumably due to an inhibitory effect of cyclooxygenase products on NOS directly rather than through altered gene transcription (66).

Despite positive changes in $PC_{20}$ in asthmatics treated with seratrodast, a $TXA_2$ antagonist, there were no differences in either exhaled NO or sputum eosinophils (67). The effect of theophylline and cromones has not yet been reported.

### C. Exhaled NO and Other Means of Asthma Monitoring

#### *Symptoms, Lung Function, and Airway Hyperreactivity*

There is accumulating evidence about the strong relationship between exhaled NO, clinical signs and symptoms of asthma, especially during acute episodes or asthma exacerbations. However, longitudinal studies are needed to confirm that exhaled NO may be used not only for a short-term management but also as a guide for long-term management and treatment of asthma of differing severity.

The traditional means of monitoring asthma are not sensitive enough to demonstrate a dose-dependent effect of inhaled steroids, especially in mild asthma. The fundamental limitation of lung function and $PC_{20}$ measurement, which reflect airway obstruction and airway hyperresponsiveness, in monitoring of asthma is that they are not directly related to airway inflammation. In addition, $FEV_1$ has little room for improvement in mild asthma and $PC_{20}$ is affected by corticosteroids and cannot be routinely performed in severe asthmatics. Both parameters are slow to change and lack a discriminating power to distinguish the effect of different doses of steroids.

For example, only moderate positive changes in $FEV_1$ and $PC_{20}$ were seen in mild asthma after 4 weeks of treatment with a high (1600 μg) dose of BUD, but these changes were not significantly different from the placebo group (42) Indirect inhaled spasmogens, such as AMP, might be more specific and demonstrate dose-dependent changes in $PC_{20}$ when compared with placebo after moderate (400 μg/day) or high (1600 μg/day) but not low (100 μg/day) doses of inhaled steroids, as has been shown for the novel corticosteroid ciclesonide (68). However, a significant reduction in exhaled NO, which was better than the placebo and which coincided with improvement in asthma symptoms and lung function, was seen after this and much lower doses of BUD—100 μg and 400 μg (22). The latter changes were also dose-dependently different.

The advantage of exhaled NO measurements is that the changes in NO during steroid treatment are dose-dependent and precede the improvement in symptoms, $FEV_1$ (17), and sputum eosinophils, (6) in asthma. Recently, we have demonstrated that 400 μg BUD rapidly reduced NO within 3 days and abolished nighttime asthma symptoms in all patients (unpublished observation). This was also associated with amelioration in $FEV_1$. The onset of action of 100 μg BUD was

slower, and its effect on NO was less marked, which was reflected in a slower improvement in nighttime symptoms.

Rapid recovery of exhaled NO levels on stopping steroid treatment precedes the reduction in lung function, with $FEV_1$ or $PC_{20}$ returning to the pretreatment level over 1 week (69). Exhaled NO measurements may therefore serve as a fast-responding indicator to assess patient compliance with therapy and to titrate steroid treatment. For example, increasing levels of exhaled NO, asthma symptoms, and use of $\beta_2$-agonists during the third week of treatment with a low dose of BUD might be an indication of loss of asthma control and a need to increase the dose of steroids. On the other hand, our data further support the fact that most patients with mild-to-moderate asthma may require low doses of steroids taken once daily to achieve or to maintain adequate control (70).

The relationship between exhaled NO and $FEV_1$ depends on the severity of asthma. There is no strong link between exhaled NO, $FEV_1$, and symptoms in mild steroid-naïve asthma measured under stable conditions (14,16). However, higher concentrations of exhaled NO were linked to recent symptoms of bronchial obstruction (71), and NO was 2.6 times higher in children with recent symptoms compared with symptom-free subjects (71). Exhaled NO correlated with symptom frequency and with rescue $\beta_2$-agonist use and is significantly higher in those patients with difficult/severe asthma who have the highest symptom score where changes in lung function may have limited sensitivity (19).

### *Induced Sputum*

The combination of exhaled NO measurements and sputum induction is the most beneficial approach for the use of these noninvasive assessments of airway inflammation in asthma. Recently it has been shown that a combination of sputum eosinophilia and increased NO levels resulted in a positive predictive value of 72% and a negative predictive value of 79% in predicting the response to a trial of oral steroid in asthma (41). Elevated levels of exhaled NO have been validated against invasive measurements of inflammation such as bronchial biopsies or BAL (4,5,30) and induced sputum (6), and a significant correlation has been found between exhaled NO and iNOS positive granulocytes in sputum eosinophils (7,8).

One of the most attractive features of exhaled NO measurements is that they can be repeated at short intervals without affecting endogenous NO production or causing discomfort to the patients. This is invaluable to study an acute effect and onset of action of a variety of drugs that influence NO production in patients of different severity and age.

Sputum induction, however, can cause an excessive bronchoconstriction despite pretreatment with salbutamol (8) and significant fall in $Sao_2$ (72) during the inhalation of hypertonic saline solution, as well as neutrophilia detectable for at least 24 hours (2) thereafter, and other changes in their cellular and biochemical composition, both in healthy subjects and mild asthmatic patients (3,73).

We have shown that after inhaled steroid dose reduction, exhaled NO and sputum eosinophil numbers are increased in parallel with loss of airway function (74). Exhaled NO has a low threshold for the effect of steroids, and therefore, even a low dose of locally applied steroids is capable of significantly reducing exhaled NO. The use of sputum eosinophil (Eos) as readout for steroid treatment efficacy might be limited. It has been shown that only high (1600 μg/day) or medium (400 μg/day) (42,68) but not low (100 μg/day) doses (22,68) of inhaled steroids were able to significantly reduce the number of Eos in sputum. No dose-dependent changes were observed in sputum Eos after either low, moderate (22), or high (68) doses of inhaled steroids. Sputum Eos may not reflect the full extent of asthma severity, or the effect of inhaled steroids, as the cellular and biochemical composition of the larger airways [higher presence of Eos, neutrophils, and eosinophil cationic protein (ECP)] is different from the peripheral airways (higher presence of macrophages, surfactant protein) and depends on the duration of sputum induction (75).

However, the combined use of exhaled NO measurements and sputum induction is of particular importance in severe persistent or steroid-resistant asthma, which is associated with elevated levels of exhaled NO (19,52), despite high-dose steroid treatment, neutrophilia (52), and oxidative stress. It has been shown that elevated ECP levels, but not Eos numbers, in induced sputum of corticosteroid-dependent asthmatics with recent exacerbations may be a more accurate assessment of airway inflammation in these patients (76). The correct identification of these patients by their profile of inflammatory cells and mediators in sputum is crucial, as they may require a different treatment.

## V. Future Directions

There has been an interesting attempt to direct treatment with steroids in patients with moderate asthma according their levels of $PC_{20}$ to methacholine (77). Apart from small changes in $PC_{20}$ (1.1 double dilution), the major limitation of this and other single parameter–based guidelines is its relatively weak link with airway inflammation. The advantage of exhaled NO is that it has a much stronger association to airway inflammation, asthmatic/atopic inflammation in particular, and is much more sensitive to anti-inflammatory treatment so that the control of the disease can be improved without the risk of overtreatment.

Measurements of lipid mediators, such as cysteinyl-leukotrienes and other eicosanoids, in induced sputum (78) and exhaled condensate are promising approaches. However, the methodological issues, such as considerable within-subject variability of most sputum eicosanoid concentrations (78), needs to be addressed. Exhaled condensate is less contaminated with saliva and proteins and is easy to collect in a controlled fashion and perhaps therefore has the advantage. Re-

cently, we have determined significantly different levels of leukotriene $E_4$, $C_4$, $D_4$, and $B_4$ in exhaled condensate of patients with asthma of different severity before and after treatment with corticosteroids.

Therefore, a combination of exhaled NO measurements with determination of other inflammatory markers and mediators in exhaled breath condensate, such 8-isoprostane (79, 80), leukotrienes, and prostaglandins, is a promising noninvasive approach towards asthma and COPD management.

Objective, noninvasive, and effort-independent monitoring of respiratory symptoms in adults and children with asthma is vital for optimizing their anti-inflammatory treatment. Recently, we used a quantitative method for tracking breath sounds overnight and during the day in mild-to-severe asthma patients. The overnight wheeze scores were over 20 times higher in moderate asthmatics on inhaled steroids when compared with mild steroid–naïve asthmatics (unpublished observation).

A combination of a cornerstone asthma sign, such as wheeze (also related to airway obstruction) with a variety of inflammatory markers in exhaled breath and exhaled condensate may be clinically useful in the detection and management of cytokine-mediated inflammatory lung disorders.

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## Discussion

**Dr. Seale:** The Bisgaard (AJRCCM, 1999) study showed a 20% reduction in NO after montelukast. Do you regard this as a clinically significant reduction in NO and what do you think is the mechanism for this reduction in NO?

**Dr. Kharitonov:** It could be regarded as a clinically significant reduction in exhaled NO, as it was associated with the positive changes in $FEV_1$ and asthma symptoms. The effect of leukotriene antagonists on exhaled NO may be due to a reduction of inflammatory cytokines and their impact on iNOS. We have shown that prednisolone (30 mg/day), for example, given to mild asthmatics produces a significant but moderate (22%) reduction in exhaled NO within 72 hours.

**Dr. Derendorf:** Can you give us a feel for the reproducibility? What is the intra- and inter-individual variability?

**Dr. Kharitonov:** The coefficients of variation of NO measurements made at two consecutive days varied between 12% for exhaled NO and from 7% to 11.8–13% for nasal NO. In a large population sample of young adults the repeatability of NO measurements, estimated by calculating the intraclass correlation coefficient (ICC), was 0.98 representing the range within which 95% of pairs of repeated measurements would be expected to lie, as 4.58 ppb. Individual variability of exhaled NO levels is low, providing the measurements were made on subjects free of URTI or without asthma exacerbation. For example, no significant difference in NO measured at 371-, 54- and 60-day intervals was seen in three normal subjects and over 55, 28 and 20 days in three clinically stable asthmatic patients. Therefore, the level of reproducibility of NO measurements is acceptable for biological measurements, which normally have coefficients of variation between 2–15%.

**Dr. Edsbäcker:** Does endogenous control affect the assessments of exhaled NO; i.e., is there any diurnal variation in NO readouts? Has NO been studied in inhaled steroid studies having an oral steroid sparing design; i.e., what is the local effect component in the overall NO reduction by steroids?

**Dr. Kharitonov:** There is no diurnal variation in exhaled NO measurements. Exhaled NO has not yet been studied in inhaled steroid/oral steroid sparing design studies.

**Dr. Selroos:** Are NO measurements useful for monitoring the disease? With high initial doses there seems to be a rapid normalization of NO levels and they remain low.

**Dr. Kharitonov:** The onset of action of inhaled budesonide (BUD), for example, on exhaled NO and the time to reach the maximal reduction are dose-

dependent. We have shown that the higher dose of BUD rapidly reduced NO (within 3 days), which coincided with the improvement in nighttime asthma symptoms and $FEV_1$ in all patients. The onset of action of 100 μg budesonide was slower and its effect on NO and symptoms was less. It has been shown that exhaled NO levels were substantially reduced, but not normalized, after a course of different doses (100 to 1600 μg) of inhaled steroids in mild asthmatics. In contrast, a large dose (1 mg/kg/day for 5 days) of oral prednisolone given to infants and children with wheezing exacerbations was able to normalize their exhaled NO. However, the same dose given to more severe asthmatic children had exhaled NO levels reduced only to the levels in mild-to-moderate asthma.

**Dr. Pedersen:** You presented the data as a percentage change from baseline without actually giving the baseline value. That makes it difficult to get a feeling for the severity calculated in this way. We found a 70% reduction in exhaled NO during treatment with 200 μg budesonide/day. That would not have fit with your data where you combine data from two studies (one with budesonide and one with FP). This raises the question of whether it is appropriate to combine the studies in the way you did?

**Dr. Kharitonov:** The baseline NO levels are obviously important. Recently, it has been shown that exhaled NO of $>10$ ppb at baseline has a positive predictive value of 83% for an improvement in $FEV_1$ of 15% and therefore may be useful in predicting the response to a trial of oral steroid in asthma. The comparison between the studies can be made by either baseline levels comparison and/or by the effect of different doses of steroids on these baseline NO levels.

**Dr. Brattsand:** Dr. Schleimer listed that the blocking activity of glucocorticoids on NOS is not restricted to iNOS. If also cNOS is blocked, that may be negative as Tulic and Sly (ERJ 1999;14(suppl 30):157s) reported that raised cNOS is coupled to reduced hyperresponsiveness (in vivo studies in challenged rats).

**Dr. Kharitonov:** The most likely effect of steroids on NOS is via reduction of the impact of cytokines on the iNOS.

**Dr. Jeffery:** Is the measurement of NO telling us more about the extent of perturbation of the epithelium rather than eosinophilic inflammation per se? What happens in response to viral infection?

**Dr. Kharitonov:** The predominant source of exhaled NO is epithelium with a contribution from eosinophils. Therefore, it is a reflection of perturbation of the epithelium. Exhaled NO is elevated during viral infection.

**Dr. Persson:** The time course of effects of steroids on established airway eosinophilia may not have been well examined, neither in animal nor in human studies. Since steroids inhibit eosinophil traffic to the asthmatic airways but may not affect the pathways of eosinophil elimination (apoptosis, cytolysis, and

airway luminal entry) the inhibition of established eosinophilia could reflect "normal" dwell times of eosinophils in the airway mucosa. Now, you compared the effect of inhaled steroids on NO and sputum eosinophilia: both were reduced after 1 week. Do you have data on the rate of steroid-induced lowering of eosinophil numbers in the sputum?

**Dr. Kharitonov:** The time-course for the exhaled NO and sputum eosinophils is different. It is slower in case of the eosinophils. I have not presented any data on 1-week reduction in eosinophils.

**Dr. Inman:** What would happen to the increased NO during a viral infection if inhaled steroids were given?

**Dr. Kharitonov:** It has not been studied yet, I'm afraid. I would expect that steroids will be less effective in reducing exhaled NO levels.

# 19

# Markers of Systemic Actions of Corticosteroids

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## I. Introduction

Asthma is characterized by airway inflammatory and remodeling processes, thought to be responsible for the variable airflow obstruction and respiratory symptoms observed in asthma (1,2). The main preventive pharmacotherapeutic agents of asthma are corticosteroids (3,4). A significant breakthrough in asthma therapy occurred about three decades ago, when corticosteroids became available as inhaled preparations, reducing the potential for systemic effects while achieving excellent therapeutic results. Inhaled corticosteroids (ICS) are the most potent of the anti-inflammatory drugs commonly used in asthma treatment. Their action is thought to result mainly from the reduction of the number and activation of inflammatory cells in the bronchial mucosa and through their inhibitory effects on different mediators and cytokine synthesis (5,6).

ICS commonly used in the treatment of asthma include beclomethasone dipropionate (BDP),* budesonide (BUD), fluticasone propionate (FP), triamcinolone acetonide (TAA), and flunisolide (FLU). Agents such as mometasone furoate

*BDP with a chlorofluorocarbon as a propellent, unless indicated otherwise in the text.

**Table 1** Markers of Systemic Effect of Glucocorticosteroids

| |
|---|
| *Changes in adrenal function* |
| Plasma cortisol level |
| Periodic plasma cortisol levels[a] |
| 24-hour urinary free or overnight cortisol excretion |
| Response to ACTH stimulation (short and long tests, high-dose and low-dose) |
| *Bone metabolism and density* |
| Bone formation: |
| Serum osteocalcin |
| Plasma level of bone-specific alkaline phosphatase |
| Procollagen peptides |
| Procollagen type 1 N-terminal propeptide (PINP) |
| Procollagen type 1 carboxy-terminal propeptide (PICP) |
| Bone resorption: |
| Urinary hydroxyproline |
| Urinary calcium |
| Urinary pyridinium cross-links |
| Pyridinoline |
| Deoxypyridinoline |
| Cross-linked carboxyterminal telopeptide of type I collagen (ICTP) |
| Tartrate-resistant alkaline phosphatase |
| Growth in children: height velocity and statural height (stadiometry, knemometry) |
| Cutaneous changes: skin thickness, bruising |
| Ocular effects: intraocular pressure, cataracts |
| Central nervous system |
| Blood and bone marrow |
| Other metabolic effects |

[a]Measured several times during day and night to evaluate diurnal variation.

(MF) and ciclesonide (CS) are currently being studied. Although these agents are considered to have a local effect on airways cells and constituents, there may be a variable degree of systemic absorption depending on the nature and dose of the agent as well as the site and degree of lung deposition. ICS are generally considered to have no clinically relevant systemic effects at doses below or equivalent to 800–1000 μg/day of BDP in adults and 400–800 μg/day in children, although at moderate to high doses they may produce variable changes in markers of systemic effect (7–9). In the following document, we will present the main markers used to determine systemic action of corticosteroids and illustrate their comparative changes following ICS administration by referring to recent studies and analyses (Table 1).

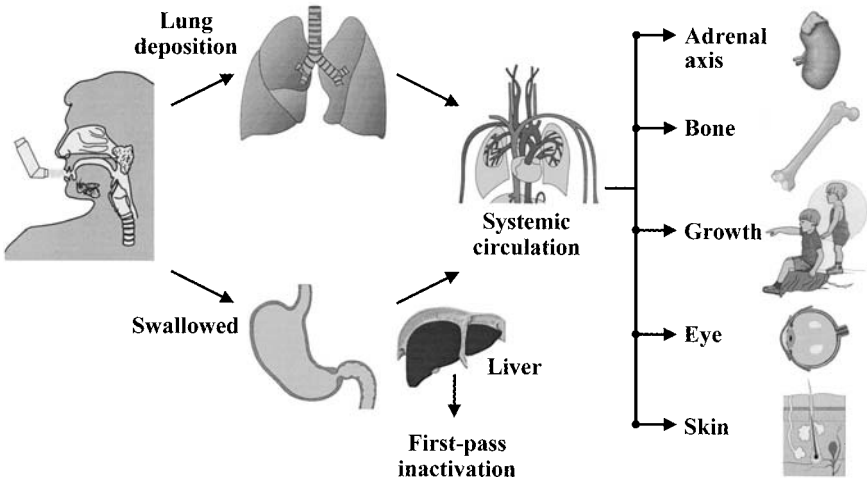


**Figure 1** Pharmacokinetics of inhaled corticosteroids.

## II. Pharmacokinetics of Glucocorticosteroids

Corticosteroids may cause systemic effects by their absorption through the gut and lungs into systemic circulation (Fig. 1). The total systemic effect of an ICS will therefore depend on the amount of drug deposited into the airways and the amount absorbed from the gastrointestinal tract, which then becomes available systemically. These vary considerably from one drug and one inhalation device to another, so that the therapeutic effect over systemic absorption (or side effects), defined as the therapeutic ratio, should be determined for each drug preparation and type of inhaler used.

Apart from the dose delivered, factors such as the extent of first-pass hepatic metabolism, the site of lung deposition, and other pharmacokinetic properties of ICS act as major determinants of the topical versus systemic effects of those agents. The available inhaled ICS show differences in systemic bioavailability, particularly at high doses. A low systemic bioavailability is preferable to reduce the risk of systemic effects. Factors such as receptor affinity, elimination half-life, and lipophilicity may affect efficacy, as well as also systemic bioavailability (10,11). A better "pulmonary targeting" of ICS, from increasing the local/systemic effect ratio, may result from low oral bioavailability, rapid systemic clearance, high plasma protein binding, and slow absorption from the lungs (12). Theoretically, a high water solubility may reduce tissue retention and thereby increase elimination of the drug, reducing the risk for systemic effects. A long elimination half-life may reduce between-dosing fluctuations of serum concentrations of the drug, although studies using oral corticosteroids suggest that it is mostly the persistent plasma levels and not the magnitude of the peak plasma concentrations that

induce detrimental systemic effects such as those on the hypothalamic-pituitary-adrenal (HPA) axis (13).

The newer lipophilic ICS have, however, shown a large total volume of distribution with systemic tissue retention and somewhat low detectable plasma concentrations. Modes of administration also influence systemic availability by altering the dose deposited in the oropharynx and the lungs, and the influence of the delivery devices, spacers, and technical factors related to administration should be considered when comparing different drugs (14).

## III. Markers of Systemic Effects

### A. Effects on Adrenal Function

#### *Adrenal Function and Systemic Corticosteroids*

Plasma cortisol levels, maintained through adrenal secretion under the regulation of adrenocorticotropin-releasing hormone from the pituitary gland, range from about 100 to 300 nmol/L during the day and show a few nocturnal peaks. Exogenous administration of systemic corticosteroids causes a negative feedback on steroid receptors in the anterior pituitary gland and hypothalamus, resulting in a suppression of corticotropin-releasing hormone and corticotropin, which in turn causes a reduction in cortisol secretion from the adrenal cortex (15). Depending on the mode of administration, the dose and duration of use, systemic corticosteroids may lead to prolonged adrenal suppression with adrenal cortex atrophy and impaired adrenal response to unusual stress (e.g., surgery) if the exogenous steroid therapy is stopped or insufficient.

Although clinically significant adrenocortical insufficiency is rare among ICS-dependent patients, the influence of ICS on the adrenal axis is nevertheless used frequently as a parameter for evaluating the systemic activity of these drugs (16–20).

#### *Methods of Assessment*

Among the tests that evaluate this axis are those that measure basal HPA secretory function, such as morning (07:00–08:00 a.m.) plasma cortisol level, 24-hour integrated plasma cortisol levels, 24-hour urinary free cortisol excretion, and overnight urinary cortisol excretion. Screening of the HPA axis function may be done from single morning plasma cortisol level determination, although this last test is less sensitive than repeated plasma cortisol measurements to detect small changes in basal cortisol excretion.

The response to stimulation testing with tetracosactrin (ACTH high dose, 250 μg, or low dose, 500 ng or 1 μg—this last being more discriminative and possibly reducing the risk of hypersensitivity reaction) may assess adrenal reserve

while insulin-induced hypoglycemia and metyrapone tests may test the entire HPA axis (15). Response to pyrogenic and vasopressin challenges may also assess HPA function. The insulin stress test is the usual "standard," but sensitivity and specificity of these tests are uncertain. The short tetracosactrin test is currently the most used.

In regard to the circadian variation in cortisol secretion, repeated plasma cortisol levels over 24 hours or overnight, and the 24 hours free urinary cortisol (or its metabolites) excretion are sensitive measures.

### *Influence of ICS on Adrenal Function*

Excellent reviews have been published of studies on the effect of ICS on the HPA axis (7,8,16–18,21–22). Studies show variable results, probably due to differences in factors such as duration of dosing of ICS and the various methods used to assess HPA axis suppression. Dose-ranging studies have shown that there is a dose-related suppression of HPA axis, the steep part of the curve being over an ICS dose equivalent to 800 μg/day BDP. High doses of ICS in asthma could induce a decrease in morning plasma levels and 24-hour urinary cortisol in proportion to the total daily and cumulative dose of ICS (23,24).

The following studies illustrate the use of different markers to determine the comparative influence of the most frequently used ICS on HPA axis parameters. First, in regard to adult asthmatics, in a study of 78 patients taking inhaled ICS (mostly BDP) at a median dose 1600 μg/day for a median duration of 13 months, 20% of patients had changes in the ACTH test and urinary-free cortisol suggesting adrenal dysfunction (25). A close correlation was observed between the maximum cortisol measured during hypoglycaemia and both urinary free cortisol and posttetracosactrin cortisol. In another study, BUD or FP, respectively, given at 800 or 750 μg/day for one week and 1600 or 1500 μg/day for another week, administered with a dry-powder inhaler, caused no significant suppression of early morning cortisol, while both drugs attenuated the posttetracosactrin serum cortisol at low and high doses (26).

Clark and Lipworth compared the effects of FP and BUD given on a microgram equivalent basis by metered dose inhaler on overnight urinary cortisol excretion and plasma cortisol levels at 08.00 hours in asthmatic patients (27). With repeated dosing across a dose range of 250–1000 μg twice daily, fluticasone propionate produced significantly greater reduction in both plasma and urinary cortisol than budesonide. The authors suggested that factors contributing to the systemic effects of fluticasone comprised enhanced receptor potency, prolonged receptor residency time, greater tissue retention, and a longer elimination half-life. Other studies also showed a greater effect of FP than BUD on adrenal axis (28,29). However, these differences are more obvious at high than at low/moderate doses of ICS, and the doses required to control asthma have been shown to be less for FP

than BUD or BDP (16,30,31). This stresses the fact that when we look at the systemic effects of ICS, the therapeutic/systemic effect ratio should always be considered.

In the child population, most studies found no significant changes in urinary free cortisol excretion in children on FLU 100 and 200 µg/day, BDP up to 400 µg/day, and BUD up to 800 µg/day (17). Lipworth et al. showed no evidence of significant adrenal suppression after repeated twice-daily administration of 200–400 µg/day of FP or BUD given via a large volume spacer, using overnight urinary cortisol excretion (32). This indicates a good safety profile in children of these ICS at conventional dose levels. Fitzgerald et al. found that in children with persistent asthma, FP 750 µg/day was as effective as BDP 1500 µg/day when both were delivered through a spacer device for 12 weeks; a mild reduction in adrenal production of cortisol, of similar degree for the two drugs, was, however, observed, as assessed with 24-hour urinary free cortisol (33).

Meta-analyses of the studies looking at the influence of ICS on systemic parameters have been done. One looked at 34 studies evaluating the effects of ICS on the HPA axis, 21 using urinary cortisol measurements, and 13 08:00 h plasma cortisol levels (7). Variable degrees of reduction of adrenal function were found and FP had a steeper dose-related systemic activity than BDP, BUD, or TAA. These effects were mostly evident with doses over 800 µg/day.

In two meta-analyses of the comparative effects of FP, BUD, and BDP, Barnes et al. also found a variable dose-related suppression of HPA axis, but no differences between FP and BUD (FP given at half-doses of BDP) at low doses in regard to serum cortisol, while at higher dosages there could be differences in favor of FP (34). In this meta-analysis, half-doses of FP were similar to BDP in regard to effects on HPA axis.

Although variable from a study to another, in general FP produced more effects on adrenal function in both adults and children than BUD on a µg-per-µg basis, although the dose required to control asthma was lower with FP. Furthermore, high doses of BUD and FP had fewer effects on adrenal function than BDP (36).

### *Confounding Factors*

When looking at the potential effects of ICS, many factors should be taken into consideration, such as dose, timing, mode and frequency of administration, duration of treatment, compliance with medication, previous or concurrent use of oral ICS, and the population studied (Table 2). The additive and much more potent influence of oral ICS is particularly important when evaluating these effects. Compliance with 24-hour urine collection may be poor and should be checked. Fractionated overnight or early morning collection may be alternatives (21).

**Table 2** Factors to Consider When Looking at Systemic Effects of Inhaled Corticosteroids

| |
|---|
| Type of ICS, dosage, duration of treatment, and compliance |
| Mode, timing, and frequency of administration |
| Inhalation device (including spacers) |
| Asthmatic vs. normal control subjects |
| Associated disease or condition |
| Asthma severity and control |
| Past or concurrent use of systemic corticosteroids |
| Population studied (age, gender, etc.) |
| Diet, sedentarity, alcohol intake, gonadal status, preexisting osteopenia (for bone metabolism) |
| Other current medications |
| Individual susceptibility |

#### *Conclusions*

Changes in adrenal function are considered to be good markers of the systemic effect of ICS. Among the tests available to assess HPA axis, measurements of the 24-hour urinary free cortisol and of serum cortisol concentrations expressed as area under the curve averaged over 24 hours are accurate markers of changes in HPA function. For this last test, single or multiple dosing of ICS may be used to assess systemic effects, as most effects are evident even after a single dose, with a variance of the response similar to multiple dosing (37). Although sensitivity of morning plasma cortisol level is low, it may be a good screening test. The influence of ICS on the HPA axis is quite variable between subjects, but doses under 1000 μg/day of BDP had little or no effect on HPA axis function, while high doses, particularly equal or greater than 1500 μg/day, could induce variable reductions in cortisol secretion.

### B. Effects on Markers of Bone Metabolism

#### *Bone Metabolism and Systemic Corticosteroids*

A dynamic equilibrium between bone formation, involving osteoblasts, and bone resorption, involving osteoclasts, exists in the bone matrix, particularly in trabecular bone, which is more metabolically active than cortical bone. Up to 10% of bone is replaced yearly, about 5% of cortical and up to 20% of trabecular bone. Systemic corticosteroids may reduce bone mass by inhibiting osteoblast function and favor bone resorption by increasing osteoclast function. They may inhibit intestinal calcium absorption and renal calcium reabsorption (secondary hyper-

parathyroidism). Corticosteroids may also potentiate the activity of parathyroid hormone on osteoblasts. Their inhibitory effects on estrogen and testosterone secretion may further contribute to osteopenia. The main effect of corticosteroids is a reduction in the formation of new bone (22,36–38). If, following long-term use of oral corticosteroids, the process is severe and/or prolonged, it may lead to osteoporosis and fractures.

### *Methods of Assessment*

Effects of ICS on bone have been evaluated with various serum or urinary markers of bone formation and resorption, and through measurements of bone density (39–41). Markers of bone formation include serum osteocalcin, plasma level of bone-specific alkaline phosphatase, and various types of serum procollagen peptides such as procollagen type I N-terminal propeptide (PINP) and procollagen type I carboxy-terminal propeptide (PICP).

Osteocalcin is a calcium-binding gamma-carboxyglutamic acid–containing noncollagenous protein called bone Gla protein (BGP) and is synthetized by osteoblasts. About 25% of osteocalcin escapes into circulation, and circulating levels reflect osteoblast activity. Different immunoessays using polyclonal or monoclonal antibodies are used for its measurement.

Many isoenzymes of alkaline phosphatase are produced by different organs. Bone-specific alkaline phosphatase is found in the membrane of active osteoblasts and preosteoblasts, and its level increases during bone formation.

PINP and PICP are released during collagen synthesis and their serum levels correlate with collagen type I synthesis in bone or soft tissue. Their levels fluctuate among subjects.

Main markers of bone resorption include urinary excretion of hydroxyproline and calcium, urinary pyridinium cross-links pyridinoline and deoxypyridinoline, cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), and tartrate-resistant alkaline phosphatase (TRAP).

Urinary excretion of hydroxyproline or calcium are measured after a 12-hour fast. Hydroxyproline is the collagen amino acid released during collagen degradation. Present in all types of collagens and other proteins, about 50% of that found in urine comes from the bone. Diet can influence this test (e.g., gelatin increases urinary levels).

Pyridinoline, present in bone and cartilage, and deoxypyridinoline, mostly from the bone, are collagen cross-links released by the osteoclast during bone resorption. TRAP is found in osteoblasts and reflect their activity. ICTP is produced during collagen degradation, and its level correlates with bone resorption.

Markers of bone formation tend to be more sensitive than those of bone resorption. In this regard, serum osteoblast–derived osteocalcin is considered a sensitive, specific, and reproducible measurement (21). The more recently devel-

oped measurements of breakdown products from collagen metabolism mentioned above are promising but remain to be further assessed as markers of systemic effects of ICS.

Bone densitometry of peripheral sites such as the radius or central ones such as the proximal femur or lumbar spine can be used to assess the effects of treatments on bone mineral density (BMD). A low BMD in adults is considered to be associated with a statistically significant increase in the risk of fractures. Dual-energy x-ray absorptiometry is currently recommended in osteoporosis management (41). Other methods such as ultrasound of the calcaneus and quantitative computed tomography have sometimes been used in adult patients.

### *Effects of ICS on Bone Metabolism and Density*

In adults, daily doses of inhaled ICS equivalent to $<800$ μg of beclomethasone usually exert no or minimal influence on markers of bone metabolism (42–45). A lower serum osteocalcin and higher urinary phosphorus level may be found in subjects using more than the equivalent of 1000 μg of inhaled BDP (38,44,45). This effect varies among ICS and in regard to each parameter, as shown by some of our recent observations (31). They showed that serum osteocalcin level was significantly lower in subjects on BDP compared with those on half-doses of fluticasone, while other markers of bone metabolism such as urinary *N*-telopeptide/creatinine ratio, serum procollagen type I carboxy-terminal peptide, and specific alkaline phosphatase were not different (31). This suggests that "classical" measures such as osteocalcin may more accurately reflect the effect of two ICS on bone metabolism compared with more recent markers, although this has to be further evaluated. Furthermore, their comparative values as predictors of clinical outcome remain to be determined.

While bone density is unaffected by low doses of inhaled ICS, variable results have been obtained with high doses (46–51). No changes in bone density were found with doses of about 1000 μg/day of BDP. While the effect seems minimal at doses up to 1000 μg/day, higher doses of ICS seem to have the potential to reduce bone density in a dose-dependent fashion (37,51,52). A recent study was also reassuring in this regard, showing minimal changes in bone density with doses of 2000 μg/day of BDP and no change with FP 1000 μg/day in patients 18–40 years old (53). In this last study, no significant difference was found between the two groups in markers of bone metabolism such as serum osteocalcin, bone alkaline phosphatase, PICP, deoxypyridinoline, and C-telopeptide of type 1 collagen.

In children, a reduced bone turnover rate has been found with high doses of ICS. However, the significance of those changes is still unknown, and there seems to be no adverse effect on markers of bone metabolism at standard pediatric doses (54–60). A dose-ranging study comparing μg-per-μg equivalent doses of FP and

BUD administered with a metered-dose inhaler (0.5–2 mg/day) showed a greater suppression of osteocalcin with FP (27 and 12%) (54). Agertoft and Pedersen performed dual energy x-ray absorptiometry (DEXA scan) in 157 asthmatic children treated with inhaled BUD at a mean daily dose of 504 μg for a mean of 4.5 years in comparison with 111 age-matched children suffering from asthma but never treated with corticosteroids (59). Measures of total body bone mineral density, total body bone mineral capacity, total bone calcium, and body composition were obtained. No statistically significant differences were found between the two groups for these parameters.

The safety of ICS in regard to bone density in subgroups at risk for osteopenia such as postmenopausal women has been evaluated (19,48). In reviews of studies on bone density in asthmatic patients on ICS, we can observe that results have sometimes been contradictory and that many studies were confounded by past or current oral corticosteroid therapy and postmenopausal status (19,52).

A decline in bone mineral density of 0.5 standard deviations on average was observed for each 1 mg increment in the daily dose of BDP or BUD inhaled with a spacer (51). At doses lower than 1 mg per day, the reduction was too small and variable to be statistically significant. This is consistent with the observations of little or no effect on bone mineral density of low to medium doses of BDP administered by CFC propellant, FP, or BUD in placebo-controlled studies (60).

Unfortunately, no reliable predictor of bone loss has been found. We recently showed that the slight reduction of bone density observed in patients using moderate to high doses of inhaled corticosteroids could not be predicted by initial measurements of either bone density or biological markers (46). Changes in these markers mostly reflect short-term changes, and their impact on long-term bone metabolism is unclear, as compensatory mechanisms seem to occur.

Finally, studies on chronic obstructive pulmonary diseases (COPD) provided further information on the potential side effects of ICS. A placebo-controlled study by Pauwels et al. in a large cohort of patients with smoking-induced mild COPD (mean age 52) treated with twice daily BUD at a dose of 400 μg/day delivered from a Turbuhaler™ reported no reduction in bone density over a 3-year period (61). There was even a small but statistically significant difference in favor of BUD. No difference was noted in the fracture rate between the two groups.

### *Confounding Factors*

Previous studies on bone density have often been difficult to interpret because control groups included nonasthmatic subjects, making it impossible to estimate the confounding effect of the disease itself on this parameter. Many other factors affecting bone metabolism include individual susceptibility, gender, age, osteopenia, diet, alcohol intake, physical activity, gonadal status, and use of other medications, such as estrogen replacement therapy, diuretics, or anticonvulsants

(62,63). Some asthmatic children never treated with corticosteroids may have decreased bone density and reduced osteoclacin levels compared to nonasthmatic children (64).

#### *Conclusions*

In conclusion, there is a dose-response effect of ICS on bone metabolism and density, although this influence seems to be small. Osteocalcin and pyridinium crosslinks are probably the best markers of bone formation and resorption, respectively, although further studies are needed to evaluate the usefulness and clinical relevance of the more recently proposed markers of bone metabolism. Bone density measurements by dual-energy x-ray absorptiometry is currently proposed as the best marker of fracture risk, although its usefulness in the follow-up of inhaled corticosteroid-dependent asthmatic patients remains to be further documented.

### C. Growth in Children

#### *Growth in Children and Corticosteroids*

As this topic is discussed in more depth elsewhere in this book and as several excellent reviews on ICS have discussed this effect thoroughly, we will only briefly review its use as a marker of systemic activity (65–69).

Growth during childhood has three phases: a rapid one during the first 2 years followed by a slower steady growth related to growth hormone production and, finally, the pubertal growth spurt controlled by growth hormones and sex steroids. In asthma, growth may be slow and associated with a delayed puberty, so that final height is normal except in those with severe disease. Systemic corticosteroids may induce different degrees of suppression of growth depending on the duration, dosage, and individual susceptibility.

#### *Methods of Assessment*

Height velocity, or statural height expressed as a height standard deviation score compared to age and sex-matched standards, are currently measured. A calibrated stadiometer can be used for this purpose. More precise and sensitive tools, such as short-term lower-leg growth measured by knemometry, have also been used. This technique, developed 15 years ago, is highly accurate and has a very low coefficient of variation. The equipment required, however, is more expensive, and the results depend on the skill of the observer.

#### *Effects of ICS on Growth*

The effects of ICS have been variable according to the groups studied and the drugs and the methodology used. Both lower-leg and overall growth velocity seem to be reduced with doses of ICS as low as 400 μg per day of BDP, but longer-term

consequences are uncertain (66–71). In a 12-month study, growth velocity of mild to moderate asthmatic children 6–16 years old was 4.4 cm/y on BDP MDI 84μg qid vs. 6 cm/y for theophylline (72). A linear growth of 3.96 vs. 5.40 cm was found in mild asthmatic children 6–14 years old on BDP 200 μg bid vs placebo (73). In a similar group, a slower growth (4.7 cm) was observed with BDP 200 μg bid than for salmeterol (6.1 cm) (74).

In regard to fluticasone, growth retardation was observed in six severely asthmatic children after introduction of high-dose FP treatment administered with a dry-powder inhaler (75). In a double-blind, randomized, parallel-group, multicenter study, 325 prepubescent children with persistent asthma and normal growth rates were treated with placebo or inhaled FP powder 50 μg or 100 μg administered twice daily by a breath-actuated device for one year (76); prepubescent children treated with FP grew at rates similar to placebo-treated control subjects and at rates equal to expected growth velocity for age.

Agertoft and Pedersen looked at the effects of BUD and FP administered with a Turbuhaler and Diskhaler, respectively, on short-term lower-leg growth measured by knemometry in 24 children aged 6–12 who were receiving 400 μg/day of drug or placebo for 2 weeks (77). No significant correlation was found between knemometry and adrenal function measurements. Agertoft and Pedersen recently reported that children with asthma who have received long-term treatment with budesonide (mean daily dose of 412 μg) attained normal adult height (78).

### *Confounding Factors*

A change in growth may be considered a marker of systemic effects of ICS in children, although many confounding factors should be considered, such as nutrition, hormonal and seasonal changes, the degree of control and severity of asthma and medication needs to achieve that control, particularly short courses of oral corticosteroids.

As for the other systemic effects, lung deposition and systemic bioavailability can be influenced not only by the nature of the ICS molecule but also by the type of inhaler used, including the propellant for metered-dose inhalers (chlorofluorocarbons vs. hydrofluorocarbons).

### *Conclusions*

In conclusion, compared to other methods, knemometry is considered a sensitive technique for studying the systemic effects of ICS on growth, but confounding factors should be considered, and it does not necessarily relate to long-term growth. Short-term knemometry was found to be more sensitive than 24-hour free urinary cortisol excretion in detecting systemic effects of exogeneous corticosteroids in children, but there was no significant correlation between those two types of measurements (79). At doses of ≥400 μg/day of BDP, short-term suppression

of growth may be observed with sensitive measures such as knemometry, but the predictive value of those changes on long-term growth seems to be poor.

### D. Skin

#### *Skin and Corticosteroids*

Skin thinning and bruising from increased collagen turnover or reduced synthesis may result from chronic systemic glucocorticosteroid activity. Changes in collagen content of the skin have also been described with ICS (80).

#### *Methods of Assessment*

Prevalence of bruising determined from questionnaire and examination and more precise methods such as ultrasound evaluation of skin thickness are among the methods used to evaluate skin changes following ICS use (81).

#### *Effects of ICS on Skin*

Capewell et al. studied 68 patients on long-term prednisolone or high-dose BDP compared to controls using ultrasound skin thickness determination and visual assessment of bruising (81). Prevalence of bruising was, respectively, 80, 48, and 12% for prednisolone, BDP, and controls. Compared with controls, skin was 28–33% thinner on prednisolone and 15–19% thinner on high-dose BDP.

Sixty-nine asthmatic subjects were enrolled in a double-blind crossover study with BDP or FP at half the dose of BDP for two 4-month periods each. The frequency of bruising reported by patient questionnaire was not different, but there were significantly more bruises on physical examination for BDP than for FP (31). Skin bruising, cortisol response to cortrosyn, and osteocalcin levels were not significantly correlated with the duration of BDP or FP intake. Other studies showed variable increases in skin bruising with high doses of ICS (7,31).

In a recent study in patients with chronic pulmonary obstructive disease (COPD), 10% patients on 800 μg/day of BUD for 3 years had skin bruising compared to 4% in the placebo group (61).

#### *Confounding Factors*

Age and concomitant use of oral corticosteroids are major confounding factors (16,80).

#### *Conclusions*

Skin bruising and reduction in its thickness may be considered markers of systemic effects of ICS, but individual susceptibility and other associated factors may induce wide variations in these measurements. Their role in evaluating systemic

effects of ICS and the correlation between this parameter and others remain to be further explored.

### E. Ocular Effects

#### *The Eye and Corticosteroids*

Long-term use of systemic corticosteroids may contribute to the development of cataracts, particularly posterior subcapsular (PSC). Topical ophthalmic corticosteroids may increase ocular tension and the incidence of secondary open-angle glaucoma in susceptible individuals.

#### *Methods of Measurement*

Slit-lamp eye examination may detect cataracts, and tonometry may be used to assess intraocular pressure.

#### *Effects of ICS on the Eye*

In regard to inhaled ICS, most data come from epidemiological studies using case-controlled or cross-sectional designs. No evidence of cataracts or glaucoma was found in children treated with inhaled ICS (82,83).

Although the causality relationship may be questioned, a large case-control study reported a slight increase in the risk of ocular hypertension and open-angle glaucoma in patients older than 65 years using high doses of inhaled ICS for at least 3 months, particularly at doses greater than 1.5 mg/day (84). There was no increase in the risk with low-moderate daily doses of ICS. However, although the results of this study were statistically significant, its clinical importance remains uncertain, and the influence of cofactors on this risk has to be determined.

An association between current and cumulative doses of ICS and PSC cataracts has been suggested in a large adult population–based study (85); the highest prevalence of PSC was in those with a lifetime dose exceeding 2000 mg of beclomethasone. However, the overall increased risk was small (1% in the non-ICS group and 2% in the ICS treatment group). The role of confounding factors cannot be excluded.

#### *Confounding Factors*

They are similar to the other markers previously discussed (Table 2). Observations should take into account the possibility of unrelated preexisting or concomitant disease. The risk of age-related cataract may increase with smoking, poor diet with insufficient antioxidant vitamin intake, ultraviolet B exposure, and diabetes.

#### *Conclusions*

In conclusion, when used at high doses, ICS may possibly increase the risk of glaucoma and cataracts in predisposed individuals, although further studies are

required to confirm those observations and evaluate their significance. As these effects are very rare and seem to occur only in certain subgroups of asthmatic subjects, measurements of intraocular pressure and evaluation for cataracts are not very useful to determine the comparative systemic action of medications in current trials.

### F. Other Effects

#### *Metabolic*

There is no evidence of significant metabolic effects of ICS on cholesterol, triglycerides, carbohydrate metabolism, or insulin concentrations (7). The rare anecdotal cases of increased blood glucose in some subjects suggest that it is unlikely to be a significant effect (86). In the previously mentionned COPD study, no increase in diabetes, myopathy, or PSC cataracts was observed with BUD (61).

#### *Influence on Blood and Bone Marrow*

ICS may reduce blood eosinophils and lymphocytes and increase circulating neutrophils. BDP was reported to cause a greater reduction of lymphocytes and eosinophils than BUD (87). ICS reduce eosinophil activation and survival by inhibiting cytokines such as GMCSF, IL-3, and IL-5 (88). Wood et al. showed that in subjects with mild stable asthma, inhaled BUD (400 µg/d) over 8 days significantly attenuated the allergen-induced early and late asthmatic responses, the degree of increase in sputum and blood eosinophils, and the baseline numbers of total bone marrow CD34+ cells, CD34+ IL-3R α− cells, and IL-5–responsive Eo/B–colony- forming units (CFU) (89). Although allergen inhalation significantly increased Eo/B-CFU grown in the presence of IL-3, GM-CSF, or IL-5 alone and, in combination, CD34+ IL-5 R alpha+ cells, these increases were not affected by budesonide treatment.

#### *Central Nervous System*

Although psychiatric disturbances have been associated with oral steroids, they are very rarely associated with ICS, and in the few cases described they were reversible with the discontinuation of the drug (90).

## IV. Which Is the Best Marker of Systemic Effects of ICS?

Further studies remain to be done to determine the best way to assess and compare systemic effects of ICS, both for safety surveillance in clinical practice and for comparative studies in view of regulatory issues. For this last, a sensitive test may be used, while for clinical follow-up, a test with a good clinical significance is preferred. Although HPA axis and bone metabolism markers are currently used to evaluate the systemic effect of ICS, the former is usually preferred as it has been

more fully documented. Although there may be correlations between markers of adrenal function and bone metabolism, these are generally weak and it is not practical to use one of these as a surrogate for the other.

Serum cortisol concentrations averaged over 24 hours are equally or more precise and sensitive than alternative measurements; single doses of about 1 mg of ICS given to healthy control subjects showed a coefficient of variation of about 20% (16–24%) for this parameter (37). It would be useful to determine if measures of bone turnover such as serum osteocalcin or the urinary production of cross-linked N-telopeptides can achieve the degree of precision offered by the 24-hour variation of serum cortisol concentrations (37).

In children it has been suggested that, when corticosteroid treatment is initiated, knemometry seems more valid as a measure of risk than 24-hour urine free cortisol excretion to assess the lowest dose at which the systemic activity of ICS can be detected (78). However, height measurement with a stadiometer is generally suggested as an adequate method for safety surveillance in clinical practice.

Finally, there are such large interindividual variability in response to various doses of ICS that mean results are difficult to extrapolate to a given patient, particularly if there is a high-risk group for systemic effects (e.g., postmenopausal women not on hormonal replacement for bone density).

With respect to the issue of comparing one ICS to another for regulatory purposes, a most interesting two-step approach has been suggested by Toogood (37,91). As a first step, the relative toxicity of the unknown test drug is compared to a standard drug in healthy subjects with ideally a precise test such as 24-hour area under the curve of plasma cortisol. Having determined the systemically equivalent doses for both drugs, in a second step, the antiasthmatic potency of the two drugs is determined in asthmatic subjects using systemically equivalent graded doses of those last in the range considered useful in clinical practice, using parameters such as expiratory flows or symptoms.

## V. Comparison of Systemic Effects of Different ICS: Some Methodological Aspects

When looking at the comparative effects of different ICS on HPA axis, studies exploring different dose levels on the steep part of the ICS dose-response curve are more revealing than single-dose studies (37). If the dose is too low, there may be a lack of detectable systemic effect of the drugs, or it may be too small to provide a reliable basis for discriminating between the treatments.

Time of measurement of the parameters used after the last dose administration may influence the results. A drug with a prolonged half-life can have more influence on its plasma levels, although it may not reflect exactly the clinically relevant systemic effects. Healthy subjects may be preferred for such comparisons, as it avoids confounding effects of past or current corticosteroid use and the influence of the disease. However, it may not reflect exactly what happens in patients with

airway disease. In fact, the same dose of ICS may possibly induce less cortisol reduction in asthmatic compared to nonasthmatic subjects, as a result of altered absorption of the drug from the airways. This favors the use of nonasthmatic subjects for preliminary safety testing; however, further testing should be done in asthmatics as results cannot be extrapolated.

Compliance to therapy during the trial is of major importance, and it has been shown that it may be low, therefore underestimating the effects of the ICS tested. Even pills counts and weighing the canisters may not be accurate if some dumping occurs during the study.

Chronopharmacology of ICS should also be considered when comparing two agents. The influence of ICS on HPA axis, for example, will be maximal when administered at the end of the day, around 10 p.m. Finally, what is a clinically important change or difference in a specific marker is still a subject of debate.

## VI. Clinical Significance of Changes in Markers of Systemic Effects

For most markers of systemic activity of ICS, the clinical and particularly the long-term influence of ICS is uncertain. Furthermore, the possibility that the observed changes in these markers decrease over time has to be studied. Although there is a potential for adverse effects on parameters such as bone metabolism when high doses of ICS are used for many years, such effects seem small and quite variable from one subject to another. In cross-sectional surveys of bone density in adults treated with ICS, contrary to what is found with oral corticosteroids, there was no evidence that increased duration of ICS therapy at usual doses was associated with an increased risk of bone loss (51, 92); in fact, the opposite may apply possibly as a consequence of bone restitution triggered by a reduction in previous prednisone exposure and possibly increased physical activity from better asthma control with ICS (51). As there is yet no reliable predictor of these changes, a reasonable approach would seem to be to monitor these effects in high-risk groups, such as postmenopausal women not receiving hormonal replacement therapy, particularly when high doses of ICS are used for prolonged periods. Nevertheless, although the usually poor clinical relevance of the markers described makes those not very useful for clinical practice safety monitoring, they may provide a means to assess the comparative systemic absorption of ICS for research and regulatory purposes.

## VII. How to Reduce Systemic Effects

Mouth rinsing and expectoration after ICS use can slightly reduce systemic absorption; because of the almost complete hepatic first-pass inactivation of agents such as fluticasone, it may not be useful to use this method to reduce its systemic

effects, although it may have other beneficial effects such as reducing oral candidiasis (93). Using the pressurized metered-dose inhaler (pMDI) with a spacer device can reduce the total available systemic dose, although it may have variable effects on the systemic absorption of inhaled ICS depending on the drug, the design of the spacer, and the way it is used (91). A likely explanation for a reduction in systemic activity when inhaled via a spacer is reduced intrapulmonary delivery.

The best option remains to use the lowest dose of ICS required to achieve adequate control of the disease and, when not sufficient, add medications such as long-acting $\beta_2$-agonists (Laβ2A) or leukotriene receptor antagonists (LTRA). In doing so, even when asthma is moderately severe, we may then be able to keep the dose of ICS $\leq$1000 μg daily in beclomethasone equivalent, as suggested by most recent guidelines (94,95). It has now been well established that with daily doses of ICS $>$1000 μg, the ratio of therapeutic effect over systemic absorption decreases rapidly (21). Although more long-term studies are required on the effects of such practice, adding other agents such as Laβ2A or LTRA generally improve asthma control and reduce the need for higher doses of ICS (96,97). Known risk factors for effects such as bone loss (e.g., age, hormonal status, concurrent diseases) should be assessed and preventative interventions may be proposed. Finally, the more recently developed ICS such as hydrofluoroalkane-beclomethasone dipropionate (HFA-BDP), and mometasone furoate provide additional options for ICS with a reduced potential of systemic effects compared to BDP with a cholorofluorocarbon propellant (98,99).

## VIII. Conclusion

A variety of markers of systemic absorption of ICS have been used to assess their comparative effects, the most common being markers of adrenal suppression and bone metabolism. However, the potential of these parameters to evaluate the specific side effects of the drugs may be influenced by many cofactors. Moreover, the magnitude of changes does not correlate with clinical outcome in most instances. Nevertheless, particularly in homogeneous groups of asthmatic patients and when confounding factors are taken into consideration, they may offer a useful estimate of the systemic effect of the ICS drugs. The identification of a simple and accurate marker of systemic effects of ICS is still needed, and the comparative value of those currently used remains to be further assessed (100).

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## Discussion

**Dr. Seale:** Are there studies (possibly with oral steroids) of both bone mineral density (BMD) and serum markers in which BMD was reduced? From such a study it may be possible to determine the magnitude of change in serum markers at which BMD is affected.

**Dr. Boulet:** To my knowledge, there is no marker of bone metabolism that could act as a predictor of reduction in bone mineral density. In a recent study we found no correlation between changes in various serum markers of bone metabolism and changes in bone density. (Boulet LP, Milot J, Gagnon, L, Poubelle PE, Brown J. Long-term influence of inhaled corticosteroids on bone metabolism and density: Are biological markers predictors of bone loss? Am J Respir Crit Care Med 1999; 159:838–844).

**Dr. Pedersen:** We have the same experience. Marker levels or steroid-induced changes in bone markers are not predictive at all for changes in bone mineral density. It seems that often a decrease in markers of bone formation is paralleled by a decrease in markers of bone degradation indicating that the turnover rate is reduced. That may not have any long-term adverse effects, even though the changes in markers were statistically significant.

**Dr. Szefler:** Most of the studies regarding adverse effects of inhaled corticosteroids have been conducted in heterogeneous, relatively normal populations. Are there studies available on patients at relative risk, for example, of reduction in bone density in elderly females?

**Dr. Boulet:** Some studies done in this particular population showed no influence of ICS on bone density, but this seemed to be due to the influence of estrogen replacement therapy. More studies are required in this type of patient.

**Dr. Georas:** I am wondering if we need to consider atopy as a confounding factor in considering the risk of osteoporosis on inhaled steroids. I am specifically reminded of a study in which IL-4 was overexpressed in transgenic mice resulting in osteoporosis (Lewis DB, Liggitt HD, Effmann EL, Motley ST, Teitelbaum SL, Jepsen KJ, Goldstein SA, Bonadio J, Carpenter J, Perlmutter RM. Osteoporosis induced in mice by overproduction of interleukin 4. Proc Natl Acad Sci USA 1993; 90:11618–11622). Are you aware of an association between atopy and the risk of osteoporosis? Would you please comment on the poor correlations between cortisol suppression and delayed bone growth? What are the mechanisms for this, and what does this tell us about the use of cortisol suppression as a surrogate marker of systemic side effects?

**Dr. Boulet:** I am not aware of a significant effect of atopy as a confounding factor in the evaluation of the risk of osteoporosis on inhaled corticosteroids.

The often poor correlation between cortisol suppression and delayed bone growth suggest that one cannot really be used as a surrogate marker of the effects of the other. This may suggest a different susceptibility of the end organ to the drug or probably more often the associated influence of other confounding factors.

**Dr. Newman:** All studies of HPA axis function must be interpreted carefully in light of the marker used. Recently we completed a study looking for the best marker of cortisol suppression as compared to the gold standard of 24-hour serum cortisol measurements. All measures, including 24-hour urine cortisols, ACTH levels, CRH stimulation, and AM serum cortisols, showed no or weak correlations with 24-hour serum cortisols. Therefore, it appears that studies that don't use serial serum cortisol measurements may lead to misleading results and incorrect conclusions.

**Dr. Boulet:** I agree with your comment. We should be careful in the interpretation of these results.

**Dr. Selroos:** AstraZeneca PMS data indicates a very low frequency of adverse events with budesonide. Based on 7 billion patient treatment days, approximately only 640 reports have been filed.

**Dr. Derendorf:** In many comparative studies, attempts are made to relate the potency in fixed ratios, e.g., 1:2. However, we are dealing here with complex relationships between dose and effects combined with circadian rhythms and different devices. This may be too much for our brains to handle intuitively, and computer simulations may be helpful.

# 20

## Patient Adherence to Inhaled Corticosteroid Therapy

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Because inhaled corticosteroids (ICS) have demonstrated high efficacy in reducing airways inflammation and controlling asthma symptoms, international asthma management guidelines recommend the use of ICS as the preferred therapy for the management of persistent, symptomatic asthma in children and adults. In clinical practice, however, the actual effectiveness of ICS therapy in controlling asthma has been shown to be significantly reduced by poor patient adherence. Clinical researchers are frustrated when they develop highly effective and safe asthma therapies that do not translate into the asthma control breakthroughs anticipated. Clinicians are often puzzled and discouraged when their patients seek medical advice for the management of their asthma and then promptly disregard their doctor's recommendations. As we have previously reviewed in *Severe Asthma* (1) and *Fatal Asthma* (2), poor adherence to asthma medication regimens is widespread, even when asthma is severe. As asthma has increasingly become recognized as a chronic disease that requires preventive treatment, it now shares many of management problems of other chronic diseases, such as hypertension and diabetes. It is a truism in the management of all chronic diseases, including asthma, that patient nonadherence is both common and a frequent cause of poor disease control. Because human behavior is the necessary interface between good therapies and

therapeutic effectiveness, it behooves both the clinical researcher and the clinician to understand the factors associated with patient adherence. This chapter updates our reviews of adherence issues in asthma published in 1995 (3) and 1998 (2), with a particular focus here on adherence to ICS therapy. We will review the relationship between adherence to ICS therapy and asthma morbidity, as well as the prevalence of nonadherence to ICS therapy recommendations. In addition, we will discuss the patient factors associated with nonadherence, including types of nonadherence and unique barriers to adherence in special populations. Finally, we will review general clinical strategies that have been identified as useful in improving patient adherence.

## I. Inhaled Steroid Adherence and Asthma Morbidity

The effectiveness of ICS therapy in real-world clinical practice should be evident in marked improvement in markers of asthma morbidity, such as asthma symptoms, urgent care utilization, and hospitalizations. Conversely, patient nonadherence to ICS should result in measurable disease exacerbations. Case-control studies, which examine the relationship between level of ICS adherence and asthma morbidity, are not only useful in providing a quantitative estimate of the effectiveness of ICS therapy, but also in defining the parameters of nonadherence that result in increased risk.

The most extreme examples of asthma risk are fatal and near-fatal asthma. Ernst et al. (4) examined the relationship between use of ICS and the risk for fatal or near-fatal asthma among 12,301 residents of Saskatchewan, Canada. The source population for this study was created from the Canadian Health Insurance file, and use of asthma medications was assessed using the Out of Hospital Prescription Drug data files. After adjusting for use of other asthma medication and other markers of high risk, results showed that dispensing of at least one canister of ICS per month for 12 months was associated with reduced risk of fatal and near-fatal asthma (O.R = 0.01; CI = 0.2–0.6). It is of interest that of the 269 patients who had been dispensed ICS in the previous year, only 37 (14%) had been dispensed amounts congruent with appropriate compliance over the course of the year (i.e., 12 or more canisters). Ernst et al. suggest that this is most likely due to erratic dispensing (i.e., failing to refill prescriptions), rather than regular use of very low doses of ICS. They conclude that their data support the value of ICS in reducing the risk of fatal and near-fatal asthma, but this reduced risk appears to be dependent on appropriate adherence with ICS therapy.

A related marker of high-risk asthma morbidity is hospitalization for asthma. Several studies have examined the relationship between ICS use and rates of hospitalization for asthma. Blais et al. (5) investigated a cohort of 2059 hospitalized asthmatic patients (5–54 years of age) and estimated the effectiveness of ICS in preventing rehospitalization for asthma (using the same database and

definitions as Ernst et al. (4) above). In this study the relative effectiveness of ICS therapy was estimated for different durations of therapy. Regular use of ICS was defined as patients who had filled at least one prescription of the medication every 90 days. This classification was based on the minimum daily recommended use of ICS (i.e., one canister every 50 days) and was expanded to account for some level of noncompliance (~55% compliant). Results showed that patients who started ICS therapy within a month of their initial hospitalization were three times less likely to be readmitted to the hospital for asthma in the 16–90 days after this initial hospitalization (RR = 0.3; CI = 0.2–0.6). ICS use during the first 15 days or 4–12 months after the initial hospitalization did not reduce risk. Initiation of ICS therapy within 2 years was associated with similar reductions in risk up to 6 months (RR = 0.6; CI = 0.4–0.9). The investigators concluded that patients may require up to 15 days of therapy before ICS begin to offer protection against readmission. They also suggest that the observed reduction in the protective effect of ICS beyond 6 months may result from a bias introduced if patients with less severe asthma are more likely to discontinue therapy in the first year while patients with higher risk asthma continue to use ICS therapy.

Donahue et al. (6) observed similar associations between ICS use and reduced risk of hospitalization among 16,941 asthmatic patients enrolled in a U.S. managed care group. After controlling for markers of asthma severity (i.e., sex, age, amount of β-agonist use, ambulatory care), they found that use of ICS therapy was associated with a relative risk of hospitalization of 0.5 (CI = 0.4–0.6). However, unlike the Ernst et al. study(4) examining risk of fatal and near-fatal asthma, they found no evidence of a dose-response relationship between ICS dispensing and reduced risk for hospitalization. In fact, as shown in Table 1, dispensing of only one canister of ICS was associated with the same relative risk of hospitalization as dispensing more than eight canisters of ICS. In contrast, increased dispensing of β-agonists was associated with increased risk of hospitalization. The failure to observe any dose-response relationship between ICS use and risk in this study may be attributable to other risk or protective factors that overwhelm the dose-response relationship. For example, patients who are prescribed ICS may be more likely to be receiving specialist care and hence better quality (rather than quantity) asthma management. These patients may also differ from those not prescribed ICS therapy in important, but unmeasured dimension of risk, such as socioeconomic factors.

## II. Rates of Adherence with ICS and/or Other Preventive Asthma Drugs

### A. Primary Adherence with ICS Therapy

Before patients can begin to adhere to ICS therapy, they must first fill their prescriptions. Research suggests that between 6 and 44% of all initial prescriptions

**Table 1** Relative Risk for Asthma Hospitalization Among Members of Harvard Pilgrim Health Care, 1991–1994

| | Relative risks (95% CI) | | | |
|---|---|---|---|---|
| Inhaled steroids | Total ($n$ = 16941) | Age 0–17 y ($n$ = 6562) | Age 18–44 y ($n$ = 7689) | Age ≥45 y ($n$ = 2690) |
| None | 1.0 | 1.0 | 1.0 | 1.0 |
| >0–1 | 0.4 (0.3–0.6) | 0.3 (0.2–0.5) | 0.5 (0.3–0.8) | 0.5 (0.3–1.0) |
| >1–2 | 0.6 (0.5–0.9) | 0.8 (0.4–1.4) | 0.4 (0.2–0.7) | 1.0 (0.5–1.8) |
| >2–3 | 0.3 (0.2–0.5) | 0.3 (0.1–0.8) | 0.2 (0.1–0.4) | 0.5 (0.2–1.2) |
| >3–5 | 0.3 (0.2–0.4) | 0.3 (0.1–0.6) | 0.1 (0.1–0.3) | 0.6 (0.3–1.3) |
| >5–8 | 0.3 (0.2–0.5) | 0.3 (0.1–0.8) | 0.2 (0.1–0.4) | 0.5 (0.2–1.1) |
| >8 | 0.4 (0.2–0.6) | 0.5 (0.1–2.0) | 0.3 (0.1–0.7) | 0.6 (0.2–1.3) |

Number of hospitalizations for asthma per person-year among recipients and nonrecipients of inhaled steroids within various categories of β-agonist dispensing for persons in the Harvard Pilgrim Health Plan from October 1991 through September 1994. Error bars indicate 95% confidence intervals. The relative risks (reference group: individuals who received no inhaled steroids or β-agonists), number of persons hospitalized, and total number of persons in each stratum are also provided.
*Source*: Ref. 6.

are not filled (7–11). The failure to fill a prescription is classified as "primary nonadherence," while the inappropriate use of prescribed medication is classified as "secondary noncompliance" (12). Once dispensed, conservative estimates indicate that almost half of the prescription medications each year are not taken as prescribed (13). These statistics suggest that estimates of adherence to inhaled corticosteroids that are drawn from clinical trial settings (where medications are provided and adherence is monitored) reflect the *maximum* probable ICS adherence levels. The real life sequlae to a clinician's prescribing of ICS therapy will include a range of less desirable patient outcomes, including a failure to fill the initial prescription, erratic or underuse of ICS therapy, and premature discontinuation of therapy.

Studies based on pharmacy databases are particularly useful for determining primary adherence with ICS therapy (14). Watts et al. (12) examined primary nonadherence in patients with asthma by matching prescriptions written with those filled over a period of 3 months. The study included both new and repeat prescriptions for asthma medication. The setting of the study was geographically isolated and therefore no prescriptions were expected to be filled outside the area. Of 359 documented prescriptions written, 251 (approximately 70%) were filled. Of the prescriptions filled, most were filled by the sixth day (91%), with the vast majority filled on the same day as the clinic visit (76%). Patients with lower socioeconomic status showed decreased relative odds of filling their prescriptions (RR = 0.84) compared to patients of high socioeconomic status. Neither gender

nor age had an influence on primary adherence in this study. In a similar study by Kelloway et al. (15), medical records were compared to data from pharmacy claims in order to calculate adherence rates for theophylline and ICS therapy. Patients had theophylline adherence rates of 73% in the younger group (age 12–17) and 80% in the older group (age 18–65). Adherence rates for ICS were 30% and 57% in the younger group and older group, respectively. Since these primary adherence estimates are based on refill rates, they represent the maximum possible levels of adherence and do not provide any information on the day-to-day patterns of medication use in the home.

### B. Secondary Adherence with ICS Therapy

#### *Self-Reported Adherence to Therapy*

Patient self-report, whether by diary card or clinical interview, is the most commonly used measure of adherence to ICS. Self-report measures have the advantage of simplicity, low cost, and appropriateness to the clinical care setting. Numerous studies and reviews have documented, however, that self-report measures have highly variable validity (16).

Despite their limitations, self-report measures are the only appropriate measures for assessing reasons for noncompliance. For example, Van Es et al. (20) used focus group interviews to collect information about adolescent experiences with adherence to asthma therapy. Results found that the teens usually reported forgetting in the morning if they were in a hurry or when their normal routines were interrupted. Participants who had no daily routine stated that they often forgot to use their daily medication "rarely gave a thought to it," or made a conscious decision not to take their medication. Some participants stated that they had deliberately decided to discontinue therapy. Reasons mentioned for these teenagers stopping treatment included not seeing any effect from their preventive treatment, bad taste of medicines, and not having asthma symptoms all the time.

Survey research that assesses patient adherence with therapy is also reliant on self-report measures. Population-based surveys, however, have the potential to measure asthma patient behavior outside the constraints (and potential demand characteristics) of the clinic setting. In a survey of those 400 adult asthma patients prescribed ICS therapy, Chambers et al. (21) found that only 38% of the respondents reported that they used their ICS therapy as prescribed on most days.

#### *Medication Measurement of Adherence with Therapy*

While more labor intensive than self-report, medication measurement is often used as an objective assessment of adherence in clinical trials. In two 6-month studies, Toogood et al. (22) investigated the influence if different dosing regimens (qid or bid) on response to inhaled budesonide among adult outpatients with chronic asthma (22,23). To monitor adherence to the protocol regimen, all budeso-

nide canisters were weighed before they were given to the participants and again upon return at each clinic visit. Toogood et al. reported adherence, by canister weight, to the prescribed budesonide regimen at 97% throughout the study. Over-adherence was also observed in some participants, with adherence ranging from 78 to 155%. In a separate study comparing qid budesonide to bid budesonide, Malo et al. (23) found that both the qid and bid treatment groups were approximately 85% adherent to their prescribed regimens, according to canister weight.

Different forms of therapy or modes of delivery may influence rates of adherence. In a pilot study of 72 adult patients with mild asthma, Hughs et al. (24) examined patient adherence to three different forms of therapy: montelukast sodium tablets, fluticasone aerosol, and budesonide dry powder inhaler. Pill counts, canister weights, and remaining doses were collected to assess adherence to the different regimens. Adherence was calculated by dividing doses taken by doses prescribed. The investigators found different levels of adherence for each therapy, with montelukast mean daily adherence at 97.3% of prescribed, inhaled fluticasone at 85.6%, and 78% of prescribed for dry powder budesonide, suggesting that mode of therapy may influence adherence.

Celano et al. (25) examined anti-inflammatory metered-dose inhaler adherence in low-income, urban, primarily African American children with asthma. Estimated metered-dose inhaler adherence was determined by weighing canisters and calculating the ratio of the number of puffs used over the study period to the number of puffs prescribed. Estimated metered-dose inhaler adherence in this study was 44% for all participants and only 12% of the children had rates above 75%.

### *Electronic Measures of Adherence*

While medication measurement provides an objective method to document total ICS use, it is not useful for assessing patterns of inhaler use and is vulnerable to deliberate emptying [i.e., "dumping" (26)]. With the advent of electronic monitors, such as the Nebulizer Chronolog (Medtrac Technologies, Lakewood, CO), investigators were given a powerful new strategy for objectively measuring adherence. The Chronolog monitoring device had the ability to measure the date and time of each metered-dose inhaler actuation. While these actuations represented presumptive uses, they provide a detailed picture of real world use of medication.

Using a Nebulizer Chronolog, Gibson et al. (27) have also studied compliance with inhaled prophylactic medication among preschool children. Electronic monitoring data revealed generally variable and poor adherence in the study group of 29 asthmatic children, ages 15 months to 5 years. Median compliance was 50% of study days having full compliance, with an overall median of 77% of the prescribed doses taken during the average 2-month monitored period. Asthma symptoms recorded on diary cards showed little relationship to medication compliance. Dosing frequency was unrelated to compliance. [This is in contrast to the study

by Coutts et al. (28) described above), in which participants who were prescribed prophylactic regimens twice a day were adherent on 71% of the days, compared to only 18% of study days for those with four times daily regimens.] The Gibson et al. study is striking in that it reflects poor adherence even among parents who had a clear understanding that adherence was being monitored and who had been provided with careful explanations of the importance of adherence with prophylactic medications. The authors note that this poor adherence might reflect persistent misunderstandings or concerns about the side effects of the medications.

In a 6-week study, Mann et al. compared adherence with bid or qid dosing of inhaled flunisolide in patients with asthma (29). All participants were initially instructed to use their flunisolide bid to establish baseline adherence (Time 1). After 3 weeks, half of the participants were instructed to change their dosing to qid for 3 weeks (Time 2). Adherence was monitored electronically in both groups throughout the study. The group that had the bid dosing for 6 weeks showed no difference in adherence between T1 and T2 and had fewer than prescribed inhalations on approximately 37% of study days. The group which went from bid to qid dosing almost tripled the percentage of days with fewer than eight inhalations from 20% during T1 to 50% during T2. Overuse was observed in over 20% of patient study days. The investigators reported on one participant who did not use the medication during the study intervals but "dumped" his medication by actuating the Chronolog repeatedly on his two clinic visit dates.

Apter et al. (30) used electronic monitoring to evaluate adherence with β-agonist and beclomethasone in a group of 13 adult patients with asthma. Twelve of the 13 patients were prescribed qid dosing. During this 9-week study patients maintained asthma diaries for symptoms and peak flow measures. The total percent of prescribed ICS adherence for each patient ranged from 11 to 106%, with mean daily beclomethasone compliance of 64% for the group. They note that if compliance were defined using a criteria of taking at least 75% of prescribed doses [as described by Mawhinney et al. (31)], then only 38% of the patients could be considered compliant.

In a series of small (9–11 patients) studies using an electronic monitor, Yeung et al. (32) examined ICS use over a period a 2–3 weeks. When patients were aware of monitoring 60% of the patients were fully compliant, 20% were partially compliant (taking just 70% of the prescribed dose), and 20% were nonadherent. When patients were unaware of the monitoring, 6 of 11 took between 30 and 51% of the prescribed doses. In both of these studies patients tended to overreport inhaled steroid use.

## III. Forms of Nonadherence

Understanding patient nonadherence to ICS therapy requires a recognition that there are different forms of nonadherent behavior with diverse contributing fac-

tors. Careful clinical interviewing can probe to uncover these problems and set the stage for identifying appropriate strategies for ameliorating these difficulties.

### A. Erratic Nonadherence

Perhaps the form of nonadherence that is most common and most acknowledged by patients and providers is missed doses because of forgetfulness, changing schedules, or busy schedules. Erratic nonadherers understand their prescribed regimen and would like to adhere appropriately, but they find that they have trouble complying because the complexity of their lives interferes with adherence or because they have not prioritized asthma management. Patients who have changing work schedules or chaotic lifestyles may have difficulty establishing the habit of a new medication regimen. For some patients Monday-to-Friday adherence is fine, but weekends or holidays disrupt medication routines. Strategies to improve erratic adherence center on simplification of the regimen (e.g., once daily dosing), establishing new habits through linking (e.g., MDI next to the toothbrush), and cues and reminder aids (e.g., pill organizers).

### B. Unwitting Nonadherence

Many patients may be inadvertently nonadherent to the prescribed therapy because they failed to fully understand either the specifics of the regimen or the necessity for compliance. Studies have found that patients frequently forget instructions given to them by a physician during a clinic visit.(33) Metered dose inhalers, unlike pill bottles, do not typically have attached labels that list dosing instructions. In asthma management it is common for patients to misunderstand the difference between PRN medication and daily medication. Or they may interpret the prescription for "ICS twice every day" as meaning "ICS twice every day—when you have symptoms." Patients may overuse their inhaled β-agonist because they have never been given clear guidelines as to when to discontinue home treatment and seek medical assistance. The ubiquity of unwitting nonadherence is illustrated in a study by Donnelly et al. (34). The investigators interviewed 128 Australian parents of children with asthma about their attitudes, beliefs, and their knowledge of asthma and asthma medications. Only 42% of parents had basic understanding of the mode of action of β-agonists, 12% of methylxanthines, 12% for cromoglycate, and 0% for inhaled steroids. Approximately half of the parents reported that sodium cromoglycate and inhaled steroids were used to prevent asthma attacks, while 40–50% were unsure of the mode of usage. The majority of parents reported using antibiotics, antihistamines, and decongestants in treating their child's asthma. The authors suggest that this poor parental understanding of asthma medications may result from inadequate doctor-patient communication, which may contribute to the high prevalence of nonadherence in asthma. In a study of adult asthmatics and COPD patients in the Netherlands, Dekker et al. (35) found that 20% of the patients using pulmonary medications admitted that they did not know

the prescribed daily intake. Twenty-nine percent thought that their regular daily medication was actually to be used "short-term" or "as needed." Only 51% correctly perceived that their medications were to be taken regularly.

### C. Intelligent Nonadherence

Sometimes patients alter, discontinue, or even fail to initiate ICS therapy on purpose. This deliberate nonadherence is called intelligent nonadherence, reflecting a reasoned choice, rather than a necessarily wise choice (36). Patients who feel better may decide that they no longer need to take prescribed medications. Fear of perceived short- or long-term side effects of ICS may cause some patients to reduce or discontinue dosing. Patients may abandon a therapy because taste, complexity, or interference with daily life may convince them that the disadvantages of therapy outweigh the benefits. Some patients may find a variation of the prescribed therapy works better than the doctor-prescribed regimen. In fact, given the well-documented underuse of ICS, the effectiveness ICS therapy in the management of asthma suggests that many patients manage quite well with altered or reduced dosing. This deliberate nonadherence, like all nonadherence, does not necessarily result in worsening asthma. In every clinical practice there are patients who have knowingly altered their prescribed therapy, yet their physician may never uncover this modification. Regardless of the source of medication nonadherence, the necessary first step to addressing the problem is identifying the problem through effective, open-ended patient-provider communication. Only through the use of careful interviewing and active listening will the asthma care provider be equipped with the information necessary to establish and reinforce appropriate medication adherence (37). The time constraints placed on clinicians time by managed care represents a serious barrier to this recommendation.

## IV. Factors Associated with ICS/Other Adherence

### A. Asthma Severity

Because of the significant burden of symptoms and the risk associated with more severe asthma, it would seem logical that patients with severe disease would have a greater incentive, and hence likelihood, of adhering with prescribed therapy. Indeed, it could conversely be argued, that for some asthmatic patients more symptomatic disease is the consequence of inadequate compliance with treatment. For example, Milgrom et al. (18) found in a study of pediatric asthmatic patients that prednisone bursts were more common in those patients who were found by electronic monitoring to be the least adherent with inhaled anti-inflammatory therapy.

It has also been suggested that active asthma symptoms should serve as a cue for improved medication adherence. Research on the relationship between asthma severity and adherence to therapy presents a mixed picture. Studies by Watts et al. (12) and Ernst et al. (4) have reported that patients with more severe

asthma are more likely to fill prescriptions for their asthma medication. However, in studies that closely examine day-to-day use of asthma medication, the occurrence of disease exacerbations (e.g., reduced peak flow asthma symptoms) does not appear to be related to improved adherence (38–40). For example, Mann et al. (41) examined this hypothesis by measuring the relationship between patient adherence with qid beclomethasone and periods of increased asthma severity. Ten adult patients with moderate to severe asthma were monitored over a 9-week period using Nebulizer Chronologs to measure inhaler adherence and peak flow monitoring to measure airflow obstruction. In addition to PEFR, daily asthma symptom scores and daily use of albuterol assessed asthma severity. Mean beclomethasone compliance for the study group was 67%, with underuse reported on 69% of the days and overuse on 11% of the days. Beclomethasone compliance decreased progressively over the 9 weeks, with 60% mean adherence at week nine. They found that variations in PEFR values, symptom scores, and albuterol use were found to be unrelated to beclomethasone adherence. The investigators concluded that compliance with inhaled steroids was not modulated by asthma severity (as measured by PEFR) or by symptoms.

### B. Patient Beliefs About ICS and Asthma

Regular adherence with ICS therapy is dependent upon the patient's acceptance that asthma is a chronic disease that requires preventive treatment. In addition, patients must feel comfortable that the prescribed therapy is effective in achieving the desired treatment goals and that therapy is safe for long-term use. A number of studies have confirmed that patient beliefs about their asthma and the prescribed therapy are strongly associated with the likelihood of adherence. When patients don't believe that their asthma is chronic or that it requires preventive treatment, compliance with therapy is generally episodic.

The relationship between asthma beliefs and adherence with preventive therapy was clearly illustrated in a study by Adams et al. (42). The investigators interviewed adult patients in Wales using qualitative interviewing strategies and identified three common self-perspectives among this group: deniers/distancers, accepters, and pragmatics. Each of these perspectives was associated with very different patient beliefs about the nature of asthma and the use of preventive medication. The half of the sample who were classified as deniers/distancers ($N = 15$) claimed that they did not have asthma (despite a doctor diagnosis and prescribed medications) or reported that they had "slight" or "not proper" asthma. These patients stated that asthma had no effect on their lives, and they rarely took their reliever medications. With additional probing, however, these patients revealed that they used their reliever surreptitiously and that they had developed complex behaviors to avoid physical symptoms (e.g., not running, staying indoors in certain seasons). None of these patients were using their prophylactic asthma medications. These patients rejected the label of "asthmatic" (which they consid-

ered stigmatizing) and instead described their breathing difficulties as an acute, situation-specific problem or as a "bad chest." Those patients classified as acceptors, on the other hand, accepted the chronic nature of asthma and had internalized the social identity of "asthmatic." These patients sought to achieve a normal life through the use of good asthma-management behaviors, rather than by denial. In contracts to the deniers, who perceived preventive medications as a source of stigma, acceptors saw these medications as an aid to normalization. Acceptors were most apt to use preventive asthma medications. Pragmatics ($N = 6$) were less neatly categorized, although in general they were closer to the acceptors than to deniers. While these individuals may not have embraced an asthma diagnosis in the proactive manner of the acceptors, they were attempting to reconcile their lives and self-image with the social identity of "asthmatic." While they used their prophylactic medications, they might not have been using them in the approved manner. These individual's level of self-disclosure and self-presentation shifted according to the relevant audience. This analysis suggests that patient beliefs may influence adherence with preventive asthma therapy.

Parents and patients who are concerned about using steroids may underdose or discontinue long-term use in a self-determined effort to be "steroid-sparing." Boulet et al. (43) conducted a telephone survey of over 600 adult asthmatic patients in Canada in order to understand patient perceptions about the role of ICS in the treatment of asthma and the potential side effects of this therapy. Thirty-nine percent of those surveyed had used intermittent or regular ICS in the past year. While the majority of patients classified their disease as "mild," the high level of symptoms reported suggested that patients might be underestimating the severity of their disease. As shown in Table 2, patients frequently had misperceptions

**Table 2** Perception of the Role of Inhaled Corticosteroids[a]

| | Ever used inhaled corticosteroids ($n = 272$) | Past 12 months corticosteroid use ($n = 235$) |
|---|---|---|
| ✗ Opens the airways—relieves constriction | 43 | 41 |
| ✓ Reduces inflammation/swelling of the airways | 22 | 24 |
| ✓ Prevents asthma attacks | 14 | 16 |
| ✗ Relieves an asthma attack | 11 | 12 |
| ✓ Gets asthma symptoms under control | 7 | 8 |
| ✗ Builds up/strengthens lungs | 3 | 3 |
| Don't know | 12 | 11 |

[a]Checkmark (✓) is considered a good answer; "✗" is considered a false answer. Answers are respectively true or false according to current knowledge.
*Source*: Ref. 43.

**Table 3** Concerns About Inhaled Corticosteroid Use

| Concern | % of subjects |
|---|---|
| Fear of side effects | 59 |
| Need for higher doses over time to match effectiveness previously experienced | 38 |
| May become less effective when used on a long-term basis | 36 |
| Causing weight gain | 29 |
| Building huge muscles | 24 |
| Causing infections | 20 |
| Making bones brittle/susceptible to fractures | 16 |
| Stunting growth | 14 |
| Causing cataracts | 8 |
| Causing diabetes | 7 |

*Source*: Ref. 43.

about the role of ICS, even patients who had recently used this treatment. Over 40% of patients believed that ICS opened up the airways to relieve bronchoconstriction, while less than a quarter of the patients reported that ICS reduced airway inflammation. This fundamental misunderstanding of the mechanism of ICS suggests that these patients may also not understand the underlying chronic inflammation that characterizes asthma and the need for preventive therapy. Forty-six percent of these patients indicated that they were reluctant to take ICS on a regular basis, and only 25% of patients reported that they had discussed their fears and concerns about ICS with their primary care provider. Misperceptions about the side effects and long-term consequences of ICS use were also common (Table 3). Thirty-eight percent believed that ICS doses would need to be increased over time to maintain effectiveness, while 36% believed that ICS therapy becomes less effective over time. Concern about side effects was high (59%), with the most frequently cited fears being weight gain (29%) and building huge muscles (24%). Boulet and colleagues concluded that information about the safety and usefulness of ICS does not seem to have reached many patients with asthma. This study also suggests that health care providers must initiate conversations with patients to proactively address possible concerns about ICS therapy that might interfere with patient adherence.

In a similar study conducted in the United States, Chambers et al. (21) surveyed 694 asthmatic patients 18–49 years of age who had been prescribed ICS in 1995–96. Patients were identified for this study by review of medical records within the TriState Primary Care Research Network, a network of family practice providers in the greater Philadelphia area. All data analysis was conducted on the 394 survey forms that were returned and eligible (i.e., within age range and asthma

**Table 4** Most Frequent Reasons for Not Using ICS[a]

| Reason | % |
|---|---|
| I use it only when I need it | 62 |
| I don't like using medicine unless I feel sick | 33 |
| I don't want to use steroids | 27 |
| I feel fine | 22 |
| I don't like the side effects | 18 |

[a]Based on 247 respondents that did not report using ICS at least twice a day almost every day; respondents were allowed to select multiple items.
*Source*: Ref. 21.

diagnosis). The majority of these patients were symptomatic and reported experiencing nighttime or daytime symptoms within the previous 4 weeks. Most notable in this survey was the low level of self-reported adherence with therapy, with 62% of patients reporting less than regular twice-daily ICS use. Thirty-six percent of these patients endorsed the option "Some days I use it at least twice, but on other days I don't use it at all," while 22% reported that they no longer used ICS. Four percent of patients claimed that they have never used ICS. Those who were less than fully adherent were asked the reasons they were not using ICS, and as shown in Table 4, the most frequent reason cited was that they only used therapy when they believed they need it. This is consistent with a patient belief that their asthma is an episodic disease, rather than chronic disease, and that therapy can and should be adjusted to match disease exacerbations. The high level of symptoms reported by this group, however, argues that this self-titrating is ineffectual in fully controlling asthma symptoms and reducing asthma risk.

Psychological models of disease management have suggested that medication compliance may be related to the patient's perceived vulnerability to the negative consequences of illness (44), with an increased sense of risk associated with better adherence. In pediatric research several studies have suggested parents who consider their children's health to be fragile or vulnerable (based on real events or not) will be vigilant and adherent with health care recommendations (45–47). Spurrier et al. (48) examined the relationship between asthma management strategies used by 101 parents of children with asthma and this parent's perceptions of their child's vulnerability to illness. The study found that after controlling for the frequency and severity of asthma symptoms, those parents who felt their child had greater vulnerability to illness were more likely to use regular preventive medications, take the child to the doctor, and keep the child home from school. The authors suggest that one possible explanation of this finding is that "parents who do not perceive their child to be medically vulnerable may discontinue administering regular medication" (48).

### C. Regimen Factors in Asthma Therapy

A number of studies across a range of chronic diseases have found that characteristics of the prescribed treatment regimen are strongly associated with patient adherence (49). In general, the longer the duration of therapy, the more frequent the dosing, and the more complex the regimen (e.g., multiple devices or tasks), the poorer patient compliance. Actual or perceived treatment side effects and the cost of therapy can also reduce adherence levels. The logic of this relationship is clear to most clinicians; however, all too often asthma therapy choices are driven by habit rather than a careful matching of regimen characteristics to each patient's preferences and abilities. Selecting medications, delivery devices, or dosing frequency should be a negotiated and individualized process between the clinician and the patient that considers not only the asthma severity, but the patient's lifestyle, past adherence history, beliefs and concerns about medication, ability to pay for medications, and other medication regimens.

Considerable interest has been directed in recent years to developing an effective and safe once-a-day therapy for asthma because of its presumed advantage for patient compliance. However, while the evidence is convincing that dosing regimens greater than twice a day lead to decreased adherence (19), the data are equivocal on the superiority of once-a-day dosing over twice-a-day dosing (50–54). For some patients once-daily dosing may simplify their daily regimen and decrease inadvertent missed doses due to forgetfulness. However, for the patient who is intentionally decreasing or discontinuing therapy because they believe that they no longer need to use it (43) or because they are concerned about side effects (21), once-daily dosing is unlikely to improve adherence (55). Apart from adherence considerations, once-daily asthma therapy appears to be preferable for most patients. Venables et al. (56) examined patient preference in asthma therapy and found that 61% of patients expressed a preference for once-a-day treatment, 12% preferred twice-a-day treatment, and 27% expressed no preference. While preference may not necessarily lead to improved compliance, it may well reduce the burden of therapy and enhance the patient's quality of life.

## V. Adherence Issues in Special Populations

### A. Children

For pediatric patients assessing and improving asthma medication, adherence requires that the clinician appreciate the family context in which medication use occurs and open communication about asthma management between the health care provider and the family. This communication starts with reviewing parent and patient concerns about the potential long- and short-term side effects (real or imagined) associated with ICS. It is important to consider ICS therapy in terms of the family's priorities, rather than the clinician's. Dismissing the potential for

negative consequences of ICS or minimizing the importance of some risks, such as minor growth suppression, can discourage parents from honestly expressing concerns. Instead of arguing with their doctor, many parents will simply not initiate ICS therapy, or use it only during acute excerbations. When a clinician is authoritarian in expressing their belief that ICS therapy is the best choice for the child, parents who alter therapy because of concerns will generally not reveal these changes for fear of being judged a bad parent or because they don't want to "disappoint" the doctor.

Another important consideration in pediatric adherence with ICS therapy is identifying who in the family is responsible for delivering asthma medications to the child. Responsibility for medication administration generally shifts as a child grows from total parent management for a young child to shared medication management for the school-age child to complete self-management for the adolescent. There can be great diversity among families in how medication management is implemented. Day care providers, grandparents, and siblings may assume responsibility for regular asthma medication delivery in some households. In chaotic, troubled families, primary responsibility for medication monitoring may be confused. The age at which a child is capable of assuming responsibility for remembering to take daily medication is highly variable. In some families children may be expected to manage their own medication early, less because the child has demonstrated sufficient responsibility, and more because the parent believes the child is "old enough to do it" (57). Because of the highly variable and often shifting family responsibility for a child's medication use, it is therefore necessary for the physician to review with both the parent and the child medication use habits in order to develop an adherence profile.

Research on adherence in pediatric chronic diseases has underscored the particular vulnerability of the adolescent to medication adherence problems (58,59). Rule testing, acting out, and rejection of parental authority may be normal and inevitable behaviors during adolescence, but they can significantly interfere with responsible asthma management. Some adolescents may deny disease severity and undertreat or ignore asthma symptoms. Family conflict and a denial of disease severity in an adolescent with severe asthma should therefore suggest a patient at a higher risk for nonadherence with ICS therapy.

### B. Elderly

Some barriers to adherence to ICS therapy are more common in older patients and warrant particular attention in clinical management. For example, while patients of any age are at risk for forgetting to take their medication, for some older patients memory difficulties may be exacerbated by other medications or early dementia. In addition, older patients are often receiving treatment for several other chronic health conditions. The resulting polypharmacy is a well-recognized problem for many seniors, presenting both pharmacological and adherence risks

(60–62). Treatment of these multiple ailments can result in complicated and burdensome medication regimens that require dosing multiple times of day. Clinicians treating treating older patients for asthma should carefully review all prescribed medications, be attentive to potential memory difficulties, and assist the patient in integrating ICS therapy into their existing regimens.

Just as with pediatric patients, older patients may be particularly concerned about the risks of steroid-related side effects. For seniors, however, the focus may be on how ICS therapy will influence the risk of osteroporosis or cataracts. Patients may hesitate to question their health care provider or voice their concerns about ICS therapy, and instead express their fears through underuse or discontinuation of therapy. Therefore, even for patients who appear accepting of therapy, physicians should encourage discussion concerning the patient's beliefs about the risks of ICS therapy.

Another barrier to adherence faced by some older patients is the cost of prescribed medication. Patients whose only form of health insurance is Medicare may be faced with unmanageably high pharmacy bills if they are on therapy for several chronic illnesses. Research suggests that these cost barriers may encourage some patients to deliberately reduce their dosing to "stretch" their medication (63). Patients may be embarrassed to raise the issue of the cost of their prescribed medications with their physician (64). Sensitive questioning on the part of the clinician can help identify this barrier—for example, "There are several factors that we can consider in selecting the right therapy for you. For many patients the cost of the medication is an important factor. How important is cost of medication for you?"

### C. Cultural Differences

A patient's culture and lay beliefs about illness and treatment can influence acceptance and adherence to ICS therapy. Patients raised in different countries or cultures may have health belief systems or health practices at variance with their health care provider. These divergent beliefs may influence asthma management through competing therapies, fear of the health care system, or distrust of prescribed therapies. This was demonstrated in a study by Pachter and Weller (65), which examined the relationship between the level of cultural integration of inner-city Puerto Rican families attending an asthma clinic and compliance with prescribed asthma therapy (65). They found that medication adherence (as measured by serum theophylline levels) was low overall, with only 15 out of 28 patients considered compliant (7–22 mg/mL). Patients' preferred language, asthma severity and chronicity, socioeconomic status, age, and gender were unrelated to adherence. However, they found that those families with a bicultural orientation were more adherent than those families with a traditional Puerto Rican orientation. Pachter and Weller suggest that integration into American culture may be an important variable to consider in achieving adherence to asthma therapy for ethnically and culturally diverse patients.

Cultural differences between patients and physicians do not require being born in another country. The culture of poverty may create different perspectives on how best to manage asthma. While income per se does not predict compliance, the multiple covariates of poverty and inner city living may make adherence with asthma self-management more difficult. Barriers to compliance related to low income can include inconsistent primary health care, inability to pay for asthma medications, lack of transportation, family dysfunction, and substance abuse (66–69).

## VI. Intervention Strategies to Improve Patient Adherence to ICS Therapy

### A. Doctor-Patient Communication

Regardless of the source or form of adherence problem experienced by a patient, the single most important strategy to address patient adherence is improved communication between the clinician and patient. Studies suggest that the usual clinic visit results in over half of all patients leaving their clinician's office uncertain of their doctor's instructions and their prescribed therapy (33). And even if a patient understands the specifics of the prescribed regimen, they often are uncertain of why they are taking particular medications. Research suggests that patients will be most likly to adhere with therapy that they believe is effective and feasible to carry out (70); thus the patient who is uncertain about what they should be doing, or why exactly it is important, is unlikely to be adherent with therapy. The quality of doctor-patient communication has been shown to be a contributing factor in levels of patient adherence (71,72). Despite the central importance of this exchange, the realities of limited time, competing demands, and cultural and educational differences frequently contribute to the communication gulf between doctors and patient. Dimatteo has suggested that "for the therapeutic relationship to be successful and for the physician's advice to improve the patient's life, doctors and patients must communicate and agree on treatment goals. Patients must be given the opportunity to assess the potential risks or drawbacks in a proposed treatment and its potential effect on the quality of their lives" (70). Table 5, drawn from the work of Roter and Hall (72a), provides a general medical model of open-ended communication between a clinician and a patient, with the goal of enhancing patient motivation for adherence. As discussed above, cultural differences based on ethnicity, race, educational level, socioeconomic status, and regional beliefs may influence the process of doctor-patient communication. Pachter and others (33,65,72,73) have noted that patient-held beliefs and behaviors about disease management may be discrepant with those of their physician. Patient beliefs about the value, role, and risks of ICS do not necessarily match those of the treating physician. These differences may result in patients failing to initiate therapy, using ICS erratically or discontining use when symptoms remit, and these prac-

**Table 5** Educating and Motivating Patients for Adherence

| Objective | Example |
|---|---|
| Assess the patient's knowledge, beliefs, and expectations about treatment so that misunderstandings and misinformation may be discussed. | "What do you know about your condition? What do you think would help?" |
| Clearly describe treatment plans and goals. Use simple, direct language. Avoid technical jargon. | "Insulin helps your body store the sugar that's in your blood. This will lower your blood glucose." |
| Discuss any concerns or reservations the patient may have about the plan, including the ability to adhere—physically, emotionally, and financially. | "Will you be able to follow this plan? Can you afford the medications?" |
| If the patient shows reluctance to commit to the plan, negotiate treatment options and modifications until you are both comfortable with a plan to which the patient can commit. | "What do you think would work better for you? Instead of taking this pill three times a day, could you take it once a day?" |
| Check understanding of the treatment plan and goals by asking the patient to repeat it back in his or her own words. | "Let me make sure you understand this. Tell me what you're going to do." |
| At every future visit, assess the patient's adherence. Instead of simply asking, "Any problems?" use an "information-intensive" approach. | "Which medications are you taking? What dose? How often? Have you had any side effects?" |
| Probe for nonadherence in a nonjudgmental and nonthreatening manner. If this is done, patients are often quite willing to acknowledge difficulties. | "Many people have trouble remembering to take their medication. Do you ever forget to take yours? Do you ever intentionally stop taking the medication?" |
| Whenever possible, provide feedback to the patient. | "You lost 5 pounds since the last time I saw you. That's great!" |

*Source*: Ref. 72a.

tices will not usually be revealed in the standard medical office visit. For some asthmatic patients and parents of asthmatic children the regular use of any medication is troubling, particularly when the asthma is not currently symptomatic. Intermittant use of ICS to treat asthma exacerbations may be considered appropriate asthma care by a patient with frequent periods of remission. Successful doctor-patient communication is dependent on a recognition and respect for patient belief systems and a willingness to both educate and work with a patient to identify a feasible, effective, and acceptable asthma therapy.

### B. Promotion of Patient Adherence

Several studies have suggested that asthma self-management programs may be one effective strategy for improving patient adherence (74). Asthma education

programs teach a range of information and skills related to control of symptoms through allergen control, self-monitoring, and use of an asthma action plan. In addition, education about the benefits of the consistent use of anti-inflammatory medication, even when not symptomatic, is an important learning objective for all asthma self-management programs. While all patients should be encouraged to participate in asthma self-management programs, many patients reject participation in such organized activities. For this reason, clinicians and their staff will need to integrate key asthma education concepts into regular clinic visits.

When a physician identifies a compliance problem, an attempt should be made to adapt the therapy to the patient's ability and lifestyle and preference. In many cases it is easier, and ultimately more effective, to change the asthma regimen than it is to change the patient behavior. For example, a spacer or alternative delivery system (such as the Turbuhaler) should be encouraged for any patient who fails to master the use of an MDI. ICS regimens should be customized for each patient, not only based on the disease profile, but also recognizing the patient behavior profile. While TID use of inhaled anti-inflammatory medications may be the optimal treatment on paper, for the patient who finds it difficult to adhere to this regimen a more "user-friendly" treatment plan should be considered. Poor compliance with even state-of-the-art therapy will not achieve effective asthma management. For this reason, alternative, viable treatment plans may need tailoring to the patient's preferences and practices. Reduced dosing, altered dose timing, "double-puffing," the use of long-acting forms of medication, and the use of non-ICS therapy should all be considered as appropriate and effective tailoring strategies.

### C. Monitoring Patient Adherence

The final component of improving patient adherence is ongoing monitoring of adherence. Physicians who rely on their "clinical judgment" to pick out nonadherent patients make one of the most common mistakes in assessing adherence. In fact, research has consistently found that physicians are poor judges of patient compliance, with accuracy rates often not much better than chance (49). Many of the sociodemographic factors that physicians and others presume are associated with compliance, such as level of education, race, income or gender, are actually poor predictors of adherence. The most common reason that clinicians make inaccurate assessments of ICS adherence, however, is the simple failure to explicitly ask the patient about their patterns of ICS use. Clinicians often do not directly discuss ICS adherence issues with patients unless their "clinical judgment" leads them to believe the patient is grossly noncompliant. And when a health care provider does question a patient about ICS use patterns, the communication strategy used may discourage patients from candidly expressing difficulties and concerns about their therapy. Queries such as "You aren't having any problems taking your inhaler are you?" or "Are you taking your inhaler the way we discussed?" may discourage

open disclosure. Use of closed-ended questions such as "Are you using your inhalers?" will tend to elicit limited yes/no responses rather than useful information about erratic adherence patterns. Research suggests that the most effective communication between doctors and patients about medication adherence requires asking questions that explore the complexity of appropriate medication adherence over time (75). Effective assessment of ICS adherence should include questions about patient beliefs about the efficacy and risks of ICS therapy, past patterns of adherence with ICS or other preventive therapies, patient criteria (as opposed to provider expectations) for adequate adherence, and discussion of any financial, physical, psychological, or social barriers to ICS adherence.

## VII. Summary and Recommendations

The benefits of appropriate adherence to ICS therapy is reflected in research that has demonstrated that ICS use is associated with improved control of asthma symptoms, decreased risk of hospitalization, and decreased risk of fatal and near-fatal asthma. Unfortunately, the potential benefits of ICS therapy are adversely impacted for many asthma patients by the frequently poor levels of ICS adherence. Noncompliance with ICS therapy can result from forgetting, misunderstanding, or deliberate alterations in prescribed regimens. The best predictors of ICS adherence include patient beliefs and characteristics of the regimen. Patients often have concerns about asthma or its treatment that they may not articulate without effective doctor-patient communication. The clinician's goals for this exchange should be to discover the patient's knowledge, beliefs, and feelings about asthma and ICS therapy, to negotiate a feasible and effective regimen that is acceptable to the patient, to assess adherence with therapy, and to reinforce and encourage appropriate asthma management.

Finally, by linking adherence with ICS therapy to the patient's own goals (e.g., sleeping through the night or participating in sports), rather than abstract medical indices such as $FEV_1$, the clinician can make the value of adherence more immediate and salient to the patient. With ongoing education and reinforcement, the clinician can help patients learn that through self-management they can control their asthma and lead relatively unrestricted lives.

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## Discussion

**Dr. Rohdewald:** Are you able to compare compliance in case of ICS with compliance to other prescriptional drugs taken on a regular basis?

**Dr. Rand:** Research suggests that average patient adherence with ICS therapy is 50% of prescribed dose or less. This is very comparable to rates of adherence seen for other forms of chronic disease therapy, such as hypertension and diabetes. Patient adherence tends to be worst for prophylactic regimens.

**Dr. Boulet:** You mentioned that education is important to increase compliance to therapy and rightfully what type of educational intervention is critical. We found that the beliefs of the educators and their ability to motivate patients to use their medication correctly is a major determinant of compliance. We should also promote "intelligent" compliance so that they can learn how to reduce or increase their corticosteroid dose according to their action plan and asthma control criteria. Finally, in our study (Chest 1998; 113:587), a large number of patient's concerns about asthma drugs had not been addressed, and when they were told about those, the majority were reassured and mentioned that they would be more inclined to follow physician instructions.

**Dr. O'Byrne:** Do once-daily drug regimens improve adherence, and are combinations inhalers likely to improve adherence?

**Dr. Rand:** While research clearly suggests an adherence advantage for therapies no more frequent than twice-daily dosing, the data is equivocal on the superiority of once-a-day dosing over twice-a-day dosing. Several studies have reported comparable rates of adherence for twice-daily therapy compared to once-daily therapy (1–4). Apart from adherence considerations, once-daily asthma therapy appears to be preferable for most patients. Venables et al. (5) examined patient preference in asthma therapy and found that 61% of patients expressed preference for once-a-day treatment, 12% preferred twice-a-day treatment, and 27% expressed no preference. While preference may not necessarily lead to improved compliance, it may well reduce the burden of therapy and enhance patients' quality of life.

**Dr. Talton:** Is there a difference in compliance/adherence when a patient knows he or she is being monitored?

**Dr. Rand:** Our research suggests that when patients in clinical trials know that they are being monitored with electronic inhaler devices, deliberate inhaler emptying prior to follow-visits (i.e., "dumping") is eliminated. In addition, when known monitoring is combined with feedback, there appears to be a beneficial effect on adherence rates (6). Known monitoring does not appear to be sufficient, however, to significantly improve overall rates of adherence.

**Prof. Dolovich:** What is the role of nontraditional herbal medicines in patient nonadherence to inhaled medication?

**Dr. Rand:** No research had been reported on the effect of use of alternative therapies (e.g., herbal remedies) on adherence to asthma therapy. Our studies suggest, however, that parents of children with asthma commonly use "home remedies," such as teas, coffees, and over-the-counter drugs (e.g., decongestants, cough medicines), along with prescribed asthma medications (7).

**Prof. Dolovich:** Patients can trigger some electronic monitors without actually inhaling the medication. Are there any monitors that are foolproof?

**Dr. Rand:** There are no foolproof inhaler monitors. The current MDILog device made by Medtrac Inc. records not only the discharge of the inhaler, but also shaking of the inhaler and the actual inhalation. While it is probably possible to fool any electronic device, in our experience patients who are nonadherent are unwilling to go to the daily effort to deceive monitoring devices. Such efforts take at least as much time as adhering to therapy.

**Dr. Schleimer:** In one slide, it was stated that 18% of patients who chose not to use ICS did so because of the side effects. Was this due to actual side effects experienced or fear of side effects? ICS are clearly very safe medications and the possibility of offering low-dose preparations over the counter should be considered. From a compliance perspective, what would be the anticipated influence of this type of change?

**Dr. Rand:** Patient beliefs about the potential side effects of medications are as powerful in effecting adherence as actual side effects. As the data from the Boulet et al. (8) study indicated, patient misperceptions about the role of side effects associated with ICS therapy are widespread and strongly argue for improved patient education for all patients prescribed ICS therapy.

**Dr. Denburg:** Are there country-by-country differences in adherence? If compliance is a problem even in clinical trials, how does this impact on guidelines for asthma management and on our estimates of safety of IS?

**Dr. Rand:** I'm not aware of any published studies that have compared adherence rates across different countries. In general, the adherence literature suggests that poor adherence with ICS therapy is a common problem internationally; however, it is possible that there are country-by-country differences. Patient beliefs about the seriousness of asthma, the risks of ICS therapy, and the role of alternative forms of therapy (e.g., herbal healers) may play a role in cross-cultural differences in adherence.

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# Part Seven

## FUTURE CHALLENGES

DRUG DEVELOPMENT

# 21

## Prospects for Developing Inhaled Steroids with Extrahepatic Metabolism

"Soft Steroids"

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### I. Introduction

As described in other chapters in this volume (see Chaps. 9 and 10), the critical inactivation of the currently used inhaled steroids (IS) occurs mainly in the liver and involves oxidative metabolism by cytochrome P450 3A enzymes. However, even when first-pass hepatic inactivation is very efficient [approaching 99% for fluticasone propionate (FP) (see Chap. 10) and mometasone furoate (MF) 1)], the large airways/lung absorbed fraction has a wide body distribution (2), before undergoing its final hepatic inactivation (see flow scheme in Fig. 1). The more lipophilic an IS is, the larger is its volume of distribution ($V_D$) and the longer its plasma half-life (see Chap. 10) (2,3). Lipophilicity also enhances the receptor affinity (2), and together these properties contribute to systemic activity of potent IS, which, depending on dose and individual sensitivity, in rare cases may reach adverse levels (see Chaps. 3, 15, and 19). Awareness of the systemic bioavailability of IS maintains steroidophobia among many doctors and patients, limiting the use of this key therapy (see Chap. 20) (4). Development of novel IS, with a profile that further limits their systemic potential, would therefore encourage a broader and earlier use of IS in mild asthma and rhinitis.

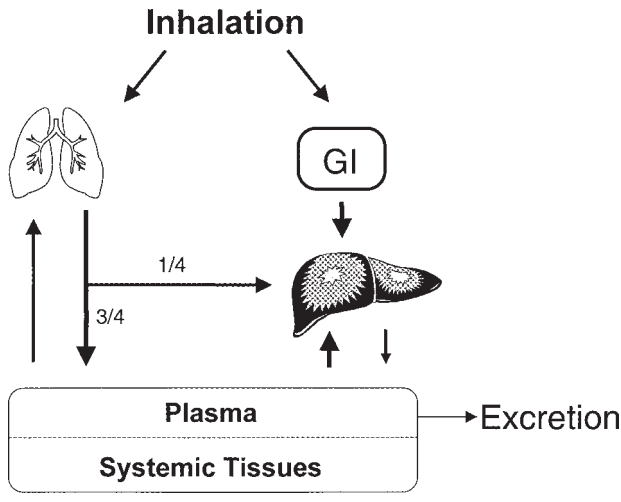



**Figure 1** Overview of the systemic disposition of inhaled steroids. (Adapted from Ref. 2.)

A more acceptable profile might be achieved if steroid inactivation is initiated already during the systemic uptake and disposition (see Fig 1). This requires that mechanisms in addition to CYP450-mediated hepatic inactivation would have to be exploited, for example, hydrolytic inactivation reactions. However, because such reactions are widespread, this introduces a risk of steroid inactivation also within airways/lung, leading to reduced therapeutic efficacy. While introducing higher steroid concentration at the administration site could to some extent compensate for that risk, an even better approach would be to exploit metabolic differences between the airways/lung target and the rest of the body. Application of such approaches to improve the topical selectivity of drugs has been termed "extrahepatic" or "soft drug" (soft steroid) design (5,6). Current IS are not true soft drugs, as they have to reach the liver for inactivation. At the application site a soft steroid is active by itself and is then predictably inactivated in vivo during its systemic distribution, preferably in a single metabolic step (see Chap. 22).

A key issue for the profile of soft steroids is whether they should be completely free of systemic steroidal activity or not. The systemic spillover of current liver-inactivated IS results in plasma concentrations of 0.1–2 nmol/L lasting for some hours (7–9). These levels are similar to the in vitro $K_D$ (50% receptor saturation) values for these compounds (2,10). This suggests that even such low levels of circulating steroid may exert some anti-inflammatory and immunosuppressive activity in the bone marrow, blood compartments, and elsewhere. As studied in vitro, modulation of growth factor, cytokine, and chemokine production/action is possible at these low steroid concentrations (see Chap. 11). However, it has been

difficult to document a therapeutic contribution by such low plasma levels of IS in vivo. Toogood et al. (11) and Lawrence et al. (12) compared the antiasthmatic efficacy of an IS regimen with that of a high oral dosing, so that both regimens gave similar area under the curve (AUC) of plasma steroid level. The clinical outcome was just a marginal efficacy by the oral route, suggesting a low therapeutic contribution by such low plasma steroid concentrations. One weakness of the study design is that inhalation and oral dosing result in different shapes of plasma level curve, even though the overall AUC is the same. Peak plasma levels are attained rapidly after inhalation, compared to low but sustained levels after oral intake. If induction of positive systemic actions requires a steroid peak in plasma (surpassing some threshold), then the relevance of the above comparitive design is unclear. A final answer as to whether soft steroids should have a systemic bioavailability or not will probably come first from clinical studies with such appropriate drugs, and the answer may well vary depending on disease severity. For example, in mild asthma a compound with a strictly topical selectivity may be effective enough, while some systemic activity may be indispensable in more severe forms to affect the systemic components of the disease.

One of the most successful approaches for the development of soft drugs (5,6) has been to start their synthesis from inactive metabolites (Fig. 2). For example, one route of cortisol metabolism is the stepwise oxidation of the 17β-hydroxyacetone side chain, forming the inactive 17β-ketocarboxylic and 17β-carboxylic acids (13). However, when such acids are properly esterified, they are transformed to active glucocorticoids. If such esters are administered to an inflamed tissue, these compounds can trigger local glucocorticoid receptors and

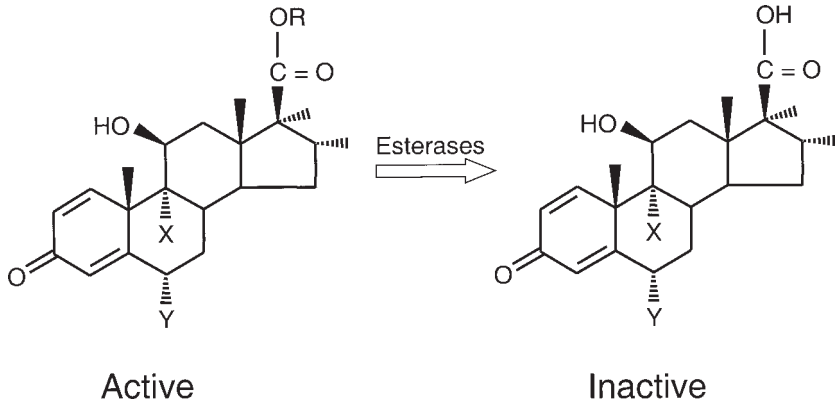



**Figure 2** The principle to design soft steroids from inactive metabolites. R is the substituent (normally acyl) that adds glucocorticoid activity to the soft steroid. Esterases will in vivo hydrolyze the active steroid to its inactive metabolite.

then subsequently undergo hydrolysis via blood and tissue esterases back to their corresponding inactive acids. Starting from a 17β-ketocarboxylic acid metabolite of the systemically active steroid fluocortolone, Laurent et al. designed the topical steroid fluocortin butyl ester (FCB), primarily intended for topical skin use (14). FCB is a rather weak steroid with a several fold lower glucocorticoid receptor affinity and topical anti-inflammatory potency compared to dexamethasone (15). In vitro, FCB is metabolized by human blood to its corresponding, inactive 17β-ketocarboxylic acid (16). This occurs also in vivo but at a slow rate, as reflected by a plasma $t_{1/2}$ of 2.5 hours after iv injection of FCB to man (16), which is not shorter than for the liver-inactivated budesonide (see Chap. 10). When given by inhalation as a dry powder formulation, FCB ameliorated allergic rhinitis (17) at daily doses of 2–8 mg (divided into two to four daily applications). Hitherto, however, there are no reports as to the efficacy of FCB when administered once daily, in contrast to proven once-daily efficacy of the currently used IS. Furthermore, in bronchial provocation tests, FCB at a dose of 8 mg daily (divided into four daily inhalations) did not protect against bronchial obstruction, as well as a 10-fold lower dose of BDP did (18).

Butixocort propionate (BXP) is a steroid that can undergo some extrahepatic metabolism (19). When the 21-thiol ester BXP is hydrolyzed, it becomes a substrate for the enzyme S-methyl transferase (located in blood and tissues), which can convert the steroid 21-thiol to a S-methyl ether. This partial deactivation of BXP, combined with its only moderate glucocorticoid potency (20), may account for its reported lack of systemic activity after inhalation (21). The antiasthmatic efficacy of BXP, as well as of FCB, proved to be too low for commercial introduction, but it was unclear whether that depended mainly on their low receptor affinity or on their local inactivation. Subsequent soft steroid development has aimed at achieving the following: (1) to enhance the receptor affinity to a level equivalent to the currently used IS, and test whether that can better compensate for the risk of a rapid local inactivation (here examplified with an Astra project); (2) to combine an enhanced receptor affinity with a more moderate rate of hydrolytic inactivation (here examplified with loteprednol etabonate (see Chap. 22); and (3) to achieve a higher metabolic stability specifically within the airways/lung, compared to blood and peripheral tissues (here examplified with a recent GlaxoWellcome project). These projects have focused on search for structures that optimize interactions with both the glucocorticoid receptor and the active site of inactivating esterases. The glucocorticoid receptor is normally very strict in its acceptance of proper ligands, but the soft steroid projects have shown that the receptor may accept steroids with bulky substituents in the 17β-position. Esterases are still rather poorly characterized, regarding both their overlapping substrate specificities and their tissue and blood distribution (22,23), making it of little use to perform structure-activity studies on individual esterases. For these reasons the soft steroid projects have been based on empirical, rather than on rational drug design.

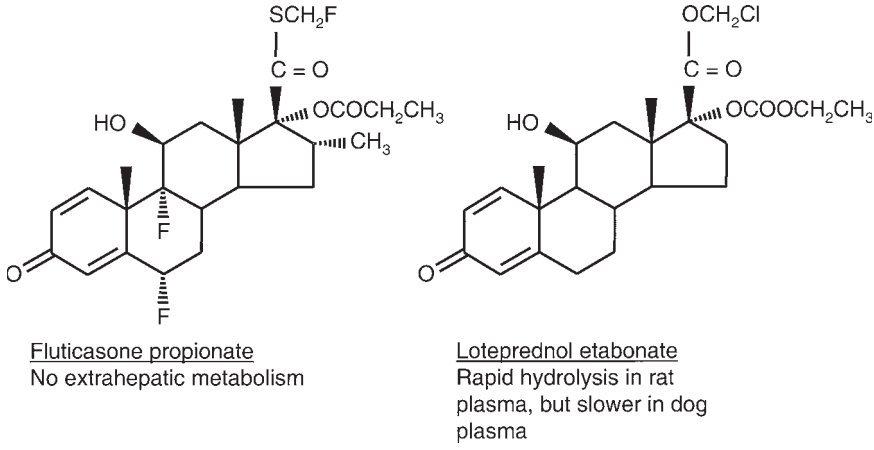


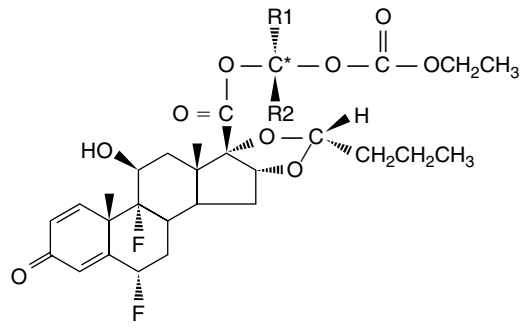

**Figure 3** The 17β-thiocarboxylic acid ester fluticasone propionate and the 17β-carboxylic acid ester loteprednol etabonate.

The main chemical approaches for new soft steroids have been based on synthesis of 17β-carboxylic and 17β-thiocarboxylic acid esters or these acids with other esterase-sensitive substituents. The R substituent in these structures (Fig. 2) was varied to enhance both the glucocorticoid receptor affinity and the rate of hydrolytic biotransformation. However, not all 17β-carboxylic or 17β-thiocarboxylic acid esters are hydrolyzed by plasma or tissue esterases. Fluticasone propionate (FP), for example, is a 17β-thiocarboxylic acid ester (Fig. 3) that is fully stable in blood and tissue, but undergoes a rapid hepatic oxidative metabolism by CYP450 3A, generating the inactive 17β-thiocarboxylic acid (9,24a). One of the pioneers of the soft drug concept, Nicholas Bodor, designed loteprednol etabonate (LE) for local use, and this drug has been approved as a safer steroid for topical ophthalmic therapy. LE has a high receptor affinity (25) and a rapid inactivation rate in rat plasma but less so in dog plasma (26,27). Its pharmacological and clinical documentation is described in Chapter 23.

## II. Astra Soft Steroid Project

In 1981 Astra started a soft drug project based upon easily hydrolyzed steroid 17β-carboxylic acid esters. This type of structure was selected because both the hydrolysis rate to the inactive 17β-carboxylic acid metabolite and the level of glucocorticoid receptor affinity were highly influenced by different substituents at the chiral center (asterisk-marked carbon atom in the structural formula of Table 1), as well as by changes to the terminal alkyl of the ester group. Fluoro substituents introduced into 6α- and 9α-positions of 16α, 17α-acetals (U.S. patent 4,950,659)

**Table 1** Structural Basis of Astra's Soft Drug Project Based on 17β-Carbonate Esters—Importance of Methyl Substitution and the Stereochemistry at the Ester Chiral Center




| R1 | R2 | RBA (Bud=1) |
|---|---|---|
| H | H | 0.05 |
| $CH_3$ | $CH_3$ | 0.12 |
| $CH_3$ | H | 0.25 |
| H | $CH_3$ | 1.03 itrocinonide |

Receptor affinity (RBA, determined in rat thymus cytosol) is given in relation to budesonide = 1. Itrocinonide (D5159) was selected as the candidate drug.

and 17α-esters (U.S. patent 4,804,656) increased their glucocorticoid receptor affinity.

The structure-activity relationship of a series of carbonate esters (Table 1) showed that their receptor affinities (studied in vitro in subcellular homogenates) varied significantly according to the substituents and the stereochemistry at the 17β-ester group chiral center. With the selected compound, itrocinonide (D5159), a receptor affinity was achieved similar to budesonide, thereby fulfilling one of the project aims. Thus, even if itrocinonide was broken down within the target tissue, it might initially mediate a strong receptor trigger, since the formation and translocation of the steroid receptor complex occur within a few minutes of agonist addition. It was thought that the steroid located deep within the conformationally changed receptor might possibly be metabolically more stable over the triggered receptor cycle than is the case for nonliganded steroid. Support for a "hit-and-run–like activity" hypothesis for glucocorticoids (i.e., maintenance of steroid activity without the continuous presence of steroid in medium) was at that time provided by Alan Munck's group using steroid-pulsed thymocytes (28). They showed that although hydrocortisone could be washed away from the thymocyte incubation medium within minutes, its ability to modulate mRNA and protein synthesis with

subsequent functional activities persisted for some hours (28). One aim of the Astra soft steroid project was to see whether a hit-and-run–like trigger would be applicable also for inducing anti-inflammatory activity, and, if so, could be exploited for soft steroid design.

While methyl substitution at the chiral center of the 17β-carbonate ester group raised the glucocorticoid receptor affinity (Table 1), it only marginally affected the rate of the hydrolytic breakdown. On the other hand, variation of the terminal alkyl of the ester group significantly influenced the rate of formation of the inactive 17β-carboxylic acid metabolite. The two diastereomers created by introduction of mono methyl substitution at the ester chiral carbon differed four-fold in receptor affinity (Table 1), while both had similar high hydrolytic rate. Itrocinonide was selected as a tool and candidate drug from a series of derivatives (U.S. patent 4,950,659), as it combined a high receptor affinity with a rapid rate of hydrolysis. The pathway resulting in the selection and evaluation of itrocinonide was as follows: measurement of receptor affinity in rat thymocyte cytosol, hydrolysis rate in rat and human lung tissue and plasma in vitro, pharmacological profiling in animal airways/lung inflammation models, systemic effects in volunteers, and finally antiasthmatic efficacy of a dry powder formulation in patients.

Itrocinonide had a similar low water solubility when compared with beclomethasone dipropionate and FP (~0.1 μg/mL). Preclinical experiments showed that the bioavailability of intratracheally instilled itrocinonide was markedly improved by including additives like Tween or lactose to the formulations. These were subsequently used in pharmacological experiments with this mode of administration.

Itrocinonide was hydrolyzed in all animal and human tissues tested. When incubated with human blood at 37°C, its half-life was 30 minutes, and in human lung homogenates its breakdown was even faster. The breakdown was enzymatic, as in vitro addition of the nonspecific esterase inhibitor PMSF largely blocked formation of the corresponding 17β-carboxylic acid metabolite (referred to in Figs. 4 and 6 as **I**). The rapid hydrolysis of itrocinonide significantly affected its in vivo pharmacokinetics (Fig. 4). When itrocinonide was intratracheally instilled into rats at a dose of 0.6 mg/kg, even plasma samples obtained at early time points contained principally the hydrolytic metabolite **I**), suggesting a very effective first-pass inactivation before or during systemic uptake/distribution.

### A. Topical Efficacy/Selectivity in Animal Models

Supporting the hit-and-run hypothesis of the project, the rapid inactivation of itrocinonide was still compatible with topical anti-inflammatory efficacy in animal airways/lung models. Acute intratracheal instillation of itrocinonide inhibited both the immediate and late allergic reactions in guinea pigs and the Sephadex-

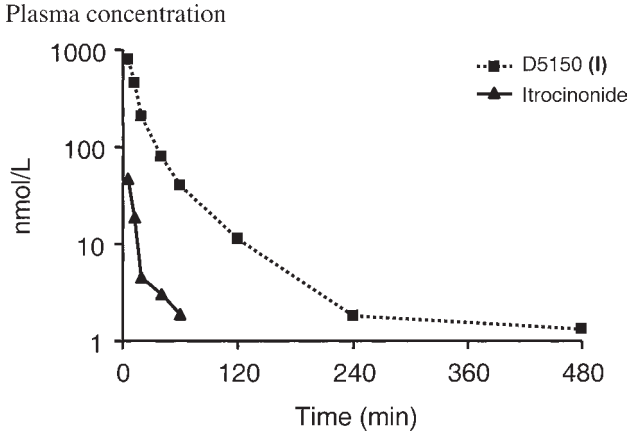



**Figure 4** Plasma concentration (nmol/L) of itrocinonide and its corresponding acid metabolite D5150 (I) after intratracheal instillation in rats.

induced pulmonary edema in the rat. The doses required for inhibition varied with the steroid sensitivity of the species, with guinea pigs needing higher steroid doses (1.6–5 mg/kg) than rats (0.3 mg/kg). Within these species itrocinonide was just a couple of times less potent than budesonide. However, more importantly, the ratio between its anti-inflammatory efficacy in airways/lung and its systemic steroid activity (i.e., in the rat thymus involution and in guinea pigs plasma cortisol depression) was much better than the corresponding ratio for budesonide. In sheep, acute inhalation of an aerosol formulation of itrocinonide (2 mg/animal) was shown to inhibit the development of the late allergic reaction (W. Abraham et al., unpublished).

The topical selectivity of itrocinonide was studied more closely in the Sephadex model. In this model, alveolitis and bronchiolitis is induced by intratracheal instillation of Sephadex beads (consisting of cross-linked dextran to which rats have an innate hypersensitivity). The Sephadex model is characterized by a rich, interstitial and intraluminal infiltration of various inflammatory cells, including a high proportion of eosinophils, and the formation of an interstitial edema raising lung weight and impairing respiration (29). The inflammation is glucocorticoid-sensitive, but liver-inactivated IS (e.g., budesonide and fluticasone propionate) lack topical selectivity in this model as their antiedematous efficacy is paralleled by reduced thymus, spleen, and body weights (30). In this model, itrocinonide achieved good topical efficacy with minimal systemic actions when administered as a single steroid instillation in the acute (one-day) test. In the subacute test (where Sephadex was instilled and steroid was inhaled the first day, followed by

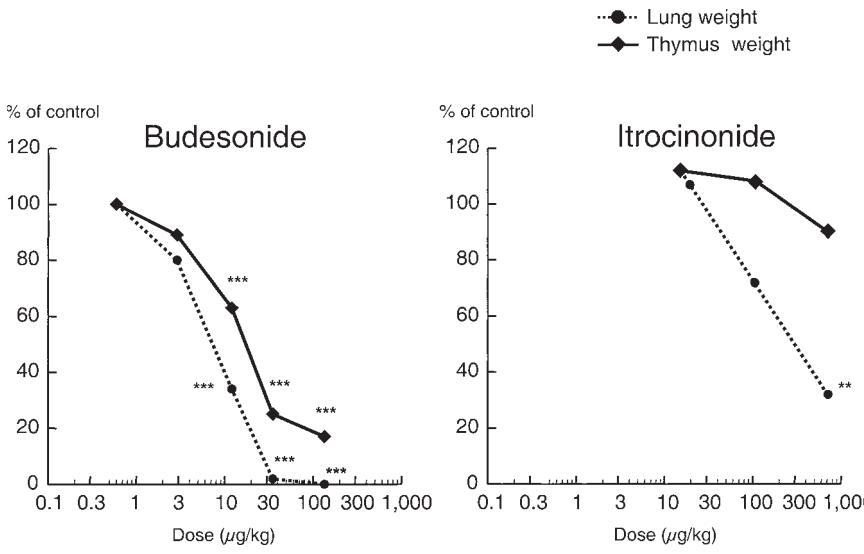



**Figure 5** Effect of repeated inhalations of itrocinonide and budesonide on lung weight gain (interstitial pulmonary edema) and thymus weight in Sephadex-treated rats. The steroids were inhaled for 10 min, once daily over 4 days. Over the inhalation period the rats were anesthetized and intubated with a tracheal tube. Micronized steroid powder was delivered via a Wright Dust Feeder. The doses relate to estimations of the airways/lung-deposited fraction. N > 8 rats/group. Significance values are calculated against nondrug–treated Sephadex controls (**$p < 0.01$; ***$p \leq 001$).

once-daily steroid inhalation for 3 further days) itrocinonide was also effective, achieving a 60% reduction of edema (Fig. 5) with minimal systemic activity (reduction of thymus weight). In contrast, inhalation of budesonide at an equipotent antiedema dose was accompanied by a 40% reduction ($p < 0.001$) of thymus weight (Fig. 5).

These in vivo results showed that: (1) topical application of a soft steroid gives a much better pulmonary selectivity than for liver-inactivated glucocorticoids, (2) selectivity can be achieved even when hydrolysis starts within the target organ, thereby supporting a hit-and-run hypothesis for glucocorticoid anti-inflammatory activity, and (3) when compared to budesonide, itrocinonide had a much lower potency by inhalation than by intratracheal instillation. The lower potency of inhaled itrocinonide may be due to impaired absorption (the inhalation formulation did not contain solubilizing additives) and/or to the wider lung distribution by inhalation, resulting in a generally shortened local dwell time of intact itrocinonide.

### B. Results in Human Testing

Itrocinonide was delivered to humans as a dry powder formulation by Turbuhaler. Its impressive systemic tolerance was confirmed in volunteers and asthmatics. Its iv kinetics revealed a plasma clearance of 4.4 L/min (threefold greater than budesonide) and a plasma $t_{1/2}$ of approximately 30 minutes (1/5 that of budesonide and FCB), thereby confirming a rapid, extrahepatic metabolism. Only very low levels of intact itrocinonide were detected in the plasma after inhalation of a 7.5 mg dose (Fig. 6), while higher levels of the hydrolytic metabolite (**I**) were present even at early time points. Furthermore, the same rapid inactivation of itrocinonide occurred in asthmatics as in healthy volunteers and remained constant after dosing by inhalation for one week. The profile of itrocinonide contrasts to that of inhaled, liver-inactivated steroids where early plasma samples contain mainly levels of intact steroid, due to their better systemic bioavailability from airways/lung (3,7–9). In accordance with its low plasma level, itrocinonide lacked measurable systemic glucocorticoid activity. In a crossover study (Fig. 7) 12 volunteers, who had received inhaled itrocinonide (20, 40, or 80 mg) at 10:00 p.m. the night before showed a normal morning plasma cortisol rise and overnight urinary cortisol, compared with the reduced readouts after taking budesonide (3.2 mg). A better systemic tolerance was also observed for itrocinonide (8 mg) following b.i.d. inhalation over 6 days, compared with budesonide (0.8 mg).

The therapeutic efficacy of dry powder (Turbuhaler) formulations of itrocinonide was tested in four trials in patients with asthma and in one trial in birch pollen–induced seasonal rhinitis. In a study of steroid-naive asthmatics, three

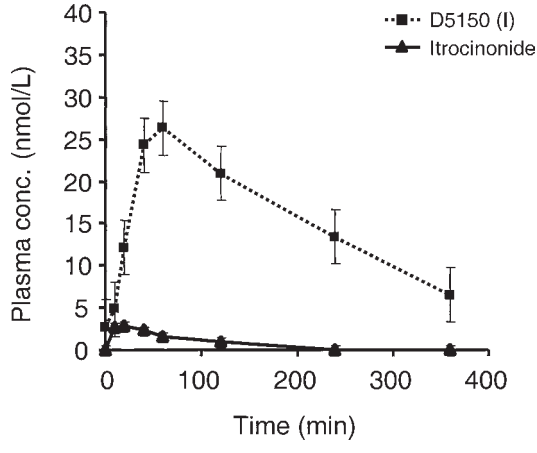



**Figure 6** Plasma concentrations of itrocinonide and its inactive acid D5150 (I) after inhalation of a 7.5 mg Turbuhaler formulation. Mean ± SEM of three volunteers.

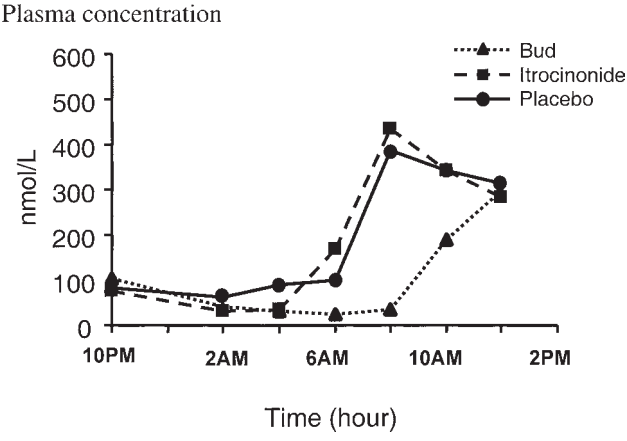



**Figure 7** Plasma cortisol concentrations after acute inhalation of 40 mg itrocinonide or 3.2 mg budesonide, both in Turbuhaler formulation, at 10 p.m. Mean of 12 volunteers.

parallel groups (>40 patients/group) were treated over a one-month period with either placebo or itrocinonide 0.5 or 2 mg b.i.d. Compared to run in, morning peak flow rates deteriorated 14 L/min in the placebo group and 5 L/min in patients taking low-dose itrocinonide (0.5 mg b.i.d.), while the peak flow rate improved by 7 L/min over the run in ($p < 0.007$, compared to the change in the placebo group) in patients taking the higher dose (2 mg b.i.d.) of itrocinonide. In a study with steroid-dependent asthmatics (requiring 0.2–1 mg conventional IS), itrocinonide (4 mg b.i.d.) was compared to placebo as substitution for the earlier steroid treatment. Following 2 weeks of treatment, itrocinonide was more effective than placebo in preventing the decline in $FEV_1$ ($p < 0.06$ between the groups). Inhalation of itrocinonide (4 mg b.i.d.) for one week afforded a slight, but nonsignificant, protective effect on exercise-provoked asthma, while protection by budesonide (0.4 mg b.i.d.) was statistically significant. In the allergen challenge test, a single inhalation of itrocinonide (8 mg, 10 min before provocation) very weakly reduced the late obstruction (31). In a rhinitis study, a total of 25 patients/group received either placebo or itrocinonide (4 mg b.i.d.) for one month during the birch pollen season. Itrocinonide improved the overall symptom score ($p < 0.01$), reduced $H_1$-antagonist consumption ($p < 0.03$) and improved the other subscores, with the exception of the "blocked nose" symptom.

Thus, itrocinonide did exert some antiasthmatic and antirhinitic efficacy, which must be strictly topical since the substance lacked measurable systemic activity. Even though itrocinonide had a much higher receptor affinity than its forerunner, FCB, and an affinity equivalent to budesonide, this advantage was not sufficient to compete with the efficacy of current IS. One pharmacological limita-

tion of these clinical studies was that the steroid was inhaled as a dry powder formulation, which due to device restrictions lacked solubilizing additives. Because such additives improved the airways/lung bioavailability of itrocinonide in preclinical models, the bioavailability of the human dry powder formulation may have been compromised. We do not know whether a more prolonged local pool of itrocinonide in tissue would have been attained, resulting in a better efficacy, if the bioavailability of the dry powder formulation could have been optimized.

By raising the dose to 7.5 mg (Fig. 6) it was possible to reach a similar initial plasma peak (approximately 2 nmol/L) for intact itrocinonide that was attained for intact budesonide after a 0.5 mg dose (7). Because even this dose had poor clinical efficacy, this suggests that for the formulation used, the local inactivation of itrocinonide in airways/lung tissue was too rapid for clinical efficay. This is supported by in vitro studies showing a short half-life for itrocinonide in human lung homogenate.

## III. GlaxoWellcome Soft Steroid Project

In a series of patent applications (WO 97/24365, WO 97/24367, and WO 97/24368) and in one short communication (32) GlaxoWellcome (GW) described a soft drug project based on compounds that are very rapidly inactivated by human plasma, but not by the S9 fraction of human lung tissue. Their chemical entities consist of γ-butyrolactone derivatives of pregnanes and androstanes. The γ-butyrolactone ring can be opened by specific plasma esterases, yielding a hydrophilic metabolite with low glucocorticoid receptor affinity. Figure 8 shows the structure of one pre-

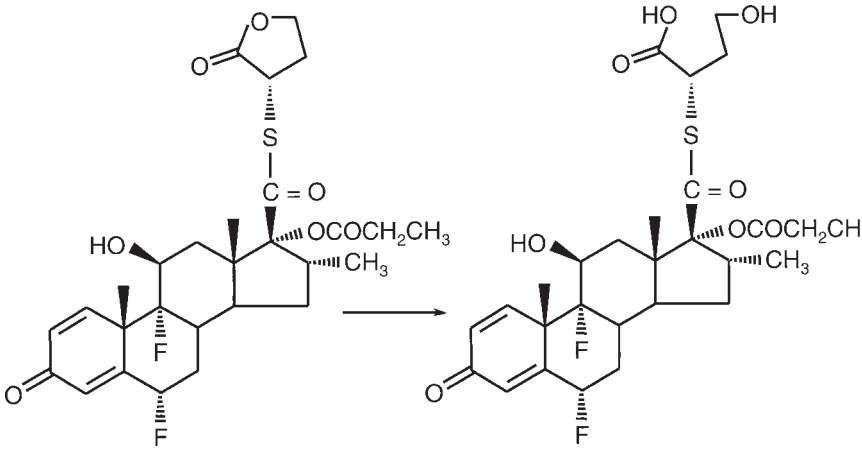



**Figure 8** Structure and hypothetical metabolism of one preferred γ-butyrolactone derivative, exemplified in WO 97/24365.

ferred compound (from WO 97/24365) and its hypothetical metabolism. The metabolising enzyme (32) is claimed to be paraoxonase EC 3.1.8.1 (WO 99/01467), an esterase having a much lower activity in lung tissue than in plasma (33,34). The better stability of γ-butyrolactone derivatives in lung tissue depends on the low paraoxonase activity there and their resistance to conventional (PCMB-, PMCF-eserine–sensitive) esterases (32).

The compounds selected by GW have high glucocorticoid receptor affinity, reaching that of current IS, and a good topical efficacy in animal models (WO 97/24365, WO 97/24367, WO 97/24368). The $t_{1/2}$ in human plasma in vitro at 37°C is just one or a few minutes (32). For the compound examplified in Figure 8, the claimed higher metabolic stability in lung tissue was confirmed as its $t_{1/2}$ was at least 10-fold higher in rat and human lung tissue than in corresponding plasma (data on file, AstraZeneca). When the in vivo profile of this compound was compared to itrocinonide in the acute Sephadex test following intratracheal steroid administration, the γ-butyrolactone derivative had a somewhat higher topical anti-edema potency, but otherwise the two soft drugs reached the same high topical selectivity (data on file, AstraZeneca).

## IV. Current Status of Soft Steroids

The clinical efficacy of the soft steroid approach based on a rapid and nondifferentiated hydrolytic breakdown has hitherto been disappointing. The clinical results with itrocinonide do not support the hypothesis that effective topical anti-inflammatory efficacy can be achieved through a short pulse of a potent, but biolabile steroid. It is likely that for these compounds insufficient levels of intact steroid will be maintained at their site of action to trigger corticosteroid receptors after their initial turnover. This kinetic profile contrasts with current IS, which have a good metabolic stability during their local tissue binding (8,35–37). In addition, for budesonide special circumstances prevail, since its reversible esterification adds an extra tissue pool (38) contributing to its once-daily efficacy (39).

Itrocinonide was found to possess a weak antiasthmatic and antirhinitic efficacy, which might correspond to a budesonide dose in a range of 50–100 μg. Because no systemic adverse actions are known for budesonide at such low doses (see Chaps. 3, 15, and 19), it is not possible to conclude whether conventional IS at these low levels or the use of soft steroids will achieve the best therapeutic ratio. However, soft steroids might still compete therapeutically with weak antiasthmatic drugs like leukotriene antagonists and cromones. Alternatively, by choosing steroid esters with a slower hydrolytic rate, it may be possible to reach some selectivity preference over current IS. The extended clinical evaluation of loteprednol etabonate will tell whether that drug at respirable doses can attain soft drug characteristics also for respiratory disorders, where the kinetical conditions differ from those in the eye.

In the GlaxoWellcome soft steroid project a differential metabolic approach is used in that these drugs can have a longer survival time in airways/lung tissue (outside blood vessels) than in plasma. However, more information is required about the paraoxonase content of the interstitial fluid, and whether that may affect drug stability around target cells. The clinical and kinetic outcomes of that project may help answer key issues about the future prospects of this novel approach. Open questions are, what level of efficacy can best be reached and is there a requirement for some systemic activity in order to attain the full steroidal efficacy, as proposed in this volume (see Chap. 11). If the latter appears true, the future of soft steroids will be restricted to milder forms of asthma.

## Addendum in Proof

A poster was presented at ATS 2000 on preclinical properties of the γ-butyrolactone soft steroid GW 215864X, reporting a lower topical potency in the rat lung, than would be awaited from its high receptor affinity and potency in cellular assays (40). According to Adis R&D Insight 2000, Accession numbers 10129 and 10130, the development of GW 250495 and GW 215864 (covered within above GW patent applications) has been discontinued for the asthma indication. This suggests that even with this interesting approach it is difficult to maintain sufficient hydrolytic stability in the inflamed airway wall, where there may be a higher rate of plasma exudation.

## Acknowledgments

Our thanks to Dr. Magnus Dahlbäck for inhalation study in rats, to Per Strandberg for preclinical kinetic analyses, and to Drs. Göran Eriksson, Stefan V. Ohlson, and Margareta Grind and external clinicians for coordination and accomplishment of clinical trials with itrocinonide.

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## Discussion

### Chapter 21

**Dr. O'Byrne:** There were at least two other "soft" steroids that were shown not to be effective in asthmatics. Were all of these esterase-sensitive steroids also?

**Dr. Brattsand:** Fluocortin butylester is broken down by tissue and plasma esterases. Butixocort propionate is partially deactivated by S-methyl transferases in blood and tissue. The metabolism of tipridane is too poorly studied to draw clear conclusions about its metabolism in lung and blood.

**Dr. Schleimer:** We still struggle with the question of whether there are systemic effects of ICS which contribute to clinical efficacy. In Judah Denburg's and Paul O'Byrne's studies, it is possible that the bone marrow effects are indirect and exerted in the lung. These drugs could be useful to answer this question. Are you aware of any studies?

**Dr. Brattsand:** No, not yet at the bone marrow level.

**Dr. O'Byrne:** We have previously shown that two of the "soft" steroids were ineffective in protecting against allergen-induced late responses, which is very sensitive to treatment with conventional inhaled glucocorticoids.

**Dr. Denburg:** Evidence that a beneficial systemic effect may be important in designing new IS comes from the clinical observation that treatment of one compartment of the airways (upper or lower) enhances management of the other in patients with asthma and/or rhinitis (Greiff et al., ERJ 1999; 11:1268–1274). The hemopoietic response may offer an explanation for this phenomenon, but other mechanisms (e.g., T-cell migration) may also be important.

### Chapter 23

**Dr. Jeffery:** In respect to the BUD-21-palmitate, have you conducted labeling experiments in vivo, experimentally, to demonstrate the major cellular localization to macrophages, Clara cells, type II cells or other cell types?

**Dr. Brattsand:** As we have not performed microautoradiographic studies within airway tissue, we don't know the distribution among these cell types. However, based on BAL we can state a much more longlasting uptake into alveolar macrophages of rats instilled with 21-palmitate liposomes than of rats given a conventional budesonide formulation.

**Prof. Dolovich:** What is the contribution of mucociliary clearance to the removal of the inhaled liposome formulation from the lung, given that it becomes an approximately 6 μm diameter aerosol postinhalation into the humid lung?

**Dr. Brattsand:** The liposomes are formed first when the formulation comes in contact with water, explaining why some liposomes can be larger than the normal size limitation for inhalation (but the inhaled formulation has naturally to fulfill this limitation). The smallest liposomes have a potential to rapidly fuse with membranes, while the larger liposomes formed at central airway level may be transported away by mucociliary clearance, if not taken up by phagocytic cells.

**Dr. Hochhaus:** The kind of liposomes you described need to be actively taken up. What is the variability in uptake among asthmatics or healthy volunteers?

**Dr. Brattsand:** This does not seem to be a major problem; as in the clinical trials performed with liposome or proliposome formulations, the individual spreading of efficacy has not deviated much from that of conventional budesonide.

# 22

# Design and Development of a Soft Corticosteroid, Loteprednol Etabonate

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## I. Introduction

A number of allergic diseases, including asthma and rhinitis, are reaching epidemic proportions within industrialized countries (1). In most of these countries, almost half of the population demonstrates sensitization to one or more environmental allergens, and in some of them, 25% of children under 14 years of age have asthma and 20% have eczema (1). It is becoming clear that these rising trends are due both to increased exposure to sensitizing allergens and to reduced stimulation of the immune system during critical periods of development (1). Even if most allergic diseases are not life threatening, they can cause considerable discomfort for the individual and a considerable total cost of treatment for the society. For example, in the United States in the early 1990s, asthma had a prevalence of around 4% and caused an estimated total yearly cost of $6.2 billion ($3.6 billion direct medical cost and $2.6 billion indirect cost; cost per patient per year being around $640) (2).

At present, corticosteroids are the most effective treatment available for atopic diseases (3,4). Inhaled corticosteroids are the mainstay of therapy for patients with chronic asthma, and nasal steroids are the most effective treatment for

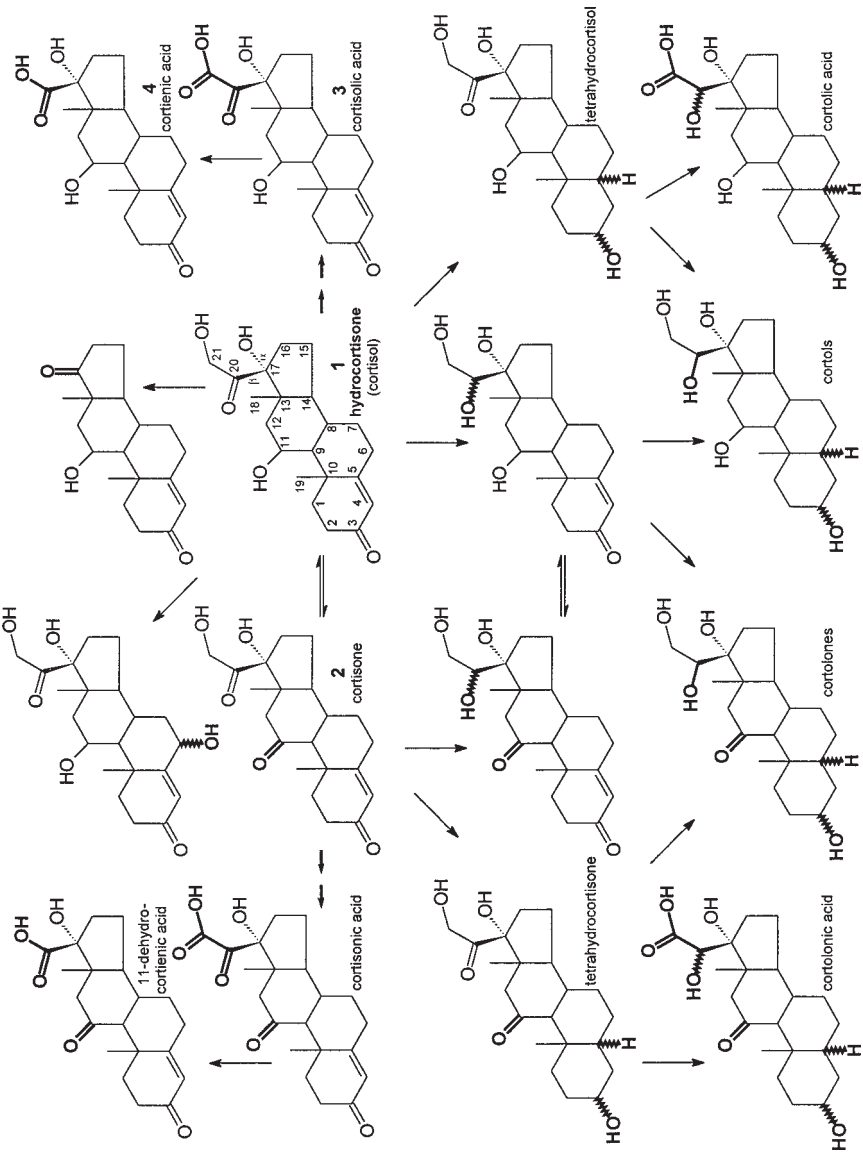


**Figure 1** Major metabolic pathways of hydrocortisone (**1**). (Adapted from Ref. 5.)

allergic rhinitis. Antigen challenge of the nose, lung, eye, or skin is known to produce a biphasic clinical reaction consisting of an early phase reaction (e.g., congestion and rhinorrhea of upper airways, wheezing, lacrimation, and edema of eyes) and a late phase response. Topically applied steroids have been shown to be able to inhibit both the early and the late phase of the allergic response to antigen challenge, and they also have proven capable of suppressing the eosinophilic inflammation underlying all allergic diseases. However, significant portions of the topically applied drugs (e.g., lung, nasal mucosa, gastrointestinal tract, or skin) will reach the general circulatory system. Consequently, resulting systemic side effects, such as adrenal suppression, effects on bone and growth, skin thinning and easy bruising, or increased risk of cataracts and glaucoma, together with local side effects, such as oral candidiasis or dysphonia, may limit their applications.

Furthermore, traditional corticosteroids are subject to different oxidative and/or reductive metabolic conversions. Formation of various steroidal metabolites, which may be active or even toxic, can lead to complex, undesirable situations, as all these compounds may be present simultaneously and in various time-dependent concentrations. An illustrative example is provided by hydrocortisone (cortisol) (**1**, Fig. 1) (5). Hence, there has been a continuous search for corticosteroids that undergo nonoxidative, extrahepatic metabolism to minimize the risk of systemic absorption.

## II. Soft Drugs

Soft drugs represent a possible solution, as they may eliminate the problems caused by the possibility of formation of various metabolites and they can minimize systemic side effects. Soft drug design approaches represent new approaches aimed to design safer drugs with an increased therapeutic index by integrating metabolism considerations into the drug design process (6,7). The soft drug concept was introduced in 1976 (8) and reiterated on a number of occasions in 1980–81 (9–14). Soft drugs are new therapeutic agents that undergo predictable metabolism to inactive metabolites after exerting their therapeutic effect. They are designed by building into the molecule, in addition to the activity, the most desired way in which the molecule is to be deactivated and detoxified subsequent to exerting its biological effects. The desired activity is generally local, and the soft drug is applied or administered at or near the site of action. Therefore, in most cases, they produce pharmacological activity locally, but their distribution away from the site results in a prompt metabolic deactivation that prevents any kind of undesired pharmacological activity or toxicity.

Inclusion of a metabolically sensitive site into the drug molecule makes possible the design and the prediction of the major metabolic pathway and avoids the formation of undesired toxic, active, or high-energy intermediates. Hence, in soft

drug design, the goal is not to avoid metabolism, but rather to control and direct it. If possible, inactivation should take place as the result of a single, low-energy, high-capacity step that yields inactive species subject to facile elimination. Most critical metabolic pathways are mediated by oxygenases that exhibit not only interspecies but also interindividual variability and are subject to inhibition and induction (15). In different individuals, half-lives of foreign compounds may vary as much as 10- to 50-fold (15). Furthermore, the rates of hepatic monooxygenase reactions are at least two orders of magnitude lower than the slowest of the other enzymatic reactions (16). Therefore, it is usually desirable to avoid oxidative pathways and slow, easily saturable oxidases and to design soft drugs that are inactivated by hydrolytic enzymes. Metabolism can be more reliably carried out by ubiquitous esterases, because they are widely distributed, nonspecific, and much less susceptible to saturation and inhibition. Because diseases can alter the organs responsible for the metabolism of bloodborne substances, it is better not to rely on metabolism or clearance by organs such as liver or kidney since blood flow and enzyme activity in these organs can be seriously impaired, especially in critically ill patients.

Carboxylic ester hydrolases (EC 3.1.1) efficiently catalyze the hydrolysis of a variety of ester-containing chemicals to the respective free acids. They exhibit broad and overlapping substrate specificity toward esters and amides, and the same substrate is often hydrolyzed by more than one enzyme. Consequently, their classification is difficult and still is in a somewhat confused state, despite the important roles that carboxylesterase (EC 3.1.1.1) and/or other carboxylic ester hydrolases, such as arylesterase (EC 3.1.1.2) and cholinesterase (EC 3.1.1.8), play in the metabolism of many xenobiotics (17–23). Humans have been shown to express carboxylesterase in the liver, plasma, small intestine, brain, stomach, colon, macrophage, and monocytes (23). It should be mentioned that esterase activity varies quite strongly between species (17–23). For example, the stability of aliphatic esters frequently employed in prodrug and soft drug designs usually increases in the rat $<$ rabbit $<$ dog $<$ human order (24,25), but there might be considerable variability. Nevertheless, we have recently succeeded in developing a quantitative structure-metabolism relationship (QSMR) model that accounts for 80% of the variability in the log half-lives of 67 noncongener carboxylic esters for in vitro human blood data and that should be useful in estimating approximate rates of hydrolysis even ahead of synthesis (26,27).

The general principles of soft drug design and their practical applications have been reviewed in the literature (6,7,28–30). A number of already marketed drugs, such as esmolol (Brevibloc™), an ultra–short-acting β-blocker, remifentanil (Ultiva™), a unique ultra–short-acting opioid analgesic, or loteprednol etabonate (Lotemax™, Alrex™), a soft corticosteroid, resulted from the successful application of such design principles (7). Within the corticosteroid field, various attempts have been made to separate local and systemic effects by integrating

moieties susceptible to facile, extrahepatic metabolism into the corticosteroid structure (31–37). Here, we will concentrate mainly on an attempt that at present can be considered as the most successful one along these lines. It is a classic, inactive metabolite-based soft drug approach that started from a true inactive metabolite of prednisolone (**9**) and ultimately yielded loteprednol etabonate (**7**) (Fig. 2).

At this point it should be mentioned that some other steroid drugs, such as fluticasone propionate, tipredane, or butixocort 21-propionate, have been erroneously called soft drugs on different occasions (4,38). Contrary to the previously mentioned soft drug design principles, these structures are metabolized primarily in the liver by oxidative processes and not by extrahepatic hydrolysis. Thiol ester corticosteroid structures have been shown to be metabolized primarily in the liver by oxidative processes, rather than by hydrolysis in the plasma (39). Fluticasone propionate (FP) itself was found to have a terminal half-life of 7.7–8.3 hours in 12 healthy male subjects after inhaled administration of 500, 1000, and 2000 μg of drug using a metered-dose inhaler. In these subjects it produced dose-related cortisol suppression; the highest administered dose of FP resulted in cortisol concentrations that were lower than the limit of detection (40). The slow elimination of FP led to accumulation during repeated dosing. This accumulation may explain the marked decrease in plasma cortisol seen during treatment with fluticasone propionate within the clinical dose range (41).

There also is some misconception regarding soft drugs, particularly soft steroids. Often, the soft nature is associated with fast hydrolytic degradation, but this is not necessarily so. Too fast hydrolysis could result in very weak activity. The desired increase of the therapeutic index can be achieved only if the drug is sufficiently stable to reach the receptor site and the target organ and to produce its desired effect, but the free, non–protein-bound drug undergoes facile hydrolysis to avoid unwanted, systemic side effects. In order to successfully separate the desired local activity from systemic toxicity, an adequate balance between intrinsic activity, solubility/lipophilicity, tissue distribution, protein binding, and rate of metabolic deactivation has to be achieved, particularly for long-term activity. In the case of slow, sustained release to the general circulatory system from the delivery site, even a relatively slow hydrolysis could result in a very low, almost steady-state systemic concentration.

## III. Loteprednol Etabonate

As mentioned, a classic soft drug approach (14,42–57) recently ended successfully and yielded loteprednol etabonate [**7**, (11β,17α)-17((ethoxycarbonyl)oxy)-11-hydroxy-3-oxoandrosat-1,4-diene-17-carboxylic acid chloromethyl ester; chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene, 17β-carboxylate], an active corticosteroid that lacks serious side effects and that

received final FDA approval in 1998 as the active ingredient of two ophthalmic preparations, Lotemax™ and Alrex™. Loteprednol etabonate became the only corticosteroid to receive FDA approval for use in all inflammatory and allergy-related ophthalmic disorders, including inflammation following post–cataract surgery, uveitis, allergic conjunctivitis, and giant papillary conjunctivitis (GPC). Currently, it is also being developed for treatment of asthma, rhinitis, colitis, and dermatological problems.

Topical corticosteroids represent an important class of drugs used to treat ocular inflammations and allergies as they are the most effective ocular anti-inflammatory compounds and offer the broadest range of treatment. However, a number of contraindications severely limit their usefulness. In addition to the general systemic corticosteroid side effects, they can also produce a number of ocular complications such as elevation of the intraocular pressure (IOP) and resultant steroid-induced glaucoma, induction of cataract formation, and secondary complications. Ocular administration of corticosteroids usually produces increased IOP as a result of increased resistance to aqueous humor outflow, but the precise mechanism of decreased outflow is not known (58). The mechanism of steroid-induced cataract is somewhat obscure (59), but the most prominent hypothesis involves the formation of Schiff bases between the steroid C-20 ketone group and nucleophilic groups such as ε-amino groups of lysine residues of proteins. Schiff base formation is potentially followed by a Heyns rearrangement (60) involving the adjacent C-21 hydroxyl group and affording stable amine-substituted adducts (61–64).

### A. Design Considerations and Development History

The soft drug approach to be described here resulted in an active corticosteroid that is void of these serious side effects. As already mentioned, hydrocortisone (**1**) undergoes a variety of oxidative and reductive metabolic conversions (Fig. 1) (5). One of its major metabolic routes is oxidation of the dihydroxyacetone side chain, which through a 21-aldehyde (21-dehydrocortisol) and a 21-acid (**3**, cortisolic acid) ultimately leads to formation of cortienic acid (**4**). Cortienic acid is a major metabolite excreted in human urine, and it lacks corticosteroid activity; therefore, it is an ideal lead for the inactive metabolite-based approach of soft drug design (6,43,65). The design process (Fig. 2) directly involves restoring the important pharmacophores found in the 17α and 17β side chains to afford soft corticosteroids **5**. We felt that suitable isosteric/isoelectronic substitution of the α-hydroxy and β-carboxy substituents with esters or other types of functions should restore the original corticosteroid activity but incorporate hydrolytic features to keep toxic levels of corticosteroids from accumulating and producing local or systemic adverse affects. Modifications of the 17β ester function and the 17α hydroxy function, together with other changes [introduction of $\Delta^1$, fluorination at 6α ($X_2$) and/or 9α ($X_1$), methylation at 16α or 16β ($R_3$)], led to a host of analogs repre-

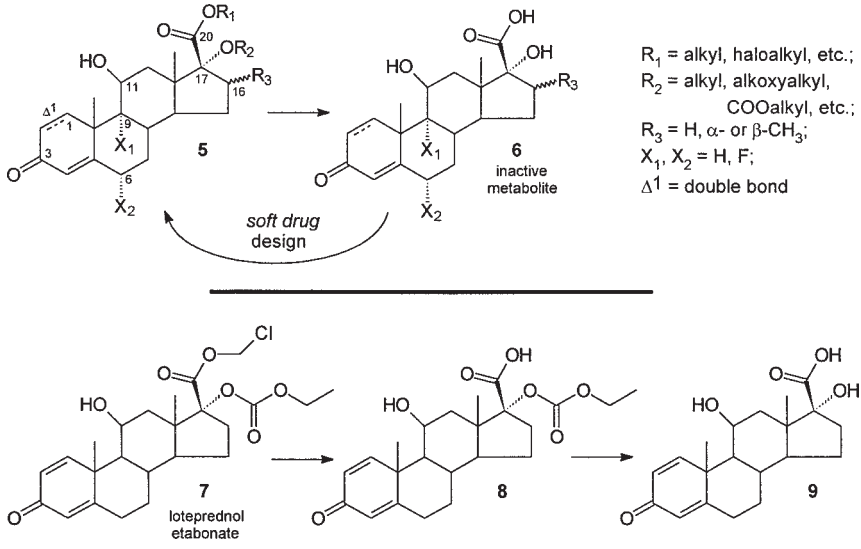


**Figure 2** Design and metabolism of soft corticosteroids (**5**). Loteprednol etabonate (**7**), a soft steroid, is an active anti-inflammatory compound that lacks the IOP-elevating side effect of the other steroids used to treat ophthalmological diseases.

sented by the general structure **5**. Over 120 of these soft steroids (**5**) have been synthesized. The first soft analogs of this kind were synthesized soon after the introduction of the soft drug concept (8) during the late 1970s, followed by a systematic synthetic study performed in collaboration with Otsuka Pharmaceutical Company (Japan) in 1980–1981 (14,42,66). Critical functions for activity are a haloester in the 17β position and a novel carbonate (42,45) or ether (67) substitution in the 17α-position.

Incorporation of 17α carbonates or ethers was preferred over 17α esters to enhance stability and to prevent formation of mixed anhydrides that might be produced by reaction of a 17α ester with a 17β acid functionality. Such mixed anhydrides were assumed toxic and probably cataractogenic. The 17α carbonates were a new class of corticosteroids, and they turned out to be difficult to obtain from normal corticosteroid derivatives. However, after oxidative removal of the C-21 carbon, their synthesis proved relatively easy (45). Initial activities were determined by classical cotton pellet granuloma tests and by human vasoconstrictor studies (6,13,66,68,69). A variety of 17β esters were synthesized, and they showed very different activities. Since this position is an important pharmacophore that is quite sensitive to small modifications, the freedom of choice was relatively limited. For example, while chloromethyl or fluoromethyl esters showed very good activity, the chloroethyl or α-chloroethylidene derivatives were very weak. Simple

**Table 1** Comparison of Loteprednol Etabonate (**7**) with Other Steroids

| Treatment | *N* | $ED_{50}$[a] | Rel. pot. | $TD_{50}$[b] | Rel. pot. | TI[c] |
|---|---|---|---|---|---|---|
| Loteprednol etabonate (0.1%) | 8 | 178.0 | 0.48 | 10,000 | 0.02 | 24.0 |
| Hydrocortisone 17α-butyrate (0.1%) | 8 | 121.0 | 0.70 | 369 | 0.57 | 1.3 |
| Betamethasone 17α-valerate (0.12%) | 8 | 84.8 | 1.00 | 212 | 1.00 | 1.0 |
| Clobetasone 17α-propionate (0.1%) | 8 | 2.9 | 29.70 | 11 | 19.30 | 1.5 |

[a]Anti-inflammatory activity in the cotton pellet granuloma test (μg/pellet).
[b]Thymolysis potency (μg/pellet).
[c]Therapeutic index: the ratio of the relative potency for the $ED_{50}$ to the relative potency for the $TD_{50}$; betamethasone 17α-valerate has been assigned arbitrarily a value of 1.

alkyl esters also proved virtually inactive. Consequently, the 17β chloromethyl ester was maintained constant and various 17α-carbonates with different substituents on the steroid skeleton were selected for further investigation. For a number of derivatives, the therapeutic index was determined as the ratio between the anti-inflammatory activity and the thymus involution activity. As illustrated in Table 1, classical steroids, regardless of their intrinsic activity, have very similar therapeutic indices, but loteprednol etabonate, the soft steroid, provides a significant improvement. Many of the other soft steroids also showed a dramatic improvement in the therapeutic index (43,70). Recent studies on binding to rat lung cytosolic corticosteroid receptors showed that some of the compounds approach and even exceed the binding affinity of the most potent corticosteroids known (Table 2).

Selection of loteprednol for development was based on various properties. In addition to the therapeutic index, availability, synthesis, and "softness" (the rate and easiness of metabolic deactivation) also had to be considered. Even if the route of development was technically easy, it involved various companies due to financial problems. First, as mentioned, it involved Otsuka Pharmaceutical Company (1980–1985) in performing synthesis, preclinical studies, animal toxicology, and limited Phase I/II human studies directed toward dermatological use. Xenon Vision Inc., which was specifically established to explore the potential ophthalmic use (1986–1991), performed regulatory animal toxicology, Phase I and Phase II human studies, and the establishment of proof of concept in giant papillary conjunctivitis and allergic conjunctivitis. Finally, the involvement of Pharmos Corporation and Bausch and Lomb Inc. (1992–1996: Phase III studies in giant papillary conjunctivitis, allergic conjunctivitis, uveitis, and post–cataract surgery) led to submission of the New Drug Applications in 1995 and 1996, respectively.

Early studies in rabbits (46,49) and rats (50) demonstrated that, consistent with its design, **7** is indeed active, is metabolized into its predicted metabolites (**8**,**9**), and these metabolites are inactive (45). The highest ratio of metabolites to unchanged drug was found in the cornea, suggesting that the primary site of me-

**Table 2** Relative Binding Affinities (RBA) of Representative Soft (**5** with $R_2 = COOR'_2$) and Reference Glucocorticoids to the Glucocorticoid Receptor of Rat Lung ($RBA_{dexamethasone} = 100$)

| No. | $R_1$ | $R'_2$ | $R_3$ | $X_1$ | $X_2$ | RBA |
|---|---|---|---|---|---|---|
| LE (**7**) | $CH_2Cl$ | $C_2H_5$ | H | H | H | 320 |
| 5602 | $CH_2Cl$ | *n*-$C_4H_9$ | H | H | H | 110 |
| 5606 | $CH_2SCH_3$ | $C_2H_5$ | H | H | H | 3 |
| 5608 | $C_2H_5$ | $C_2H_5$ | H | H | H | <1 |
| 5613 | $CH_3$ | $CH_2Cl$ | H | H | H | <1 |
| 5614 | $C_2H_5$ | $C_2H_5$ | H | F | H | <1 |
| 5618 | $CH_2Cl$ | $CH_2OCH_3$ | H | H | H | 16 |
| 5702 | $CH_2Cl$ | $CH_3$ | H | H | H | 180 |
| 5628 | $CH_2Cl$ | $C_2H_5$ | α-$CH_3$ | F | H | 740 |
| 5649 | $CH_3$ | *i*-$C_3H_7$ | H | H | H | 3 |
| 5651 | $CH_2OC_2H_5$ | *i*-$C_3H_7$ | H | H | H | <1 |
| 5673 | $CH_2Cl$ | $C_2H_5$ | α-$CH_3$ | F | F | 2100 |
| 5685 | $CH_2CH_2Cl$ | *i*-$C_3H_7$ | H | H | H | 1 |
| 5711 | $CH_2F$ | $C_2H_5$ | H | H | H | 200 |
| Acid metabolites (substituted cortienic acids) are inactive: | | | | | | |
| | H | $R_2$ | $R_3$ | $X_1$ | $X_2$ | <1 |
| Dexamethasone (DEX) | | | | | | 100 |
| Beclomethasone dipropionate (BDP) | | | | | | 50 |
| Beclomethasone monopropionate (BMP) | | | | | | 1300 |
| Budesonide (BUD) | | | | | | 940 |
| Fluticasone propionate (FP) | | | | | | 1900 |
| Triamcinolone acetonide (TA) | | | | | | 200 |

tabolism is the cornea (46). Loteprednol etabonate concentrations in the aqueous humor paralleled the concentration-time profile found in the cornea but were about 100 times lower, suggesting that the origin of loteprednol in the aqueous humor is the cornea. As high levels of corticosteroids in the aqueous humor are believed to lead to ultrastructural changes in the trabeculum leading to decreased outflow and increased IOP, this should also make loteprednol less likely to raise IOP.

In rats, the metabolism and excretion of loteprednol etabonate was found to be dose-dependent (54). As the intravenous (i.v.) administered dose increased from 1 mg/kg to 20 mg/kg, the half-life ($t_{1/2}$) increased from 16 to 49 minutes, and the total clearance ($CL_{tot}$) decreased from ~120 to 60 mL/min/kg, all values higher than the physiological hepatic blood flow in rats (58 mL/min/kg) (54). In dogs, after i.v. administration of the relatively high dose of 5 mg/kg, loteprednol etabonate had a terminal half-life of 2.8 hours, a mean residence time of 1.7 hours,

and a total clearance of about 1 L/h/kg (51). Loteprednol showed a plasma protein binding of >90% and, according to limited studies in dogs and rats, a very low oral bioavailability of close to 0% (51). Its pharmacokinetic profile indicated that, when absorbed systematically, it is rapidly transformed to the inactive metabolite **8** and eliminated from the body mainly through the bile and urine (50,51,54). In dogs, both oral and i.v. administration resulted in over 90% excretion, mostly as acidic metabolites in feces, an observation supporting the facile elimination of the metabolites to the bile. In vitro plasma hydrolysis data are misleading due to the high concentrations used. In whole body cases, multiple hydrolytic (esterases) metabolism sites and much lower concentrations generated by therapeutic concentrations result in facile hydrolysis of the free, non–protein-bound loteprednol etabonate.

It did not effect the intraocular pressure in rabbits (49), an observation confirmed later in various human studies (71,72). A study in known corticosteroid responders (71) showed that subjects ($n = 10$) receiving loteprednol etabonate (0.5%) had a mean IOP elevation of 3.0 mmHg over a period of 42 days, whereas subjects receiving prednisolone acetate ($n = 9$) had a mean IOP elevation of 7.4 mmHg (Fig. 3). Because the protocol required discontinuation of the treatment upon significant IOP elevation, it is likely that the IOP would have continued to increase in the prednisolone acetate group had several patients not been intercepted with topical β-blocker therapy. A long-term use (≥28 days) study based on pooled data showed that IOP elevation greater than 10 mmHg, a dreaded side effect of steroid therapy, occurred in 1.7%, 0.5%, and 6.7% of patients taking loteprednol etabonate (formula concentrations of 0.5% or 0.2%, $n = 901$), vehicle

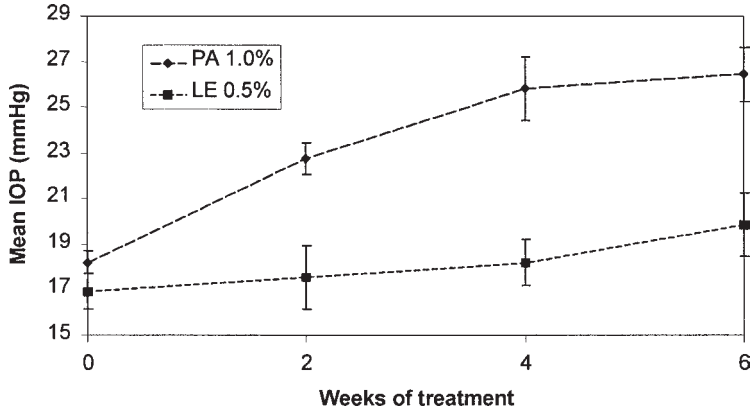


**Figure 3** Mean intraocular pressure (IOP) in known corticosteroid responders receiving loteprednol etabonate (LE, 0.5%; $n = 10$) or prednisolone acetate (PA, 1.0%, $n = 9$). (Modified from Ref. 71.)

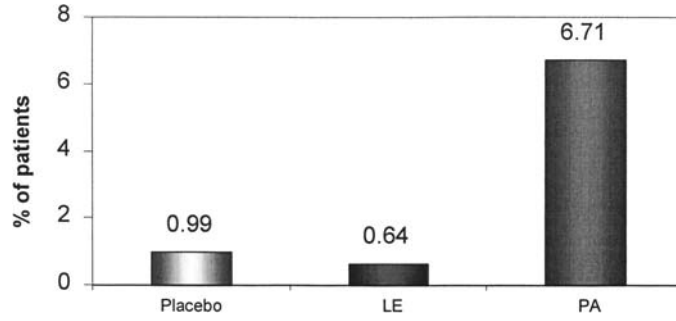


**Figure 4** Pooled data showing the percent of patients with IOP elevation greater than 10 mmHg among patients not wearing contact lenses and treated for more than 28 days. The number of patients within each group was as follows: placebo, $n = 304$; loteprednol etabonate (LE), $n = 624$; prednisolone acetate (PA, 1%), $n = 164$. (Data from Ref. 72.)

($n = 583$), and prednisolone acetate (formula concentrations of 1%, $n = 164$), respectively (72). For loteprednol etabonate, 11 out of the 15 patients with IOP elevation greater than 10 were in the GPC studies, where the higher incidence is likely due to the reservoir effect of soft contact lenses absorbing the drug. For patients who did not wear contact lenses, the same numbers were 0.6%, 1.0%, and 6.7%. Loteprednol etabonate has, therefore, a lower propensity to cause clinically significant elevations in IOP than prednisolone acetate and, in patients not wearing contact lenses, this propensity is similar to that found in subjects receiving vehicle (Fig. 4). Recent Phase IV data (Bausch & Lomb Pharmaceuticals, presented at the 3rd International Symposium on Ocular Pharmacology and Pharmaceutics (ISOPP), Lisbon, Portugal, February 10–13, 2000) demonstrated that, consistent with the soft nature, long-term use of loteprednol etabonate (with an average of 100 days) still did not show any typical corticosteroid side effects such as IOP elevation.

Consistent with the soft nature of this steroid, systemic levels or effects cannot be detected even following chronic ocular administration (73). Plasma levels of loteprednol etabonate and its primary metabolite (**8**) were below the 1 μg/L detection limit in 10 healthy volunteers who received the drug (one drop, 0.5%) in both eyes eight times daily for 2 days and four times daily for a further 41 days (73).

### B. Ophthalmological Applications

Clinical studies proved that loteprednol etabonate is a safe and effective treatment for contact lens–associated giant papillary conjunctivitis (GPC) (74–76), seasonal allergic conjunctivitis (77–79), postoperative inflammation (Fig. 5) (80,81), or uveitis (82). Most of these clinical results were recently reviewed in detail by

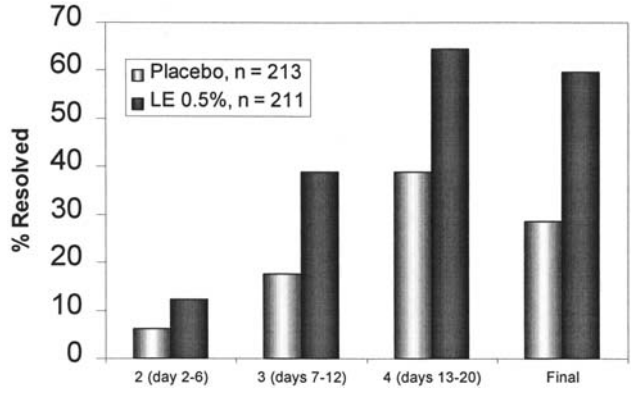


**Figure 5** Resolution of anterior chamber inflammation in postcataract inflammation with intraocular lens implantation. The percent of resolved cases are shown for each visit during placebo ($n = 213$) and loteprednol etabonate 0.5% ($n = 211$) treatment. (Data from Refs. 80,81.)

Noble and Goa (83) and Howes (84). In addition to its already approved uses, loteprednol etabonate is also being developed for the treatment of colitis, atopic dermatitis, and asthma based on promising results from animal studies (53,54).

## IV. Effect of Loteprednol Etabonate on Airway Activity

Recent studies demonstrated that loteprednol etabonate (LE, **7**) can advantageously be used in many other inflammatory conditions where separation of activity and side effects is very important, including asthma and rhinitis (85–89).

### A. In Vitro Studies

In vitro studies in human blood and nasal polyp cells, which are considered to be an adequate model of chronic respiratory mucosal inflammation, from six patients aimed to compare the effects of LE and other corticosteroids showed that LE has activity similar to dexamethasone (DEX) or beclomethasone dipropionate (BDP). The corresponding $IC_{50}$ values for inhibition of endotoxin (lipopolysaccharide, LPS)-induced release of tumor necrosis factor-α (TNFα) in 1:5 diluted human blood and for inhibition of anti-IgE–induced granulocyte-macrophage colony-stimulating factor (GM-CSF) release in dispersed nasal polyp cells are shown in Figure 6 (86). Inhibition of cytokine biosynthesis is thought to be an important mechanism through which steroids exert their anti-inflammatory effects (89). TNFα is a proinflammatory cytokine released from several inflammatory/immunocompetent cells, and there is considerable evidence suggesting that TNFα up-

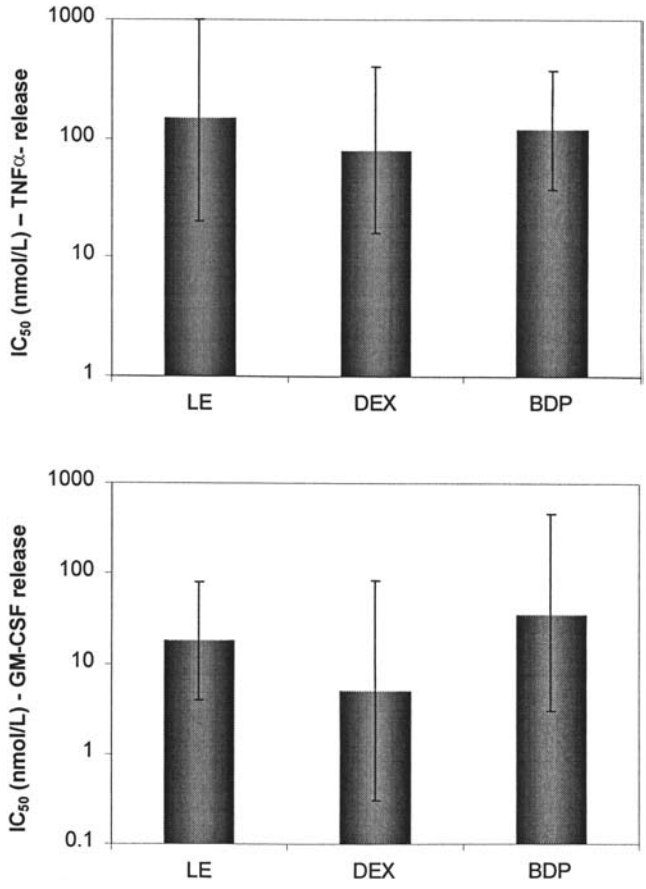


**Figure 6** $IC_{50}$ values (nmol/L) with 95% confidence limits for inhibition of endotoxin (LPS)–induced release of TNFα in 1:5 diluted human blood and for inhibition of anti-IgE–induced GM-CSF release in dispersed nasal polyp cells for loteprednol etabonate (LE), dexamethasone (DEX), and beclomethasone dipropionate (BDP). (Data from Ref. 86.)

regulation occurs in asthmatic patients. GM-CSF has been shown to be produced by cells present at inflammatory response sites, such as T lymphocytes, macrophages, and endothelial and mast cells and is assumed to be an important mediator for inflammatory reactions.

### B. In Vivo Studies

Loteprednol etabonate was shown to be equipotent with the highly used beclomethasone dipropionate (BDP), in various in vivo experimental models of aller-

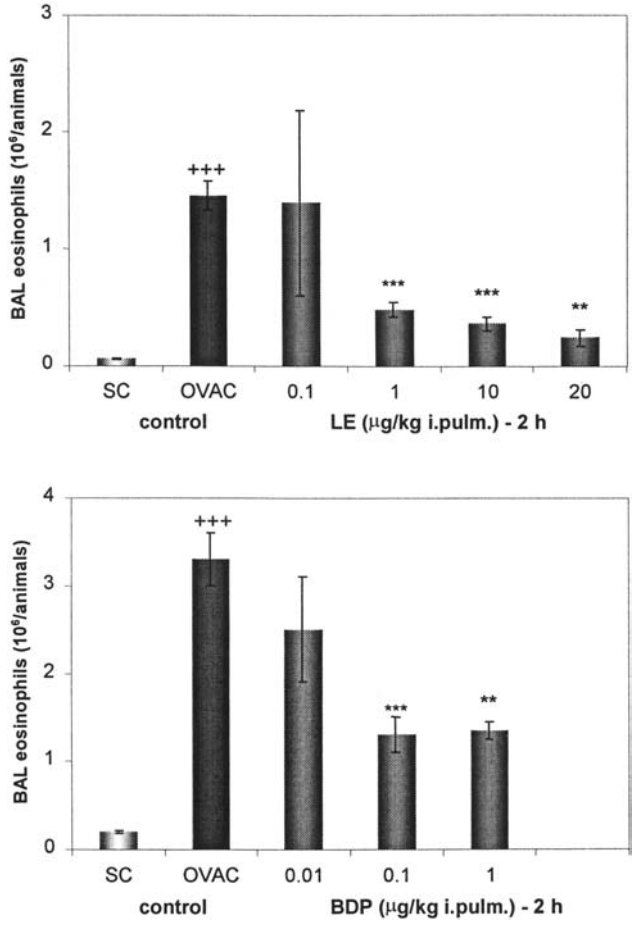


**Figure 7** Effect of loteprednol etabonate (LE, 0.1–20 μg/kg) and beclomethasone dipropionate (BDP, 0.01–1 μg/kg) on allergen-induced eosinophilia in bronchoalveolar lavage fluid (BALF) 48 h after allergen-challenge in actively sensitized Brown-Norway rats by single intrapulmonar dry powder administration 2 h prior to challenge. Data are mean ± SEM. Notation: SC = lactose-treated/saline-challenged control group; OVAC = lactose-treated/OVA (ovalbumin)-challenged control group; $^{+++}p < 0.001$ compared to animals sham-challenged with saline (SC); $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to vehicle-treated, allergen-challenged animals (OVAC).

gic diseases, such as ovalbumin (OA)-induced rhinorrhea and OA-induced lung eosinophilia in actively sensitized Brown-Norway (BN) rats (Fig. 7). This is a well-characterized animal model for allergic asthma, as these animals respond to sensitization by developing high levels of circulatory antigen-specific IgE (see the response of the placebo-treated/OA-challenged OVAC control group in Fig. 7). In these experiments of OA-induced airway eosinophilia in actively sensitized BN rats, compounds were given 2 hours prior to OA challenge intratracheally as a dry powder. The animals were challenged by inhaling OA aerosol. The number of eosinophils in bronchoalveolar lavage fluid (BALF) were counted for 48 hours postchallenge. Eosinophilia in BALF was reduced dose dependently by LE (Fig. 7) with an $ID_{50}$ of 0.44 μg/kg i. pulm., comparable to that of BDP (0.11 μkg i. pulm.) and significantly better than that of dexamethasone (DEX, 10 μg/kg i. pulm.) (85,87,89).

In similar studies involving late phase allergic eosinophilia in guinea pigs, eosinophilia in BALF was reduced dose dependently by LE ($ID_{50}$ = 29 μg/kg), by DEX ($ID_{50}$ = 134 μg/kg), and by BDP ($ID_{50}$ = 19 μg/kg) (Fig. 8) (85,89). In this set of experiments where the OA challenge was applied 2 hours after the dose with steroids, fluticasone propionate (FP), a highly potent steroid, showed the highest activity ($ID_{50}$ = 0.89 μg/kg). It was found, however, that when LE and FP were compared in a study (85) with longer separation (6 hours) between administration of steroids and OA challenge, LE still showed a strong activity with $ID_{50}$ = 77 μg/kg, but FP showed only a very weak and not dose-dependent effect (Fig. 9). These data indicate that LE produced a strong anti-inflammatory effect in vivo after

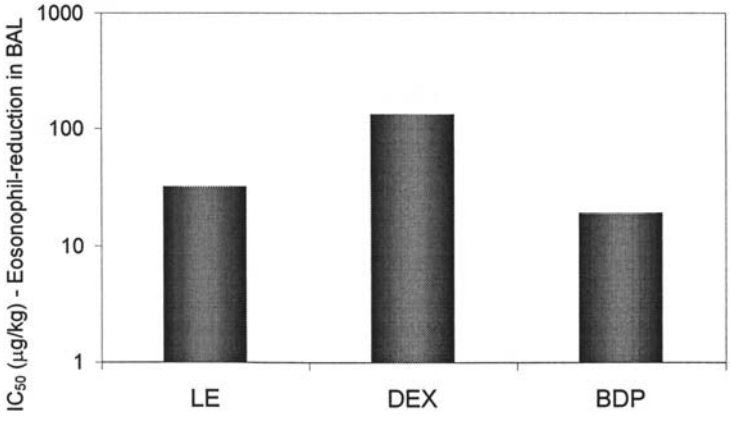


**Figure 8** $ID_{50}$s (μg/kg) for reduction of eosinophilia in BALF in late phase allergic eosinophilia in guinea pigs for loteprednol etabonate (LE), dexamethasone (DEX), and beclomethasone dipropionate (BDP). (Data from Ref. 85.)

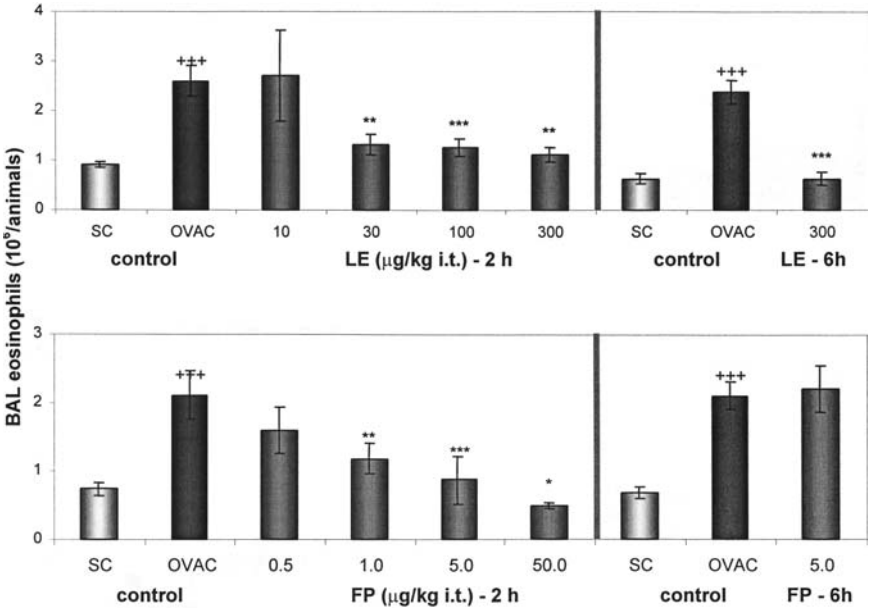


**Figure 9** Comparison between the effects of loteprednol etabonate (LE, 10–300 μg/kg) and fluticasone propionate (FP, 0.5–50 μg/kg) on allergen-induced eosinophilia in BAL-fluid 24 h after allergen challenge in actively sensitized guinea pigs by single intratracheal dry powder administration 2 h prior to challenge (left side) and 6 h prior to challenge (right side) (LE 300 μg/kg, FP 5.0 μg/kg i.t.). When the corticosteroids were administered 6 h prior to challenge, a strong effect was still observed for loteprednol etabonate, but no effect was observed for fluticasone propionate. See Figure 7 for notation. (Data from Ref. 85.)

intranasal and intrapulmonary administration. In comparison to FP, LE showed significantly longer-lasting action.

LE also has been evaluated in a sheep model for asthma (T. Ahmed, personal communication). The same six sheep were used in all three studies. After measurement of baseline values of specific lung resistance ($SR_L$), the sheep were treated with equivalent doses of either LE or BDP dissolved in tetraglycol as 5% solutions and delivered as aerosols. $SR_L$ measurements were made, and one hour later the animals were challenged with the *Ascaris suum* antigen. After the challenge, measurements were made hourly between 1 and 6 hours and every 30 minutes from 6½ to 8 hours. As summarized in Figure 10, both LE and BDP inhibited the late phase response. Loteprednol etabonate is being now developed for the treatment of allergic conditions, such as rhinitis and asthma.

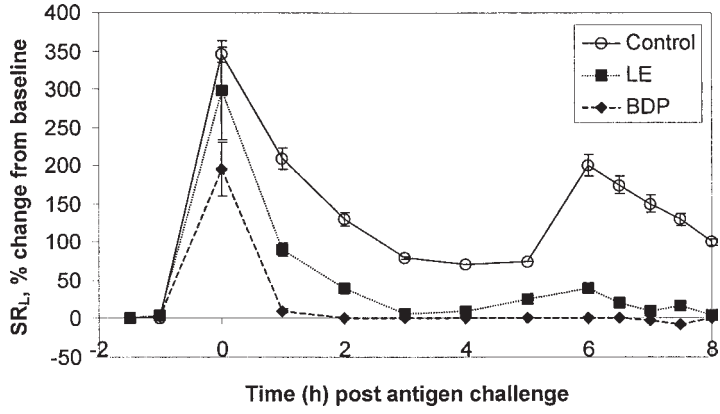


**Figure 10** Comparative results obtained in a sheep model of asthma for equivalent doses of loteprednol etabonate (LE) and beclomethasone dipropionate (BDP) showing that both drugs inhibited the late phase response.

Understandably, a reduced risk of steroid-related side effects is an important aspect of intranasal or intrapulmonary administration. Preliminary studies in domestic pigs indicated that LE has no influence on plasma cortisol levels in this species following intranasal or intrapulmonary administration even at high doses (89). The influence of LE on thymus involution was extensively investigated in rats (88,89). Following 5-day intrapulmonary treatment (drugs were directly blown into the lungs), LE caused only minimal thymus involution even at a high intrapulmonary dose of 20 mg/kg/day, whereas BDP (1 mg/kg/day), budesonide (BUD) (0.5 mg/kg/day), and FP (1 mg/kg/day) all caused significant ($p < 0.05$) thymus involutions at considerably lower doses. A quantitative comparison of the therapeutic indices for intrapulmonary administration in rats, calculated, for example, as the ratio of the toxic dose $TD_{25}$ that causes 25% thymus involution to the effective dose $ED_{50}$ that causes 50% BAL eosinophilia inhibition, clearly shows LE as being superior to FP and much superior to BDP, even when the $ID_{50}$s for the effects with the shorter separation between steroid administration and OA challenge are used for comparison. However, a reliable quantitative comparison could not be made at this stage, because essentially all doses tested for LE (0.1–20 mg/kg/day) produced no significant thymus involution, and because no sufficient data (only at two concentrations) are available for BDP and FP to estimate $TD_{25}$ for thymus involution. Another study based on once-daily s.c. dosing for 5 days also found that, in contrast to BDP (1 mg/kg), BUD (2 mg/kg), and FP (1 mg/kg), loteprednol etabonate (1 and 10 mg/kg) does not cause significant ($p < 0.01$) thymus involution (Fig. 11) (89).

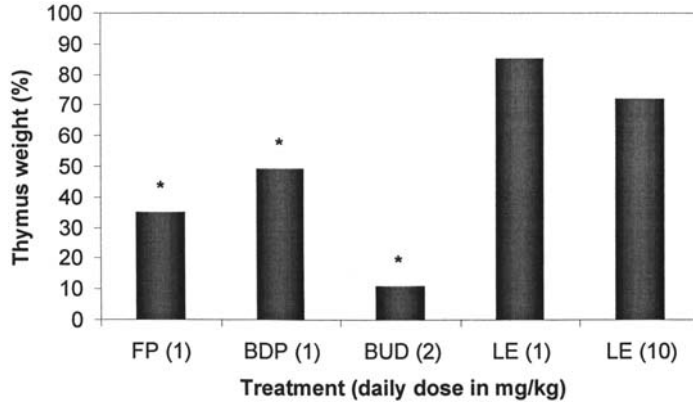


**Figure 11** Effect of glucocorticoids on thymus weight in rats after daily subcutaneous administration for 5 days. A star (*) denotes significant ($p < 0.01$) thymus involution. (Data from Ref. 89.)

## V. Conclusions

Topical application of active corticosteroids that undergo nonoxidative, extrahepatic metabolism can provide improved, safer treatments of allergic diseases by minimizing the risk of systemic absorption and, therefore, the occurrence of side effects. Loteprednol etabonate, a soft corticosteroid that contains 17α carbonate and 17β ester side chains and that was designed by using an inactive metabolite-based approach, lacks serious side effects and already received FDA approval for use in all inflammatory and allergy-related ophthalmic disorders. Since experimental evidence indicates that it also produces strong and long-lasting anti-inflammatory effect after intranasal or intrapulmonary administration, currently it is being developed for the treatment of allergic conditions, such as rhinitis and asthma.

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# 23

# Development of Inhaled Steroids Based Upon Prodrugs with Prolonged Intraluminal Retention Time

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## I. Introduction

A weakness of current inhaled steroids is that the bulk of the inhaled substance is rapidly absorbed from airways/lung. Thus, the $C_{max}$ of budesonide, for example, is reached approximately 0.5 hour after inhalation, indicating a rapid absorption (1,2). In the central airways, however, esterification of budesonide into lipids generates a reservoir that is only slowly hydrolyzed back to the active compound. This can to some extent compensate for the rapid absorption (3,4). A substantial systemic uptake of inhaled steroids occurs very rapidly from the peripheral part of the respiratory tract, where it contributes mainly to the adverse effects of steroids. One approach to improve the topical efficacy and reduce the undesirable systemic effects of inhaled steroids would be to prolong the intraluminal deposition time. The water solubility of fluticasone propionate (FP) is 100-fold lower than budesonide (0.1 and 16 μg/mL, respectively) (5) which may explain why inhaled FP also has a longer mean absorption time (6–8 hours compared to 40 min for budesonide). However, because of its higher systemic activity, FP obviously would require an even longer intraluminal deposition time in order to decrease the plasma concentration even further to reduce its unwanted systemic activity. In the central

airways a very long dissolution time may be a problem as it can reduce the topical efficacy because insoluble particles will be removed by mucociliary clearance. By contrast, in the more distal airways and in the lung, where mucociliary clearance is less pronounced, a prolonged deposition time might result in better topical efficacy and reduced systemic activity and therefore improved selectivity of inhaled steroids. In this case, glucocorticoid-sensitive airway diseases like COPD and interstitial lung diseases like alveolitis/granulomatosis may be better treated by slow release steroid formulations.

## II. Aim of Astra Project

One approach to improve the topical efficacy/selectivity of inhaled GCS was to decrease their water solubility in the aqueous epithelial lining fluid and thereby prolong the local deposition time and prevent rapid absorption from distal airways and lung. The substitution of a lipophilic acyl chain in the 21-position of the active GCS, D5119, enhanced the compound's lipophilicity (Fig. 1). D5519 possesses a higher receptor affinity and faster hepatic metabolism (6) than budesonide and a receptor affinity and low oral bioavailability similar to FP and mometasone furoate. Although the water solubility of D5519 is similar to budesonide, the palmitic acid ester, D5522, is more than 1000 times lower. As such, D5522 has a negligible apparent receptor affinity due to its fatty acid acylation and can thus be considered a prodrug (7). Hydrolysis of the prodrug by lipases in vivo, however, yields the very potent glucocorticoid D5519. Based on in vitro studies the extracellular lipase activity of the epithelial lining fluid does not allow substantial hydrolysis and uptake of D5522.

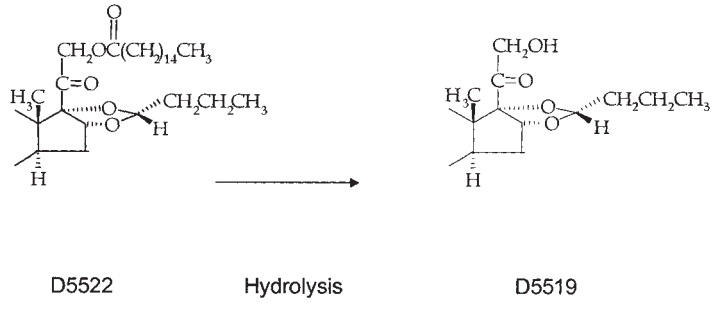


**Figure 1** Structure of the D-ring of the lipophilic prodrug D5522, and the active glucocorticosteroid D5519.

## III. Lipid Formulation for Attaining Bioavailability of D5522

The very low water solubility of D5522 (<1 ng/mL) implies that the prodrug requires special formulation to achieve bioavailability from the airways/lung. If absorption is incomplete or inefficient, compound accumulation in lower parts of the lung may be a problem due to a lack of mucociliary clearance in that region. By using phospholipids as a co-solvent for D5522, a bioavailable, lipase-sensitive formulation has been developed. The critical importance of this formulation is shown in Figure 2, where the lipase activity toward D5522 in crystalline form or in the phospholipid formulation was compared. While there was almost complete release of D5519 from the lipid formulation, crystalline D5522 was not subject to lipase metabolism. The lipid formulation comprises the phospholipids, dipalmitoylphosphatidyl choline, and dimyristoylphophatidyl choline and is similar to one used previously in a liposomal formulation of the palmitic acid ester of budesonide (8). However, the latter comprised preformed liposomes, while the dry D5522 formulation is a molecular mixture of D5522 and lipids that spontaneously form liposome-like structures when hydrated. In the subsequent presentation the

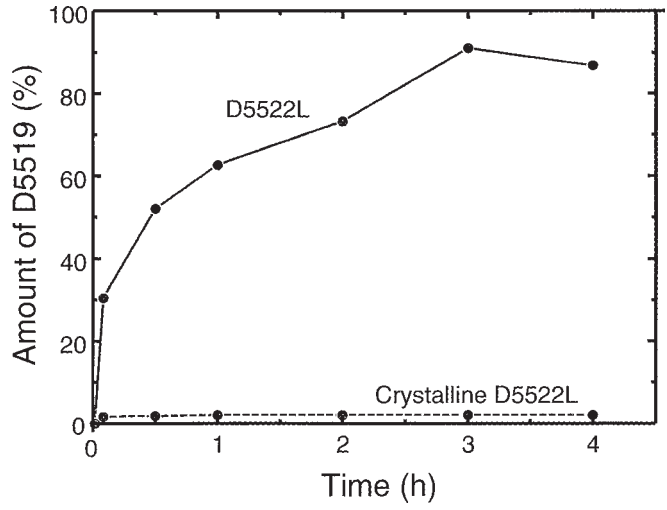


**Figure 2** Generation of D5519 from D5522 after exposure to pancreatic lipase. D5522L (solid line) and crystalline D5522 (dotted line) were incubated with pancreatic lipase in vitro. At different time periods an aliquot of the incubation mixture was analyzed and the amount of D5519 determined. The amount of D5519 is expressed as a percentage of added D5522.

lipid formulation is given the suffix L (D5522L) while D5522 refers to the compound in general.

## IV. Pharmacological Testing

The functional importance of prolonged intraluminal deposition time has been tested in a model of Sephadex-induced lung inflammation in rats. In this model, bronchiolitis and alveolitis are induced following intratracheal instillation of Sephadex beads (see Refs. 9–11). The model is characterized by:

A bronchiolitis with infiltration of white blood cells, which include a high proportion of eosinophils
An alveolitis, which develops as an interstitial edema, followed by granuloma formation
Impaired respiration
Bronchial hyperresponsiveness

Using edema formation as an easily quantifiable marker of inflammation, D5522L administered by inhalation demonstrated a 10-fold better anti-inflammatory potency than budesonide (Fig. 3). Furthermore, D5522L achieved a superior

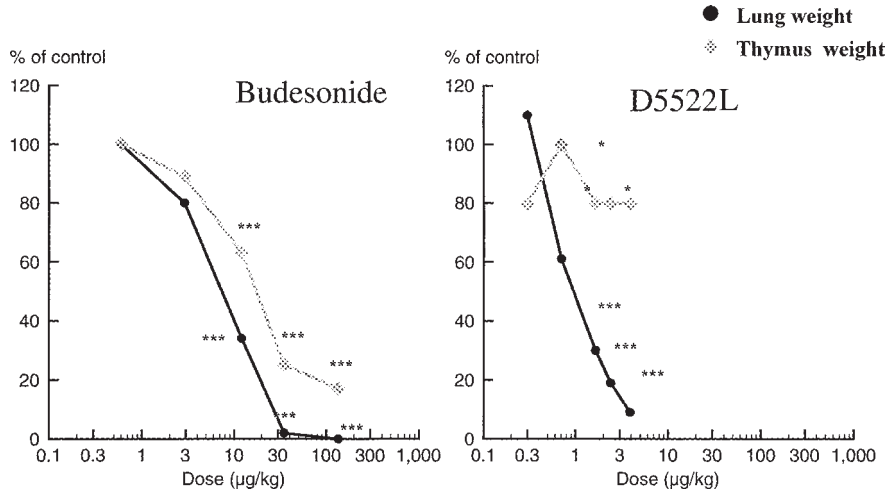


**Figure 3** Local and systemic effects of inhaled budesonide and D5522L in a Sephadex-induced lung alveolitis/bronchiolitis model in rats. Sephadex was instilled on day 1, and the rats were administered steroids by inhalation daily up to day 4 and then sacrificed on day 5. The data represent mean values, $n = 6$. *$p < 0.05$; ***$p < 0.005$.

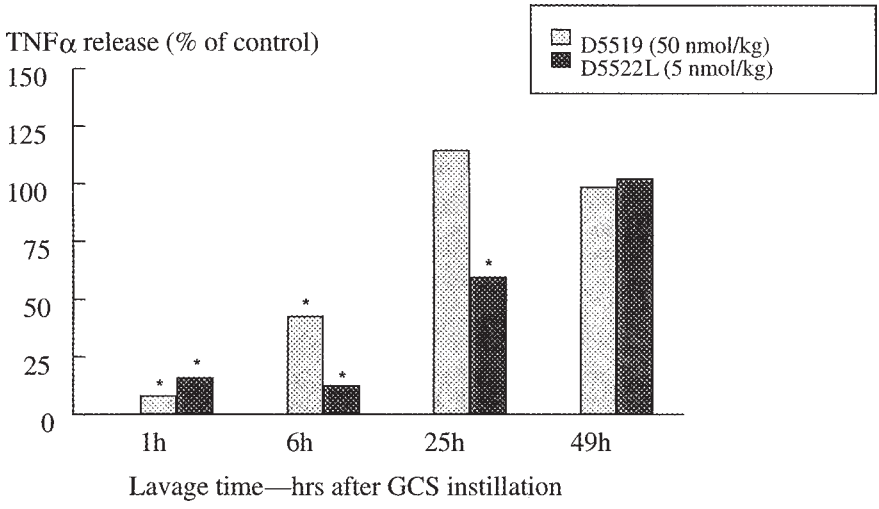


**Figure 4** Effect of hydrated D5522L and D5519 on LPS-induced TNFα release from BAL cells ex vivo after in vivo dosing. Lavages were performed 1, 6, 25, and 49 hours after i.t. instillation of the GCS. The data represent mean values, $n = 8$. $^*p < 0.05$ compared with saline-treated animals.

therapeutic ratio when the antiedema and systemic effects were compared. Thus, D5522L inhibited lung edema formation by 90% with just a 20% reduction of thymus weight. By contrast, an equieffective anti-inflammatory dose of budesonide was accompanied by an 80% reduction in thymus weight. These in vivo studies therefore confirm the topical selectivity of D5522L compared with budesonide.

The alveolar macrophage is an important inflammatory cell in the lung, and consequently a likely target for steroid action. To determine whether alveolar macrophages contribute to the hydrolysis and efficacy of D5522, BAL cells (consisting predominantly of macrophages) from rats instilled with D5522L were investigated ex vivo with regard to distribution, duration, and function of the compound. When steroid uptake and distribution into alveolar macrophages were compared some hours after intratracheal instillation of either radioactively labeled and hydrated D5522L or D5519, there was significantly higher incorporation of D5522L than of D5519 (data not shown). To study the functional relevance of this enhanced and prolonged cellular disposition, BAL macrophages were stimulated ex vivo with LPS and the release of TNFα was measured. Both D5522L and its active component D5519 completely inhibited TNFα release by cells collected 60 minutes after steroid instillation. D5522L was 10-fold more potent in this respect (Fig. 4). In BAL cells taken 6 hours after steroid administration, complete inhibition of TNFα release by D5522L was still apparent, but only weak inhibi-

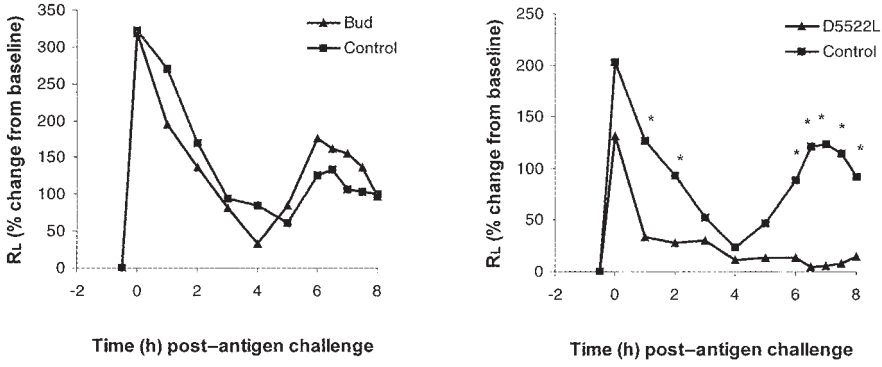


**Figure 5** Effect of *Ascaris suum* challenge on lung resistance (RL) following administration of GCS (triangles) or placebo (squares) 24 and 36 hours before antigen challenge. The data represent mean values, $n = 4$. *$p < 0.05$ compared with control animals.

tion was observed in cells from animals administered with D5519. When the cells were assayed for TNFα release 25 hours after steroid administration, only the inhibitory effect of D5522L was significant. Both compounds were inactive when BAL cells were tested 2 days following steroid administration.

The prolonged, topical antiallergic efficacy of D5522L compared to budesonide has also been documented in the allergic sheep model (W. Abraham, unpublished). Development of the early and late phase reactions after allergic provocation of *Ascaris suum*–sensitive sheep were studied in this well-characterized model (12,13). Pretreatment with budesonide (300 μg by inhalation 0.5 and 12 hours before challenge) resulted in 50% inhibition of the early phase reaction and total inhibition of the late phase reaction. A similar level of inhibition effect was obtained with D5522L but at lower doses (2 × 100 ug) (data not shown). However, when compounds were administered 36 and 24 hours prior to allergen challenge, the effect of budesonide was lost, whereas D5522L still retained its inhibitory activity in both early and late phase reactions (Fig. 5). This clearly demonstrates that D5522 in lipids possesses a prolonged duration of action compared with conventional GCS.

## V. Deposition, Luminal Spreading, and Uptake Mechanisms of D5522L

Inclusion of the naturally occurring phospholipid components of D5522L permits a rapid hydration of the steroid in the phospholipid bilayer. Figure 6 shows the hydration of a D5522/lipid powder after administration to rat trachea. Five minutes

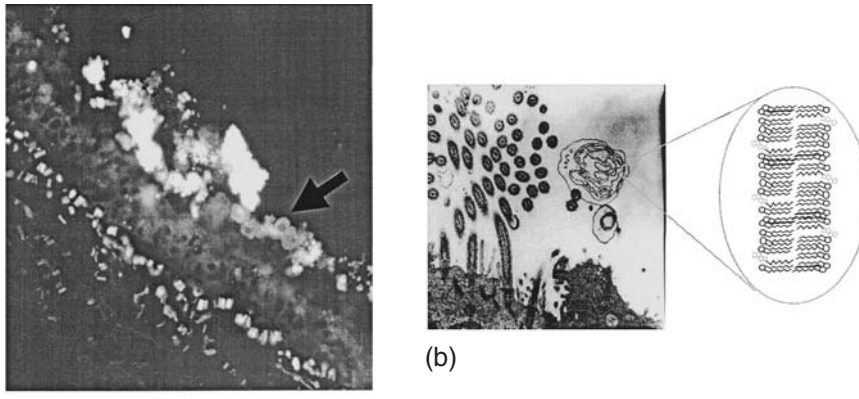


**Figure 6** (a) Image showing hydration of fluorescent D5522L powder after administration to rat trachea. The fluorescent probe was located in the phospholipid moiety. The black arrow indicates the presence of circular liposome-like structures and the bright white area is D5522L powder. The dot-like appearance in the subepithelial region is caused by autofluorescence. (b) Electron micrograph showing formed liposomes in the trachea.

after exposure, a substantial amount of fluorescent lipid material was observed in the upper and lower trachea (Fig. 6a). The material adhered to the epithelial surface, most probably embedded in the mucus layer (invisible in these images). The presence of large liposome-like structures formed from partly hydrated powder is clearly observed in Figure 6b.

The time course for the airways/lung distribution of radioactively labeled and hydrated D5522L (labeled in steroid moiety) following instillation into rat trachea is shown in Figure 7. Samples from different areas of airway/lung were excised at 5 minutes (the earliest possible time point) and 60 minutes, and the total radioactivity was measured. Sixty minutes after administration, the total amount of radioactivity was similar to the total amount measured at 5 minutes. This demonstrates the slow uptake of D5522 and is in accordance with previous studies with liposomal preparations of budesonide palmitate (8). Analysis of individual lung sections, however, revealed significant differences in the distribution of D5522 at 5 minutes and 1 hour. D5522 was found to redistribute over this time period from its initial deposition in the upper and lower trachea to more peripheral lung regions. The mechanism of redistribution is unknown, but the surface properties of the lipid formulation, and the small volume applied (1.5 μL), suggest that it may occur via the lung surfactant layer.

D5522 requires hydrolysis to the active compound D5519 in order to exert its anti-inflammatory activity. Therefore, a kinetic comparison between the con-

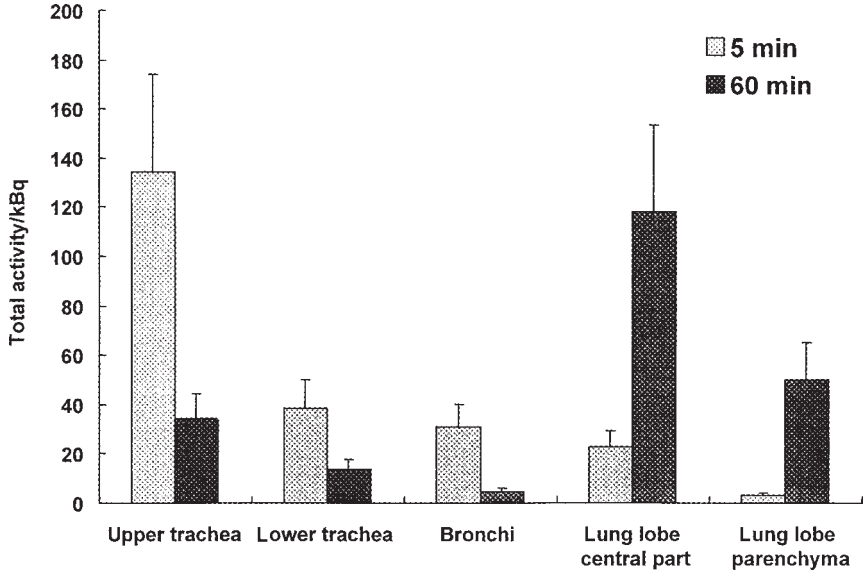


**Figure 7** Distribution of radioactively labeled and hydrated D5522L (labeled at the steroid moiety) after intratracheal administration to rats. Samples from different areas of the lung were excised 5 and 60 minutes after steroid administration, and associated radioactivity was measured.

centration of *active* GCS in the trachea and lung following inhalation of D5522L and those obtained following inhalation of budesonide was carried out in rats (Fig. 8). Following administration of equimolar doses of compound, the animals were sacrificed and the trachea and lung excised. D5519 and budesonide were extracted from the tissues and the concentrations of steroids were assayed by a combination of LC-MS/MS. During the first 4 hours following inhalation, the tracheal budesonide concentration was markedly higher than that of active D5519, but at later time points the concentrations equalized. However, there was a marked difference in the lung tissue levels. With the exception of the first hour following administration, a much higher peripheral lung concentration of active D5519 was found compared with budesonide. This observation agrees with the data reported above (Figs. 3 and 4) of a significantly higher topical anti-inflammatory efficacy and selectivity of the prodrug D5522L in the peripheral lung compared to a conventional inhaled steroid.

The disposition of the D5522 and the phospholipid moieties of hydrated D5522L have been analyzed at various time points in the rat. In two separate experiments, the disposition of radiolabeled D5522 (labeled in the steroid moiety)

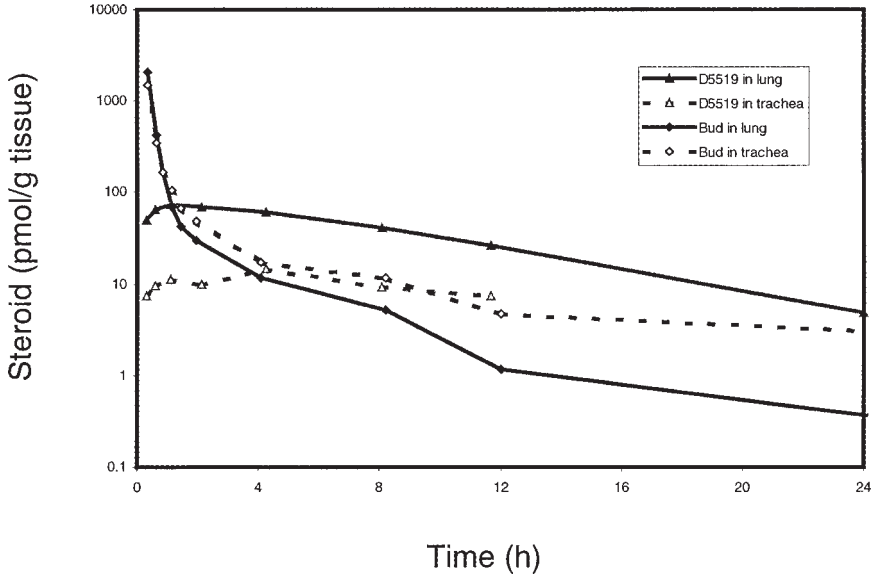


**Figure 8** Tissue concentration of D5519 and budesonide in lung and trachea after inhalation of D5522L and budesonide, respectively. D5519 and budesonide were extracted from the tissues and the amount of steroid was quantified using LC-MS/MS.

and dipalmitoyl-phospatidylcholine (labeled in the choline moiety) were determined at different times in tracheal and lung samples (Fig. 9). The similar distribution of the two compounds suggested that the clearance of D5522 from the airways was mechanistically coupled to that of the carrier lipid and could therefore explain the prolonged deposition and slow tissue uptake/release of D5522/D5519. It might also imply that the two compounds behave similarly due to the similar physiochemical properties of the phospholipid and lipophilic prodrug.

The similar uptake and disappearance of D5522 and phospholipid in the absence of lipase activity (in the BAL fluid) suggested that a significant proportion of D5522 is taken up and activated in the form of a "lipid complex." The low transport of hydrated D5522L across epithelial cells in vitro (data on file at AstraZeneca) suggests that the release and uptake of D5522 from the lipid complex requires the participation of target cells with phagocytic or endocytic capacity. In view of the likely fusion of inhaled endogenous lipid with natural surfactant (14), the target cells are those involved in the turnover of natural surfactant, i.e., alveolar macrophages, Clara cells, and type II cells (14, 15). The current hypothesis for the distribution and uptake mechanisms of D5519 from the D5522L formulation are summarized in Figure 10.

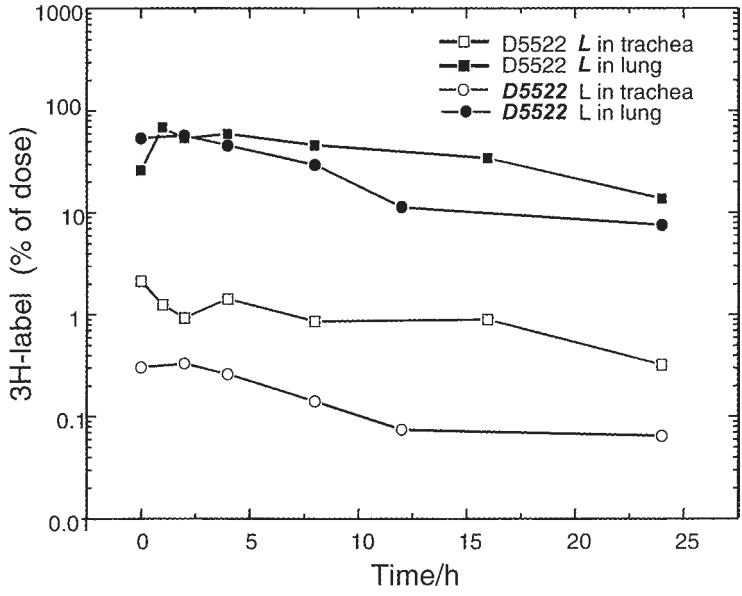


**Figure 9** Tissue levels over time following intratracheal instillation of hydrated D5522L to rats. Tritiated phosphatidyl choline (squares) and D5522 (circles) were administered at separate occasions as D5522L. The amount of radioactivity in lung and tracheal tissues is expressed as percentage of administered radioactive dose.

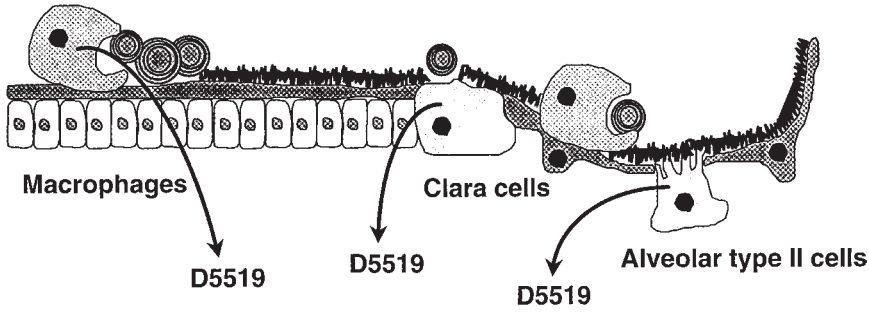


**Figure 10** Schematic presentation of proposed cells involved in the activation of D5522 to D5519 in the lung from hydrated D5522L.

## VI. Conclusion and Prospects

The potent and prolonged anti-inflammatory efficacy of the D5522L formulation results from the slow activation of the prodrug and localized release of the very potent steroid, D5519. Administration of the D5522L powder to the respiratory tract causes a rapid hydration of this formulation and leads to the formation of a dispersed, mobile, slow-release reservoir of D5522 in the airway lumen. Pharmacokinetic studies in dog and humans suggest that the majority of D5522 deposited in the airways/lung is absorbed as D5519 over several hours (mean absorption time in humans is 14 hours). The resulting plasma steroid levels are quite low and comprise very little intact D5522, indicating the efficiency of the hydrolytic process (data in files of AstraZeneca). A minor fraction of the airway-deposited steroid may be transported, by mucociliary clearance, to the oropharynx and swallowed, ultimately being inactivated in the liver and/or gut. BAL fluid does not release D5519 from D5522L to a significant extent (in vitro data), which suggests that the uptake/hydrolysis of D5522 is tissue-associated. Consequently, low levels of active steroid are slowly released in the peripheral part of lung. The kinetics of this process differs from steroid disposition following administration of a conventional inhaled steroid. However, even at central airway level the D5522L formulation exerts anti-inflammatory activity in human airways as it in trials in asthma mediates an anti-asthmatic efficacy similar to budesonide. The kinetic and dynamic results with D5522L in rat lung models suggest, however, that optimal exploitation of this new formulation principle would be in conditions characterized by peripheral airways inflammation, for example, COPD and lung parenchymal disorders, where there is an urgent unmet need for improved and safer therapy. Here, the D5522L formulation serves as a novel lead for the future development of lipophilic prodrugs, where this formulation principle can markedly enhance both topical efficacy and selectivity of local steroid action.

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See page 538 for a joint discussion of Chapters 21 and 23.

MEDICAL DOCUMENTATION

# 24

# Transcription Factors AP-1 and NF-κB as Targets for Development of Anti-inflammatory Drugs

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## I. Introduction

During the past decade there has been a major paradigm shift in the approach to drug development. The commonly used approach has been based to a large extent on the generation of applicable animal models for human disease and the use of such animal models for screening of drug candidates. In rare situations, the cellular or molecular basis for the disease has been understood at a level that allowed the development of drugs based on defined cellular or molecular targets. The new approach that is slowly taking over the drug development field is based on identification of well-defined molecular targets whose inhibition or activation are expected to result in a therapeutic effect. Once such molecular targets are defined, they are used for establishment of high throughput assays that are used for screening of chemical libraries to identify potential drug candidates. The inflammation field provides excellent examples for this paradigm shift. While early efforts were mostly focused on the development of useful animal models for human inflammatory diseases, most of the current effort has been invested in the identification of relevant molecular targets, which can be used to develop high throughput screens for potential anti-inflammatory drugs.

Because most of the long-term effects of inflammation are due to changes in gene expression, much effort has been placed on the identification of transcription factors involved in the activation of inflammation-induced genes and genes encoding inflammatory mediators. Although several transcription factors involved in the induction of such genes have been identified, this review is focused on only two: AP-1 and NF-κB.

## II. Mechanisms of AP-1 and NF-κB Activation

AP-1 is a dimeric, sequence-specific transcriptional regulator composed of members of the Jun and Fos families of basic region-leucine zipper (bZIP) DNA-binding proteins (1). AP-1 is not a single protein but a collection of Jun:Jun and Jun:Fos dimers that can bind to the AP-1 recognition site. Owing to the large number of *jun*, *fos*, and related genes, the regulation of AP-1 activity is complex and occurs at several distinct levels (2). Most relevant to inflammation is the regulation of AP-1 activity by members of the mitogen-activated protein kinase (MAPK) family. These protein kinases, which include the ERKs, the JNKs, and the p38s, migrate to the nucleus upon their activation in response to proinflammatory stimuli and phosphorylate transcription factors, including the Jun proteins, that are involved in the induction of AP-1 activity (3–5). All MAPKs appear to be capable of phosphorylating members of the TCF transcription factor family at their C-terminal activation domain and thereby enhance their ability to induce *fos* gene transcription (6,7). Members of the JNK group, but not other MAPKs, phosphorylate the activation domains of Jun and ATF2 proteins and enhance their ability to activate target genes, including the *c-jun* gene, which codes for a major component of AP-1 (8–11). The p38s, on the other hand, phosphorylate and activate members of the MEF2 group of transcription factors, some of which are also involved in induction of *c-jun* transcription (12,13). Thus MAPKs contribute to induction of AP-1 activity both by enhancing the transcription of *jun* and *fos* genes, leading to higher levels of Jun and Fos proteins, which bind AP-1 sites as dimers, and by increasing the transcriptional activity of both newly synthesized and preexisting Jun proteins (2).

Like AP-1, NF-κB is also a dimeric, sequence-specific transcriptional regulator. NF-κB dimers are composed of members of the Rel family of DNA-binding proteins and, like AP-1, represent a heterogeneous collection of dimers with different activities that bind to a common DNA sequence—the κB site (14,15). NF-κB activity is regulated at several different levels, through a number of distinct mechanisms, but one form of regulation based on cytoplasmic-nuclear distribution predominates: In nonstimulated cells, NF-κB dimers are kept in the cytoplasm through interaction with specific inhibitors—the IκB proteins. All IκBs contain a common core domain composed of seven ankyrin repeats, which di-

rectly contact the core domain of all NF-κB proteins—the Rel homology domain (16–18). Through steric hindrance, the first two ankyrin repeats of IκB prevent the recognition of the nuclear localization sequence (NLS) present in the C-terminal portion of the Rel homology domain (16,17). The major IκB proteins (IκBα, IκBβ, and IκBε) also contain an N-terminal regulatory domain with two conserved serine residues (S32 and S36 in IκBα) that are rapidly phosphorylated in response to different pro-inflammatory stimuli (18). Once N-terminally phosphorylated, the IκBs, still bound to NF-κB dimers, are recognized by an E3 ubiquitin ligase complex, $E3^{I\kappa B\alpha}$ (19). The binding of the $E3^{I\kappa B\alpha}$ complex results in recruitment of an E2 ubiquitin conjugating enzyme, which catalyzes the transfer of ubiquitin to a conserved arginine found just N-terminally to the IκB phosphorylation sites (18). Polyubiquitinated IκB is then recognized by the 26S proteasome, resulting in its rapid degradation. The released NF-κB dimers are then transported to the nucleus, where they bind κB sites on target genes to induce their transcription.

Although this elaborate pathway relies on the concerted action of a large number of different proteins, it is the activation of a single enzyme, the IκB kinase (IKK), that sets the entire process in motion. IKK is a complex composed of three subunits, IKKα, IKKβ, and IKKγ (20–23). Whereas IKKα and IKKβ are the catalytic subunits of the complex, IKKγ is the regulatory subunit. IKKα and IKKβ form homo- and heterodimers via a leucine zipper motif, and this dimerization is essential for their activity and/or activation (24,25). The IKKα/IKKβ dimers associate through an interaction with an as-yet undetermined number of IKKγ molecules to form a large IKK complex, which is a dimer of IKKα/IKKβ dimers (25). In addition to formation of this large complex, the IKKγ subunit is required for responsiveness to yet-to-be-identified IKK activators (23,26). These activators are most likely IKK kinases (IKK-Ks), which activate IKK via phosphorylation of two conserved serines at the activation loops of IKKα and IKKβ (27).

Although purified recombinant forms of IKKα and IKKβ display the same substrate specificity, phosphorylating the two serines that are needed for IκB ubiquitination, IKKβ is a more efficient IκB kinase than IKKα (Y. Chen and M Karin, unpublished results). These results are consistent with those of genetic analysis that point out clear functional differences between IKKα and IKKβ (28–30). Although IKKα-deficient mice exhibit severe developmental abnormalities, which indicate a role for IKKα in the differentiation of epidermal keratinocytes and possibly other cell types, the activation of IKK and NF-κB in response to proinflammatory stimuli seem to be perfectly normal in $Ikka^{-/-}$ mice and cells (28). By contrast, IKKβ-deficient mice die at mid-gestation due to massive liver apoptosis, a phenotype that is essentially identical to that of mice with targeted disruption of the *RelA* gene, which codes for the major NF-κB p65 subunit (29,30). The liver apoptosis in both of these mutants is due to the absence of NF-κB activity which is normally required for counteracting the pro-apoptotic effect of tumor necrosis

factor (TNF)α, which is produced in large amounts by fetal liver (31). Consistent with these results, cells derived from *Ikkβ*$^{-/-}$ mice exhibit severe defects in activation of IKK and NF-κB in response to proinflammatory stimuli (29).

## III. Involvement of AP-1 and NF-κB in Inflammatory Responses

Several lines of evidence indicate that AP-1 and NF-κB are instrumental in gene induction responses triggered by proinflammatory stimuli. First, the protein kinases involved in the activation of AP-1 and NF-κB, the MAPKs and IKK, respectively, are rapidly activated in cells exposed to proinflammatory stimuli, including interleukin (IL)-1, TNFα, components of bacterial cell walls, and double-stranded (ds) RNA, a product of viral infections (32,33). Second, transgenic or knockout mice and cells that are defective in activation of AP-1 or NF-κB exhibit deficiencies in induction of cytokines, chemokines, and other molecules involved in inflammatory responses (33–36). For instance, T cells derived from *Jnk1*$^{-/-}$ or *Jnk2*$^{-/-}$ mice exhibit defects in expression of several cytokines, including IL-2, IL-4, and γ-interferon (IFN), that are normally induced in response to antigenic stimulation (34,35). *Jnk2*$^{-/-}$ fibroblasts exhibit defective induction of type I IFN in response to dsRNA and IL-6 and IL-12 in response to dsRNA, IL-1, or LPS. Defects in induction of type I IFN and other cytokines are also exhibited by *Ikkβ*$^{-/-}$ fibroblasts (33,37), and lethally irradiated mice whose hematopoietic system was reconstituted with *Ikkβ*$^{-/-}$ stem cells exhibit major defects in the development and activation of B and T cells in response to antigenic stimulation (U. Senftleben, Z.-W. Li, and M. Karin, unpublished results).

Although the proinflammatory stimuli that lead to activation of AP-1 and NF-κB are diverse, at least some of them act through common mechanisms. The binding of IL-1 to its heterodimeric receptor results in the recruitment of several signaling proteins, including a protein called TRAF6, to the cytoplasmic domain of the signaling subunit of the IL-1 receptor (38,39). The latter protein is a member of the Toll family, which includes several other proteins, called Toll-like receptors (TLR), that function as the signaling subunits of receptors for components of bacterial cell walls, such as lipopolysaccharide (LPS) and lipoteichoic acid (40). The signals delivered both by IL-1R and the TLRs depend on the recruitment of TRAF6 (41). Two other members of the TRAF family, TRAF2 and TRAF5, are recruited to TNF receptors and are required for activation of various effector functions, including JNK and IKK activation. The mechanism of TRAF2- and TRAF6-mediated signaling was examined in detail and found to be based on the activation of various downstream effector proteins via a N-terminal effector domain (32). One of the proteins that interact with the N-terminal effector domain of TRAF2 is the MAPKKK MEKK1, whose catalytic activity is stimulated through

this interaction (32). Through the generation of *Mekk1*$^{-/-}$ cells, MEKK1 was shown to be required for JNK activation by TNFα, IL-1, LPS, and dsRNA (Y. Xia et al., unpublished).

Other evidence for the importance of AP-1, JNK, NF-κB, and IKK comes from the genetic analysis of innate immune responses in *Drosophila*, which are functionally similar to innate immune and inflammatory responses in mammals (5,40). Genetic and functional analysis has provided ample evidence for the conservation of the signaling pathways leading to activation of members of the AP-1 and NF-κB families in response to challenge of *Drosophila* larvae with various proinflammatory stimuli, including LPS.

## IV. Physiological Inhibitors of Inflammation, AP-1 and NFκB

Further evidence for the importance of AP-1 and NF-κB in activation of inflammatory responses comes from the effect of physiological inhibitors of inflammation on AP-1 and NF-κB or on the pathways involved in their activation. First and foremost among these inhibitors are glucocorticoid (GC) hormones. GC are released by the adrenal gland and for a long time have been known as potent negative regulators of inflammation and immune responses (42). Both naturally occurring and synthetic GC have been used since the late 1940s as anti-inflammatory and immunosuppressive drugs (43,44). Despite severe side effects, GC are still the drug of choice for treatment of certain chronic inflammatory diseases, such as asthma (45). GC are known to inhibit the induction of most cytokines and chemokines, including IL-1, IL-2, IL-3, IL-6, γ-IFN, TNFα, and GM-CSF (46). They also block the induction of metalloproteinases, such as collagenase and stromelysin, in response to proinflammatory stimuli (47,48).

Analysis of the mechanism responsible for inhibition of collagenase gene induction revealed that GC inhibit the activity of AP-1, the transcription factor responsible for collagenase induction in response to proinflammatory stimuli (47,48). The inhibition of AP-1 activity is most likely due to direct interaction between Jun proteins and the activated GC receptor (46–48). A dimerization defective mutant of the GC receptor ($GR^{Dim^-}$) fails to bind DNA and induce expression of GC-regulated genes but can still inhibit the activity of AP-1 and block the induction of collagenase and other genes that are induced in response to proinflammatory stimuli (49). Mice that carry the $GR^{Dim^-}$ mutation in their genome are viable, although they fail to induce classical GC target genes, such as tyrosine aminotransferase, in response to hormonal treatment (50). Most importantly, however, in these mice GC can still inhibit AP-1 activity and prevent the induction of AP-1 target genes (50). These results suggest that the most important function of the GR is negative regulation of inflammation-induced genes rather than induc-

tion of GC response genes (46). However, while these results confirm the in vivo importance of the inhibition of AP-1 activity, they shed little new light on the mechanism by which GR exerts this function. Although initially it was believed that the interaction with GCR inhibits the binding of AP-1 to DNA (48), further work suggested that AP-1 remained bound to DNA in GC-treated cells in which the induction of AP-1 target genes is inhibited (48). It is possible that docking of GR onto AP-1 prevents a productive interaction between AP-1 and components of the basal transcriptional machinery. Alternatively, GR can compete with AP-1 for binding of an important co-activator.

Another gene induced by various proinflammatory and antigenic stimuli is the IL-2 gene, which codes for a major T-cell mitogen. Induction of IL-2 transcription is also inhibited by GC and part of this effect is due to inhibition of AP-1 activity (51). However, detailed analysis of targets for GC-mediated inhibition within the IL-2 promoter revealed that some of the inhibitory activity is also targeted towards an NF-κB binding site (51). Indeed, GC were found to inhibit the activation of NF-κB by a variety of proinflammatory stimuli (51,52). Two mechanisms were proposed to account for the observed inhibition. First, GC were found to induce the expression of IκBα (51,52). Once IκBα accumulates it translocates to the nucleus to induce the export of NF-κB to the cytoplasm. In support of this mechanism, the inhibition of NF-κB by GC was shown to require new protein synthesis (51). A second mechanism was proposed to be based on direct interaction between GR and NF-κB, similar to the interaction between GR and AP-1 (53). However, in those cell lines where the subcellular distribution of NF-κB was examined, it was noted that whereas the activated GC receptor resides in the nucleus, the inhibited NF-κB dimers are located in the cytoplasm as long as the cells are exposed to GC (51). Regardless of the details of the mechanism by which GC inhibit AP-1 and NF-κB, this inhibition is likely to account for most of their anti-inflammatory and immunosuppressive activity (46).*

Another class of compounds recently identified as physiological inhibitors of inflammation are cyclopentenone prostaglandins (PGs), most notably 15-deoxy $PGJ_2$ (54,55). Cyclopentenone PGs have been known for quite some time to be capable of inhibiting NF-κB activation or activity (56). Neither the mechanism nor the physiological significance of this inhibition was clear until recently. Paradoxically, like other PGs, the synthesis of $PGJ_2$ depends on the activity of cyclooxygenase (COX), especially COX2, which serves as a major target for nonsteroidal anti-inflammatory drugs (NSAIDs). It was recently observed, using a rat model for lung inflammation (pleurisy), that administration of a COX2 inhibitor

*Despite this important realization, so far it has been rather difficult to separate the transactivating function of GC from their transrepressing function through structural alteration of the GC molecule itself.

prior to the induction of inflammation prevented the onset of inflammation, exactly as expected (54). However, when the COX2 inhibitor was given 24 hours after induction of inflammation, it prolonged, rather than terminated, the inflammatory episode (54). This effect was attributed to inhibition of 15-deoxy $PGJ_2$ synthesis, which unlike the synthesis of PGE, an established inflammatory mediator, peaks at later stages of inflammatory episodes (54). The analysis of the mechanism by which 15-deoxy $PGJ_2$ inhibits NF-κB activation revealed that 15-deoxy $PGJ_2$ is a potent inhibitor of IKK activity (55). Strong but indirect evidence suggests that 15-deoxy $PGJ_2$ directly reacts with a cysteine residue within the activation loops of IKKα and IKKβ and thereby prevents their activation. A mutant of IKKβ in which this cysteine has been replaced with an alanine is no longer sensitive to inhibition, and its transient expression prevents the inhibition of NF-κB activation by 15-deoxy $PGJ_2$ (55). These findings suggest the existence of a negative regulatory loop that promotes resolution of inflammation. Activation of NF-κB results in induction of various target genes, including the one coding for COX2 (57). Elevated expression of COX2 results in increased production of PGs. Early in inflammation, COX2 catalyzes mostly the production of inflammatory PGs, such as PGE, but later in inflammation, due to an unknown switch in specificity or subcellular localization, it catalyzes mostly the production of anti-inflammatory cyclopentenone PGs, such as 15-deoxy $PGJ_2$ (54). As 15-deoxy-$PGJ_2$ forms covalent adducts with IKK catalytic subunits, its increased production results in slow but sustained inhibition of IKK, thereby promoting the resolution of inflammation (55).

Another negative regulator of inflammation is the cytokine IL-10, which can suppress the synthesis of IL-1, IL-2, IL-6, IL-8, TNFα, IFNγ, and other cytokines by activated leukocytes (58). This inhibition occurs mostly at the transcriptional level (59). Examination of the effect of IL-10 on various transcription factors involved in inflammation revealed that it inhibits the activation of NF-κB but has no effect on AP-1 or another bZIP transcription factor, NF-IL6 (60). So far, the mechanism by which IL-10 inhibits NF-κB activation is not understood. Nevertheless, IL-10 is a promising anti-inflammatory drug for treatment of chronic disorders, such as inflammatory bowel disease (61).

## V. Development of AP-1 and NF-κB Inhibitors

The findings discussed above suggest that inhibitors of AP-1 and NF-κB or of the signaling pathways that lead to their activation can be developed into clinically useful anti-inflammatory drugs. The development of such drugs, however, depends on the identification of potent and specific AP-1 and NF-κB inhibitors. Several approaches can be taken to the development of such inhibitors. At first glance, the simplest approach would be to target the DNA-binding domains of these tran-

scription factors and screen for or develop small molecules that will inhibit their binding to DNA. It has been possible to develop DNA-based decoys that titrate sequence specific transcriptional regulators and thereby prevent their binding to target genes. However, such decoy molecules are relatively large and, being some type of an oligonucleotide and therefore highly polar, they do not enter cells very well. Furthermore, due to the high affinity with which AP-1 and NF-κB bind to their specific DNA sites, it is rather unlikely that small, cell-penetrating molecules will be able to interact with their DNA-binding domains with the same or higher affinity and specificity as the natural DNA target. As DNA binding by both AP-1 and NF-κB factors requires the formation of protein dimers, it seems plausible that another approach to inhibit their binding to DNA could be based on targeting of their dimerization domains. However, structural analysis indicates that both AP-1 and NF-κB proteins use rather large and hydrophobic interaction surfaces to mediate dimerization, and it is therefore rather unlikely that small molecules will be able to disrupt such highly efficient interactions.

In light of these considerations it appears that the protein kinases involved in the activation of AP-1 and NF-κB may be the preferred and most susceptible targets for drug-mediated inhibition. Indeed, protein kinases are enzymes and therefore act catalytically rather than stoichiometrically. Also, as enzymes, protein kinases possess a well-defined catalytic pocket that is engaged in binding of a relatively small molecule—ATP (62,63). Although all protein kinases bind ATP, the residues that line up the ATP-binding site differ from one kinase to the other, thus providing an opportunity for the development of specific inhibitors, all of which compete with the binding of ATP. Initially, protein kinase inhibitors were identified through screening of natural product libraries as well as defined chemical libraries. Such screens have resulted in the identification of quite a number of different and relatively specific protein kinase inhibitors. Biochemical analysis of the mechanism by which such inhibitors affect their protein kinase targets revealed that most of them are competitive inhibitors of ATP binding and therefore are likely to target the ATP-binding site (64). Indeed, in a few cases structural studies have confirmed the binding of protein kinase inhibitors at the ATP-binding site (64). Such results have led to the development of a more specific and systematic approach to the identification of protein kinase inhibitors. This approach is based on the preparation of highly diverse combinatorial libraries based on an adenine nucleus (65). Such libraries contain millions of different ATP analogs and therefore should be a very rich source for competitive inhibitors of ATP binding. Preliminary analysis of such libraries have already resulted in the identification of relatively potent and specific protein kinase inhibitors (65). Using such libraries as sources of inhibitors and purified recombinant forms of MAPKs and IKK, it is only a matter of time until specific MAPK and IKK inhibitors will be identified and tested for their ability to inhibit the activation of AP-1 and NF-κB and thereby prevent the induction of inflammatory responses.

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## Discussion

**Dr. Brattsand:** Do you see JNK inhibitors mainly as a substitute for or as a complement to glucocorticoids? Do you think that JNK activation contributes to glucocorticoid resistance?

**Dr. Karin:** JNK inhibitors can be used alone as well as as a complement to glucocorticoids.

**Dr. Schleimer:** I was interested to see that the JNK inhibitor blocked TNF and IL-1 production but not IL-8. Does this imply that TNF and IL-1 are not important inducers of IL-8 production? Is the lack of effect on IL-8 production true for other chemokines? Have you used your unique compound libraries to look for inhibitors of JAK enzymes involved in STAT6 activation? Since STAT6 seems to be so important in Th2-cell development, this seems worthwhile. Do you suspect the JNK inhibitors will prove to be equally effective against Th1 and Th2?

**Dr. Karin:** These data are preliminary and certainly don't mean that TNF and IL-1 do not regulate IL-8. No work on JNK inhibitors has been done. In my mind, JNK inhibitors should inhibit both Th1 and Th2 functions.

**Dr. Busse:** The observation that JNK activation could occur in $PKR^{-/-}$ mice is intriguing because this may be a pathway not affected by steroids. The questions I have are, what cell line was this process evaluated in, and what secretory events were evaluated in response to dsRNA?

**Dr. Karin:** The dsRNA work was done in fibroblasts. We found that JNK activation contributes to IL-6, IL-8, and IL-12 induction.

**Dr. Stellato:** It has been shown recently that the ability of JNK inhibitor to downregulate LPS-induced TNFa production from monocytes was lost in mice in which the AURE of the 3′UTR of TNFa mRNA was mutated, implying that JNK targets AURE-dependent posttranscriptional and translational regulatory elements. Do you have any information about the potential targets of JNK in these pathways?

**Dr. Karin:** We are working on the role of JNK in mRNA stabilization, but this work has not progressed to the point where I can answer your question.

**Dr. Georas:** What exactly is the role of calcineurin in mediating activation of JNK, and how do you think JNK inhibitors will compare with some of the selective calcineurin antagonists being developed?

**Dr. Karin:** Calcineurin-mediated Ca affects JNK activity in T cells. JNK inhibitors may generate similar effects to the calcineurin inhibitors in T cells.

**Dr. Denburg:** TNFα has some positive effects on hemopoiesis and also protects against nephritis in lupus-prone MRL mice. Have you examined the effects of JNK inhibitors on hemopoiesis or in MRL mice?

**Dr. Karin:** Not yet, but we know that the related MAPK, p38b is required for erythropoiesis.

**Dr. Brattsand:** When you think forward about potential JNK inhibitors for respiratory diseases, do you think they will be applied by systemic or local administration? From the drug development point of view, it would be easier to reach efficacy and topical selectivity by local therapy.

**Dr. Karin:** Depending on the actual inhibitor eventually developed, it should be useful to use them both systemically and topically. Of course, when possible, topical application may be desired.

MEDICAL DOCUMENTATION

# 25

# Remodeling and the Effects of Steroids in Asthma

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## I. Introduction

Asthma is a chronic inflammatory condition of both "large" and "small" conducting airways: activated T-helper lymphocytes, eosinophils, and mast cells are increased in number, and this is linked to structural changes occurring in the airway wall, the last referred to as airway wall remodeling (1,2). There is an associated increase in airway responsiveness to a variety of stimuli not normally causing such reaction in normal healthy individuals. In addition to the chronic inflammation of asthma, there may be acute bouts of inflammation with episodic symptoms, including wheezing, breathlessness, chest tightness, and cough, usually associated with variable airflow obstruction that is at least partially reversible either spontaneously or following treatment. The reliance on reversibility for the clinical definition of asthma as distinct from the airflow obstruction associated with chronic obstructive pulmonary disease (COPD) is not without difficulty (Fig. 1). While the clinical and tissue differences between the nonsmoking asthmatic with reversible airflow obstruction and the smoker with COPD are reasonably clear (3), a significant proportion of asthmatics (particularly in the older age group) demonstrate relatively poor reversibility. Likewise, many patients with COPD may show sig-

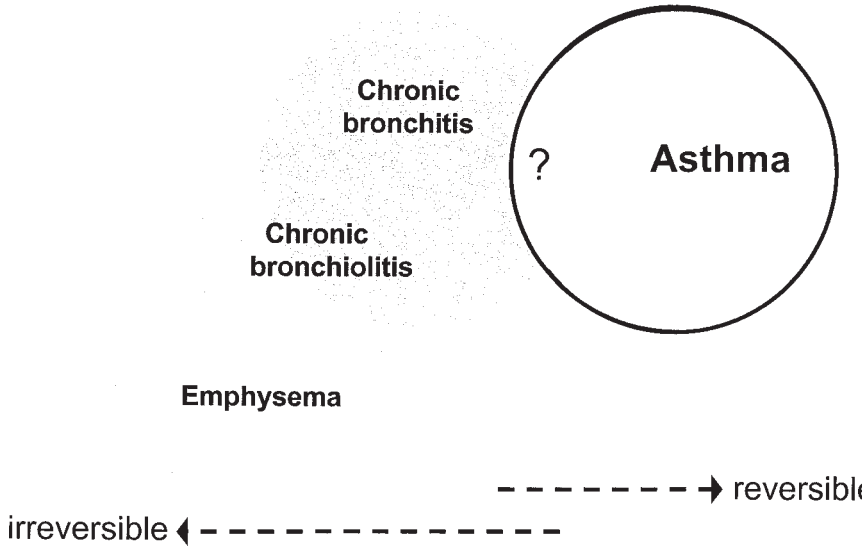



**Figure 1** Interrelationship between asthma and chronic obstructive lung disease (COPD).

nificant airway reversibility, associated with some of the inflammatory and airway structural features of asthma (4).

Inhaled corticosteroids are highly effective agents in the treatment of mild to moderate asthma: they reduce airway inflammation, decrease symptoms, and improve lung function (5–7). However, about 10–15% of asthmatics respond poorly to inhaled or oral corticosteroids, and (as in COPD) this is often associated with accelerated decline in lung function even in the absence of a history of smoking (8,9): symptoms persist and inflammation continues in spite of steroid treatment (Fig. 2). The current premise is that the accelerated decline in forced expiratory flow over time is the result of a shift from episodic acute to chronic inflammation and consequent airway wall remodeling. The remodeling results in a thickening of the airway wall, reduction of the airway lumen, and a marked increase in resistance to airflow (Fig. 3) (10,11).

Acute inflammation is the response of a vascularized tissue to injury: it protects the host and restores tissue to normal by a process of healing in which there is a process of transient remodeling. The concept of "remodeling" implies that a process of "modeling" must have preceded it. For example, the lung in utero undergoes extensive modeling and remodeling: these processes are entirely appropriate to the normal process of lung development. Accordingly, the working definition of remodeling proposed herein is "an alteration in size, mass or number of tissue structural components that occurs during growth or in response to injury and/or inflammation. It may be appropriate, as in lung development or the acute

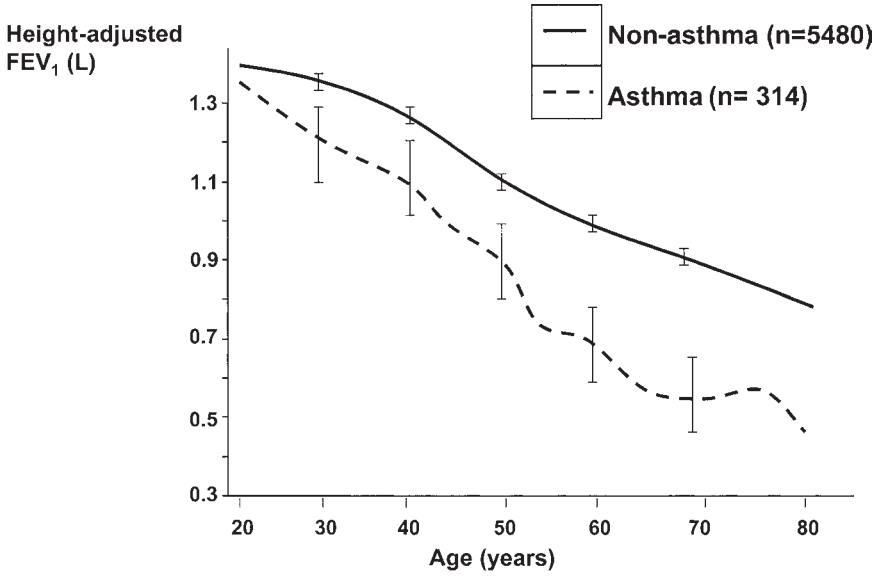



**Figure 2** Decline of lung function ($FEV_1$) in a subgroup of male nonsmokers with severe asthma. (Adapted from Ref. 9.)

reaction to injury, or 'inappropriate' when it is chronic as, for example, in: asthma, COPD or fibrosing alveolitis." For example, in wound healing (in the skin) there is swelling/edema, rapid restitution of the denuded areas by epithelial dedifferentiation, proliferation, and migration from the margins of the wound. This is associated with an inflammatory reaction: i.e., early infiltration of the injured tissue by neutrophils and later by lymphocytes and macrophages. Reticulin is deposited within days, and this may mature to form interstitial collagen, a scar, by 2–3 weeks. In addition, healing may involve contraction of the surrounding tissue by myofibroblasts that may proliferate transiently in relatively large numbers (12,13). Thus, normal tissue architecture is restored consequent to an inflammatory and appropriate remodeling process in which a number of inflammatory cytokines and growth factors are involved. All of the components of wound healing and many of the cytokines involved appear also in asthma, but in asthma both the inflammation and remodeling persist and the consequences are inappropriate to the maintenance of normal (airway) function. The reasons for the persistence are unknown but may be the result of repeated inhalation of allergen or exposure to high concentrations of allergen or a genetically influenced abnormal host inflammatory response or defect in the repair process. It is currently presumed that there is a continuum between the chronic inflammation, remodeling and loss of function in asthma (Fig. 4), but as yet there is no evidence for this. It could be that the

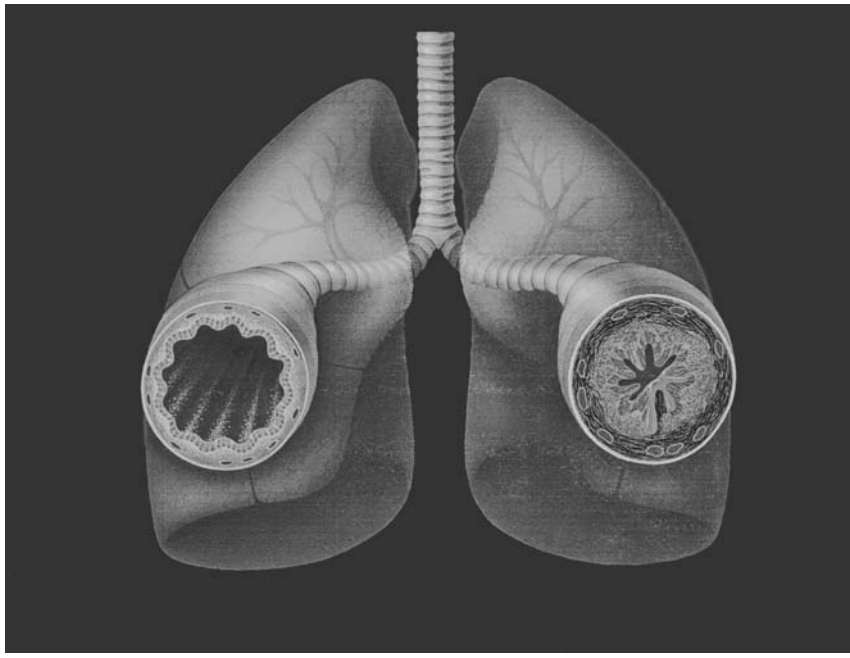


**Figure 3** Diagrammatic representation of the thickened airway wall in asthma (on right) as compared with the normal.

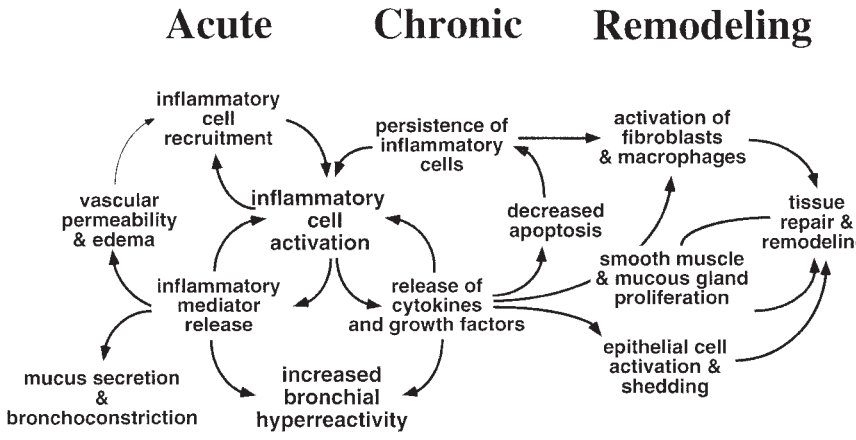



**Figure 4** Interrelationship between acute and chronic inflammation and airway wall remodeling.

mechanisms responsible for the maintenance of chronic inflammation and those that perpetuate remodeling are distinct. Thus it should not be assumed that corticosteroids, acknowledged as powerful anti-inflammatory agents, should also attenuate or reverse the remodeling process. Second, theoretically it would not be helpful to reduce that component of (acute) inflammation that is protective to the host. Thus, the targets for steroid treatment in asthma should ideally be (1) the *chronic* inflammatory elements, e.g., lymphocytes, monocytes/macrophages, and the chronic tissue eosinophilia, and (2) any aspect of the remodeling process that is inappropriate to normal airway and lung function, e.g., goblet cell hyperplasia, reticular basement membrane thickening, and an increase in the mass of bronchial smooth muscle.

The following considers the distinct components of the airway wall that are considered to be "injured" and inappropriately remodeled in asthma and the current evidence and controversies regarding the effects of corticosteroids in influencing such remodeling.

## II. Surface Epithelium

Histologically, damage and shedding of the airway surface epithelium are reported in asthma postmortem (Fig. 5), but this change is highly variable, with some airways having intact surface epithelium even in the presence of marked inflammation and other structural changes. Loss of the epithelium induces a healing process that either results in complete restoration of the columnar/cuboidal epithelium with normal proportions of goblet and ciliated cells or, if the injury is repeated, squamous cell metaplasia and/or goblet cell hyperplasia. Histologically, damage and shedding of airway surface epithelium appears to be an early feature of asthma as it is prominent in biopsy specimens of patients with stable mild disease: it is not a usual feature of smokers with bronchitis or COPD (Fig. 6) (14–16). Loss of superficial epithelium is normally accompanied by mitotic activity in the remaining cells (see Ref. 17), albeit there are unpublished data indicating that the mitotic response in asthma may be abnormally suppressed. Aggregations of platelets together with fibrillary material thought to be fibrin have been observed in association with the damaged surface. The greater the loss of surface epithelium in biopsy specimens, the greater is the degree of airway responsiveness (14). It is recognized that there is inevitably artefactual loss of surface epithelium during the taking and preparation of such small biopsy pieces, even normally, which makes interpretation of the epithelial loss seen in bronchial biopsies difficult (18). In the author's opinion, the observed loss in biopsies reflects the fragility (but not loss) of the epithelium in vivo and the consequent loss of an already loosened epithelium that occurs during the bronchoscopy procedure. The fragility of the epithelium in vivo in asthma is supported by the frequent appearance of clusters of sloughed epi-

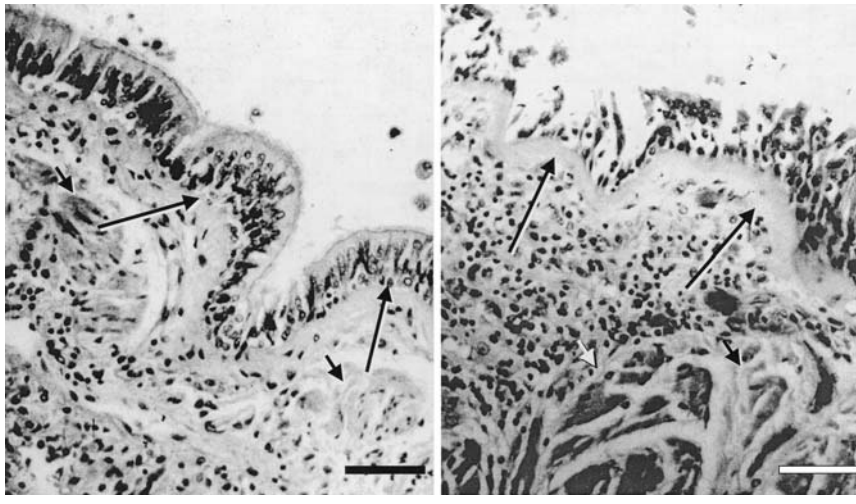
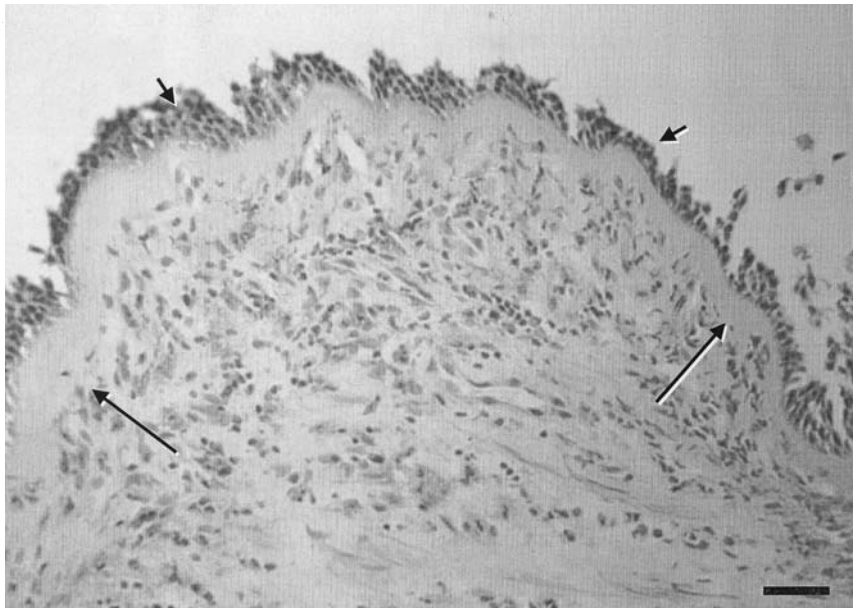



**Figure 5** Histological sections stained with haematoxylin and eosin (H&E). (a) Part of the airway wall of an accidental nonasthmatic death showing intact airway epithelium, a thin reticular basement membrane (RBM) (arrow) and small blocks of airway smooth muscle (ASM) (small arrow). (b) A case of fatal asthma showing sloughing of the surface epithelium, a homogeneously thickened and hyaline RBM (arrow), a marked infiltration of the mucosa by inflammatory cells and enlarged ASM (small arrow). Scale = 80 μm.

thelial cells in sputa (19) and the increased presence of bronchial epithelial cells in bronchoalveolar lavage of asthmatics with mild disease (15).

There are few studies of the effects of steroids on epithelial integrity and epithelial cell composition. The biopsy study by Lundgren and colleagues (20) demonstrated that periods of up to 10 years of treatment of asthmatic patients with inhaled beclomethasone (400 μg daily) were associated with repair of damaged ciliated epithelium as assessed by scanning electron microscopic examination. In another study in which tissues had been prepared for transmission electron microscopy (TEM), bronchial biopsies obtained before randomization were compared with those obtained from patients after 3 months of treatment with 750–1200 μg daily of budesonide (21). Compared to the biopsies taken from subjects given the placebo, TEM of biopsies taken from the steroid-treated group demonstrated a restored ciliated to goblet cell ratio, greater numbers of intraepithelial nerves, and fewer inflammatory cells (21,22).

Epithelial goblet cell hyperplasia and submucosal gland enlargement is a feature of fatal asthma (23). Many asthmatics suffer from excessive production of mucus, which, together with the inflammatory exudate, forms highly tenacious plugs that block the airways (24). Goblet cell hyperplasia is a feature of asthma



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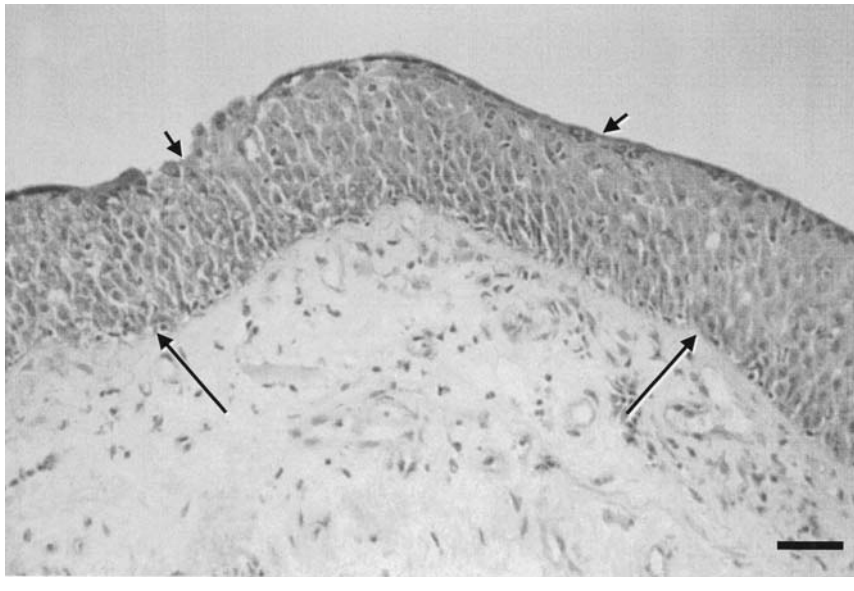


(b)

**Figure 6** H&E-stained sections of bronchial biopsies. (a) Section from a case of mild atopic asthma showing the sloughing of the epithelium (small arrow) and the early thickening of the RBM (arrow). (b) In contrast, a case of a smoker with COPD has an intact epithelium that shows squamous metaplasia (small arrow) and a RBM (arrow) of normal thickness. Scale = 60 μm.

(25,26) and also of bronchitis (27): this aspect of remodeling can also be reproduced in animal (rodent) "models" of these two human conditions following repeated exposure to inhaled allergen or to cigarette smoke, respectively (28–30). For example, 2 weeks of passive daily exposure of laboratory rats to cigarette smoke induces airway goblet cell hyperplasia. The effect of cigarette smoke can be attenuated by concurrent (intraperitoneal) administration of dexamethasone, prednisolone, or hydrocortisone (in order of descending effectiveness) (31). In the sensitized atopic mouse model, repeated intratracheal allergen challenge induces a dose-related goblet cell hyperplasia in the large airways and a mucous metaplasia in the smaller airways that normally lack this cell type: there is also an IL5-dependent eosinophilia (32). When a "subclinical" infection with a strain of human respiratory syncytial virus is superimposed on the model of atopic asthma, there is recruitment of monocytes and lymphocytes to the airways associated with discharge of mucin from goblet cells. Daily systemic treatment with dexamethasone during the period of allergen challenge results in a reduction of the numbers of inflammatory cells and a dose-related suppression of the developing increase in goblet cells (Fig. 7): moreover, similar treatment with the steroid during the resolution phase shortens the time taken to recover to normal (33).

In respect to the epithelium's role in inflammation, there is also experimental evidence in vitro that steroids can attenuate the production of epithelial-derived pro-inflammatory molecules such as eotaxin and monocyte chemoattractant pro-

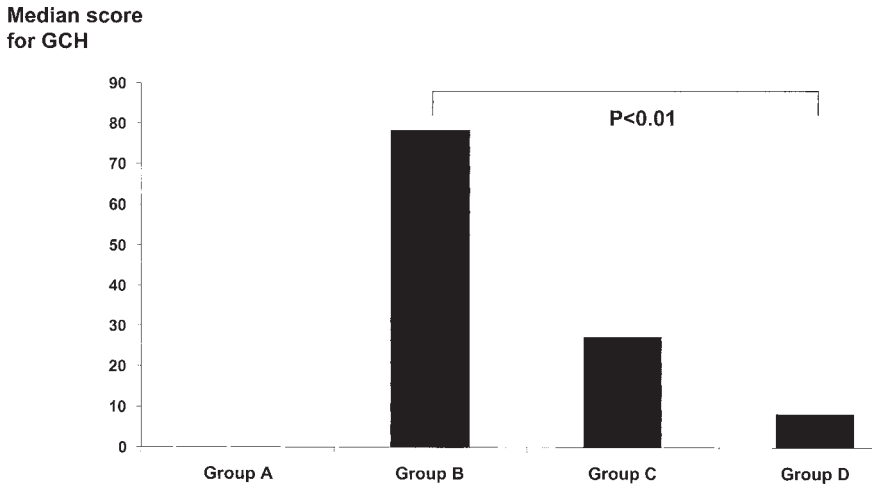
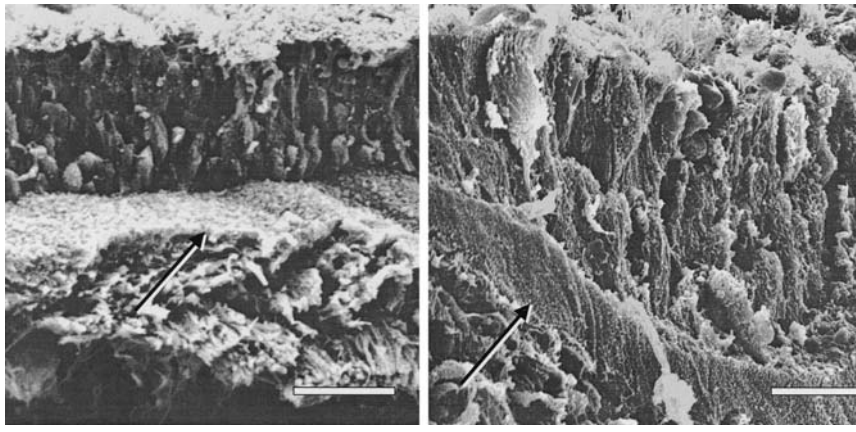



**Figure 7** Bar chart showing reduction by dexamethasone (dex) of ovalbumin (OA)–induced goblet cell hyperplasia (GCH) in the mouse. Groups: A, saline challenge + saline treatment; B, OA + saline; C, OA + dex (0.1 mg/kg/day); and D, OA + dex (1 mg/kg/day). (Adapted from Ref. 33.)

tein (34,35). Moreover, dexamethasone dose-dependently inhibits adenovirus-induced ICAM-1 expression by epithelial cells responsible for inflammatory cell/epithelial cell interaction and adhesion (36). Thus, the effects of inhaled steroids would be expected, in vivo, to reduce the attraction, retention, and accumulation of inflammatory cells in the epithelium and protect against consequent epithelial injury and remodeling.

## III. Reticular Basement Membrane

Recurrent epithelial injury or failure of the epithelium to repair normally would result in the continued release of epithelial-derived factors (such as transforming growth factor beta), which would affect not only the epithelium per se but also structures lying deeper in the airway mucosa. Thickening of the subepithelial reticular basement membrane (RBM) (i.e., the lamina reticularis) has long been recognized as a consistent change in extrinsic, intrinsic, and occupational forms of asthma (14,37–40) (see Figs. 5b and 6a). This example of remodeling, when homogeneously thickened and hyaline in appearance, is very characteristic of asthma and is not found in COPD (see Fig. 6b). The reticular basement membrane is not present in the fetus (at least up to 18 weeks of gestation) but develops in normal, healthy individuals, presumably during childhood: its thickening in asthma occurs very early on (41), even before asthma is diagnosed (42). The thickening remains even when asthma is mild and well controlled by antiasthma treatment (43), and it is present postmortem in patients with a long history of asthma but who have not died of their asthma (39) (Fig. 8). The extent of thickening is maximal early on in the development of asthma, and it does not appear to increase significantly in thickness with time or severity of disease, albeit the last is debated. The reticular basement membrane is immunopositive for collagen types III and V, for fibronectin, but not laminin: consequently its thickening in asthma has been referred to as "subepithelial fibrosis" (38). In the author's opinion this is an unfortunate application of the term "fibrosis" as the thickened layer of reticulin is ultrastructurally distinct from the "banded" (65 nm periodicity) collagen, which lies deeper in the interstitium of the airway wall, or that which comprises a scar. In contrast to interstitial collagen the reticular basement membrane is composed of thinner fibers of reticulin linked to a tenascin-rich matrix in which there are sugars together with entrapped molecules such as heparin sulfate and serum-derived components such as fibronectin. These entrapped molecules may modulate the state of differentiation, integrity, and function of the overlying surface epithelium and may, in the author's opinion, provide an osmotic gradient that encourages its thickening by swelling. Interestingly the thickened layer does not behave as a barrier to the transmigration of inflammatory cells, which presumably by the release of enzymes (e.g., metalloproteases) pass through it. The thickening shows a positive association with airways hyperresponsiveness and the frequency of asthma



(a) (b)

**Figure 8** Scanning electron micrographs (SEM) of the epithelium and its RBM. (a) Ciliated epithelium with an RBM (arrow) of normal thickness. Scale = 40 μm. (b) A thickened RBM (arrow) in a case of a man with 25-year history of asthma but who did not die of his asthma. Scale = 10 μm.

attacks and with the numbers of fibroblasts and "myofibroblasts" that lie external and adjacent to it (44,45).

Can the RBM thickening of asthma be reversed? Evidence to support the capacity for the RBM to reduce in thickness comes initially from studies of subjects with occupational, toluene diisocyanate–induced asthma (46). In this study bronchial biopsies were taken at diagnosis and 6 months later, after cessation of the occupational exposure when the thickness of the RBM was found to be significantly reduced in spite of evidence of continued inflammation. In another study, the results of a placebo-controlled bronchial biopsy study of inhaled fluticasone (250 μg) given for 6 weeks showed a significant withingroup reduction in RBM thickness in the FP-treated group (47). However, in the author's opinion, comparison of the FP-treated group with the placebo renders the reductive effect of FP questionable. The randomization starting levels in the active group were unfortunately much higher than the placebo, and the final value after reduction with FP was similar to that of the placebo (see Fig. 9). Tenascin and fibronectin are extracellular matrix components expressed normally during morphogenesis and in repair after injury. Interestingly, there is increased immunoreactivity for tenascin in the RBM of patients with chronic and seasonal asthma, and in the latter group, inhaled budesonide (800 μg daily) prevented the birch pollen–induced increase in tenascin (48). Further support for the efficacy of inhaled steroids in reversing RBM thickening comes from a prospective, randomized, single-blind, parallel

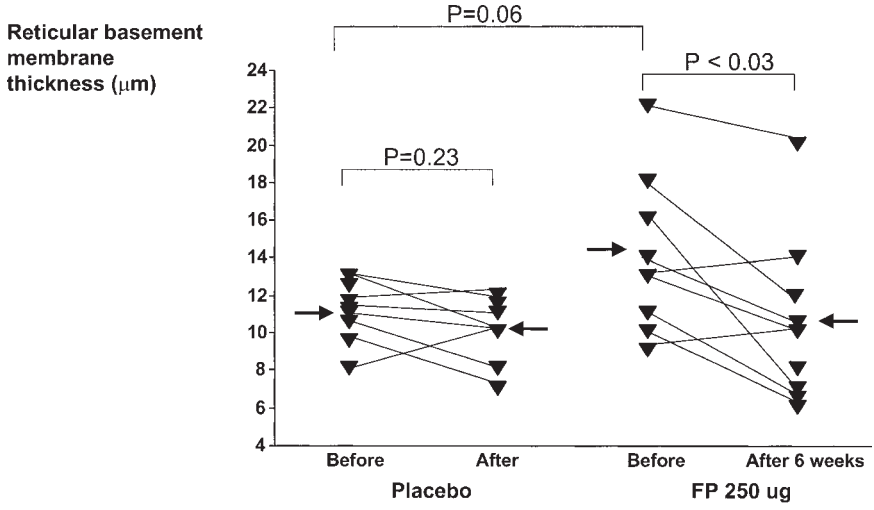



**Figure 9** Graph showing the effects of 6 weeks of treatment with an inhaled steroid on the thickness of the RBM. (Adapted from Ref. 47.)

trial with a 2-year follow-up of the effects of inhaled corticosteroid (budesonide or beclomethasone) given at a range of doses to mild to moderate asthmatics (49). A treatment strategy aimed at reducing airway hyperresponsiveness (AHR) (i.e., the AHR strategy) in addition to the recommendations of the then current guidelines was more effective in controlling asthma and reducing inflammation than that of the then current guidelines alone (i.e., the reference strategy). Quantification of bronchial biopsies demonstrated that the thickness of the RBM was significantly reduced after 2 years of treatment, but only in the AHR strategy group, and the difference was significant when compared with the reference strategy group (Fig. 10). In another placebo-controlled study of 1000 μg BDP daily given over 4 months, there were significant reductions reported in the thickness of the subepithelial components that immunostained for type III collagen (50). However, the interpretation of these results is equivocal, as the measurements of RBM thickness were more than three times higher than the average of values obtained by all other investigators in the field. It is likely that the authors were measuring the RBM as well as the deeper interstitial collagen, which also stains for type III collagen.

In contrast, an earlier TEM study of mild asthmatics biopsied before and after treatment with either steroid or beta-agonist, demonstrated no significant reductive effect on the RBM thickness following inhaled budesonide (400 μg daily) or terbutaline given over 4 weeks (51). The same study also reported the results of a cross-sectional analysis of a group of more severe asthmatics and found no

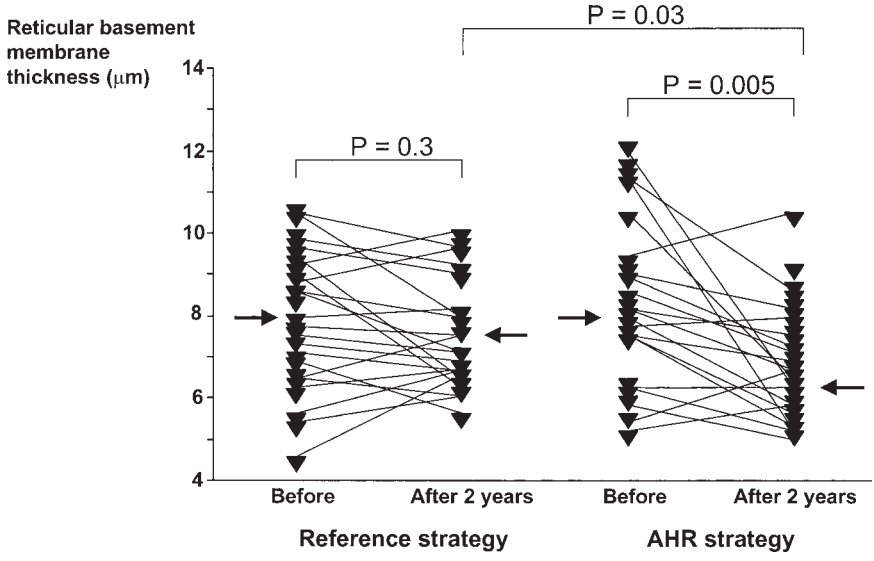



**Figure 10** The effects of two distinct treatment regimens given over 2 years to asthmatics, using the current guideline recommendations (reference-strategy) and using the additional endpoint of reduction in airways hyperresponsiveness (AHR strategy). The latter is associated with a significant reduction of the RBM thickness. (Adapted from Ref. 49.)

significant effect of inhaled steroids given for up to 10 years (average 3.5 years) in spite of marked reductions in the numbers of inflammatory cells. The same group of workers recently demonstrated that the RBM of asthmatics who required regular inhaled steroids for adequate long-term control had a RBM that was significantly thicker than a group of subjects with COPD free of steroid treatment but matched for age and disease severity (43) (see Table 1).

Thus, the effects of steroids on RBM thickness in asthma are still debated. On balance, the evidence appears to favor a reductive effect, but more confirma-

**Table 1** Thickness (μm) of Reticular Basement Membrane ± SEM

| Airway level | Normal | CB alone | CB + COPD | Asthma |
|---|---|---|---|---|
| Lobar | 3.7 ± 0.3 | 4.9 ± 0.2 | 4.1 ± 0.4 | 8.3 ± 0.5* |
| Subsegmental | 4.3 ± 0.4 | 4.6 ± 0.2 | 4.3 ± 0.3 | 8.3 ± 0.6* |

*$p < 0.05$ compared with normal, CB, or CB + COPD.
*Source*: Ref. 43.

tory studies are required and the mechanism by which this could be brought about needs to be elucidated.

## IV. Interstitial Collagen and Elastic Tissue

In contrast to the fibrotic changes recognized to occur in COPD, the evidence for fibrosis in asthma is, at best, controversial. There are reports of increased airway wall collagen in mild asthma (52). The amount of subepithelial interstitial collagen, referred to by the authors as "subepithelial fibrosis," is reported to increase with the severity of asthma and to correlate with loss of lung function (53). However, others researchers demonstrate little or no change in collagen content in asthma and no relationship of collagen mass to the severity of disease (45).

Most researchers agree that transforming growth factor beta (TGF-β) is a potent pro-fibrotic cytokine: associations between TGF-β and the number of fibroblasts in asthma have been demonstrated (53,54). Yet the data as to whether or not tissue TGF-β is increased in asthma are contradictory (45,53–55). The immunohistochemical results of examination of biopsies in mild asthma by Trigg and colleagues (50) for the identification of collagen type III likely included measurements of interstitial collagen as well as components of the RBM. The steroid-related reductive effects these authors reported indicate that steroids may reduce overall collagen mass (at least in the mucosa) in asthma. In contrast, there was no effect of corticosteroid on either the amount of collagen or elastic tissue of the airway wall when electron microscopy and stains for elastic are applied to the airways and lungs of both mild and fatal cases of asthma. As a positive control for the procedure, there were, however, reductions found in the cases of cystic fibrosis (56).

Fibroblasts are the main cells responsible for the production of connective tissue matrices, and they are key cells involved in normal repair. Their number, activity, and contractility are regulated by a variety of pro-inflammatory mediators and growth factors. One much implicated factor is, again, TGF-β, whose source may be, initially, the damaged epithelium (referred to above) and later the fibroblast per se. The concentrations of TGF-β are reported to be increased in bronchoalveolar lavage fluid (BALF) of asthmatics, and the increases are reduced in asthmatic subjects following 3–12 months of treatment with FP (750 μg bd). During this time there are significant improvements in airways responsiveness and lung function (i.e., $FEV_1$) (57). It appears also that the auto-induction of TGF-β1 by fibroblasts in culture can be inhibited by steroid (budesonide) and that the effect of a single dose can be sustained for 24 hours (58). Interestingly, steroids augment fibroblast contraction by inhibition of the release of prostaglandin E, a prostanoid that normally inhibits fibroblast contraction (59). The biological relevance of the last observation is presently unclear: it may be of greater relevance in the treat-

ment of small airways of subjects with COPD where scarring and irreversible contraction of airways is known to occur. Fibroblast function in asthma does appear to be altered: those harvested from biopsies of asthmatics are reported to proliferate less well and have a shorter life span than those obtained from normal individuals. In addition, those from asthmatics respond by proliferation (i.e., incorporate tritiated thymidine into their DNA) to either platelet-derived growth factor-BB or dexamethasone, while fibroblasts from the biopsies of nonasthmatic individuals do not (60). These data require confirmation, and their biological significance needs to be understood.

## V. Airway Vessels

Dilatation of bronchial mucosal blood vessels, congestion, and wall edema are also consistently reported features of fatal asthma, and these can account for considerable swelling of the airway wall (see Figs. 11 and 12) (61–63). Vasodilatation, congestion, and mucosal edema are cardinal signs of inflammation (64). The

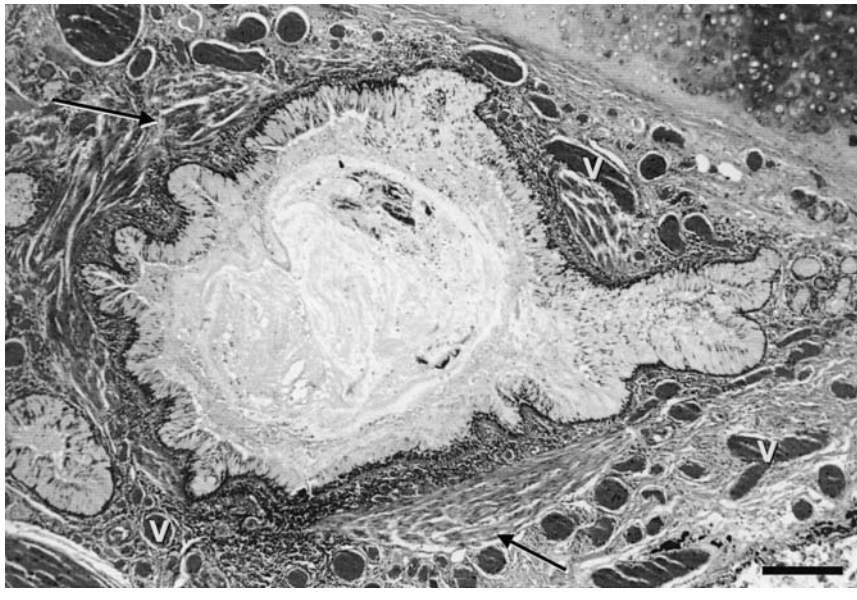



**Figure 11** Transverse section of a bronchus in a case of fatal asthma stained with H&E to show lumenal plugging, many dilated and congested bronchial vessels (V), inflammation and areas of ASM (arrow) in the airway wall. Scale = 180 μm. (Courtesy of Professor Heard.)

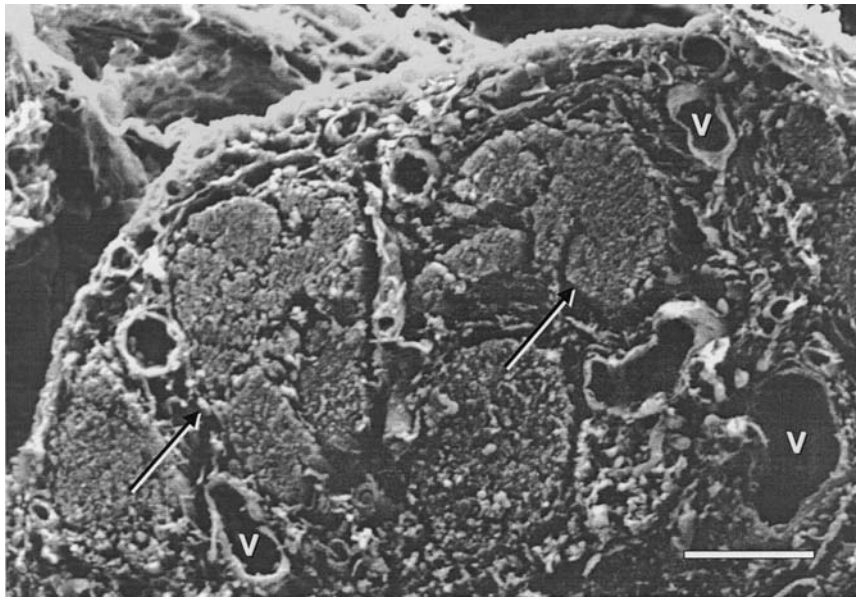



**Figure 12** SEM of part of the airway wall in fatal asthma showing the thickening of the wall due to dilatation of bronchial vessels (V) and enlargement of airway smooth muscle (ASM) (arrow). Scale = 50 μm.

formation of granulation tissue in which new vessels appear is a normal part of the healing response. There are indications that there may be new growth of bronchial vessels that contribute to the increased vascularity of the airway wall in mild asthma (65) and particularly when asthma is severe (66). In the former mild group, 7 asthmatics who did not receive inhaled corticosteroids had a significantly greater number of and greater area of the mucosa occupied by bronchial vessels than 11 nonasthmatic controls: these changes correlated with the degree of airway responsiveness and percentage change in $FEV_1$ after bronchodilator. Those receiving inhaled corticosteroid (beclomethasone, 200–1500 μg daily) had a statistically significantly reduced vessel area and a trend to fewer vessels than those not treated (67).

## VI. Bronchial Smooth Muscle

The percentage of bronchial wall occupied by bronchial smooth muscle is increased in fatal asthma (23) (Fig. 12). The absolute increase in muscle mass is reported to be particularly striking in large intrapulmonary bronchi of lungs ob-

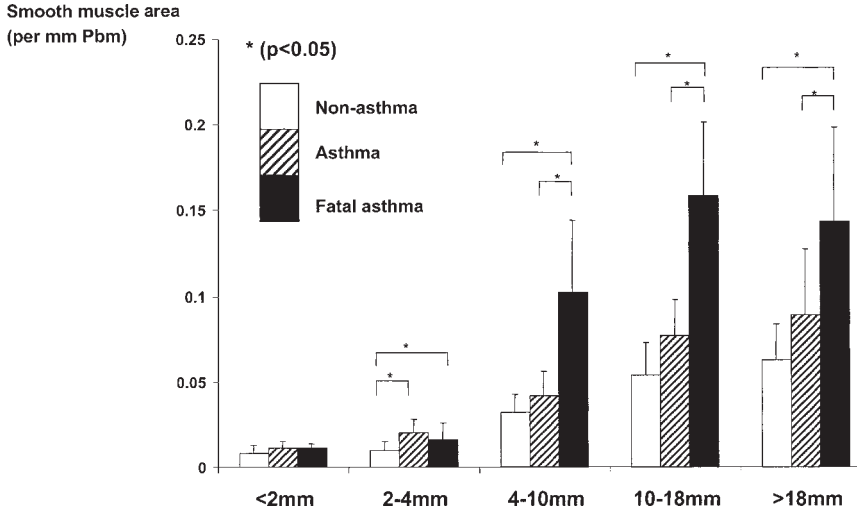


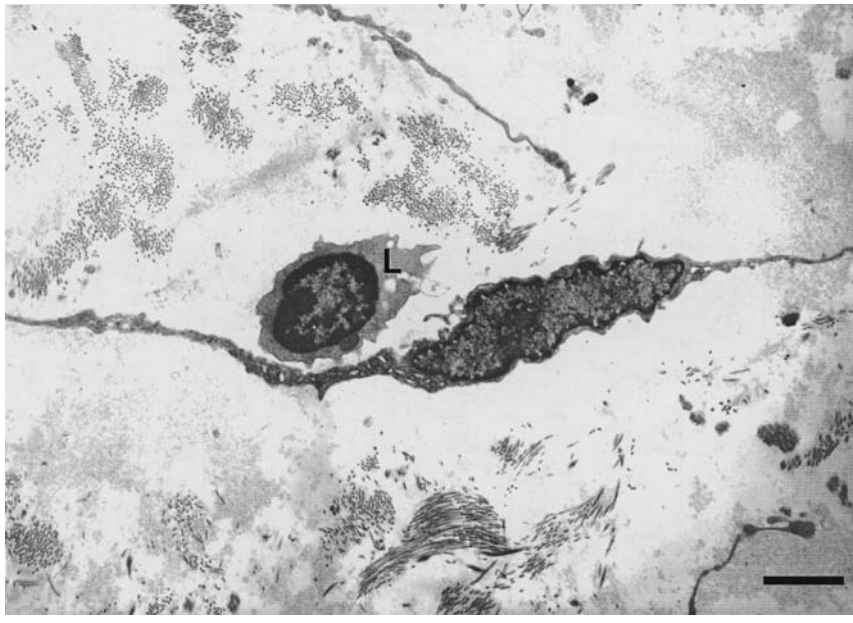

**Figure 13** Bar chart demonstrating the area of the airway wall occupied by airway smooth muscle (ASM) in three groups of subjects: nonasthmatics, asthmatics who did not die of asthma, and those who died in status asthmaticus. The enlargement of ASM is prominent in the large airways of fatal asthma. Pbm = millimeter perimeter basement membrane. (Adapted from Ref. 11.)

tained following a fatal attack as compared with that in asthmatic subjects dying of other causes (11). The increase in muscle mass is an important contributor to the thickening of the airway wall and hence to the marked increase in resistance to airflow, which may become life-threatening (10,68–70). Using a morphometric technique applied to tissue sections of airways, Dunnill and coworkers (23) showed that approximately 12% of the airway wall in segmental bronchi obtained from cases of fatal asthma was comprised of muscle compared to about 5% in normals. Hogg and colleagues (71) confirmed this trend in airways larger than 2 mm in diameter and demonstrated a three- to fourfold increase over normal in the area of the wall occupied by bronchial smooth muscle. In asthma the increase in muscle mass, in absolute terms, is not as striking in airways of less than 2 mm in diameter (72), albeit the relative contribution of airway wall muscle to overall airway wall thickness in small airways is greater than that in large airways (Fig. 13). The relative contribution of muscle fiber hyperplasia (73), hypertrophy (74,75), or other mechanisms to the increase in muscle mass in asthma is unclear. Two patterns of distribution of increased muscle mass have been described in asthma: one in which the increase occurs throughout the airways and another in which the increase is restricted to the largest airways (74,75). It is suggested that in the former there is muscle fiber hyperplasia as well as hypertrophy, in the latter hypertrophy

alone. Thus, smooth muscle cell proliferation (an increase in fiber number) is likely to be a major contributor to the increase in smooth muscle mass.

The mechanisms that control airway smooth muscle proliferation may be studied in vitro using the uptake of tritiated thymidine as a marker of DNA synthesis, and the number of cells can be counted to assess the degree of muscle cell proliferation. A number of agents including cytokines and growth factors influence the growth of airway smooth muscle (ASM). These include epidermal growth factor (EGF), insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), tumor necrosis factor (TNF), fetal calf serum (FCS), thrombin, and leukotriene $D_4$ (LTD4). In vitro pretreatment of human ASM with dexamethasone (100 nM for 60 min) inhibits the mitogenic response (both $^3$H-thymidine and cell number) of ASM to thrombin, bFGF, FCS, and EGF, with the greatest and least inhibitory effects of the steroid seen with thrombin and EGF, respectively. Following thrombin, addition of dexamethasone up to 19 hours (but not after 21 hours) following thrombin treatment produces a similar degree of inhibition as that seen with pretreatment of ASM by steroid (76). There is also a dose-dependent steroid (methyl-prednisolone) inhibition of LTD4-induced ASM proliferation reported (77). TNF at low doses (0.3–30 pM) appears to have a mild mitogenic effect on ASM, which can be inhibited by dexamethasone: however, at higher doses (300 pM, i.e., in the range of concentration detected in BALF) TNF blocks the stimulatory effects of thrombin (78). Interestingly, the TNF blockade of thrombin-induced ASM proliferation can also be inhibited by dexamethasone (78). Steroids have been shown to block ASM proliferation by reduction of intracellular cyclin D1, both mRNA and protein, via their action at an intracellular site downstream of or parallel to the ERK (extracellular regulated kinase) pathway (79).

In respect to other mechanisms contributing to the increase in airway smooth muscle mass, we have observed that solitary contractile cells (referred to as "myofibroblasts") appear in substantial numbers during the late phase response to allergen challenge (Fig. 14). We have suggested that, with repeated exposure to allergen, these "myofibroblasts" or "fibromyocytes" contribute not only to the increased production of reticulin (i.e., reticular basement membrane) but may also be precursors of the increased mass of bronchial smooth muscle seen in the airway wall in severe asthma (80). We propose that dedifferentiation of existing smooth muscle and its migration, in the form of a myofibroblast phenotype, to a subepithelial site occurs in asthma (80): this process may parallel the changes of vascular smooth muscle described in atherosclerosis (81). This late phase "remodeling" response to allergen challenge may also be responsive to pharmacological intervention. Such clinical experiments to determine the effects of treatment are ongoing: there are no data, as yet, in humans concerning the effectiveness of steroids, β-agonists, or leukotriene antagonists in inhibiting the initiation of the myofibroblast response to allergen challenge in asthma.

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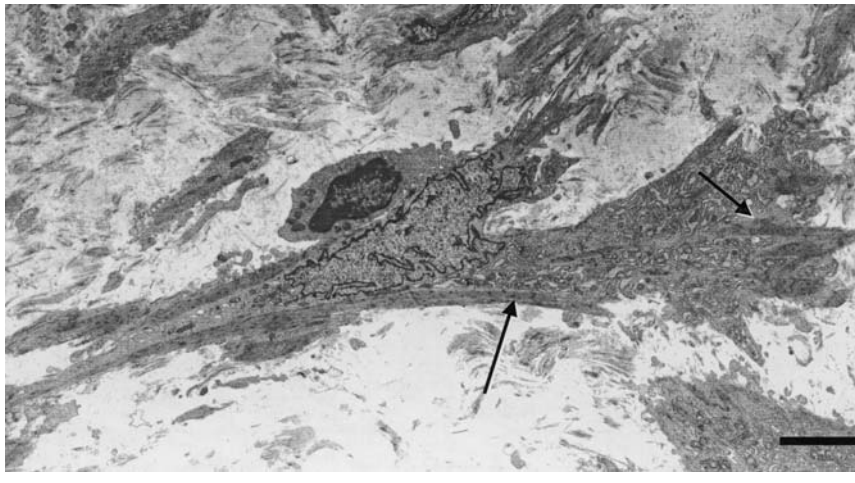

(b)

**Figure 14** Transmission electron micrograph of the mucosa in bronchial biopsies from cases of atopic stable asthma. (a) A subepithelial fibroblast lying close to a lymphomononuclear cell (L). Scale = 2.5 μm. (b) A myofibroblast taken from an allergic asthmatic 24 hours after allergen challenge. The myofibroblast is significantly larger than the fibroblast and the cytoplasm has filaments organized as bundles running along the length of the cell membrane. There are bundles of electron-dense condensations (arrows) as in the contractile apparatus of bronchial smooth muscle cell. Scale = 5.0 μm. (From Ref. 80.)

## VII. Airway Wall Nerves

The topic of airway wall innervation and its relevance to asthma is a large one (82,83). There are unconfirmed data showing that in *fatal* asthma there is an absence of (relaxant) vasoactive intestinal polypeptide (VIP)–containing nerve fibers and an increase in the numbers of substance P–containing fibers (stimulatory to bronchial smooth muscle). The alteration contrasts markedly with the innervation of "disease" control lungs taken at resection from chronic smokers (84,85). The reduction reported in fatal asthma has not been confirmed by examination of bronchial biopsies in milder groups of asthmatics (86). The biopsy study of Laitinen and colleagues (21) reported an increase in the number of intraepithelial nerves in asthmatics treated for 3 months with inhaled budesonide, but the significance of these findings is not yet clear.

## VIII. Remodeling and Airflow Limitation

Thickening of the airway wall due to chronic inflammation and inappropriate remodeling of the airway wall (87) results in an increased resistance to airflow due to encroachment of the airway lumen by the airway wall, particularly when bronchial smooth muscle contracts (see Fig. 13). James and colleagues have shown that airway wall thickening need only be relatively minor to have dramatic consequences on airflow limitation (68). The relevance of the airway wall thickening to reduced airflow and AHR in asthma has been discussed (88,89). It has been suggested that for a given degree of smooth muscle shortening, the effect on reduction of airway radius and increased resistance to airflow (to the power 4) would be considerably greater if the airway wall were thickened. The concept and link of airway geometry to AHR has been confirmed by computer modeling. The model predicts that when the airway wall is thickened in the absence of muscle contraction, there will be a relatively moderate increase in baseline airflow resistance, but, in contrast, there will be profound increase in resistance when bronchial smooth muscle shortens even normally (10,69,70). Indeed, James and colleagues have shown that smooth muscle need only shorten by about 40% to completely occlude the airway lumen. A further consideration is that airway smooth muscle is not truly circular, but rather it encircles the airway arranged as two opposing spirals (a so-called geodesic pattern). Normally muscle contraction thus has the effect of both shortening and constricting the airway. Stiffening of the airway wall and prevention of airway shortening by thickening of the reticular basement membrane or wall edema may result in a greater proportion of the force, generated by bronchial smooth muscle shortening, being redirected to airway constriction. Thus, chronic inflammation and remodeling of the airway wall may be responsible for the airway hyperresponsiveness of asthma. If small as well as large

airways become involved, such airway wall remodeling may also contribute to the accelerated decline in lung function ($FEV_1$) observed in more severe subsets (10–15%) of asthmatics (8). While inhaled corticosteroids improve markedly peak flow and $FEV_1$ in mild asthmatics, there are presently no data on the long-term capacity of steroids to improve lung function of those asthmatics who decline more rapidly than normal.

## IX. Summary and Conclusion

Asthma is a chronic inflammatory condition of conducting airways, and this is associated with remodeling of the airway wall in both large and small airways. Remodeling is considered to relate in some way to airway hyperresponsiveness and accelerated decline in lung function in asthmatics, but a causal relationship is unproven. The remodeling process is driven most probably by persistent epithelial injury or a failure to repair the epithelium normally. The reparative response includes (1) squamous and goblet cell metaplasia/hyperplasia following epithelial loss, (2) hypertrophy of mucus-secreting glands (which originally develop from the epithelium), (3) thickening of the epithelial reticular basement membrane (i.e., the laying down of reticulin), (4) increases in the numbers of bronchial vessels, and (5) an increase in the numbers of myofibroblasts and in the mass of bronchial smooth muscle. Steroids are clearly effective in reducing much of the chronic (particularly eosinophilic) inflammation of asthma and the relevant pro-inflammatory cytokines and chemokines. There is evidence that steroids may also attenuate aspects of the remodeling response, but as the current data are conflicting, further work on this topic is required. It seems clear that if irreversible alterations are to be prevented from developing, then early introduction of antiasthma treatment, including steroids, would be a logical approach, providing that any of the recognized side effects of treatment are minimized.

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## Discussion

**Dr. Schleimer:** The term *remodeling* implies an ongoing process. What is the evidence that the changes you have described are progressive? When do they occur during the natural history of the disease?

**Dr. Jeffery:** Remodeling involves alterations to epithelium, its reticular basement membrane (RBM), and to deeper components including interstitial collagen, mucus-secreting glands, bronchial blood vessels, and smooth muscle. The thickening and hyaline appearance of the RBM appears to be a very early change that occurs in children at least as early as 8 years. Drs. Bush, Payne, and I have demonstrated a statistically significant thickening in children with symptoms of asthma, and the results of Pohnek and coworkers indicate that the thickening begins even before the diagnosis of asthma is made (Pohnek et al., 1997; Eur Respir J 11:160S). What has impressed me is that the thickening appears to be maximal at an early time point and is not progressive. Adults with a long history of asthma and even those who die of their asthma do not appear to have a thicker RBM than those with mild or newly diagnosed asthma. Similarly with interstitial collagen deposition, there seems to be no clear association with severity (Chu et al., AJRCCM 1998; 158:1936–1944). However, the increase in airway smooth muscle (ASM) mass is likely progressive but only in the significant minority of asthmatics whose lung function goes into a more rapid decline than is normal and in those who have a fatal attack. Those who die of their asthma have a significantly greater proportion of their airway wall occupied by ASM than longstanding asthmatics that die of non-respiratory cause (Carroll et al., ARRD 1993; 147:405–410).

**Dr. Boulet:** We previously reported that nonasthmatic patients with allergic rhinitis or with mild asymptomatic already had evidence of abnormal subepithelial collagen deposition, suggesting that airway remodeling starts before asthma happens. In that last group, as published recently (Eur Respir J 1999), when these patients develop symptomatic asthma they show a marked increase in subepithelial collagen deposition in addition to increased airway inflammation. However, when we studied recently diagnosed vs. longstanding asthmatic patients, there was no difference between these two groups in apparent reticular basement membrane thickness both on baseline and following a high-dose inhaled corticosteroid treatment. Do you think airway remodeling plays a different role at different stages of the natural history of asthma and responds differently to inhaled corticosteroids?

**Dr. Jeffery:** There is some in vitro evidence that corticosteroids are less effective in inhibiting ASM proliferation, induced by growth factors such as EGF and IGF, than leukotriene receptor antagonists. I believe there is evidence from

your own laboratory that dexamethasone actually increases myofibroblast proliferation. This may be of relevance to one mechanism by which muscle mass increases in asthma.

**Dr. Inman:** When you said that you would not want inhaled steroids to interfere with acute inflammatory processes, did you mean these processes in asthma or in nonasthmatic tissue injury?

**Dr. Jeffery:** Our airways, whether normal or asthmatic, are repeatedly bombarded with particulates, gases, and infection. They respond by mounting an acute inflammatory response that is designed to protect the host. If steroids inhibit this, then I would interpret this as bad. However, in asthma the host response to injury or allergen appears to be inappropriate, either in type or duration. It is this latter aspect we need to treat selectively, as it is currently understood to induce the process of airway wall "remodeling."

**Dr. Rand:** What if we don't treat mild asthmatics and the remodeling continues, what would be the long-term effect?

**Dr. Jeffery:** I speculate that the long-term effect is irreversible (or difficult-to-reverse) thickening of the airway wall and reduced lung function associated with more rapid decline in $FEV_1$ than is normal.

**Dr. Persson:** Airway permeability should not be listed as evidence for epithelial damage in asthma in that increased absorption permeability has not been demonstrated and the epithelial permeability involved in plasma exudation involving luminal entry of even the largest proteins (such as $\alpha_2$-macroglobulin) is not (necessarily) associated with nor does it cause any epithelial derangement. Indeed, the laying down of plasma proteins in the lamina propria, the epithelium, and on the mucosal surface would perhaps be considered part of airway remodeling in asthma. My question concerns the thickening of basement membrane that is examined so frequently. Is it a good surrogate marker of airway remodeling? Does it predict or correlate with other airway effects especially other remodeling changes?

**Dr. Jeffery:** As I have stated, RBM thickening occurs early and appears to be maximal at this early time point. Other changes such as increases in vessel number, mucus-secreting gland, and ASM mass appear to take much longer. No statistical correlation has been undertaken, but I would predict that there would be no associations.

**Dr. Stellato:** What is the cause of epithelial fragility? Alteration of tight functions or other adhesive molecules? What is the role of epithelial fragility in relation to smooth muscle hypertrophy?

**Dr. Jeffery:** Epithelial fragility is unlikely the result of alterations to tight junctions (zonula occludens) whose major role is to selectively regulate the transepithelial movement of water, ions, and macromolecules. Instead, it is the disruption of adhesive intercellular, desmosomal junctions and E-cadherin, particularly along a plane of cleavage between superficial ciliated and goblet cells and basal cells attached to the basal lamina. Epithelial disruption caused by irritant or allergen induces the release of growth factors such as GM-CSF, TGF-β, IGF, and PDGF. These likely diffuse through the subepithelial tissue to induce phenotypic changes to muscle blocks and fibroblast. Our continuing investigations at the electron microscopic level of biopsies obtained during the late phase reaction to allergen challenge show signs of smooth muscle de-differentiation, the appearance of a "synthetic" smooth muscle phenotype that appears to be in the process of migration to the subepithelial zone. At this site adjacent to the epithelium, myofibroblasts become associated with areas of the RBM that have dissolved or thinned. Several aspects of this airway response mimic the atheromatous change described in the vasculature in cardiovascular disease, and there may be similarity in the mechanisms, which should be explored.

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# Inhaled Corticosteroids and the Natural History of Asthma

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Inhaled corticosteroids (ICS) are the most potent form of controller medication available for the treatment of childhood asthma (1). When administered regularly and in adequate doses, ICS significantly improve symptoms, bronchial hyperresponsiveness to methacholine and other stimuli, and quality of life in school-aged children and adults with persistent forms of the disease (2,3). ICS have also been shown to be effective in controlling symptoms in preschool children, but effects are usually not as dramatic and consistent in this age group as they are in older children (4–6). What may cause these differences in the efficacy of ICS in infants and young children with respect to that in older children will be discussed later in this chapter.

## I. Asthma and Airway Inflammation

The effectiveness of ICS in controlling asthma symptoms and bronchial hyperresponsiveness appears to confirm some of the basic tenets of the inflammatory theory of asthma. During the last quarter of twentieth century, our concept of asthma evolved from that of a disease characterized by intermittent attacks of bronchial obstruction and airway smooth muscle contraction to one in which the

defining characteristic of asthma was now considered to be the presence of a chronic inflammatory process of the bronchi (7). According to this theory, asthma is characterized by a particular type of inflammatory response, usually mediated by Th-2–type cytokines (8). These Th-2–like responses are associated with IgE-mediated immunity and with the presence of eosinophil-mediated responses, as suggested by the presence of eosinophils in the airways of most asthmatics (9). It was also believed, however, that by itself, this inflammatory response could not be responsible for the asthma phenotype. Individuals with allergic rhinitis but no asthma also develop a similar immune response in the airways when challenged with antigens that they are sensitized against (10). It thus appeared that, together with a local inflammation, some other factor that was specific to the lung needed to be present that made subjects susceptible to the type of airway inflammation that is characteristic of asthma. Nevertheless, since both components seem to be necessary, many authors strongly believed that by controlling inflammation, the natural course of the disease could also be reversed.

The basic premise behind this assertion is that chronic airway inflammation, on the one hand, predisposes to acute and chronic asthma symptoms and, on the other hand, determines the development of chronic changes in airway structure and function, a process that has been called "airway remodeling" (11). This process was believed to be a progressive and ongoing one, in the sense that it was active in subjects with asthma as long as their airway inflammation was not controlled. It was thus quite natural to conclude that chronic administration of a potent anti-inflammatory agent such as ICS should hamper the progressive nature of the disease. Given the fact that the chronic structural changes believed to be associated with airway remodeling were also thought to contribute significantly to the chronicity of symptoms in asthma, it was legitimate to believe that children treated with ICS in a systematic manner would show fewer deficits in lung function growth. Similarly, it was plausible to surmise that adults could show reversibility of lung function alterations after prolonged treatment with ICS.

The evidence supporting this hypothesis is very scanty (12). Very few studies have assessed long-term effects of inhaled corticosteroid in asthma, and even fewer have determined if these effects persist beyond the active treatment period. Studies by Juniper and coworkers in the early 1990s (13) convincingly showed that subjects with asthma who were put on regular inhaled budesonide showed substantial improvements in airway responsiveness, in levels of lung function, and in clinical asthma severity. These same authors evaluated whether these improvements were maintained when the dose of budesonide was reduced (14). Although subjects with the reduced dose did not show worsening of bronchial hyperresponsiveness, they did show worsening in lung function. Symptoms also started redeveloping in the group placed on a reduced dose as compared with that kept on full dose treatment. Haahtela and coworkers treated with inhaled budesonide for 2 years 37 patients with newly diagnosed asthma at a dose of 1200 μg per day (15).

After this treatment, half were assigned to treatment with 400 µg of budesonide per day and half with treatment with placebo. Much like in the studies by Juniper et al. (13,14), treatment with reduced dose of budesonide was effective in maintaining bronchial hyperresponsiveness at a level similar to that achieved with a higher dose. However, improvement was maintained in only 33% of the patients receiving placebo, and in this latter group lung function significantly deteriorated as did bronchial hyperresponsiveness and morning peak flow (15).

These studies were performed in adults, most of who had mild persistent or moderate persistent asthma. More recently, the results of the Childhood Asthma Management Program (CAMP) have been reported (16). This study was designed to evaluate whether continuous, long-term treatment (over a period of up to 6 years) with either budesonide or an inhaled noncorticosteroid drug (nedocromil) could improve lung function as compared with treatment for symptoms only (16). The main hypothesis of this study was that school children with asthma treated for long periods of time with inhaled corticosteroids would not show the deficits in lung function thought to be characteristic of active asthma. The authors randomly assigned over 1000 children aged 5–12 years of age with mild to moderate persistent asthma to receive either 200 µg of budesonide, 8 mg of nedocromil, or placebo twice daily. The study showed no significant difference between either treatment and placebo in the primary outcome, which was the degree of change in forced expiratory volume in one second ($FEV_1$) after administration of a bronchodilator. The children given budesonide showed significant improvement in airway responsiveness to methacholine and in clinical outcomes and symptoms. Of particular interest was the fact that, during the study period, growth in lung function was very similar and not statistically different between subjects treated with ICS, nedocromil, or placebo.

## II. Challenging the Inflammatory Theory of Asthma

The results of the studies quoted earlier clearly challenge the hypothesis that uncontrolled, persistent bronchial inflammation *occurring at any age* by itself can determine structural changes in the airways in adults and deficits in lung function growth in children with asthma. Long-term administration of inhaled corticosteroids clearly improves bronchial hyperresponsiveness (17) and is able to control airway inflammation as assessed by bronchoalveolar lavage and by airway biopsies (18,19). These effects are associated with significant clinical improvements in both children and adults with asthma. However, in spite of these remarkable effects, the data do not support the contention that such a potent anti-inflammatory drug is able to change the natural course of asthma in children aged 5 or more or in adults. In these two age groups, suspension of treatment very rapidly reverses improvements in bronchial hyperresponsiveness (3,15), and levels of lung func-

tion seem to return to those observed at the beginning of even prolonged periods of treatment (3,15).

These results suggest to us that, although the inflammatory theory of asthma has been extremely useful in providing a strategy for the treatment of asthma that allows one to effectively control symptoms with minimal side effects by use of inhaled medication, it cannot explain the chronic nature of the disease. In other words, control of inflammation in schoolchildren and adults with asthma markedly improves their clinical status and airway responsiveness but is unable to reverse functional and structural changes in the airways that have probably already occurred in the airways at the time treatment is started.

## III. Natural History of Asthma Symptoms During Childhood and Early Adult Life

The results of these studies thus suggest the need for a new paradigm to approach asthma treatment. We propose that this approach will need to be solidly based on a better knowledge of the natural history of the disease. Fortunately, results have recently been reported from several long-term prospective studies of asthma in which follow-up was initiated either at birth or shortly thereafter. The picture that emerges is clearly complex but offers new very important insights into the factors that determine the initiation and the chronicity of symptoms in asthma.

We will briefly summarize the main results of these longitudinal studies in the next few sections.

### A. Most Cases of Asthma Begin During the Preschool Years

Perhaps one of the most intriguing results of longitudinal studies of asthma has been the observation that, in most cases of asthma, the first asthma-like symptoms occur during the first years of life (20). This observation does not seem to surprise most pediatricians, but it is not easily accepted by experts in adult asthma. There are many adult subjects with asthma who, when questioned thoroughly, do not recall having ever had symptoms before their adult years. However, when studies are based on objective data and not on retrospective questionnaires, results clearly show that most adult subjects with asthma do have reports of asthma symptoms during the first years of life. Most of these subjects have either forgotten that they ever had such symptoms or were never told by parents or caregivers about them. Since remission is a very frequent occurrence during the natural history of asthma (21), it is not surprising that many adults report to their physicians that their symptoms started only a few years earlier. This certainly does not exclude the possibility that true incident cases of asthma may occur during the adult years. However, most frequently the first symptoms can be tracked back to early childhood.

The awareness that most cases of asthma begin in early life has focused the attention of many investigators on this particular age group. Once again, the

picture that emerges is one of great complexity. Although most future chronic asthmatics start having symptoms during the first years of life, a large proportion of children who have symptoms of bronchial obstruction during these years are not destined to be the future asthmatics (22). The majority of these children have transient conditions that may also be quite severe, but that are destined to subside with time. These conditions are usually manifested with symptoms that occur almost exclusively during viral infections, with little symptomatology between acute attacks (23). These transient conditions seem to be associated with either alterations in immune responses to viral infections (24), in airway and lung size (25,26), or in airway responsiveness not associated with chronic inflammation of the bronchi, as observed in persistent asthma (27). Therefore, the future chronic asthmatics coexist in early life with a much larger population of young children who are having very similar symptoms but who do not have atopic asthma. It is thus not surprising that studies that do not attempt to distinguish between different groups of wheezing infants and young children have been unable to show very strong associations between symptoms in this age groups and asthma later in life. Moreover, children with transient forms of wheezing may be less likely to respond to ICS than persistent wheezers (28). This may explain the apparent less effectiveness of ICS in this age group.

### B. Symptoms in Childhood Asthma Are Usually Very Variable

A second factor that needs to be taken into account is that controlled clinical trials are almost invariably based on populations recruited in tertiary care institutions, in which by definition cases of asthma are more severe than those in the community as a whole. Studies based on general population samples invariably show that most cases of asthma are mild (29), with symptoms that tend to remit and relapse frequently (30). It is thus very important for the purpose of determining a strategy for the treatment of asthma to appropriately identify the subjects in whom certain forms of treatment may be useful because if subjects with mild forms of the disease are grouped with subjects with more severe forms, important changes occurring in the latter group may be masked by the more benign course occurring in the former.

### C. Severity of Asthma Symptoms Tracks with Age

In spite of this considerable variability within subjects, group analysis shows that asthma symptoms track markedly with age (21,31). As a consequence, school-age children with severe asthma are most likely to show continued symptoms up to their late thirties, with over 80% of these children having moderate or severe asthma in adult life. Conversely, a large proportion of school-age children with mild asthma symptoms will have remitted or will show mild symptoms by early adult life, with only a small minority progressing towards more severe forms of the disease.

Interestingly, the same degree of tracking is not apparent between symptoms occurring during the first 3 years of life and those of school-age children (32). In this case, although many children with frequent asthma symptoms during the school years do have symptoms in early life, these may be quite mild. Similarly, although infants and young children with severe asthma-like symptoms are more likely to have severe symptoms during the school years, the association is not as strong as that between symptoms during the school years and in adult life (32). These results suggest that progression towards chronic asthma is more likely to occur early in life and, particularly, during the preschool years.

### D. Deficits in Lung Function Growth Occur Mainly During the Preschool Years

The recently published results from the CAMP study discussed earlier clearly show that school-age children treated with placebo for prolonged periods of time do not show significantly larger deficits in lung function growth than those observed among children treated with effective anti-inflammatory therapy (16). These results strongly support recent observational studies of lung function development in asthma. Perhaps one of the longest and most informative of such studies is the Melbourne Longitudinal Study of Asthma (33). This study was started more than 30 years ago by Williams and McNicol (34), with the enrollment of groups of children with different degrees of asthma severity at the age of approximately 7 years. A group of subjects with more severe asthma and significant deficits in lung function was enrolled a few years later, at the age of 10. This study has provided a significant wealth of information, but perhaps its most important result is the observation that between the ages of 7 and 35, levels of lung function ran in parallel in the different groups of asthmatics (31). In other words, in spite of continued, even severe symptoms of asthma, no deficits in lung function growth was observed after enrollment and up to mid-adulthood. Even the group with severe asthma enrolled at the age 10 and who started follow-up with marked deficit in lung function showed no further deterioration in lung function level, and this was independent of treatment with inhaled corticosteroids during follow-up (31). It is important to stress here that decline in lung function has been reported to be more steep in subjects with self-reported asthma after the age of 40 (35).

Two scenarios may explain the rather stable lung function observed in subjects with asthma up to mid-adult life. A first possibility is that the basic factor determining asthma chronicity is present at the time of birth, with little influence of environmental factors acting after birth. This scenario is plausible but unlikely. There are very wide variations in the prevalence of asthma among populations with relatively similar genetic backgrounds (36,37). These variations are particularly true for the more severe forms of asthma. Although it has been suggested that events occurring in utero may have a strong influence in the subsequent risk for

asthma (38), it is improbable that only factors acting in utero are important determinants of asthma inception.

In support of this contention are the results of the Tucson Children's Respiratory Study (39). This longitudinal survey of the risk factors and natural history for asthma was started in the early 1980s when over 1000 newborns without neonatal lung disease were enrolled. These children have now been followed for two decades. In a fraction of these children, lung function was measured with the so-called chest compression technique (40) during the first months of life, before any manifestation of airway obstruction had occurred. By use of this technique, maximal flows using partial expiratory maneuvers were obtained and subsequently compared with similar flows obtained through voluntary maneuvers at ages 6, 11, and 16 (41). This study had the characteristic that, for the first time, a distinction was made between subjects who were having respiratory illnesses with wheezing during the first 3 years of life but whose symptoms had remitted by the age of 6 from those who were also wheezing in early life but whose symptoms had not remitted by the early school years (persistent wheezers). The results showed that persistent wheezers had levels of lung function shortly after birth that were not significantly different from those of children who never wheezed during the first 6 years of life. However, by the age of 6 years, persistent wheezers showed significant deficits in lung function development when compared with their peers who either had no asthma-like symptoms during the first 6 years of life or who started having such symptoms after the age of 3 years (42). Interestingly, these deficits in lung function did not progress any further between the ages of 6 and 11 years or up to the age of 16 years (41). This occurred in spite of a much higher risk of continued asthma symptoms at ages 11 and 16 in persistent wheezers than in children who started wheezing after the age of 3 years.

It is important to stress here that only a small minority of children enrolled in the Tucson Children's Respiratory Study and who had asthma during the first 11 years of life were treated with inhaled corticosteroids. Moreover, those in whom inhaled corticosteroids were prescribed received such treatment for only short periods of time. This is mainly attributable to the prevailing patterns of asthma treatment in the United States until the early 1990s, when inhaled corticosteroids began to be used much more widely in general practice. The results of the Tucson Children's Respiratory Study, therefore, are in agreement with those of the CAMP study cited earlier, and suggest that the deficits in lung function present in asthmatic subjects at the beginning of the school years show no marked progression even in the absence of treatment with anti-inflammatory drugs.

## IV. A Developmental Approach to Asthma Treatment

The results of longitudinal studies of the natural history of asthma and those of well-designed, long-term studies of continuous treatment with ICS suggest the

need for new conceptual framework of asthma based on our understanding of the natural history of the disease. As explained earlier, there is little doubt that active airway inflammation is crucial in determining persistence of asthma symptoms in all age groups. However, a form of treatment that has been shown to be extremely effective in controlling airway inflammation and asthma symptoms seems to have no effect on the natural history of the disease when started during the school years or later. It is our contention that the still scanty data available on the natural history of asthma are compatible with this conclusion. It appears that most of the deficits in lung function present in subjects with asthma up to mid-adult life are established very early in life, as are the patterns of disease expression. Moreover, most subjects with severe asthma in childhood and early adult life start having symptoms during infancy and early childhood and disease progression seems to occur mainly during the preschool years.

This latter period of life is characterized by a very fast growth of both airway and lung size (43). Moreover, marked changes also occur in the regulation of airway tone: while most newborns show marked responsiveness to histamine and other bronchoconstrictors, airway responsiveness decreases markedly with age in normal subjects (44). It is thus plausible to surmise that a chronic inflammatory process, persistently activated by continuous exposure to local aeroallergens to which subjects with asthma become sensitized very early in life (45), may disrupt the normal process of lung development during this crucial phase.

It is important to stress here that very little is known about the potential growth effects of the cytokines released during the IgE-mediated immune responses that are elicited by aeroallergens in young asthmatics. Very recent data suggest that, at least in animal models of asthma, both IL-13 (46) and IL-10 (47,48) may have direct effects on airway smooth muscle. It is also possible that these and other cytokines may have important effects on the deposition of collagen and elastin in the growing lung (49). This suggestion is supported by recent data derived from a rat model of childhood asthma (50). In this model, rat strains that are genetically predisposed to respond to IgE-mediated mechanisms and in which these mechanisms are activated very early in life show marked disruption of lung growth and early development of airway hyperresponsiveness (51).

## V. Recognizing Early Asthma

The above scenario suggests that chronic, persistent asthma is a developmental disease that begins in early life and is progressive mainly during the period of fast lung structural and functional growth that is characteristic of the preschool years. We postulate that controlling airway inflammation during crucial periods of lung development may be an effective strategy of secondary prevention of the disease. Recognizing early asthma and distinguishing it from other forms of airway ob-

struction occurring during the preschool years becomes therefore a very important challenge both for epidemiologists and clinicians. We have recently proposed a simple asthma predictive index (32) in which young children at high risk of having chronic asthma were identified during the first 3 years of life by use of clinical data easily available to any general practitioner. Although such indices may become useful tools for the clinician, their predictive capacity may still be insufficient: approximately one third of all children who did develop frequent asthma symptoms during the school years had a negative predictive index during the first 3 years of life (32). We are convinced, however, that advances in studies of the genetics of asthma will allow for the development of more accurate predictive indices in the near future.

Studies are underway in which children at high risk for the development of asthma and adults with mild asthma (52) are being treated with anti-inflammatory drugs for prolonged periods of time in order to determine if such treatment can change the natural history of the disease. These studies may provide crucial information not only about the potential role of inhaled corticosteroids in the secondary prevention of asthma but also about the factors that determine the natural history of the disease.

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# 27

# Combination Therapies Using Inhaled Corticosteroids

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## I. Introduction

The reappraisal of bronchial asthma as being a chronic inflammatory disease of the airways (1) resulted in the late 1980s and early 1990s in a number of asthma treatment guidelines. Before the introduction of long-acting inhaled bronchodilators and leukotriene receptor antagonists, the treatment guidelines recommended a stepwise increase (from step 2 to step 4) in the daily doses of inhaled corticosteroids. With increasing knowledge about the efficacy and safety profiles of inhaled corticosteroids, it became apparent that their benefit/risk ratio peaked somewhere around daily doses of 1000 μg. Up to this dose level a dose-dependent increase in efficacy can be demonstrated, but increasing the daily dose to 2000 μg per day and higher increases the risk of systemic side effects and does not result in additional benefits for the majority of patients with asthma (2). The first guidelines to mention a treatment alternative for step 3—a moderate dose of an inhaled corticosteroid plus a long-acting inhaled $\beta_2$-agonist—was the Swedish guidelines published in 1992 (3). This treatment alternative is also recommended in the most widely distributed document, the Global Initiative for Asthma (GINA) guidelines (4).

A number of clinical studies in adults and children with asthma have indicated that early introduction of an inhaled corticosteroid results in better asthma control, better airway function, and greater effect on bronchial hyperresponsiveness than a delayed initiation of therapy (5–8). In a recent study 335 patients with asthma were followed for 3–5 years after the introduction of budesonide, usually at a daily dose of 400 μg twice daily ($n = 272$), although a starting dose range from 100 to 800 μg twice daily could be applied (9). When starting the treatment with budesonide 206 patients (mean age 34 years) had asthma symptoms for less than 2 years (median duration 14 months) and 129 patients (mean age 39 years) for longer periods of time (median duration 5 years). Their mean $FEV_1$ was 80.3% and 75.6% of predicted normal values and mean morning peak expiratory flow (PEF) was 83.1% and 74.0% of predicted normal values in the two groups, respectively. At the end of the follow-up period, 83% of the patients in the early introduction group were using inhaled steroids in a mean daily dose of 411 μg. In the delayed introduction group, 99% of the patients required inhaled steroids in a mean daily dose of 836 μg. In an attempt to achieve stated treatment goals (4) 6% of the patients in the early introduction group had received the addition of an inhaled long-acting $\beta_2$-agonist. In the late introduction group 61% of the patients had to be treated with an inhaled long-acting $\beta_2$-agonist, 26% received theophylline, and 21% some other additional treatment, mainly a leukotriene receptor antagonist. Table 1 shows how the treatment goals were achieved, clearly indicating the benefit of early introduction with inhaled corticosteroids. These figures also indicate that roughly speaking some 25% of the patients who receive early anti-inflammatory treatment may need additional treatment in later years in order to be as well controlled as possible in their asthma, whereas this percentage increases to approximately 75% if inhaled steroids are introduced later than 2 years after the first symptoms and signs of the disease.

This review covers results of controlled clinical studies investigating the efficacy and safety of combination therapies when inhaled long-acting $\beta_2$-agonists, theophylline and leukotriene receptor antagonists have been added to doses of inhaled corticosteroids in comparison with higher doses of inhaled steroids.

**Table 1** Achievement of Treatment Goals

| | Early treatment ($n = 206$) | Delayed treatment ($n = 129$) |
|---|---|---|
| $FEV_1 > 90\%$ predicted normal | 78% | 41% |
| Exercise tolerance normal | 77% | 24% |
| Normal sleep | 83% | 78% |
| Use of rescue medication <4 times per week | 66% | 34% |
| Exacerbations per patient per year requiring prednisolone | 0.03 | 0.4 |

## II. Inhaled Corticosteroids Plus Long-Acting Inhaled $\beta_2$-Agonists

### A. Beclomethasone Dipropionate Studies

Greening et al. investigated the efficacy of 200 μg beclomethasone dipropionate (BDP) plus 50 μg salmeterol twice daily in comparison with a twofold higher dose of BDP, 500 μg twice daily, in a 6-month parallel-group study in 429 asthma patients with remaining symptoms on 400 μg daily BDP (10). The combination treatment resulted in significantly greater improvements in airway function (morning and evening PEF; Fig. 1), and in reductions in diurnal variation of PEF, asthma symptoms, and use of rescue salbutamol. No differences in asthma exacerbations or adverse events were found between the two treatment groups.

Woolcock et al. performed a similar type study over 6 months in 738 patients with more severe asthma, comparing BDP 500 μg twice daily plus either 50 μg or 100 μg salmeterol twice daily with BDP 1000 μg twice daily (11). The results were very similar to those reported by Greening et al. (10): addition of salmeterol provided greater improvements in lung function and symptom control without alterating the degree of bronchial hyperresponsiveness or exacerbation rates than did doubling the dose of BDP. No differences were found between the two salmeterol dose groups.

Wilding et al. (12) determined the effect of adding salmeterol 50 μg twice daily for 6 months to current treatment in 101 patients with asthma, who were using at least 200 μg daily of BDP (or budesonide), on the possibility of reducing their inhaled corticosteroid dose according to a management plan. The study was double-blind and placebo-controlled. Compared with placebo, salmeterol treat-

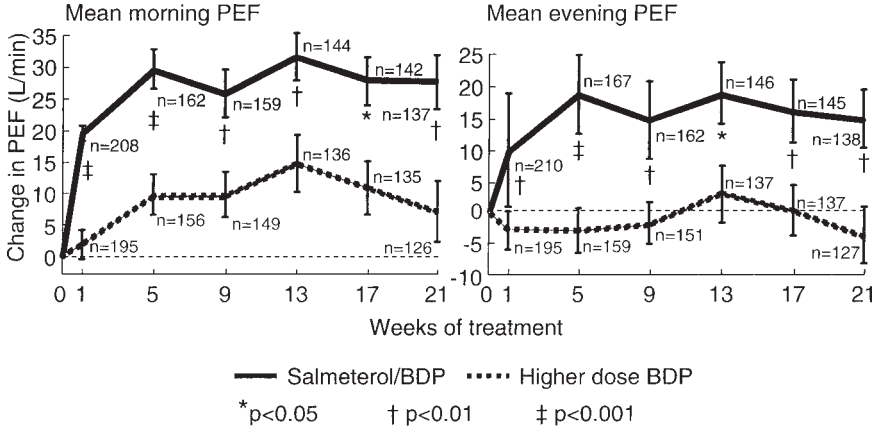


**Figure 1** Changes from baseline (±SE) in mean morning and evening PEF during 6 months of study treatment. (From Ref. 10.)

ment resulted in a 17% reduction in inhaled steroid doses and improved lung function and symptom control. No differences in number of exacerbations or in use of oral prednisolone courses were found between the two groups.

A fourth BDP study was reported by Bouros et al. (13). In an open-label randomized, parallel-group 12-week study in 132 patients symptomatic on 500 μg daily BDP, addition of formoterol 12 μg twice daily delivered via a pressurized metered dose inhaler was significantly more effective in improving lung function and reducing asthma symptoms and need of rescue bronchodilators than doubling the dose of BDP to 1000 μg daily.

### B. Budesonide Studies

Pauwels et al. (14) evaluated the effects of adding formoterol 12 μg twice daily to either a low dose of budesonide (100 μg twice daily) or a four times higher dose of budesonide (400 μg twice daily) delivered via Turbuhaler in a double-blind, randomized, placebo-controlled, parallel-group 12-month study in 852 patients—the FACET study. The fourfold difference in inhaled corticosteroid dose in this study is important because dose-response studies with inhaled steroids have failed to demonstrate statistically significant differences in any outcome variables between doubling doses (2). Therefore, in the FACET study the higher dose of the inhaled steroid was given a fair chance in comparison with the combination treatments.

Because the primary variable of efficacy in the FACET study was the frequency of severe and mild asthma exacerbations, the study started with a 4-week run-in period during which the patients received a high dose of budesonide, 800 μg twice daily, in order to be maximally improved and stabilized before randomization. In fact, 1114 patients entered the run-in phase, but 262 patients were excluded as being ineligible.

At entry, patients were using mean daily maintenance doses of inhaled corticosteroids between 818 and 856 μg in the four treatment groups. Before the run-in period their mean $FEV_1$ values were between 75.4 and 76.3% of predicted normal values, and after the run-in period they were between 81.8 and 84.0%.

As in the previously reported studies with BDP and salmeterol, the addition of formoterol to budesonide resulted in significant improvements in airway function ($FEV_1$ and PEF) and reductions in asthma symptoms, episode-free days, awakenings, and need for rescue bronchodilators. The important finding, however, was the statistically significant reductions in severe and mild asthma exacerbations seen when formoterol was added to both low- and high-dose budesonide compared to budesonide treatment alone (Fig. 2). Also important was the finding that the fourfold higher dose of budesonide without formoterol was significantly more effective in reducing the rate of severe asthma exacerbations compared with low-dose budesonide plus formoterol. Adding formoterol to budesonide did not affect the pattern of onset or recovery of the 425 severe exacerbations noticed dur-

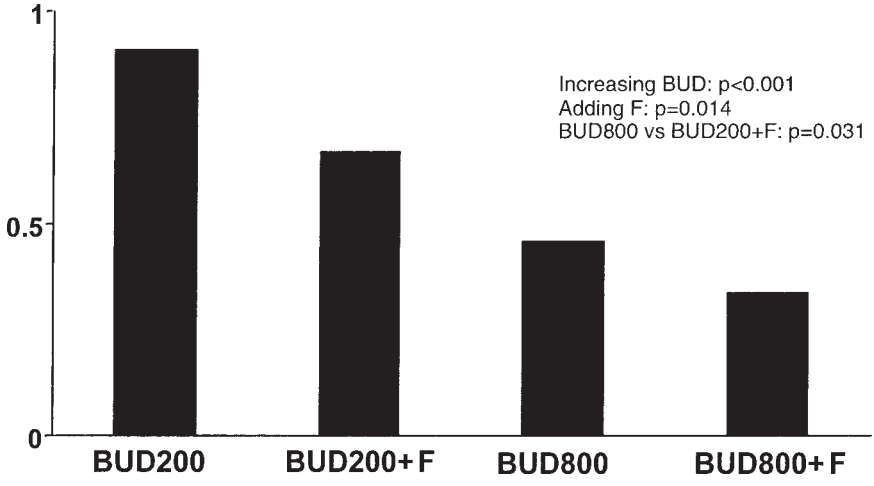


**Figure 2** Rate of severe asthma exacerbations per patient per year in patients treated with budesonide Turbuhaler 100 μg or 400 μg twice daily with or without formoterol Turbuhaler, 12 μg twice daily. (Adapted from Ref. 14.)

ing the FACET study as measured as changes in PEF values and the use of rescue bronchodilators before and after an exacerbation (15).

The FACET study thus demonstrated an additive effect of increasing the dose of budesonide and adding an inhaled long-acting $\beta_2$-agonist to the budesonide treatment. The beneficial effects of the two treatment options were independent and additive. But most importantly, the study demonstrated that adding formoterol to budesonide treatment did not result in deterioration of asthma control (14).

In a separate 12-month study with the same design as the FACET study, the influence on inflammatory markers was evaluated by studying 100 μg budesonide plus 12 μg formoterol twice daily versus 400 μg budesonide twice daily (16). No difference in eosinophil numbers in induced sputum could be found. The clinical variables behaved very similarly compared to those in the main study.

### C. Fluticasone Propionate Plus Salmeterol

The studies by van Noord et al. (17), Baraniuk et al. (18), Pearlman et al. (19), and Condemi et al. (20) all evaluated the efficacy of a low dose of fluticasone propionate (FP) plus salmeterol 50 μg twice daily in comparison with a twofold higher dose of FP. The results were consistent and very similar to those reported for BDP plus salmeterol versus a twofold higher dose of BDP (10).

The study by Pearlman et al. (19) had six treatment arms and included monotherapy with salmeterol, 50 μg twice daily, in addition to placebo, the FP

monotherapies (100 μg and 250 μg twice daily) and the combination treatments (adding salmeterol 50 μg twice daily to both doses of FP). Salmeterol alone improved airway function more than the monotherapies with FP.

### D. Fixed Combinations Products

Because of the favorable results seen in the studies with the combination of an inhaled corticosteroid and an inhaled long-acting $\beta_2$-agonist cited above, fixed combination products have been developed consisting of FP plus salmeterol (Seretide® Advair®) and budesonide plus formoterol (Symbicort®).

## III. Inhaled Corticosteroids Plus Theophylline

Evans et al. (21) performed a 3-month double-blind, placebo-controlled, randomized, parallel-group study in patients still symptomatic when using 67–1000 μg of inhaled corticosteroids daily (mean doses around 700 μg per day). They received budesonide 400 μg daily plus 250 or 375 mg of theophylline depending on body weight ($n = 31$), or 800 μg of budesonide ($n = 31$). The patients' mean morning PEF values (SE) were 383 L/min (16) and 397 L/min (19) and mean $FEV_1$ values were 78 and 72% (4) of predicted normal values in the combination group and the higher budesonide dose group, respectively. All treatments resulted in improvements in lung function, but the lower dose of budesonide together with theophylline improved lung function ($FEV_1$ and FVC) more than the double dose of budesonide, which, on the other hand, reduced asthma symptoms significantly, which was not seen in the combination treatment group. No statistically significant differences in PEF values were observed. The mean theophylline plasma concentration was 8.7 μg/mL in patients receiving theophylline.

In another randomized, double-blind, parallel-group study conducted in several European countries, 69 patients were treated for 6 weeks with theophylline plus BDP 400 μg per day, compared to 64 patients treated with BDP 800 μg per day (22). The mean serum theophylline concentration was 10.1 μg/mL. The study demonstrated clinical equivalence of theophylline plus BDP 400 μg per day and BDP 800 μg per day in patients whose asthma is not controlled on BDP 400 μg per day.

## IV. Inhaled Corticosteroids Plus Leukotriene Receptor Antagonists

The effects of adding a leukotriene receptor antagonist to treatment with inhaled corticosteroids have been reported in a number of studies. However, many studies have so far been published only as abstracts. Hui and Barnes (23) demonstrated in

10 patients, of whom 9 were receiving inhaled steroids (median dose 800 μg/day), that adding a single dose of zafirlukast 40 mg (old formulation corresponding to 16 mg of the marketed product) improved $FEV_1$ by 7% compared with placebo. The study had a double-blind, randomized, crossover design. After 4 hours a high dose of salbutamol was given. This resulted in a total 26% improvement in $FEV_1$ in the zafirlukast plus salbutamol group compared with 18% in the placebo plus salbutamol group. These results indicate that the bronchodilating effect of zafirlukast was additive to that of a $\beta_2$-agonist.

In a placebo-controlled study, patients treated with pranlukast were able to tolerate a 50% reduction in their inhaled corticosteroid dose from an average of 1900 μg per day of BDP without loss of asthma control measured as unchanged morning PEF values (24). Furthermore, the placebo-treated patients, but not the pranlukast patients had a significant rise in serum ECP concentrations and in exhaled nitric oxide. In a 12-week, double-blind, randomized, placebo-controlled study in 226 patients, the addition of montelukast 10 mg once daily resulted in a 47% reduction in the inhaled steroid dose compared to a 30% reduction in the placebo group. Fewer patients on montelukast (16%) versus placebo (30%) required discontinuation because of treatment failure (25).

In another study 27 patients not well controlled on 3 months of treatment with 800–1200 μg of BDP received pranlukast 225 mg twice daily or placebo for 4 weeks (26). Morning PEF improved significantly by 21 L/min in the pranlukast group compared with 3 L/min in the placebo group. No differences were found for daytime or nighttime asthma symptoms, use of rescue $\beta_2$-agonists, $FEV_1$, or quality of life measurements.

Virchow et al. added zafirlukast, 80 mg twice daily, or placebo to the treatment of 368 asthmatics still symptomatic despite high-dose inhaled corticosteroid therapy (mean dose 1600 μg per day of BDP) in a 6-week, double-blind, randomized study (27). Their mean age was 48.3 years and mean $FEV_1$ was 64% of predicted. A gradual improvement in morning and evening PEF was seen, reaching a statistically significant difference versus placebo at week 6, when morning PEF had improved 18.7 L/min in the zafirlukast-treated group compared to 1.5 L/min in the placebo group. Statistically significant differences were also found for evening PEF, $FEV_1$, and asthma symptomatology (daytime asthma score, $\beta_2$-agonist use). An effect on asthma exacerbations was also demonstrated: a 37% decrease for mild exacerbations compared with placebo (49 in the placebo and 31 in the zafirlukast group) and a 59% reduction for moderate to severe exacerbations (33 in the placebo and 13 in the zafirlukast group).

Nayak et al. performed a 13-week, double-blind, parallel-group study in 394 patients (12 years of age) symptomatic on standard doses of BDP (28). They were randomized to treatment with 400 μg per day of BDP plus 40 or 80 mg twice daily of zafirlukast or 800 μg per day of BDP. After 13 weeks of treatment, morning PEF, $FEV_1$, daytime asthma symptom scores, $\beta_2$-agonist use, and night-

time awakenings improved in all groups compared with baseline. However, no differences between the three treatments were found. Equivalence analysis demonstrated that zafirlukast was at least as effective as a double dose of BDP. Similar results in another 12-week double-blind, randomized, parallel-group study was reported by Ringdal et al. evaluating 440 patients (12 years of age) remaining symptomatic on 400–500 μg per day of BDP treatment (29). They were randomized to treatment with the previous dose of BDP plus zafirlukast 40 mg or 160 mg per day or to a double dose of BDP. Thirty-six to 38% of the patients in all three groups improved more than 10% in morning PEF values without a difference between the groups. Twenty to 26% of the patients experienced an asthma exacerbation without differences between the groups.

Miyamoto reported the results of a 6-week, four-arm, placebo-controlled, double-blind, randomized, parallel-group study evaluating the efficacy of zafirlukast 10, 20, and 40 mg twice daily in 340 patients (16 years old) (30). Of these patients, 243 were eligible for analysis and 161 (66%) were on treatment with inhaled or oral corticosteroids. Morning PEF increased significantly in all zafirlukast groups by approximately 20 L/min above placebo. The global assessment of efficacy (based on composite scores) indicated moderate-to-marked improvements in 43, 56, 62, and 20% of patients not using corticosteroids and in 43, 57, 67, and 23% of patients on treatment with steroids in the 10, 20, and 40 mg twice-daily zafirlukast groups and placebo groups, respectively. Thus, the degree of improvement appeared to be independent of treatment with corticosteroids.

## V. Trials Comparing Combination Treatments

In a small 2-week double-blind, double-dummy, crossover study in 15 patients with nocturnal asthma, Selby et al. (31) evaluated the efficacy of salmeterol, 50 μg twice daily, and theophylline in individually adjusted doses on sleep quality and cognitive performance. Twelve of the patients were on treatment with inhaled corticosteroids. Overnight PEF falls were similar during the two treatments, but salmeterol treatment resulted in more nights without awakenings, in fewer nocturnal arousals, and in improved quality of life. Visual vigilance improved on salmeterol, but otherwise daytime cognition was unaffected. Otherwise no differences between salmeterol and theophylline were found.

A meta-analysis of nine randomized, controlled clinical studies, containing a total of 1330 patients, compared the effect of salmeterol or theophylline in patients with asthma, the majority being already treated with inhaled corticosteroids (32). The main outcome measurements were morning and evening peak expiratory flows rate (PEFR), morning and evening symptom scores, use of salbutamol as rescue medication, and withdrawal from treatment for any cause. During the second week of treatment, salmeterol patients had a 10 L/min greater increase in mean morning PEFR from baseline than theophylline patients ($p < 0.001$). Simi-

larly, in the second week the increase in mean evening PEFR from baseline observed with salmeterol was significantly greater ($p < 0.01$) than that observed with theophylline. Salmeterol also produced a significantly greater increase in mean morning and evening PEFR than theophylline at weeks 3 and 4. Patients receiving salmeterol were free from daytime symptoms for a mean of 51% of days in the second week compared to 39% for theophylline patients ($p < 0.001$). Salmeterol patients experienced a mean of 63% symptom-free nights compared to 52% for theophylline patients ($p < 0.001$). Rescue medication with salbutamol was not required on 49% of days for salmeterol patients and 34% of days for theophylline patients. All results were maintained in the third and fourth weeks of treatment. Withdrawal and incidence of adverse events leading to withdrawal were significantly less frequent in patients receiving salmeterol ($p < 0.001$).

Two studies have compared the effects of salmeterol or montelukast added to treatment with inhaled corticosteroids on adenosine monophosphate (AMP) bronchial challenges in patients not adequately controlled on inhaled steroids alone (33,34). One 2-week study in 20 patients investigated salmeterol 50 μg twice daily and montelukast 10 mg once daily with AMP challenges after the first and the last dose. Compared with placebo, both treatments resulted in significant protection after the first dose, but only montelukast maintained its protective effect up to the last dose. Montelukast treatment also gave a significant reduction in blood eosinophil counts indicating an additive anti-inflammatory effect (33). In the other single-dose, crossover study 12, mild-moderate asthmatics received montelukast 10 mg, salmeterol 50 μg alone, montelukast 10 mg plus salmeterol 50 μg or 100 μg, and placebo. AMP challenge was performed 12 hours after salmeterol and 24 hours after montelukast administration. For AMP-$PC_{20}$ there were significant protection after all treatments. There was a numerical trend to suggest an additive effect when montelukast and salmeterol were combined (34).

Busse et al. administered salmeterol 50 μg ($n = 214$) or zafirlukast 20 mg ($n = 215$) twice daily for 4 weeks to patients using fixed doses of inhaled corticosteroids (35). Salmeterol increased mean morning PEF by 30 L/min as compared to an increase of 13 and 13.2 L/min with zafirlukast. The differences between the two treatments were statistically significant. Salmeterol also significantly increased the percent of symptom-free days, the percent of days with no rescue medication, as well as the daily use of rescue medication. No difference between the treatments was found for asthma exacerbations. Similar safety profiles were reported. This study indicates that salmeterol is more effective than zafirlukast when added to inhaled corticosteroids in patients with persistent asthma.

## VI. Conclusions

Asthma treatment guidelines recommend the addition of long-acting inhaled or other controller medication as an alternative to increasing doses of inhaled cor-

ticosteroids in patients in whom treatment goals are not achieved with inhaled corticosteroids in daily doses up to approximately 1000 μg. This chapter reviewed the results of published controlled studies with inhaled long-acting $\beta_2$-agonists (formoterol, salmeterol), theophylline, and leukotriene receptor antagonists when added to treatment with inhaled corticosteroids.

Addition of all three mentioned treatment alternatives to doses of inhaled corticosteroids was more or equally effective than increasing doses of inhaled corticosteroids in terms of mean improvements in airway function, symptom control, and the use of rescue bronchodilator medication. The most effective addition was the long-acting inhaled $\beta_2$-agonist. The inhaled corticosteroid-theophylline combination is less effective and has a greater risk of side effects. The combination with an antileukotriene has not been widely compared with other possible combinations.

Addition of formoterol to a low dose of budesonide was as effective as a four times higher dose of budesonide in reducing the frequency of mild asthma exacerbations. However, a four times higher budesonide dose resulted in a significantly greater reduction in severe asthma exacerbations compared with the low-dose budesonide plus formoterol. Nevertheless, addition of formoterol to the high dose of budesonide further significantly reduced the rate of severe exacerbations.

From available data it appears that patients already treated with inhaled corticosteroids and with an airway function clearly below predicted normal values, with nighttime awakenings or with a high use of short-acting $\beta_2$-agonists for treatment of breakthrough symptoms, the addition of an inhaled long-acting $\beta_2$-agonist would be the first choice. Patients on inhaled corticosteroids having repeated severe asthma exacerbations need increased anti-inflammatory medication: an increase in the inhaled steroid dose or the addition of an leukotriene receptor antagonist. The value of adding theophylline needs further investigation.

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## Discussion

**Dr. Jeffery:** In a collaboration with my Danish and Swedish colleagues, and supported by GlaxoWellcome, we have completed two crossover studies comparing the effects of placebo, salmeterol, and fluticasone. Firstly, in stable, mild asthma, salmeterol (given at 50 μg b.d. for 6 weeks) reduces the numbers of neutrophils in biopsies and also of neutrophil markers in blood. Secondly, in response to allergen challenge, fluticasone (250 μg b.d.) increases neutrophil numbers in biopsies whilst salmeterol reduces neutrophils. These actions might be considered complementary.

**Dr. Selroos:** I have seen the results of your biopsy studies. The next obvious step would be to perform a biopsy study evaluating the combined effects of an inhaled steroid plus a long-acting $\beta_2$-agonist.

**Dr. O'Byrne:** The study design used in many of the "add-on" studies is inappropriate as doubling doses of inhaled glucocorticoids have not been shown to significantly improve the outcomes measured in the studies.

**Dr. Selroos:** I agree. A fourfold difference in inhaled steroid dose, as in the FACET study, is required in order to give the inhaled steroid limb a fair chance to compete in endpoint measurements.

**Prof. Dolovich:** It is possible that delivery of steroids to the distal lung may be enhanced if the lung is pretreated with bronchodilator, as I showed in severe asthmatic subject on ICS with PET imaging. My observations support your findings using formoterol/budesonide combination therapy, but perhaps the effect only occurs in the moderate-severe asthmatic.

**Dr. Selroos:** Your point is well taken. However, the studies in asthmatics that I am aware of have not been able to demonstrate a significantly improved deposition when a bronchodilator has been given prior to inhalation of an inhaled steroid. The main reason why we previously recommended the $\beta_2$-agonist to be inhaled some 10 minutes before the inhaled steroid dose was the fact that inhalation of the steroid pMDI (lubricants and propellants) often resulted in cough, and sometimes even in bronchospasm—which could be avoided by giving the $\beta_2$-agonist in advance. With dry powder inhalers, we do not have this problem.

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