FACTORS AFFECTING PULMONARY TARGETING OF INHALED CORTICOSTEROIDS

By

KAI WU

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006
For my Waipo, Baba, Mama, Gege and Laopo.
ACKNOWLEDGMENTS

I extend my appreciation and grateful thanks to Dr. Guenther Hochhaus for guiding me through the four years of Ph.D. study, giving me excellent suggestions and always supporting me. I would like to thank the members of my supervisory committee, Dr. Hartmut Derendorf, Dr. Jeffrey Hughes, and Dr. Saeed R. Khan, for their valuable and kind advice throughout my research. I take this opportunity to express my gratitude to Yufei Tang for her invaluable technical assistance.

I would like to thank all the secretaries, Jame Ketcham, Andrea Tucker and Patricia Khan, for their technical and administrative assistance. I thank my group members, Yaning, Manish, Intira, Vikram, Sriks, Zia, Elanor, Keerti, Navin, Nasha and other graduate students and post-docs in the department for their assistance and friendship. Especially I want to thank all my friends, Weihui, Chao, Jeff, Victor, Aixin, Kai and Zhen, Chengguan and Qin, Yipeng and Hongxiao and all my other friends for their help and support that have made my stay in Gainesville a pleasant experience.

I want to express my deep gratitude and love for my parents, brother and other family members for their unconditional support and encouragement of my academic pursuits over the years.

Last, but not least, I want to thank my lovely wife, Ling, for her 120% support, full understanding and timely encouragement. Without her, I would not have been able to achieve any of what I have achieved.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Mechanism of Action of Corticosteroids in Asthma</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Disposition of Inhaled Corticosteroids</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Inhalation Device</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Physical Characteristics of Patient</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Pharmacokinetic and Pharmacodynamic Properties of ICS</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Pharmacokinetic Properties</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Prodrug</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Mucociliary Clearance</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Pulmonary Residence Time</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Bioavailability</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Clearance</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Drug Distribution</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Half-life</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Protein Binding</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Objectives of the Study</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>IN-VITRO COMPARISON OF FLUTICASONE RESPIRABLE DOSE FROM A METERED DOSE INHALER AND THREE RIGID VALVED HOLDING CHAMBERS</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Materials and Methods</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Chemicals and Devices</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Sample Collection</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Fluticasone Propionate Assay</td>
<td>26</td>
</tr>
</tbody>
</table>
# PLASMA CONCENTRATIONS AND PHARMACOKINETICS PROFILES OF INHALED CORTICOSTEROIDS AND THEIR RELATION TO LUNG FUNCTION IN ASTHMA

## 3 Introduction

Methods

Subjects

Measurements

Lung function

Plasma drug assays

Protocol

Analysis

Results

Plasma drug concentrations and pharmacokinetic profiles

Relation between lung function and AUC

Discussion

Plasma Drug Concentrations and Pharmacokinetic Profiles

Relation Between Lung Function and AUC

Conclusion

## 4 FLUTICASONE AND BUDENOSIDE CONCENTRATIONS AFTER INHALATION EFFECT OF BRONCHOCONSTRICTION

## 5 EVALUATION OF THE ADMINISTRATION TIME EFFECT ON THE CUMULATIVE CORTISOL SUPPRESSION AND CUMULATIVE LYMPHOCYTES SUPPRESSION FOR ONCE-DAILY INHALED CORTICOSTEROIDS

Introduction

Methods

Subjects

Measurements

Spirometry

Methacholine inhalation challenge

Drug assays

Protocol

Analysis

Results

Discussion

Historic Data
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1</td>
<td>Baseline characteristics</td>
<td>36</td>
</tr>
<tr>
<td>3-2</td>
<td>Mean (SD) pharmacokinetic parameters for beclometasone \ monopropionate(BMP), \ budesonide(BUD), fluticasone propionate(FP) and \ mometasone furoate (MF)</td>
<td>38</td>
</tr>
<tr>
<td>3-3</td>
<td>Partial correlation coefficients (r) for the relationship between FEV1 % \ predicated and AUC after adjusting for age or gender</td>
<td>40</td>
</tr>
<tr>
<td>3-4</td>
<td>Predicted AUC values at 50 and 100% predicted FEV1 and PEF and the ratio of \ these values</td>
<td>40</td>
</tr>
<tr>
<td>4-1</td>
<td>Baseline characteristics</td>
<td>49</td>
</tr>
<tr>
<td>4-2</td>
<td>Pharmacokinetic parameters for fluticasone and budesonide when inhaled with \ and without prior methacholine-induced bronchoconstriction</td>
<td>50</td>
</tr>
<tr>
<td>4-3</td>
<td>Area under the curve (AUC) values for fluticasone and budesonide when \ inhaled with and without prior methacholine-induced bronchoconstriction</td>
<td>52</td>
</tr>
<tr>
<td>5-1</td>
<td>Summary of BUD pharmacokinetic model parameter estimates</td>
<td>65</td>
</tr>
<tr>
<td>5-2</td>
<td>Summary of FP pharmacokinetic model parameter estimates</td>
<td>67</td>
</tr>
<tr>
<td>5-3</td>
<td>Summary of FP and BUD pharmacokinetic/pharmacodynamic model parameter \ estimates</td>
<td>70</td>
</tr>
<tr>
<td>5-4</td>
<td>Summary of the mean cumulative cortisol suppression of the 75 subjects of \ the 8 simulations</td>
<td>73</td>
</tr>
<tr>
<td>5-5</td>
<td>Summary of the mean cumulative lymphocytes suppression of the 75 subjects of \ the 8 simulations</td>
<td>73</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Molecular structures of several inhaled corticosteroids.</td>
<td>2</td>
</tr>
<tr>
<td>1-2</td>
<td>Cellular interactions after antigen inhalation in asthma</td>
<td>4</td>
</tr>
<tr>
<td>1-3</td>
<td>Classical model of glucocorticoid (GCS) action</td>
<td>8</td>
</tr>
<tr>
<td>1-4</td>
<td>Disposition of ICSs after inhalation</td>
<td>11</td>
</tr>
<tr>
<td>2-1</td>
<td>Schematic diagram of the cascade impactor with aerosol aerodynamic size range at each impactor stage 0–7 and at the final filter</td>
<td>24</td>
</tr>
<tr>
<td>2-2</td>
<td>Mean ± SD respirable dose of fluticasone propionate from metered-dose inhaler (MDI) and valved holding chamber (VHC) devices</td>
<td>28</td>
</tr>
<tr>
<td>2-3</td>
<td>Mean ± SD oropharyngeal dose of fluticasone propionate from metered-dose inhaler (MDI) and valved holding chamber (VHC) devices</td>
<td>29</td>
</tr>
<tr>
<td>3-1</td>
<td>Mean (SE) plasma concentration-time curves for beclometasone monopropionate (BMP), budesonide (BUD), fluticasone propionate (FP) and mometasone furoate (MF) following inhalation</td>
<td>37</td>
</tr>
<tr>
<td>3-2</td>
<td>Relation between FEV$_1$ % predicted and the area under the plasma concentration-time curve (AUC) for beclometasone monopropionate (BMP), budesonide (BUD), fluticasone propionate (FP) and mometasone furoate (MF) following inhalation</td>
<td>39</td>
</tr>
<tr>
<td>4-1</td>
<td>Mean (SE) plasma drug concentrations following inhalation of 1000 µg fluticasone (Accuhaler®) and 800 µg budesonide (Turbohaler®) in 20 subjects with asthma with and without prior methacholine-induced bronchoconstriction</td>
<td>51</td>
</tr>
<tr>
<td>5-1</td>
<td>Relationship between population predicted (open triangle) and observed BUD (open circle) concentrations, individual predicted (open square) and observed BUD (open circle) concentrations as function of time</td>
<td>64</td>
</tr>
<tr>
<td>5-2</td>
<td>Relationship between population predicted (open triangle) and observed FP (open circle) concentrations, individual predicted (open square) and observed FP (open circle) concentrations as function of time</td>
<td>66</td>
</tr>
</tbody>
</table>
5-3  Diagnostic plots of BUD PK/PD model .................................................................68
5-4  Diagnostic plots of FP PK/PD model .................................................................69
5-5  Relationship between administration time and corresponding mean (+ SE) cumulative suppression ...............................................................71
5-6  Relationship between administration time and corresponding mean (+ SE) cumulative suppression ...............................................................72
FACTORS AFFECTING PULMONARY TARGETING OF INHALED CORTICOSTEROIDS

By

Kai Wu

December 2006

Chair: Guenther Hocchaus
Major: Pharmaceutical Sciences

Understanding the factor affecting pulmonary targeting would help us improve inhaled corticosteroids (ICSs) therapy. The three factors important for pulmonary selectivity are inhalation device, characteristics of patients, and pharmacokinetic/pharmacodynamic properties of ICSs. Certain aspects related to these factors were evaluated in the current dissertation.

The effect of different valved holding chambers on in vitro aerosol deposition from a fluticasone MDI was assessed using an in vitro cascade impactor method. It was found that the mean fluticasone respirable dose from AeroChamber-Plus™ and OptiChamber® was no different (p > 0.05) from the respirable dose produced by the MDI alone, while the OptiChamber®-Advantage respirable dose was significantly less compared with the dose from the MDI alone (p < 0.05).

The overall goal of the second and third study was to test the hypothesis that the pulmonary systemic availability of ICSs is related to lung function of the patients while
the extent of lung function affecting the bioavailability depends on the physicochemical properties of ICSs. In the second study, it was found that the bioavailability for all four drugs (beclomethasone, budesonide, mometasone and fluticasone) tended to be higher in the asthmatic patients with higher lung function, while the associations between the bioavailability and lung function differed for the four drugs. In the third study, it was found that $\text{AUC}_{0-5\text{hour}}$ values for fluticasone and budesonide were lower by a median of 60% and 29% respectively when bronchoconstriction was induced in the asthmatic patients.

Finally, the effect of administration time on cumulative cortisol (CCS) and cumulative lymphocytes suppression (CLS) was evaluated for once-daily ICS and a population pharmacokinetic/pharmacodynamic modeling/simulation approach was used. It was found that the optimal time for administration of ICS depends on the choice of the biomarker: afternoon in terms of minimized CCS, and morning in terms of minimized CLS.
CHAPTER 1
INTRODUCTION

More than 7% of adults and 12% of children in the United States (US) are affected by asthma, a chronic inflammatory disease of the airways [1]. Over the world, asthma afflicts 100 to 150 million people. The prevalence of asthma in the US has been increasing by 5% to 6% over the past decades [2]. Asthma is responsible for 100 million days of restricted activity and 470,000 hospitalizations each year [1]. In 1998, asthma related cost was estimated at $11.3 billion in the U.S. alone.

Inhaled corticosteroids (ICSs) have revolutionized the treatment of asthma in the past 30 years and now represent the first-line therapy for patients with chronic disease [3]. Numerous clinical studies have demonstrated the high anti-asthmatic efficacy (e.g. prevention and control of symptoms) and reduced systemic side effects of ICS therapy. Introduction of beclometasone dipropionate in 1972 as a pressurized metered-dose inhaler (MDI) marked the beginning of the targeting treatment for asthma [4]. Since then, other potent topical corticosteroids have been developed, and currently flunisolide (FLU), triamcinolone acetonide (TA), budesonide (BUD), fluticasone propionate (FP), mometasone furoate along with BDP (Figure 1-1) are available in the US. Additionally, ciclesonide (Figure 1-1) is another ICS currently under development for the treatment of asthma of all severities.

The use of inhaled corticosteroids for asthma allows, in effect, local delivery of the drug to the lung. The goal of inhaled corticosteroids is to produce long-lasting therapeutic effects at the pulmonary target site and to minimize systemic effects (pulmonary
selectivity). In general, pulmonary targeting of ICS is determined by their pharmacokinetic and pharmacodynamic (PK/PD) properties, the biopharmaceutical aspects of the delivery device and the characteristics of the patient. In this chapter, an overview of factors affecting pulmonary targeting of ICS will be discussed. In addition, the current literature on asthma pathogenesis and the mechanism of action of ICS in asthma therapy is reviewed.

Figure 1-1. Molecular structures of several inhaled corticosteroids [5, 6].
Asthma

Asthma is a chronic disorder of the airways characterized by airway inflammation, reversible airflow obstruction and airway hyperresponsiveness. Studies have shown that airway inflammation is an integral element in the pathogenesis of asthma and a driving force in airway hyperresponsiveness and a contributing factor for variable levels of airflow obstruction [7].

Asthma inflammation involves many cells, with mast cells, eosinophils, basophils, neutrophils, macrophages, epithelial cells, and lymphocytes all being active participants (Figure 1-2). The interactions of these cells result in the release of different mediators leading to bronchoconstriction, microvascular leakage, mucus hypersecretion, epithelial damage, and stimulation of neural reflex. For example, activation of the mast cell by specific antigen through cell-bound IgE releases histamine and causes synthesis of leukotrienes, which can precipitate an acute episode of airway obstruction [8]. Mast cells also release inflammatory enzymes or proteases, which can contribute to airway inflammation and airway hyperresponsiveness. Finally, mast cells express and release a wide variety of proinflammatory cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3, 4 and 5 [9]. These cytokines change the immunologic environment of the airway and are likely instrumental in initiating the inflammatory response in asthma through changes in adhesion proteins, cell recruitment, and inflammatory cell survival. Eosinophils are recruited to the lung in asthma after antigen inhalation and are the main effector cells in the resulting inflammatory process. Eosinophils contribute to inflammation by release of mediators (i.e., leukotrienes and toxic oxygen products), by degranulation and release of granular proteins, and by generation of cytokines [8, 10]. Granular proteins, such as major basic protein, directly
damage airway tissue, promote airway responsiveness, and injure airway epithelium. In general, the degree of airway eosinophilia is usually proportional to the severity of asthma [11]. Activated lymphocytes are another important element of airway inflammation. One of the T helper (T<sub>H</sub>) lymphocytes, T<sub>H2</sub>, shows a cytokine profile that contains IL-4 and IL-5, two cytokines that are essential for IgE synthesis (IL-4) and eosinophil production (IL-5). Since lymphocytes are long lived, they are believed to be crucial to the persistence of the inflammatory process in asthma. Epithelial cells are another source of proinflammatory mediators in asthma including eicosanoids, cytokines, chemokines, and nitric oxide [12].

![Figure 1-2. Cellular interactions after antigen inhalation in asthma [13]](image)

The current concept of asthma therapy is based on a stepwise approach, depending on disease severity, and the aim is to reduce the symptoms that result from airway obstruction and inflammation, to prevent exacerbations and to maintain normal lung function [14]. Controlling the allergic response to the local environment due to smoking and allergens found at home or in the workplace, is the first priority in minimizing
asthma hyperresponsiveness. Traditionally, there are five classes of medications for asthma therapy: corticosteroids (e.g., beclomethasone dipropionate), short-acting (e.g., albuterol) and long-acting (e.g., salmeterol) beta-agonists, theophylline, cromolyn sodium and nedocromil sodium, and anticholinergics (e.g., ipratropium bromide) [15]. Corticosteroids, cromolyn sodium and nedocromil sodium can be classified as anti-inflammatory medications. Beta-agonists and theophylline are both bronchodilators. Anticholinergics work by blocking the contraction of the underlying smooth muscle of the bronchi. Among these medications, inhaled corticosteroids are the most effective for the treatment of asthma. Their efficacy is related to many factors including a diminution in inflammatory cell function and activation, stabilization of vascular leakage, a decrease in mucus production, and an increase in beta-adrenergic response. The strong scientific rationale behind the combination therapy of long-acting beta-agonists and corticosteroids has led to the development of two mixed combination inhalers: salmeterol/fluticasone and formoterol/budesonide. They are increasingly used as a convenient controller in patients with persistent asthma. Advances in the knowledge of the pathophysiology of asthma in the past decade have opened the way to the development of novel therapeutic strategies that target more directly on the pathophysiology of asthma than those currently in use [16]. Various compounds able to interfere with the complex network of proinflammatory mediators, cytokines, chemokines, and adhesion molecules involved in the pathogenesis of asthma have been identified, such as leukotriene modifiers, tachykinin antagonists, endothelin antagonists, adenosine receptor inhibitors, tryptase inhibitors, cytokine and chemokine inhibitors, direct inhibitors of T-cell function and adhesion molecule blockers. New forms of immunotherapy, such as anti-IgE therapies,
gene vaccination with plasmid DNA and mycobacterial preparations, are also emerging
to aim at blocking the unbalanced T_{H2} response that characterizes the pathophysiology of
asthma. Despite the significant expansion of the experimentally available treatment
options, only the leukotriene modifiers are on the market, among which leukotriene
antagonists are now most frequently used in asthma treatment [17, 18]. The leukotriene
antagonists have been shown to inhibit exercise-provoked bronchospasm, to modify the
airway response to inhaled antigen, and to improve airway function in patients with
chronic asthma. But in head-to-head trials with inhaled corticosteroids, the leukotriene
antagonists are less effective in terms of improvement in lung function and reduction in
exacerbations [7]. Thus far, inhaled corticosteroids still represent the cornerstone of
treatment for asthma.

**Mechanism of Action of Corticosteroids in Asthma**

Corticosteroids are able to affect many of the inflammatory pathways involved in
the pathogenesis of asthma, including the complex cell to cell communications mediated
by the so-called "cytokine network" [19]. Corticosteroids start their action when the
lipophilic corticosteroid molecule crosses the cell membrane and binds to a specific,
intracellular glucocorticoid receptor (GR) (Figure 1-3). The GR is located in the
cytoplasm and belongs to the steroid thyroid/retinoic acid receptor superfamily [20].
Prior to binding corticosteroids, GR forms a large heteromeric complex with several
other proteins, from which it dissociates upon ligand binding. 90 kDa heat shock protein
(hsp90) plays a central role in this complex [21]. It binds as a dimer to the C-terminal
ligand binding domain (Figure 1-3). The association with hsp90 has been shown to be
required to maintain the C-terminal domain of GR in a favorable conformation for ligand
binding [22], but GR is transcriptionally inactive at this stage. Binding of corticosteroids
to GR induces dissociating hsp90 and other heat shock proteins, resulting in a conformational change which allows the GR complex to translocate to the nucleus or interact with cytoplasmic transcription factors. Once in the nucleus, the GR complex binds as a dimer to specific DNA sites, a specific nucleotide palindromic sequences termed “glucocorticoid response elements” (GRE) (Figure 1-3) [23]. Then the transcription of specific genes can be increased (transactivation) or decreased (transrepression) depending on whether the GRE is positive or negative. In particular, corticosteroids increase the expression of anti-inflammatory proteins such as lipocortin-1, interleukin-1 receptor antagonist (IL-1ra), interleukin-10 (IL-10), secretory leukocyte inhibitory protein, neutral endopetidase and the inhibitory protein (IκB) of nuclear factor-κB (NF-κB) [3]. It is known that lipocortin-1 inactivates the enzyme phospholipase A₂ thus inhibiting the production of lipid mediators (i.e., platelet-activating factor, leukotrienes, and prostaglandins) and NF-κB positively regulated inflammatory and immune responses [24, 25]. Corticosteroids are also able to enhance the transcription of the gene encoding the β₂-adrenergic receptor, thus reversing and/or preventing the down-regulation possibly induced by long-term treatments with β₂-agonist bronchodilators [13]. However, the genomic mechanism of transactivation is believed to be mainly responsible for the unwanted side effects of corticosteroids, rather than for their anti-inflammatory and immunoregulatory actions. The very effective control of airway inflammation exerted by corticosteroids in asthma is largely mediated by inhibition of the transcriptional activity of several different genes encoding pro-inflammatory proteins such as cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, IL-13, TNF-α, GM-CSF), chemokines (IL-8, RANTES, MIP-1α, MCP-1, MCP-3, MCP-4, eotaxin), adhesion
molecules (ICAM-1, VCAM-1, E-selectin), and mediator-synthesizing enzymes (i-NOS, COX-2, cytoplasmic PLA2), which are mostly due to GR-dependent repression of pro-inflammatory genes [19]. Corticosteroids also exert their repressive action by direct interaction with transcription factors and post-transcriptional regulation [19].

Figure 1-3. Classical model of glucocorticoid (GCS) action. The glucocorticoid enters the cell and binds to a cytoplasmic glucocorticoid receptor (GR) that is complexed with two molecules of a 90kDa heat shock protein (hsp 90). GR translocates to the nucleus where, as a dimer, it binds to a glucocorticoid recognition sequence (GRE) on the 5-upstream promoter sequence of steroid-responsive genes. GREs may increase transcription and nGREs may decrease transcription, resulting in increased or decreased messenger RNA (mRNA) and protein synthesis (Adapted from Barnes [26]).

**Disposition of Inhaled Corticosteroids**

Understanding the disposition of ICS after inhaled administration will help us understand why PK/PD properties, the choice of delivery device and the physical characteristics of the patient are important for achieving pulmonary targeting of ICS (Figure 1-4).
Upon inhalation of a glucocorticoid, only 10% to 60% of the administered dose is deposited in the lungs. The other of the dose (40% to 90%) impacts on the oropharyngeal region and is swallowed. The swallowed dose is absorbed from the gastrointestinal tract and after undergoing first pass inactivation by the liver only orally bioavailable fraction enters the systemic circulation. The drug particles deposited in the lungs dissolve according to the rate of dissolution of the drug. If not readily dissolved, the drug will be removed by mucociliary clearance back into the oropharyngeal region and then is swallowed. Only the dissolved drug interacts with the glucocorticoid receptors (GR), as described in the previous section, present in the lung to produce the anti-inflammatory effects. Following the interaction with the GR, the drug is absorbed into the systemic circulation. The absorption processes are rapid and it is believed that dissolution is the rate-limiting step involved in pulmonary absorption of IGCs. Hence, the total systemic bioavailability of an inhaled glucocorticoid is the sum of the oral and pulmonary bioavailable fraction. The free fraction of ICS in the systemic circulation binds to the systemic GR to produce the systemic side effects. The drug is eventually eliminated from the systemic circulation mainly by hepatic clearance mechanisms.

**Inhalation Device**

One factor to be considered for distinct pulmonary selectivity is efficient delivery to the lungs. As mentioned earlier, about 10% to 60% of nominal inhaled dose will deposit in the lung. The exact percentage and the distribution of the dose in the lung depends great deal on the biopharmaceutical aspects of the delivery device. It also depends on the patient since every inhaler requires certain level of technique for optimal delivery.
There are three main classes of inhalation devices for ICSs: metered-dose inhaler (MDI), breath-activated dry powder inhaler (DPI) and nebulizer. Inside MDI the drug exists as a suspension in a carrier liquid or a solution delivered through a chlorofluorocarbon (CFC) or hydrofluoroalkane (HFA) propellant, respectively [27]. However, CFC-MDI’s are gradually phased out because of their ozone-depleting potential. In addition to their environmentally friendly property, HFA solutions also seem to have the advantage of delivering a much greater mass of fine particles, with a diameter of less than 5 µm. Fine particles are more likely to be deposited in the tracheo-bronchial and pulmonary regions in the lung. On the other side, larger particles are deposited mostly in the oropharynx where they are swallowed and increase the risk of systemic absorption [28]. The average particle diameter delivered by a CFC-MDI is 3.5-4.0 µm whereas the average particle diameter delivered by a HFA propellant is around 1.1 µm. This difference in particle diameter might have a clinical significance as the average diameter of small airways is around 2 µm, resulting in a greater lung deposition [29]. This increased proportion of fine particles with the HFA-MDI results in an improved lung deposition. A previous study showed the lung deposition of beclometasone dipropionate (BDP) was increased from 4-7% using CFC-MDI to 55-60% using a newly developed HFA-MDI [30]. Another study showed with the CFC-free solution MDI, a mean lung deposition of 52% for ciclesonide could be obtained [31]. In a single-dose study comparing HFA flunisolide and CFC flunisolide, the drug deposition in the lung could even be increased to 68% (HFA) compared to 19.7% (CFC) [32]. Hence, replacing the propellant CFC with HFA leads to an increase in lung deposition. Lung deposition can also be increased by use of spacer and valved holding chamber(VHC) devices, which can
alter the amount of fine particles and therefore, increase the respirable fraction and
decrease the amount of drug deposited in the oropharynx, [33]. Moreover, use of VHC
with MDI helps the patients who lack coordination between actuation and inhalation.
However, it also needs to be kept in mind that a greater lung deposition might result in a
greater possibility of systemic adverse effects because of the lack of first-pass
metabolism after direct absorption from the lung.

![Diagram of drug disposition](image)

Figure 1-4. Disposition of ICSs after inhalation (Adapted from [34])

The other inhaler type used for ICSs is the dry powder inhaler. Use of DPI requires
easier delivering technique that requires less coordination than the MDI. However, it
requires a forceful deep inhalation to trigger the inhalation device to help break up the
aggregates of the micronized powders into respirable particles in the oropharynx and
larger airways. Therefore, lung deposition is flow-dependent and the higher the inhalation
flow, the smaller the particles will be [35]. An inspiratory flow of 60L/min is considered
to be optimal [33]. Hence, it should be ensured that asthmatic patients in all asthma stages are able to achieve an inhalation flow that is enough to achieve the required effect [35]. In a lung deposition study it was shown that reducing the inhalation flow from 58 L/min to 36 L/min reduces the lung deposition of budesonide from around 28% to around 15% [36].

The standard and most common used jet nebulizer is based on a constant output design, run by compressed air or oxygen with supplemental air drawn in across the top of the nebulizer. The nebulizer contains the drug either as a suspension or a solution. There have been also new developments in the field of nebulizers and liquid formulations. Among those are the inhalation device Mystic™ from Batelle, which is based on electro-hydrodynamic principles, employing electrostatic energy to create fine aerosols from formulated drug solutions or suspensions thereby increasing the pulmonary tract deposition to about 80% [37] and the RESPIMAT device from Boehringer Ingelheim uses a high-pressure micro-spray system of nozzles to release a metered dose to the patient. This system generates a slow release of the drug with a high concentration of respirable particles[38].

Improved pulmonary deposition will lead to improved pulmonary targeting, especially for drugs with significant oral bioavailability such as BDP, as efficient pulmonary delivery will reduce the amount of swallowed drug and, subsequently, oral absorption. In addition, the new more efficient pulmonary delivery devices reduce oral deposition which may also reduce oropharyngeal adverse effects.[39] However, efficient pulmonary deposition, with respect to systemic adverse events, is not as important for
drugs with low oral bioavailability, such as ciclesonide, mometasone furoate and fluticasone propionate, as very little drug will be absorbed through the oral route anyway.

**Physical Characteristics of Patient**

Many aspects of physical characteristics of the patient could affect pulmonary targeting of a ICS therapy. One of them is age. Usually, a proper technique for use of inhalation device is much easier to be learned and employed among adults. On the contrary, children and elderly might not learn to use the device properly, which could leads to less or more dose than right dose taken and reduced pulmonary selectivity of ICS therapy. The other issue related with age is the compliance to dosing regimen. In a study evaluating compliance with ICSs, it was found that the children between 8 and 12 years only averaged 58% of compliance [40]. Furthermore, only 32% of those doses were actually received at the correct time. A non-compliance with dose is highly likely to associated less efficacy of the therapy. Moreover, non-compliance with timing of the dose would probably affect the pulmonary selectivity of therapy, as it has been shown by some other studies that administration time is an important factor to maximize the efficacy and minimize the systemic side effects of ICSs [41-48]. The important of administration time on systemic side effects of ICSs will be discussed in later chapter of this dissertation.

Another important aspect of patient characteristics affecting pulmonary selectivity of ICSs is the lung function. It has been suggested that the pulmonary systemic bioavailability of certain ICSs is associated with the lung function of patient [49]. In one study with a single dose fluticasone dry powder in patients with asthma of varying degrees of severity there was a highly significant linear correlation between the absolute magnitude of adrenal suppression and the lung function expressed as percentage
predicted forced expiratory volume in 1 second (FEV₁) [50]. This is consistent with pharmacokinetic data where there was 62% lower plasma fluticasone concentrations (as AUC) in patients with moderately severe asthma (FEV₁ = 54% predicted) than in healthy volunteers receiving inhaled fluticasone. However, in another study, there was no difference in plasma budesonide concentrations between healthy volunteers and patients with mild asthma after inhalation budesonide, a much less lipophilic compound than fluticasone [51]. The correlation between lung bioavailability, lung function and drug’s lipophilicity will be discussed in later chapters.

**Pharmacokinetic and Pharmacodynamic Properties of ICS**

**Pharmacokinetic Properties**

**Prodrug**

A prodrug is a pharmacologically inactive compound that is activated in the body after its administration. To exert a local effect, a prodrug needs to be activated in the target tissue, e.g. lung. As an example, the ester functional group in ciclesonide needs to be cleaved by esterases in the lungs, before the active compound desisobutyryl-ciclesonide can interact with the receptor [6, 52]. The use of a prodrug might be beneficial as high lipophilicity may increase the pulmonary residence time. However, activation predominantly in the lungs is required to maintain pulmonary selectivity. One additional advantage of drugs that are delivered in an inactive form is that they might reduce certain oropharyngeal adverse effects such as oral candidiasis. It has been shown that the negligible activation of ciclesonide in the oropharyngeal region reduce the oropharyngeal adverse effects [39, 53].
Mucociliary Clearance

The mucociliary transporter is able to remove those slowly dissolving solid drug particles deposited to the upper part of the respiration tract. This phenomenon will be less pronounced for rapidly dissolving ICS or those present in solution. Mucociliary clearance mechanism is more pronounced in the central airways of the lung compared to the peripheral airways and plays an important role in reducing the overall availability (systemic + pulmonary) of highly lipophilic glucocorticoids that have a slow rate of dissolution. Studies have shown that the systemic availability of more slowly dissolving ICS, such as fluticasone propionate, is lower in patients with asthma when compared with healthy volunteers [54, 55]. The reason is that deposition in patients with asthma tends to be more central in the lungs.

Pulmonary Residence Time

The large surface area, thin membranes and the existence of pores allow an efficient exchange between the outer part of the lung and the circulatory system. Once dissolved in the lung lining fluid, ICS, as lipophilic drugs, will easily cross cell membranes and be absorbed quickly and efficiently into the systemic circulation [56]. Therefore, to achieve pulmonary selectivity requires the drug efficiently delivered to the lung, however a fast absorption into the systemic circulation immediately after delivery shall be prevented. Triamcinolone acetonide, when given as solution into rat lungs, was unable to produce pulmonary targeting as the receptor occupancy in lung and liver was very similar [57]. Extending the residence time of the drug in the lung (e.g. through controlling the release of the drug in the lung) will increase pulmonary selectivity as high drug concentrations are present in the lung for a longer period of time [58]. Various approaches to extend the pulmonary residence time of drugs have been evaluated in the
To achieve increased pulmonary residence time, two approaches are currently utilized for ICSs. Highly lipophilic drugs, such as fluticasone propionate, will dissolve slowly after deposition in the lung as particles and insure high lung concentrations over a longer period of time. A pharmacokinetic parameter used to quantify pulmonary residence time is the mean pulmonary absorption time (the average time the drug will need to leave the lung). Commercially available drugs differ in this property, with fluticasone propionate showing the highest pulmonary residence time of almost 5 hours while flunisolide is absorbed very rapidly. An alternative biological “slow release system” has been identified for budesonide and potentially ciclesonide [61-63]. In pulmonary cells, these two drugs form lipophilic esters that are biologically inactive but are so lipophilic that they are unable to cross membranes therefore they are trapped inside the cell to form a reservoir of active drug since the esters will be slowly cleaved back to the active drug. This esterification/ester-cleavage system, therefore, ensures a long residence time in the lung which might provide sufficiently prolonged asthma control for a reduced dosing regimen with budesonide and ciclesonide.

**Bioavailability**

The bioavailability of an inhaled corticosteroid is the rate and extent at which the drug reaches its site of action (pulmonary bioavailability) as well as the blood (systemic bioavailability). As mentioned earlier, a large fraction (approximately 40-90%) of the inhaled dose is swallowed and subsequently available for systemic absorption. This bioavailability of the orally delivered part is dependent on absorption characteristics of the drug from the gastro-intestinal tract and the extent of intestinal and hepatic first-pass metabolism. The oral bioavailabilities of currently used corticosteroids range from less than 1% for fluticasone propionate to 26% for 17-beclomethasone monopropionate [64-
However, the main determinant of systemic bioavailability after inhalation is direct absorption from the lung, where for the currently available inhaled corticosteroids there is no first-pass effect. All of the drug that is deposited in the lung will be absorbed systemically [49]. The percentage of the drug dose that is deposited in the lung is greatly influenced by the efficiency of the delivering device. Since the orally absorbed fraction of the drug does not contribute to the beneficial effects but can induce systemic side effects, it is desirable for the oral bioavailability of inhaled corticosteroids to be very low. Therefore, the pulmonary bioavailability is rather a function of the delivery device used for inhalation than a property of the drug itself. The pulmonary bioavailability will depend on the amount deposited in the lungs and will differ with the delivery device used [49, 71]. Fluticasone propionate, for example, has an oral bioavailability of <1% due to a high first-pass metabolism (see above). When administered to the lungs using a dry powder inhaler (DPI), the absolute bioavailability (systemic + pulmonary) is reported to be approximately 17% and compared to 26% to 29% when using a metered dose inhaler (MDI) [27, 72]. After mometasone furoate administration via a dry powder inhaler the absolute bioavailability was reported to be 11% [72].

**Clearance**

Clearance is a PK parameter describing the efficiency of the body to eliminate the drug and quantifies the volume of the body fluid that is cleared of drug over a given time period. For most drugs showing linear protein binding and non-saturated elimination this value is a constant. The higher the clearance of an ICS the lower the systemic adverse effects will be and the more pronounced the pulmonary selectivity. If drugs are mainly metabolized and cleared by the liver, the maximum clearance possible is that of the liver blood flow of ~90 L/hour. Several of the available ICS (budesonide, fluticasone...
propionate) show clearance values close to the liver blood flow [73, 74]. The clearance of such high extraction drugs is independent of protein binding. The active metabolites of ciclesonide and beclomethasone propionate have been reported to express higher clearance values when given intravenously [65, 75]. While these data suggests extra-hepatic metabolism, future studies with the active metabolites need to be performed to clearly confirm the high clearance of these two drugs, especially as this would represent a clear advantage for these drugs over other commercially ICS.

**Drug Distribution**

The volume of distribution ($V_d$) is a PK parameter that describes the distribution of drug in the body. The higher the $V_d$, the greater the concentration of drug present in the tissues and the lower the plasma concentration. For drugs such as ICS that can cross membranes easily, the $V_d$ is determined primarily by how much drug is bound to plasma proteins and how much drug is bound to tissue components. Thus, corticosteroids with a very large volume of distribution (300-900L) are extensively distributed and bound to the tissues. The more drug that is bound in the tissue compartment (the smaller the fraction unbound in tissue $f_{ut}$), the higher will be the $V_d$. Similarly, a weak plasma protein binding (fraction unbound drug in plasma $f_u$ is large) will allow more drug to enter the tissue compartment. Because the $V_d$ is determined by the ratio of protein binding in plasma and tissue, it is possible for two drugs with significant differences in the plasma protein binding to show the same $V_d$. This indicates that the $V_d$ is a parameter that is difficult to evaluate. Computer simulations did show that an increase in $V_d$, although affecting the half-life of a drug, does not affect the pulmonary selectivity [76].
**Half-life**

Half-life \( (t_{1/2}) \) is a secondary PK parameter that is determined by clearance and the \( V_d \) [77]:

\[
t_{1/2} = \frac{0.693 \times V_d}{\text{clearance}}
\]

A short half-life, because of a high clearance, is beneficial for achieving pronounced pulmonary selectivity. However, a short half-life due to a small \( V_d \) does not affect the pulmonary selectivity [76]. The half-life is therefore of little value if an ICS has to be evaluated with respect to its pulmonary selectivity.

**Protein Binding**

Protein binding is one of the important PK properties of ICS. The major protein that is involved in binding with ICSs is albumin. Albumin comprises about 55-62% of the protein present in plasma and also occurs plentifully in the tissues of mammals. Protein binding is seen as storage form of the drug because it is reversible and a rapid equilibrium is established. High plasma protein binding is considered as a desirable characteristic for ICSs. Once ICSs reach the systemic circulation, binding to plasma proteins decreases the free unbound fraction in the systemic circulation and thus decreases the risk for systemic side effects, since only the free, unbound drug is pharmacologically active. Reported clinical studies showed that ciclesonide, a novel inhaled corticosteroid with plasma protein binding of 99% for itself and its active metabolite had an incidence of adverse effects that was not significantly greater than that observed with placebo [31, 78, 79]. The extent of plasma protein binding differs among ICSs and it has been positively associated with their lipophilicity. Current ICSs in development tend to be relatively lipophilic. Plasma protein binding has been reported to be 80% for flunisolide, 87% for beclomethasone dipropionate, 88% for budesonide, 71% for triamcinolone acetonide,
90% for fluticasone propionate, 98% for mometasone furoate and 99% for active principle of ciclesonide [79-82].

As discussed above, those ICSs with higher plasma protein binding would have a safety margin over those ICSs with lower plasma protein binding. However, the increased plasma protein binding may not necessarily improve the risk-benefit ratio. As lipophilic compound, ICSs could penetrate cells and bind to both plasma and tissue proteins. It is reasonable to expect the tissue protein binding for those ICSs with high plasma protein binding would be high too because of their high lipophilicity. The fraction of free drug at the target site would be reduced. The high tissue protein binding is also indicated by ICSs’ high volume of distribution. The steady state volume of distribution (Vss) of a drug can be calculated according to:

\[ V_{ss} = V_p + \left( \frac{f_{up}}{f_{ut}} \right) \times V_t \]

where \( f_{up} \) is the unbound fraction of drug in plasma, \( f_{ut} \) is the unbound fraction of drug in tissue, \( V_p \) is the plasma volume and \( V_t \) is the volume of tissue compartment. The Vss of ICSs could also be positively associated with their lipophilicity. Thus, for those relative lipophilic IG with higher plasma protein binding and Vss, as we can see from above equation, they would have increased tissue protein binding as well. An increase in tissue protein binding of just a few percent can translate into a significant reduction in the amount of efficacy. For example, if one was 99% bound in the lung and another was about 98% bound in the lung, the one with 98% bound would present two folds of unbound concentration for interaction with receptor to produce beneficial effects. A higher dose may be needed for the ICSs with higher tissue binding to achieve efficacious free concentration, thus the safety margin obtained by their high plasma protein binding
would be offset. Therefore, it is essential to determine both plasma protein binding and
tissue binding, especially the target tissue, lung protein binding for a complete evaluation
of efficacy and safety profile of ICSs.

**Objectives of the Study**

Pulmonary targeting is essential for successful inhaled corticosteroids (ICS). Many
factors determine the pulmonary targeting of ICS and these factors include
pharmacokinetic and pharmacodynamic (PK/PD) properties of ICS, inhalation device and
patients characteristics. Understanding of these factors has contributed significantly to
improve pulmonary targeting of modern ICS. This research has been focusing on
evaluation of some aspects related to these factors and the overall objectives could be
summarized as followings:

Aim 1: Compare the *in-vitro* respirable doses of fluticasone from a metered dose
inhaler and three rigid valved holding chambers.

Aim 2: Assess pharmacokinetic profiles of inhaled beclomethasone, budesonide,
fluticasone and mometasone in asthmatic subjects with a broad range of lung function.

Aim 3: Compare the systemic exposure change between inhaled budesonide and
fluticasone after lung function decrease induced by methacholine in asthmatic subjects.

Aim 4: Evaluate administration time effect on the cortisol suppression and
lymphocytes suppression for once-daily inhaled corticosteroids.
CHAPTER 2
IN-VITRO COMPARISON OF FLUTICASONE RESPIRABLE DOSE FROM A METERED DOSE INHALER AND THREE RIGID VALVED HOLDING CHAMBERS

Introduction

There are several metered-dose inhaler (MDI) valved holding chambers (VHCs) available in the United States. The primary purpose of VHC is to hold the MDI puff very shortly before inhalation to minimize the detrimental effects of poor timing between MDI actuation and patient inhalation. Another important feature of VHC is to allow the MDI propellant time and distance to evaporate after actuation. Evaporation of propellant promotes the formation of smaller aerosol particles that are likely to be carried into small airways. Moreover, VHC collect “large” aerosol particles that would otherwise deposit into the oropharynx. VHC has been recommended for all patients who can not effectively use MDI alone, such as children and for all asthma patients prescribed an ICS to reduce the risk of topical side effects of corticosteroid such as thrush and dysphonia [1].

In vitro aerosol data indicated that the VHC choice has no impact on the aerosol characteristics of bronchodilators from an MDI [83]. Further, clinical studies have been unable to discern measurable difference in bronchodilator effect among various albuterol MDI-VHC combinations [84, 85]. Hence, many clinicians and third-party payers conclude that all VHCs available in the USA markets are equivalent. However, this statement may not stand for ICS as several in vitro studies indicate that the quantity of ICS available for delivery to human lung can vary as many as three folds, depending on the choice of VHC [83]. Therefore, interchanging one VHC with another may result in
pronounced fluctuations in the aerosol dose reaching the lungs and potentially result in therapeutic failures. Fluticasone propionate MDI (Flovent, GlaxoSmithKline, Research Triangle Park, NC) is the most often prescribed ICS in the USA for asthmatic patients. Hence, to assess the aerosol characteristics of fluticasone propionate from various VHCs is very important for delivery of successful therapy to asthmatic patients.

**Materials and Methods**

**Chemicals and Devices**

Acentone nitrile and trifluoroacetic acid were obtained from Fisher Scientific. Beclomethasone dipropionate was kindly provided by 3M. Double distilled deionized water was prepared in our lab (Gainesville, FL). The MDI tested was 110µg Flovent® (GlaxoSmithKline, Research Triangle Park, NC). The VHCs tested were AeroChamber-Plus™ (Monaghan Medical, Plattsburgh, NY), OptiChamber® (Respironics, Murrysville, PA) and OptiChamber®-Advantage (Respironics, Murrysville, PA). Andersen Mark-Π, eight-stage cascade impactor equipped with a USP induction port was purchased from Thermo Andersen (Smyrna, GA).

**Sample Collection**

The deposition characteristics of fluticasone propionate aerosol delivered from the MDI alone (110µg Flovent®), the MDI attached to AeroChamber-Plus™ VHC, OptiChamber® VHC, and OptiChamber®-Advantage VHC were examined by using the well-established in-vitro cascade impactor method [86, 87]. Fluticasone propionate aerosol particles that entered the cascade impactor were sorted by aerodynamic diameter onto eight different stages (Figure 2-1).
Aerodynamic diameter does not refer to actual aerosol droplet diameter, rather it is a means of categorizing particles that have different shapes and densities with a single dimension based on how those particles behave when settling out. Aerosol deposition onto the “throat” intake port was reflective of in vivo oropharyngeal deposition [88]. Aerosol deposition on stages 0, 1, and 2 of the cascade impactor corresponded to particles of 5.1–10 µm. These particles are too large for therapeutic use in asthma and likely would deposit into the large upper airways such as the trachea [89-91]. Aerosol that deposited on stages 3, 4, and 5 of the cascade impactor corresponded to particles of 1–5 µm, a range considered ideal for deposition and retention within the small human airways (bronchi and bronchioles) [89]. Aerosol collected on stages 6, 7, and in the final filter of the cascade impactor corresponded to particles smaller than 1 µm, a range considered too
small for therapeutic use in asthma and one that favors deposition into the respiratory bronchioles and alveoli [89].

Fluticasone propionate aerosol from the MDI connected to each of six spacer configurations and aerosol from the MDI alone were sampled directly into the cascade impactor (Mark IIACFM eight-stage nonviable ambient sampler; Andersen Instruments Inc., Atlanta, GA) through an artificial throat manufactured according to United States Pharmacopeia (USP) standards [87]. Aerosol was drawn into the impactor by continuous airflow generated by a vacuum pump (model 20-709; Graseby-Andersen, Atlanta, GA). Airflow through the impactor was calibrated to conform to the manufacturer’s requirements (28.3 L/min ± 5%) and simulated a tidal inspiratory flow of about 30 L/minute. Before each sampling run, continuous airflow through the impactor was allowed to equilibrate for 5 minutes, and each fluticasone propionate MDI canister was primed 5 times in a separate room to ensure uniformity among actuations. Before each sampling run, the MDI valve was washed twice with methanol and dried to remove any trace drug that resulted from priming. Each MDI canister was shaken for at least 10 seconds before each actuation. Each of the above devices was tested five times, and during each run, 5 MDI puffs were sampled through the cascade impactor without delay between actuation and sampling. After each actuation into the impactor, airflow was maintained for 60 seconds before the next actuation. After the final actuation in each run, flow through the impactor was maintained for an additional 60 seconds. Laboratory personnel wore antistatic outer clothing containing carbon fibers during sample collection to minimize transfer of electrostatic charges to the VHCs. All VHCs were washed in a liquid detergent solution and allowed to air dry before use, to minimize electrostatic
attraction of fluticasone propionate aerosol during sample collection [92]. Ambient temperature (22 ± 2°C) and humidity (46 ± 4%) in the aerosol laboratory were monitored to minimize inter-day variability in sample collection.

**Fluticasone Propionate Assay**

After each trial, fluticasone propionate was washed from the USP throat and from each of eight collection plates in the cascade impactor with methanol and an equal volume of distilled water. The volume used to wash each section of the apparatus varied from 5–20 ml to eliminate concentrations of fluticasone propionate below the assay limit of detection (0.5 µg/ml). Two 1-ml aliquots of the resultant solutions were collected for high-performance liquid chromatography (HPLC) analysis. Fluticasone propionate content from all washings was determined by injecting duplicate 100-µl samples into a reverse-phase HPLC system with a 254-nm ultraviolet detector. The HPLC mobile phase consisted of acetonitrile and water (53:47 volume:volume ratio [v:v]) with 0.03% trifluoroacetic acid. The flow rate through the system was 1.0 ml/minute. Fluticasone propionate standard curves and quality controls were prepared in a methanol and water mixture (50:50 v:v) Budesonide, as an internal standard was added to all solution before analysis. Correlation coefficients for the standard curves were 0.998–1.000. Assay interday coefficient of variation for fluticasone propionate quality control solutions ranged from 11.4% for the 2.0-µg/ml control to 3.2% for the 20.0-µg/ml control.

**Data Analysis**

The amount of fluticasone propionate deposited on each stage and throat of the cascade impactor was measured and normalized per 110 mg MDI actuation. A log probability plot constructed from these data was used to determine the distribution of particle sizes, i.e., mass median aerodynamic diameter (MMAD) and geometric standard
The quantity of fluticasone propionate aerosol emitted within the ideal size range (1–5 μm) for deposition and retention in the small human airways (i.e., respirable dose) and the quantity collected in the USP throat intake port (i.e., oropharyngeal dose) were also calculated. Differences among outcomes were determined by using one-way analysis of variance. The Tukey multiple comparison procedure was used to compare individual means when the overall test results were statistically significant (p<0.05). All data analysis was performed with InStat statistical software, version 3.01 (GraphPad Software Inc., San Diego, CA).

**Results**

MMAD of aerosols produced by the tested VHC devices were slightly smaller than MMAD of aerosol produced by MDI alone (p < 0.05, Table 2-1). Similarly, the tested devices reduced the GSD of aerosol compared to aerosol produced by the MDI alone (p < 0.05, Table 2-1). The differences in MMAD and GSD among the VHCs were not significant (p > 0.05, Table 2-1).

<table>
<thead>
<tr>
<th></th>
<th>MDI</th>
<th>AeroChamber-Plus™</th>
<th>OptiChamber®</th>
<th>OptiChamber®-Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMAD</td>
<td>2.84 (0.32)</td>
<td>2.50 (0.05)</td>
<td>2.42 (0.14)</td>
<td>2.30 (0.06)</td>
</tr>
<tr>
<td>GSD</td>
<td>1.26 (0.04)</td>
<td>1.22 (0.01)</td>
<td>1.22 (0.01)</td>
<td>1.22 (0.01)</td>
</tr>
</tbody>
</table>

The fluticasone propionate respirable dose from all devices is shown in Figure 2-2.

The mean ± SD fluticasone propionate respirable dose (i.e., aerosol size 1–5 mm) from AeroChamber-Plus™ (45.3 ± 1.8 μg/ actuation) and old OptiChamber® (38.1 ± 8.7 μg/ actuation) were not significantly different (p > 0.05) from the respirable dose produced by MDI alone (46.7 ± 9.7 μg/ actuation). However, OptiChamber®-Advantage produced
a mean respirable dose (32.1 ± 1.6 µg/ actuation) that was significantly less than that produced by MDI alone or AeroChamber-Plus™ (p < 0.05) but not significantly different from old OptiChamber® (p > 0.05).

Figure 2-2. Mean ± SD respirable dose of fluticasone propionate from metered-dose inhaler (MDI) and valved holding chamber (VHC) devices.

The fluticasone propionate oropharyngeal dose from all devices is shown in Figure 2-3. The mean ± SD oropharyngeal dose from MDI alone (50.5 ± 7.2 µg/ actuation) was significantly greater (p < 0.001) than the oropharyngeal dose from all three VHC devices: AeroChamber-Plus™ (0.9 ± 0.3 µg/ actuation), OptiChamber® (0.6 ± 0.8 µg/ actuation) and OptiChamber®-Advantage (0.17 ± 0.1 µg/ actuation). The difference in oropharyngeal dose between the three VHCs was not significant (p > 0.05).
Figure 2-3. Mean ± SD oropharyngeal dose of fluticasone propionate from metered-dose inhaler (MDI) and valved holding chamber (VHC) devices.

Discussion and Conclusion

All three VHCs tested significantly decreased the oropharyngeal dose compared with MDI alone. This reduction in oropharyngeal dose results from that large, unrespirable aerosol particles are collected in the VHC rather than in the impactor throat intake port when either VHC is attached. These data suggest that either VHC tested would reduce the risk of topical adverse effects induced by fluticasone.

The highest respirable dose observed was from fluticasone propionate MDI alone. However, the respirable dose from fluticasone propionate MDI alone was not significantly larger than the respirable dose from the MDI attached to AeroChamber™-Plus or OptiChamber®. The respirable doses from MDI alone, MDI attached to AeroChamber™-Plus or OptiChamber® observed in this study were not significantly different from previously reported values, respectively obtained in a nearly identical manner [93, 94]. Our results confirmed that using AeroChamber™-Plus or OptiChamber®
in combination with a fluticasone propionate-110 MDI does not alter the respirable dose available to the asthmatic patients. Further, the differences in delivered particle size distribution of fluticasone aerosol, as reflected by both MMAD and GSD, between the two VHCs and MDI alone were small. This suggests that fluticasone MDI can be used successfully with AeroChamber™-Plus or OptiChamber®. However, the respirable dose of fluticasone from OptiChamber®-Advantage was 40-45% less than from the MDI alone or the AeroChamber™-Plus VHC. Although the dose-response relationship in the treatment of asthma has been difficult to establish, a 40-45% decrease in respirable dose of inhaled fluticasone is likely to be a clinically relevant difference.

In vitro testing has some potential limitations. The most prominent of these is that the correlation between the in vitro respirable dose of fluticasone and clinical response has not been fully evaluated. Variability in patient inhalation technique and adherence with the therapy are often the biggest determinants in clinical response and an in vitro deposition study cannot account for these variables. Future work should include measurement of aerosol deposition under more physiologic conditions likely to be encountered in asthmatic patients. Another limitation of present study is that our results are only reflective of the performance characteristics of the tested VHCs with the current fluticasone MDI formulated with a chlorofluorocarbon propellant. Our results may not be reflective of performance with other inhaled formulations of the same medication or of different ICS. Although our study was conducted at only one flow rate (28.3 L/min), other studies have shown that flow rates of 60 and 90 L/min have little effect on the respirable dose from several corticosteroids MDIs when compared with that achieved at 30 L/min [21, 95].
There are differences in the in vitro performance characteristics of VHCs tested in our study. Our results suggest that an asthmatic patient would receive the same dose of fluticasone to the lung when using a MDI alone with proper inhalation technique or the same MDI attached to AeroChamber-Plus™ or OptiChamber®. However, the same MDI attached to OptiChamber®-Advantage, a newer VHC compared with the others in this study, would result in 40-45% less fluticasone reaching the lung than that from the MDI alone. This difference is likely to be clinically relevant. Hence, in asthmatics on fluticasone MDI needing a VHC, AeroChamber-Plus™ or OptiChamber® would be a better choice over OptiChamber®-Advantage since they do not change the respirable dose compared to MDI alone.
CHAPTER 3
PLASMA CONCENTRATIONS AND PHARMACOKINETICS PROFILES OF INHALED CORTICOSTEROIDS AND THEIR RELATION TO LUNG FUNCTION IN ASTHMA

Introduction
As we discussed in a previous chapter, inhaled corticosteroids are absorbed into the systemic circulation, predominantly from the lung and more variably from the gastrointestinal tract. Therefore they have the potential to produce adverse systemic effects. The risk of such adverse effects will depend on the extent of systemic (overall) absorption of ICS and may differ between inhaled corticosteroids due to their different physicochemical and pharmacokinetic properties. Recent studies have shown that absorption and systemic effects of one inhaled corticosteroid, fluticasone propionate, are greater in healthy volunteers than in subjects with asthma and airflow obstruction [54, 55, 96, 97]. However it was not true for budesonide [55, 98]. Another study showed that the extent of adrenal suppression following 500µg fluticasone propionate was closely related to lung function with greater cortisol suppression with increasing FEV$_1$ [50]. Whether the systemic absorption of other inhaled corticosteroids relates to lung function is unknown. To explore this and to provide comparative data on the pharmacokinetic profiles of the four drugs in the same subjects, we compared plasma concentrations of beclometasone monopropionate, budesonide, fluticasone propionate and mometasone furoate following inhalation of a single dose in subjects with asthma who had a wide range of FEV$_1$ values.
Methods

Subjects

Thirty non-smoking subjects with asthma aged between 18 and 70 were recruited between October 2003 and February 2004 from our volunteer database. Subjects were selected to provide a range of forced expiratory volume in one second (FEV₁) values from 30% predicted upwards. All had to have stable asthma defined as no change in asthma symptoms or treatment for at least two months. There were no restrictions based on treatment but subjects were excluded if they had significant co-morbidity, were pregnant or lactating or had a greater than 20 pack/year smoking history. The study was approved by Nottingham Research Ethics Committee and written informed consent was obtained from all subjects.

Measurements

Lung function

FEV₁ and forced vital capacity (FVC) were measured with a dry bellows spirometer (Vitalograph, Buckingham, UK) as the higher of two successive readings within 100ml. The same spirometer was used for all measurements and was calibrated weekly. Peak expiratory flow (PEF) was measured with a mini-Wright flowmeter as the best of three readings.

Plasma drug assays

The plasma concentrations of budesonide, beclometasone monopropionate (the active metabolite of beclometasone dipropionate), fluticasone propionate and mometasone furoate were quantified with validated high performance liquid chromatography/tandem mass spectrometry methods using a Micromass Quattro LC-Z triple quadrupole mass spectrometer (Beverley, MA) [99-102]. The lower limits of
detection for the assays were 15 pg/ml for beclometasone monopropionate, fluticasone propionate and mometasone furoate and 50 pg/ml for budesonide.

Protocol

Subjects were screened at an initial visit to measure FEV<sub>1</sub>, assess their suitability for the study and to familiarize themselves with the placebo dry powder inhalers. Subjects taking fluticasone propionate, budesonide or mometasone furoate were changed to an equivalent dose of beclometasone dipropionate at least four days before the study day; all subjects were then asked to omit the beclometasone dipropionate from the evening before the study day to allow time for beclometasone monopropionate to be cleared from the plasma before the study. Subjects attended the department in the morning of the study day, a venous cannula was inserted and blood taken for baseline drug assay. After ten minutes rest, FEV<sub>1</sub>, FVC and PEF were measured on two occasions five minutes apart. Subjects then inhaled two doses from each of four inhalers in random order with a mouth rinse after each drug. The drugs and total doses administered were beclometasone dipropionate 800µg (Becodisks®, Allen & Hanburys), budesonide 800µg (Turbohaler®, Astra-Zeneca), fluticasone propionate 1000µg (Accuhaler®, Glaxo-Wellcome) and mometasone furoate 800µg (Asmanex Twishtaler®, Schering-Plough). Venous blood samples were taken into sodium fluoride/EDTA tubes at intervals over the next eight hours, centrifuged at 1500 rpm for ten minutes and plasma samples extracted and frozen at -70°C.

The main study endpoint was the relation between the subject’s % predicted FEV<sub>1</sub> and the area under the plasma concentration/time curve (AUC<sub>0-8</sub>) for each drug. Power calculations based on a linear regression model showed that 30 patients would give 90% power to detect a correlation between FEV<sub>1</sub> % predicted and AUC of 0.5 or more.
Analysis

Plasma concentrations of beclometasone monopropionate, budesonide, fluticasone propionate and mometasone furoate were plotted against time for each subject; pharmacokinetic parameters (maximum plasma concentration (Cmax), time to Cmax, AUC\textsubscript{0-8}, mean residence time and terminal half-life) were calculated using a software package (WinNonlin® Professional Version 3.1 Pharsight Corporation, Mountain View, CA). Concentrations below the limit of quantification were reported as missing and extrapolated during non-compartmental analysis.

Plasma drug AUC\textsubscript{0-8} values were plotted against the mean of the two baseline FEV\textsubscript{1} measurements, FEV\textsubscript{1} % predicted and FEV\textsubscript{1}/FVC ratio, and against PEF and PEF % predicted. The correlation coefficient (r) was calculated and trendlines fitted using linear regression. Partial correlation coefficients were calculated for the relationship between lung function and AUC for the four drugs after controlling for two potential confounding factors, age and gender. To estimate the magnitude of the effect of lung function on AUC values for each drug the equation derived from the linear regression model was used to predict the AUC value at 100 and 50% predicted FEV\textsubscript{1} and PEF and the ratio of these two values was calculated.

Results

All 30 subjects (mean age 57, 16 men) completed the study as per protocol. Baseline characteristics of the subjects are shown in table 3-1. FEV\textsubscript{1} prior to drug inhalation ranged from 36 to 138% predicted. Four subjects had detectable plasma concentrations of one inhaled corticosteroid in their baseline sample. In two subjects this was beclometasone monopropionate (1152pg/ml and 46pg/ml), in one who usually took budesonide it was budesonide (215pg/ml) and in one who usually took beclometasone
dipropionate, fluticasone propionate was detected (19 pg/ml). Data for these drugs only from the four subjects were excluded from the analysis.

Table 3-1. Baseline characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>FEV1 (liters)</th>
<th>FEV1 (% pred)</th>
<th>FVC (liters)</th>
<th>FEV1/FVC</th>
<th>PEF (liter/min)</th>
<th>PEF (% pred)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>70</td>
<td>1.3</td>
<td>48</td>
<td>3.6</td>
<td>0.35</td>
<td>270</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>52</td>
<td>3.1</td>
<td>90</td>
<td>4.3</td>
<td>0.71</td>
<td>480</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>61</td>
<td>1.3</td>
<td>36</td>
<td>3.9</td>
<td>0.32</td>
<td>245</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>61</td>
<td>2.3</td>
<td>98</td>
<td>2.9</td>
<td>0.79</td>
<td>425</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>53</td>
<td>2.1</td>
<td>86</td>
<td>2.8</td>
<td>0.74</td>
<td>360</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>49</td>
<td>2.7</td>
<td>78</td>
<td>4.2</td>
<td>0.65</td>
<td>460</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>48</td>
<td>2.2</td>
<td>66</td>
<td>3.7</td>
<td>0.59</td>
<td>360</td>
<td>79</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>61</td>
<td>3.3</td>
<td>106</td>
<td>4</td>
<td>0.82</td>
<td>555</td>
<td>96</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>45</td>
<td>2.7</td>
<td>100</td>
<td>3.1</td>
<td>0.88</td>
<td>425</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>61</td>
<td>2.5</td>
<td>82</td>
<td>3.2</td>
<td>0.78</td>
<td>450</td>
<td>78</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>55</td>
<td>1.4</td>
<td>42</td>
<td>3</td>
<td>0.47</td>
<td>350</td>
<td>58</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>46</td>
<td>3.2</td>
<td>74</td>
<td>4.3</td>
<td>0.74</td>
<td>530</td>
<td>82</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>56</td>
<td>3.5</td>
<td>138</td>
<td>4.1</td>
<td>0.86</td>
<td>460</td>
<td>101</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>68</td>
<td>1.2</td>
<td>62</td>
<td>1.9</td>
<td>0.63</td>
<td>255</td>
<td>62</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>44</td>
<td>3.2</td>
<td>113</td>
<td>4.3</td>
<td>0.74</td>
<td>455</td>
<td>95</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>61</td>
<td>1.8</td>
<td>72</td>
<td>2.15</td>
<td>0.81</td>
<td>310</td>
<td>70</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>62</td>
<td>2.2</td>
<td>96</td>
<td>3</td>
<td>0.72</td>
<td>465</td>
<td>107</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>50</td>
<td>2.6</td>
<td>75</td>
<td>4.2</td>
<td>0.63</td>
<td>430</td>
<td>70</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>35</td>
<td>2.4</td>
<td>93</td>
<td>3.3</td>
<td>0.73</td>
<td>460</td>
<td>97</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>67</td>
<td>1.9</td>
<td>65</td>
<td>3.1</td>
<td>0.61</td>
<td>390</td>
<td>71</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>58</td>
<td>0.9</td>
<td>47</td>
<td>2</td>
<td>0.44</td>
<td>195</td>
<td>45</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>60</td>
<td>1.7</td>
<td>53</td>
<td>3.2</td>
<td>0.53</td>
<td>235</td>
<td>40</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>41</td>
<td>2.3</td>
<td>57</td>
<td>3.8</td>
<td>0.59</td>
<td>455</td>
<td>71</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>58</td>
<td>2.1</td>
<td>87</td>
<td>2.6</td>
<td>0.78</td>
<td>370</td>
<td>83</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>66</td>
<td>1.5</td>
<td>77</td>
<td>2.3</td>
<td>0.66</td>
<td>300</td>
<td>72</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>51</td>
<td>1.7</td>
<td>46</td>
<td>3.2</td>
<td>0.54</td>
<td>415</td>
<td>67</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>63</td>
<td>1.3</td>
<td>43</td>
<td>2.8</td>
<td>0.46</td>
<td>275</td>
<td>48</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>63</td>
<td>2.5</td>
<td>99</td>
<td>3.2</td>
<td>0.8</td>
<td>485</td>
<td>110</td>
</tr>
<tr>
<td>29</td>
<td>F</td>
<td>66</td>
<td>0.8</td>
<td>44</td>
<td>1.5</td>
<td>0.55</td>
<td>180</td>
<td>43</td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>68</td>
<td>1.0</td>
<td>40</td>
<td>1.5</td>
<td>0.55</td>
<td>180</td>
<td>43</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>57</td>
<td>2.1</td>
<td>74</td>
<td>3.2</td>
<td>376</td>
<td>73</td>
</tr>
<tr>
<td>(SD)</td>
<td></td>
<td></td>
<td>(9)</td>
<td>(0.8)</td>
<td>(25)</td>
<td>(0.8)</td>
<td>(104)</td>
<td>(21)</td>
</tr>
</tbody>
</table>
Plasma drug concentrations and pharmacokinetic profiles

All four inhaled corticosteroids were present at detectable plasma concentrations following inhalation in all subjects and plasma concentrations were above the lower limit of quantification in the majority of samples (99%, 97%, 95% and 93% of samples for budesonide, beclometasone monopropionate, fluticasone propionate and mometasone furoate, respectively).

Figure 3-1. Mean (SE) plasma concentration-time curves for beclometasone monopropionate (BMP), budesonide (BUD), fluticasone propionate (FP) and mometasone furoate (MF) following inhalation (Note that Y axes have different scales)

The shape of the mean plasma drug concentration-time curves and pharmacokinetic parameters varied considerably between drugs as shown in figure 3-1 and table 3-2. Data from two subjects for one inhaled corticosteroid (one beclometasone dipropionate, one
mometasone furoate) were unsuitable for pharmacokinetic modelling and excluded from the calculations.

Table 3-2. Mean (SD) pharmacokinetic parameters for beclometasone monopropionate(BMP), budesonide(BUD), fluticasone propionate(FP) and mometasone furoate (MF)

<table>
<thead>
<tr>
<th></th>
<th>BMP</th>
<th>BUD</th>
<th>FP</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (h)</td>
<td>2.93 (0.87)</td>
<td>0.16 (0.12)</td>
<td>1.19 (0.91)</td>
<td>1.28 (0.96)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>12.3 (13.9)</td>
<td>3.6 (0.8)</td>
<td>11.4 (14.6)</td>
<td>8.4 (5.8)</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>7.6 (9.7)</td>
<td>2.7 (0.5)</td>
<td>7.8 (10.0)</td>
<td>5.6 (4.1)</td>
</tr>
<tr>
<td>Cmax(ng/ml)</td>
<td>0.34 (0.15)</td>
<td>1.59 (0.88)</td>
<td>0.10 (0.04)</td>
<td>0.09 (0.06)</td>
</tr>
<tr>
<td>AUC(ng/ml/h)</td>
<td>1.79 (0.78)</td>
<td>3.43 (1.29)</td>
<td>0.44 (0.23)</td>
<td>0.43 (0.32)</td>
</tr>
</tbody>
</table>

Peak plasma concentrations following inhalation occurred within 10 minutes with budesonide, and after 1.2, 1.3 and 2.9 hours with fluticasone propionate, mometasone furoate and beclometasone monopropionate respectively. Mean pulmonary residence times showed a similarly wide variation from 3.6 hours with budesonide to 12.3 hours with beclometasone monopropionate. The terminal half-life ranged from 2.7 hours for budesonide to 7.8 hours for fluticasone. The magnitude of the peak plasma concentration also differed markedly between drugs being highest for budesonide at 1.6 ng.ml$^{-1}$ and 18, 16 and 5 times lower for mometasone furoate, fluticasone propionate and belcometasone monopropionate, respectively. AUC values ranged from 3.43 ng/ml/h for budesonide to 0.43 ng/ml/h for mometasone furoate (table 3-2).

**Relation between lung function and AUC**

AUC$_{0-8}$ values tended to be higher in patients with a higher FEV$_1$ % predicted for all four drugs as can be seen in figure 3-2. The correlation coefficients (r) for the relation between FEV$_1$ % predicted and AUC values for fluticasone propionate, budesonide, beclometasone monopropionate, and mometasone furoate were 0.30 (p = 0.11), 0.36 (p = 0.05), 0.39 (p = 0.04) and 0.55 (p = 0.002) respectively. The same pattern was seen for
the relation between PEF % predicted and AUC values with r values for fluticasone propionate, budesonide, beclometasone monopropionate, and mometasone furoate being 0.26 (p = 0.17), 0.35 (p = 0.07), 0.42 (p = 0.03) and 0.60 (p < 0.001) respectively. The correlation between other measures of lung function (absolute FEV₁, PEF and FEV₁/FVC ratio) and AUC was similar but generally weaker than that between FEV₁ and PEF % predicted and AUC. Both age and gender caused a 10% or greater change in the correlation coefficients. The correlation between FEV₁ % predicted and AUC was stronger after adjusting for age but weaker after adjusting for gender (Table 3-3).

![Graph showing the relation between FEV₁ % predicted and AUC for beclometasone monopropionate (BMP), budesonide (BUD), fluticasone propionate (FP) and mometasone furoate (MF) following inhalation.](image)

Figure 3-2. Relation between FEV₁ % predicted and the area under the plasma concentration-time curve (AUC) for beclometasone monopropionate (BMP), budesonide (BUD), fluticasone propionate (FP) and mometasone furoate (MF) following inhalation (Note that Y axes have different scales)
Table 3-3. Partial correlation coefficients (r) for the relationship between FEV1 % predicted and AUC after adjusting for age or gender

<table>
<thead>
<tr>
<th>Drug</th>
<th>Unadjusted r</th>
<th>Age r</th>
<th>Gender r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclometasone monopropionate</td>
<td>0.39</td>
<td>0.48</td>
<td>0.35</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>0.01</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Budesonide</td>
<td>0.36</td>
<td>0.37</td>
<td>0.28</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>0.3</td>
<td>0.45</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mometasone furoate</td>
<td>0.55</td>
<td>0.63</td>
<td>0.48</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.63</td>
<td>&lt; 0.001</td>
<td>0.099</td>
<td></td>
</tr>
</tbody>
</table>

Estimated AUC values were higher at 100 % compared to 50% predicted FEV₁ for all four drugs, the ratios for budesonide, fluticasone propionate, beclometasone monopropionate and mometasone furoate being 1.3, 1.4, 1.4 and 2.3 respectively. The same pattern was seen with PEF % predicted (Table 3-4).

Table 3-4. Predicted AUC values at 50 and 100% predicted FEV1 and PEF and the ratio of these values

<table>
<thead>
<tr>
<th>Drug</th>
<th>AUC at 50% predicted</th>
<th>AUC at 100% predicted</th>
<th>Ratio of AUC values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclometasone monopropionate</td>
<td>1.52</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>1.45</td>
<td>2.22</td>
<td>1.5</td>
</tr>
<tr>
<td>Budesonide</td>
<td>3.01</td>
<td>3.93</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>2.95</td>
<td>4.08</td>
<td>1.4</td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>0.37</td>
<td>0.51</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>0.51</td>
<td>1.4</td>
</tr>
<tr>
<td>Mometasone furoate</td>
<td>0.27</td>
<td>0.61</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
<td>0.68</td>
<td>3</td>
</tr>
</tbody>
</table>

**Discussion**

This is the first study to compare plasma concentration-time curves and pharmacokinetic profiles of beclometasone monopropionate, budesonide, fluticasone
propionate and mometasone furoate following inhalation in the same patients and to explore the extent to which systemic absorption of all four drugs relates to lung function.

**Plasma Drug concentrations and Pharmacokinetic Profiles**

The plasma drug concentration-time curves and pharmacokinetic profiles of fluticasone propionate and mometasone furoate were broadly similar to each other but differed markedly from the other two drugs, beclometasone monopropionate and budesonide. Budesonide, the most water-soluble of the four drugs, had the shortest pulmonary residence time, time to peak plasma concentration (Tmax) and terminal half-life; beclometasone monopropionate had the longest pulmonary residence time and Tmax and its terminal half-life was also longer and similar to that of fluticasone propionate.

The pharmacokinetic parameters determined for budesonide are in agreement with previous data [55, 96, 97, 103, 104] and the rapid absorption is related to its lower lipophilicity and greater solubility in bronchial fluid [105]. The shorter terminal half-life of budesonide primarily reflects its lower volume of distribution since high clearance is a feature of all the inhaled corticosteroid[106]. Budesonide had a much higher peak plasma concentration than the three other drugs, an effect attributed to high pulmonary deposition by the Turbohaler®, its rapid absorption and low volume of distribution [104].

The long pulmonary residence time and Tmax with beclometasone monopropionate reflects its greater lipophilicity and greater oral bioavailability (41% compared to 11% for budesonide and <1% for fluticasone propionate and mometasone furoate [80, 106-108]) since absorption from the gastrointestinal tract is slower than that from the lungs [107]. Our values for pulmonary residence time and terminal half-life for beclometasone monopropionate were longer than those reported in studies using metered dose inhalers, [109-111] perhaps due to differences in particle characteristics and deposition pattern.
with dry powder inhalers. The lower peak plasma concentrations for beclometasone monopropionate compared to budesonide is related to its slower absorption and greater volume of distribution.

Fluticasone propionate and mometasone furoate had fairly similar absorption profiles. The long terminal half-life of fluticasone propionate may be due to its large volume of distribution or ‘flip-flop pharmacokinetics’ due to slow release from the lungs. Both drugs had particularly low peak plasma concentrations, even though fluticasone propionate was given at a slightly higher dose than the other three inhaled corticosteroids. Slow absorption from the lungs, increased mucociliary clearance and high volumes of distribution are likely to explain these findings. Our pharmacokinetic data for fluticasone propionate are similar to those reported previously [55, 96, 97, 112, 113], whilst the only published data on mometasone furoate are difficult to compare since a relatively insensitive assay was used [114].

**Relation Between Lung Function and AUC**

We found that AUC values tended to be higher in patients with better lung function for all four drugs. Our estimates suggest that the systemic absorption of budesonide, fluticasone propionate, beclometasone monopropionate and mometasone furoate is some 1.3, 1.4, 1.4 and 2.3 times higher at 100% predicted FEV$_1$ compared to 50% predicted FEV$_1$. The effect appears to be greatest for mometasone furoate although the study was not powered to compare the magnitude of effect between drugs.

The inhaled corticosteroids were delivered by dry powder inhalers in our study, and reduced inspiratory flow may have caused less drug to be delivered to the airways in subjects with greater airflow obstruction. Furthermore, the drug that enters the airways is more likely to be deposited in central airways in patients with airflow obstruction, and
hence more likely to be removed by mucociliary clearance. This would be expected to affect drugs that dissolve slowly in airway lining fluid such as mometasone furoate and fluticasone propionate most and drugs with high water solubility, such as budesonide, least [105].

When we explored the effect of age and gender on the relation between lung function and AUC values we found the relation to be stronger after adjusting for age but weaker after adjusting for gender. This highlights the fact that factors other than lung function affect the systemic absorption of inhaled corticosteroids thus reducing the strength of the relation between lung function and plasma concentration AUC values in cross-sectional studies. This conclusion is supported by our later study, presented in next chapter, in which we compared plasma concentrations of budesonide and fluticasone propionate following inhalation with and without prior methacholine-induced bronchoconstriction (mean fall in FEV₁ of 33%). Mean AUC values were reduced for both drugs after lowering FEV₁, i.e. when we looked at within subject changes, but not when we compared differences in FEV₁ on AUC between subjects. Studies of the effect of lung function on the systemic absorption of inhaled corticosteroids across populations need to allow for between-subject variability.

**Conclusion**

In conclusion our study has shown marked differences in plasma concentration-time curves and pharmacokinetic profiles between beclometasone monopropionate, budesonide, fluticasone propionate and mometasone furoate following inhalation. The net effect of these differences on the benefit to risk ratio of inhaled corticosteroids is complex and needs to be explored further. The systemic absorption of all four inhaled corticosteroids appears to be greater in patients with a higher FEV₁ % predicted although
other factors, including age and gender affect this relationship. Our findings re-enforce the importance of reviewing the need for higher doses of an inhaled corticosteroid, particularly in patients with relatively normal lung function and in patients whose lung function improves with treatment.
CHAPTER 4
FLUTICASONE AND BUDESONIDE CONCENTRATIONS AFTER INHALATION
EFFECT OF BRONCHOCONSTRICTION

Introduction

Adverse systemic effects from inhaled corticosteroids have been recognized increasingly over recent years. With around 5% of the population in more economically developed countries prescribed an inhaled corticosteroid, often for many decades, understanding the factors that determine these adverse effects is important. All currently available inhaled corticosteroids are absorbed into the systemic circulation and hence have the potential to cause adverse systemic effects. The risk may differ between inhaled corticosteroids, however, due to their different physicochemical and pharmacokinetic properties and there is evidence that these drug related differences interact with patient factors such as airflow obstruction [115]. Plasma drug concentrations following inhalation of 1000 µg fluticasone were considerably lower in people with airflow obstruction than in healthy volunteers but this was not the case for budesonide [51, 55, 97]. The differences between fluticasone and budesonide were attributed to differences in lipophilicity between the two drugs [55]. Whether changes in airflow obstruction within an individual affect systemic absorption in the same way is unknown, but important in view of the fluctuations in airway caliber seen in asthma. To explore this we compared the plasma concentrations of fluticasone and budesonide following inhalation of a single dose, with and without prior methacholine-induced bronchoconstriction.
Methods

Subjects

Twenty non-smoking subjects with asthma aged between 18 and 70 were recruited from our volunteer database. To be included subjects had to have a forced expiratory volume in one second (FEV$_1$) of at least 80 % predicted and 1.5 litres, a provocative dose of methacholine causing a 20 % fall in FEV$_1$ (PD$_{20}$) below 8 µM, and stable asthma, defined as no change in asthma symptoms or treatment for two months. Subjects were excluded if they had significant co-morbidity, were taking any medication known to alter the metabolism of corticosteroids, were pregnant or lactating, or had a greater than 20 pack year smoking history. The study was approved by Nottingham Research Ethics Committee and written informed consent was obtained from all subjects.

Measurements

Spirometry

FEV1 and forced vital capacity (FVC) were measured with a dry bellows spirometer (Vitalograph, Buckingham, UK) as the higher of two successive readings within 100 ml.

Methacholine inhalation challenge

Subjects inhaled three puffs of normal saline followed by doubling doses of methacholine from 0.048 µM to a maximum of 24.5 µM from DeVilbiss nebulizers and FEV1 was measured one minute after each dose [116]. The test was stopped once FEV1 had fallen by 20 % from the post-saline value during screening, for calculation of PD20 by interpolation, and once FEV1 had fallen by 25 % in the main study.
Drug assays

Plasma concentrations of fluticasone propionate and budesonide were quantified with previously validated high performance liquid chromatography/tandem mass spectrometry methods using a Micromass Quattro LC-A triple quadrupole mass spectrometer (Beverley, MA) [99, 100]. The lower limits of detection for the assays were 15 pg/ml for fluticasone propionate and 50 pg/ml for budesonide. The intra- and inter-assay coefficients of variation for the assays were below 13.6 for both fluticasone and budesonide at concentrations between 0.015 and 0.75 ng/ml for fluticasone and between 0.15 and 2.5 ng/ml for budesonide.

Protocol

Subjects were screened to assess suitability and to measure FEV\textsubscript{1} and PD\textsubscript{20} methacholine. Subjects familiarized themselves with the two dry powder inhalers (Accuhaler®, Glaxo-Wellcome and Turbohaler®, Astra-Zeneca) to ensure optimal inhaler technique during the study and those taking fluticasone or budesonide were changed to an equivalent dose of beclometasone dipropionate for four days before and until study completion. Subjects were asked to avoid short and long acting β\textsubscript{2} agonists for 12 hours, and to avoid exercise and caffeine on the morning of the study.

Subjects attended for two study visits at the same time of day ± one hour, 7 ± 3 days apart. A venous cannula was inserted and after ten minutes rest, FEV\textsubscript{1} was measured. 1000 µg fluticasone (Accuhaler®), given as two 500 µg doses, and 800 µg budesonide (Turbohaler®), given as two 400 µg doses, were then inhaled in random order; after each drug subjects rinsed their mouths with water which was then discarded. Venous blood samples were taken into heparinised tubes at intervals over the next 5 hours (0, 5, 15, 30, 60, and 90 minutes, 2, 3, 4 and 5 hours), centrifuged at 1500 rpm for 10 minutes and
plasma samples were then frozen at –70 °C. The protocol for the two study visits was identical except that on one occasion prior to inhalation of the drugs, a methacholine challenge was carried out to induce a fall in FEV$_1$ of at least 25 % from baseline FEV$_1$. The two studies were carried out in random order according to a computer generated code.

Power calculations showed that 20 patients would give 90 % power to detect a 0.7 SD difference in the area under the plasma concentration time curve over five hours, the primary outcome, between the two study visits.

**Analysis**

Plasma fluticasone and budesonide concentrations were plotted against time for each subject and the maximum plasma concentration (C$_{max}$), time to maximum plasma concentration (T$_{max}$) and area under the curve up to five hours (AUC$_{0-5}$) calculated using a software program (WinNonlin® Professional Version 3.1, Pharsight Corporation, Mountain View, CA). C$_{max}$, T$_{max}$ and AUC$_{0-5}$ values for the two study visits were compared for each drug by paired t-test. To allow for the differences in concentrations of the two drugs we calculated the ratio of the AUC$_{0-5}$ values on the days with and without bronchoconstriction for each subject for each drug and compared the values between drugs by Wilcoxon signed rank test. This analysis was repeated for C$_{max}$ values.

**Results**

All 20 subjects (mean age 48, 12 men) completed the study and their baseline characteristics are shown in table 4-1. All were non-smokers and only two had ever smoked.

Subjects had a geometric mean PD$_{20}$ methacholine of 1.2 µM (range 0.06 to 6.1). Mean (SD) FEV$_1$ was 2.9 (0.7) litres (91 % predicted) on screening and 2.8 (0.7) and 2.9 (0.6) litres on arrival for the visits with and without bronchoconstriction. All subjects had
a fall in FEV$_1$ of at least 25 % (range 25 to 47 %) following methacholine giving an FEV$_1$ of 1.9 (0.5) litres (60 % predicted) prior to drug inhalation and a mean difference in FEV$_1$ prior to drug inhalation on the two days of 0.9 litres (range 0.5 to 1.35) or 33 % (range 20 to 45 %).

Table 4-1. Baseline characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>FEV$_1$ (liters)</th>
<th>FEV$_1$ % pred</th>
<th>PD$_{20}$ (micromol)</th>
<th>Usual ICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>55</td>
<td>192</td>
<td>78</td>
<td>3.8</td>
<td>91</td>
<td>2.7</td>
<td>BDP*</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>50</td>
<td>173</td>
<td>78</td>
<td>2.8</td>
<td>80</td>
<td>3.4</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>50</td>
<td>160</td>
<td>86</td>
<td>2.55</td>
<td>103</td>
<td>5.7</td>
<td>BDP</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>63</td>
<td>174</td>
<td>77</td>
<td>2.8</td>
<td>89</td>
<td>2.7</td>
<td>BDP</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>67</td>
<td>155</td>
<td>68</td>
<td>1.55</td>
<td>84</td>
<td>0.1</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>23</td>
<td>170</td>
<td>79</td>
<td>4</td>
<td>96</td>
<td>2.3</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>39</td>
<td>161</td>
<td>63</td>
<td>3.5</td>
<td>126</td>
<td>0.5</td>
<td>BDP</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>38</td>
<td>189</td>
<td>92</td>
<td>3.8</td>
<td>84</td>
<td>0.9</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>41</td>
<td>182</td>
<td>96</td>
<td>3.8</td>
<td>92</td>
<td>0.4</td>
<td>BUD</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>41</td>
<td>176</td>
<td>87</td>
<td>3.8</td>
<td>98</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>52</td>
<td>172</td>
<td>88</td>
<td>3.05</td>
<td>90</td>
<td>0.1</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>62</td>
<td>166</td>
<td>105</td>
<td>1.95</td>
<td>81</td>
<td>0.06</td>
<td>BDP</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>66</td>
<td>173</td>
<td>78</td>
<td>2.4</td>
<td>80</td>
<td>6.1</td>
<td>BDP</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>48</td>
<td>166</td>
<td>79</td>
<td>2.75</td>
<td>84</td>
<td>1</td>
<td>FP</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>49</td>
<td>164</td>
<td>68</td>
<td>2.8</td>
<td>89</td>
<td>5.1</td>
<td>BUD</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>42</td>
<td>179</td>
<td>67</td>
<td>3.55</td>
<td>89</td>
<td>0.4</td>
<td>BDP</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>65</td>
<td>161</td>
<td>64</td>
<td>2</td>
<td>93</td>
<td>6.1</td>
<td>None</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>27</td>
<td>157</td>
<td>57</td>
<td>2.8</td>
<td>96</td>
<td>2.2</td>
<td>FP</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>34</td>
<td>161</td>
<td>71</td>
<td>2.9</td>
<td>100</td>
<td>1.1</td>
<td>FP</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>44</td>
<td>166</td>
<td>60</td>
<td>2.35</td>
<td>82</td>
<td>0.6</td>
<td>FP</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>48 (13)</td>
<td>167 (10)</td>
<td>77 (13)</td>
<td>2.9 (0.7)</td>
<td>91</td>
<td>1.2**</td>
<td></td>
</tr>
</tbody>
</table>

* Beclometasone dipropionate
** Geometric mean

Data for budesonide plasma concentrations were lost for two subjects due to a computer problem during drug analysis. Complete data were therefore available for 20 subjects for fluticasone and 18 subjects for budesonide.
The shape of the fluticasone and budesonide plasma concentration-time curves showed marked differences on the study day without bronchoconstriction. Peak plasma concentrations of budesonide were 14 times higher than those of fluticasone and occurred considerably earlier as shown in figure 4-1; mean (SD) time to maximum concentration ($T_{\text{max}}$) values were 0.21 (0.24) hr and 1.21 (0.91) hr for budesonide and fluticasone respectively. The shape and time course of the plasma drug concentration curves for each drug were similar on the two study days although plasma levels were lower following methacholine-induced bronchoconstriction (figure 4-1 and table 4-2).

Table 4-2. Pharmacokinetic parameters for fluticasone and budesonide when inhaled with and without prior methacholine-induced bronchoconstriction

<table>
<thead>
<tr>
<th>Drug</th>
<th>Without bronchoconstriction</th>
<th>Following bronchoconstriction</th>
<th>Without bronchoconstriction</th>
<th>Following bronchoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax (ng.ml$^{-1}$) (SD)</td>
<td>Cmax (ng.ml$^{-1}$) (SD)</td>
<td>Cmax (ng.ml$^{-1}$) (SD)</td>
<td>Cmax (ng.ml$^{-1}$) (SD)</td>
</tr>
<tr>
<td>Fluticasone</td>
<td>0.12 (0.07)</td>
<td>0.06 (0.02)</td>
<td>1.67 (0.82)</td>
<td>1.16 (0.48)</td>
</tr>
<tr>
<td>Budesonide</td>
<td>1.21 (0.91)</td>
<td>0.78 (0.62)</td>
<td>0.21 (0.24)</td>
<td>0.23 (0.24)</td>
</tr>
<tr>
<td></td>
<td>MRT (h)</td>
<td>MRT (h)</td>
<td>MRT (h)</td>
<td>MRT (h)</td>
</tr>
</tbody>
</table>
| Cmax – Maximum plasma concentration
| Tmax – Time of maximum plasma concentration
| MRT – Medium residence time

Mean $C_{\text{max}}$ values for both fluticasone and budesonide were lower when the drugs were inhaled following bronchoconstriction (table 4-2). $C_{\text{max}}$ values were a median 44 % lower (interquartile range (IQR) 21 to 60) for fluticasone and 33 % lower (IQR -7 to 51) for budesonide following bronchoconstriction but the ratio between study visits for fluticasone was not significantly different to the ratio for budesonide, $p=0.18$.

Mean AUC$_{0-5}$ values, the primary endpoints, for both fluticasone and budesonide were lower when the drugs were inhaled following bronchoconstriction (table 4-3). AUC$_{0-5}$ values were a median 60 % lower (IQR 36 to 75) for fluticasone and 29 % lower (IQR 2 to 44) for budesonide following bronchoconstriction. The ratio for AUC$_{0-5}$ values...
between study visits for fluticasone was significantly greater than the ratio for budesonide, p=0.007.

![Graph showing plasma concentrations of fluticasone and budesonide](image)

**Figure 4-1.** Mean (SE) plasma drug concentrations following inhalation of 1000 µg fluticasone (Accuhaler®) and 800 µg budesonide (Turbohaler®) in 20 subjects with asthma with ( ) and without ( ) prior methacholine-induced bronchoconstriction (Note: Y axes have different scales).

**Discussion**

This is the first study to explore the effect of change in airflow obstruction within an individual on plasma concentrations of fluticasone and budesonide following drug
inhalation. There was an average 33 % difference in FEV\(_1\) prior to drug inhalation on the two study visits as a result of methacholine challenge. Plasma concentrations of both fluticasone and budesonide were lower when FEV\(_1\) was reduced but the magnitude of this effect was greater for fluticasone.

Table 4-3. Area under the curve (AUC) values for fluticasone and budesonide when inhaled with and without prior methacholine-induced bronchoconstriction

<table>
<thead>
<tr>
<th></th>
<th>Fluticasone</th>
<th>Differences (95% CI), p value</th>
<th>Budesonide</th>
<th>Differences (95% CI), p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without bronchoconstriction</td>
<td>Following bronchoconstriction</td>
<td>Without bronchoconstriction</td>
<td>Following bronchoconstriction</td>
</tr>
<tr>
<td>AUC (ng.ml(^{-1}.h))</td>
<td>0.40 (0.23)</td>
<td>0.16 (0.10)</td>
<td>0.23 (0.14 to 0.33), p &lt;0.001</td>
<td>2.87 (1.19)</td>
</tr>
</tbody>
</table>

The greater effect of bronchoconstriction on plasma concentrations of fluticasone compared to budesonide is similar to the findings in patients with and without airflow obstruction [55, 96, 97, 105]. Bronchoconstriction will cause a greater proportion of both drugs to be deposited in more central airways, where mucociliary clearance is better able to remove drug [117]. This would be expected to affect fluticasone more than budesonide because fluticasone, being more lipophilic, dissolves more slowly in airway lining fluid and hence is available for clearance for a longer time [105]. Furthermore little of the fluticasone that is removed by mucociliary clearance will reach the systemic circulation due to its low oral bioavailability (<1 % compared to around 10 % for budesonide) [105].

Peak plasma drug concentrations occurred earlier and were considerably higher following inhalation of budesonide than fluticasone in keeping with previous data [55]. These differences are likely to reflect high pulmonary deposition of budesonide from the Turbowhale®️, its higher oral bioavailability, and greater water solubility and its lower volume of distribution [105, 115, 118].
We gave fluticasone and budesonide at the same time to ensure that they were studied under identical conditions. This approach was validated by Agertoft and Pederson who found similar plasma fluticasone and budesonide concentrations following inhalation whether the drugs were given separately or together [119]. Drug concentrations were measured over five hours since our previous study showed that the major differences in plasma concentrations between subjects with and without airflow obstruction occurred during this time [55]. We did not seek to obtain pharmacokinetic parameters other than $C_{\text{max}}$ and $T_{\text{max}}$, since these are already available and a longer sampling period would have been required [115]. We believe our findings are due to reduced rather than delayed, drug absorption in view of the shape of the plasma concentration-time curves and the fact that the changes within subjects in this study are very similar to the differences seen over 8 and 12 hours between subjects with and without airflow obstruction[51, 55, 97].

By using a methacholine challenge we were able to study the effect of change in lung function within an individual on plasma concentrations of fluticasone and budesonide under controlled conditions. This is likely to be a reasonable model for changes in airflow that occur as asthma control deteriorates. A 33 % reduction in FEV$_1$ in our study caused a 60 % reduction in $\text{AUC}_{0-5}$ values for fluticasone suggesting a 60 % reduction in systemic exposure. Equally, as FEV$_1$ increases with treatment, systemic exposure would be expected to increase in a similar way. The risk of adverse systemic effects from inhaled fluticasone is likely, therefore, to vary considerably in relation to lung function within an individual while that from budesonide is unlikely to be affected to the same extent. These findings re-enforce the importance of reviewing the need for
higher doses of inhaled corticosteroids and particularly fluticasone, as lung function
improves and in patients with relatively normal lung function.
CHAPTER 5
EVALUATION OF THE ADMINISTRATION TIME EFFECT ON THE CUMULATIVE CORTISOL SUPPRESSION AND CUMULATIVE LYMPHOCYTES SUPPRESSION FOR ONCE-DAILY INHALED CORTICOSTEROIDS

Introduction

Inhaled corticosteroids (ICS) continue to remain the first-line therapy for the treatment of asthma [12]. Clinical studies have demonstrated the high anti-asthmatic efficacy (e.g. prevention and control of symptoms) and reduced systemic side effects of ICS therapy. However, potential systemic side effects induced by orally (swallowed) or pulmonary absorbed drug remain a concern in the eyes of the patient [13, 64, 69]. The major systemic effects include bone demineralization, growth suppression in children, hypothalamic-pituitary-adrenal (HPA) axis suppression and etc sec. Since many of these effects are either difficult to quantify or not possible to measure during short-term observations, the use of biomarkers is common in clinical pharmacology studies of corticosteroids. Two such markers have been identified for the systemic effects of corticosteroids: suppression of endogenous cortisol and blood lymphocytes [120, 121].

Cortisol serum levels are very sensitive to the presence of exogenous corticosteroids and the release of serum cortisol is often dramatically suppressed after drug administration. Thus, cortisol suppression as monitored by serum or urinary cortisol is currently used in clinical pharmacology studies for the assessment of systemic side effects, as these parameters are sensitive and relatively easy to determine while other more clinically relevant parameters (e.g. growth suppression) is used less frequently in specific patient populations. Twenty-four hour cortisol suppression has been used as the
most sensitive marker [122] and adequate PK/PD models have been developed [123]. Endogenous serum cortisol concentrations follow a circadian rhythm. Peak concentration is reached in the morning between 6AM and 10 AM, trough concentrations are reached at night between 8PM and 2AM [124-126]. Therefore, cortisol suppression after ICS is expected to undergo diurnal variation dependent on the administration time in the day [127].

It is known that the administration of corticosteroids results in a transient depletion of blood lymphocytes, which is called lymphocytopenia [128, 129]. Lymphocytopenia seems to be an appropriate choice as biomarker since the decline in lymphocytes is directly related to the suppression of immune system often observed in chronic corticosteroid therapy, whether it is side effect or desired outcome [130-134]. Endogenous cortisol, like exogenous corticosteroids, also affects the number of lymphocytes in the blood. Since the endogenous serum cortisol concentration follows a circadian rhythm, in the absence of additional stimuli the lymphocytes count shows intraday fluctuations inversely related to cortisol concentrations [121]. The overall extent of lymphocytopenia observed after exogenous corticosteroid administration is the combined effect of a direct suppression of blood lymphocytes by the exogenous glucocorticoid and a reduction of this effect due to the decrease in serum cortisol level induced by exogenous glucocorticoids. Therefore, the extent of lymphocytes suppression in the blood after ICS administration may also undergo diurnal variation dependent on the administration time of the ICS during the day. The importance of the administration time of corticosteroids for lymphocytes suppression has not yet been recognized.
Newer inhaled glucocorticoids are currently being dosed once a day. Clinical studies in recent years have tried to evaluate the effect of morning or evening dosing on the effect and systemic side effect with contradicting results [41-48]. In this study, a population modeling/simulation approach was developed and applied to evaluate the effect of daily administration time on 24-hour cumulative cortisol suppression (CCS) and cumulative lymphocytes suppression (CLS).

**Methods**

**Historic Data**

Results of a previously published study comparing the single-dose and steady-state (day 5) pharmacokinetics and pharmacodynamics of inhaled fluticasone propionate and budesonide in healthy subjects were used to develop the necessary population pharmacokinetic and pharmacodynamic model [103]. The study was conducted in accordance with the revised Declaration of Helsinki (Hongkong Revision 1989) and to Good Clinical Practice guidelines. A total of 14 healthy male volunteers, at mean age of 26.4 years (range 22-32), height of 179.2 (range 172-187) cm and weight of 72.7 (range 62-85) kg completed the study. The study evaluated the single dose and steady state pharmacokinetic and pharmacodynamic properties for inhaled fluticasone propionate (200 and 500 µg via Diskus®, FP-DSK) and budesonide (400 and 1000 µg via Turbohaler®, BUD-TBH) in healthy subjects. During this double blind, double dummy, randomized, placebo controlled, five-way crossover study single doses were administered at 8 am on day 1 followed by twice-daily dosing at 8 a.m. and 8 p.m. on days 2-5. During day 1 and 5 blood samples for drug, cortisol and lymphocytes analysis were collected at
frequent intervals. On days 2-4, only trough samples (8 a.m. and 8 p.m.) were obtained. The detail of the study has been reported elsewhere [103].

**Population Pharmacokinetic/Pharmacodynamic Analysis**

A sequential modeling approach was taken to facilitate the data analysis. In the first step, the population pharmacokinetic analysis was performed. This was followed by the population pharmacodynamic analysis with individual pharmacokinetic parameter estimates as part of input. Log-transformed data were used in the analysis since it was found that log-transformation of the data stabilized the model. The analysis was carried out using the NONMEM program (Version V, Globomax, Hanover, Md).

**Pharmacokinetic analysis**

Based on previously performed standard two-stage pharmacokinetic analysis, 1 and 2 compartment body model with first-order absorption were both tried to identify the optima structure model for BUD and FP, respectively [135, 136]. The inter-subject variability of PK parameters was modeled as exponential error model, as follows:

\[ P_i = \theta \cdot \exp(\eta_i) \]

where \( P_i \) is the parameter for ith subject, \( \theta \) is the typical population value of the parameter, and \( \eta_i \) is a random inter-subject effect with mean 0 and variance \( \omega^2 \). The intra-subject variability was modeled as additive error model, as follows:

\[ \ln(y_{ij}) = \ln(y_{pij}) + \epsilon_{ij} \]

where \( y_{ij} \) and \( y_{pij} \) represent the ith subject’s jth observed and predicted concentration, respectively, and \( \epsilon \) is the random residual error, which is normally distributed with mean 0 and variance \( \sigma^2 \). Fist-order conditional method (FOCE) was used for parameter estimation. Individual subject PK parameters were calculated using the posterior conditional estimation technique of NONMEM. After determination of structure
model, inter-occasion variability was explored for all PK parameters [137]. The significance level required to keep inter-occasion variability on a parameter in a hierarchical model was set to $p < 0.01$, which corresponds to a drop in the objective function by 6.64. Finally, a bootstrap resampling technique was applied as internal validation of the model [138]. Wings for NONMEM (Version 3.04, developed by Dr. N. Holford) was used for execution of bootstrap resampling analysis.

**Pharmacokinetic/Pharmacodynamic analysis of effects of BUD and FP on cortisol**

In the absence of exogenous stimuli, the circadian rhythm of endogenous cortisol was described by a linear release model, as follows [139]:

$$R_c = \frac{R_{\text{max}}}{Vd \cdot (t_{\text{max}} - t_{\text{min}} - 24)} \cdot t - \frac{R_{\text{max}} \cdot t_{\text{min}}}{Vd \cdot (t_{\text{max}} - t_{\text{min}} - 24)}, \ t_{\text{max}} < t < t_{\text{min}}$$

$$R_c = \frac{R_{\text{max}}}{Vd \cdot (t_{\text{max}} - t_{\text{min}})} \cdot t - \frac{R_{\text{max}} \cdot t_{\text{min}}}{Vd \cdot (t_{\text{max}} - t_{\text{min}})}, \ t_{\text{min}} < t < t_{\text{max}}$$

$$\frac{dC_{\text{tot}}^{\text{Cort}}}{dt} = R_c \cdot k_c^{\text{Cort}} \cdot C_{\text{tot}}^{\text{Cort}}$$

where $R_c$ is the release rate of cortisol, $R_{\text{max}}$ is the maximum cortisol release rate, $t_{\text{max}}$ is the time of maximum release, $t_{\text{min}}$ is the time of minimum release of cortisol, $Vd$ is the volume of distribution of cortisol and fixed at literature value 33.7 L, $C_{\text{tot}}^{\text{Cort}}$ is the cortisol concentration, $k_c^{\text{Cort}}$ is the elimination rate constant of cortisol and fixed at literature value $0.56 \ h^{-1}$. The change in total cortisol plasma concentration and its modulation by the free plasma concentration of an exogenous corticosteroid was described with an indirect response model, as follows [140]:
where $E_{\text{max}}$ is the maximum suppressive effect and fixed at 1, $C_f^{\text{ECS}}$ is the unbound exogenous corticosteroid concentration and $E_{50}^{\text{ECS} \rightarrow \text{Cort}}$ is the unbound exogenous corticosteroid concentration to achieve 50% of the maximum suppression [139, 140]. The unbound faction in plasma is 0.12 for BUD, and 0.1 for FP [80].

The inter-subject variability of PD parameters was modeled as exponential error model, as described in previous section, and residual error was modeled as additive error model, as described in previous section. First-order (FO) method was used for parameter estimation. Individual subject PD parameters were obtained as empirical Bayes estimates using the POSTHOC option of NONMEM. The cortisol data after placebo were used to calculate $t_{\text{max}}$ and $t_{\text{min}}$. The cortisol data after active treatment were modeled with PK parameters, $t_{\text{max}}$ and $t_{\text{min}}$ fixed to estimate $R_{\text{max}}$ and $E_{50}^{\text{ECS} \rightarrow \text{Cort}}$.

Inter-occasion variability was explored for all PD parameters. The significance level required to keep inter-occasion variability on a parameter in a hierarchical model was set to $p < 0.01$, which corresponds to a drop in the objective function by 6.64. The final model was validated by prediction check: the serum cortisol concentrations were simulated 100 times, then 24-hour CCS at steady state and observed 24-hour CCS at steady state were compared using Kruskal-Wallis test followed by Dunn’s multiple comparison. The 24-hour CCS is quantified as follows [127]:

$$\%\text{CCS} = \frac{AUC^{\text{Supp., 24}}}{AUC^{\text{Base., 24}}} = \frac{AUC^{\text{Base., 24}} - AUC^{\text{Therapy, 24}}}{AUC^{\text{Base., 24}}}$$
where AUC\textsuperscript{Supp,24} is the difference between the area under the cortisol plasma concentration-time curve at baseline (AUC\textsuperscript{base,24}) and during therapy (AUC\textsuperscript{therapy,24}) over 24 hour.

**Pharmacokinetic/Pharmacodynamic analysis of effects of budesonide and fluticasone propionate on lymphocytes**

The combined pharmacodynamic effects of cortisol and exogenous corticosteroid on lymphocyte can be described as [141]:

\[
\frac{dN}{dt} = K_{in} \left( I - \frac{E_{max}}{EC_{ECS\to Lym} + C_f^{ECS} + \frac{E_{50}^{ECS\to Lym}}{E_{50}^{Cort\to Lym}} \cdot C_f^{Cort}} \right) - k_{out} \cdot N
\]

where N is the number of lymphocytes in blood, C\textsubscript{f}\textsuperscript{Cort} is the unbound cortisol concentration, K\textsubscript{in} is the influx rate constant of cells from the extravascular space, K\textsubscript{out} is the efflux rate constant of cells out of the vascular space, E\textsubscript{max} is the maximum effect and fixed at 1, E\textsubscript{50}\textsuperscript{Cort\to Lym} is the unbound concentration of cortisol to produce 50% of the maximum effect and E\textsubscript{50}\textsuperscript{ECS\to Lym} is the unbound concentration of to achieve 50% of the maximum effect. Cortisol exhibits non-linear binding to serum protein in the physiological concentration range and the unbound concentration can be calculated by solving following equation [120]:

\[
C_{tot}^{Cort} = \frac{K_{Tc} \times Q_{Tc} \times C_f^{Cort}}{1 + K_{Tc} \times C_f^{Cort}} + K_{Alb} \times Q_{Alb} \times C_f^{Cort} + C_f^{Cort}
\]

where K\textsubscript{Tc} is the affinity constant between cortisol and transcortin (3×10\textsuperscript{7} 1/mol), K\textsubscript{Alb} the affinity constant between cortisol and albumin (5000 l/mol), Q\textsubscript{Tc} the total transcortin concentration (0.7 \textmu mol/l), Q\textsubscript{Alb} the total albumin concentration (550 \textmu mol/l) and C\textsubscript{f}\textsuperscript{Cort} the unbound cortisol serum concentration [142].
The inter-subject variability of PD parameters was modeled as exponential error model, and residual error was modeled as additive error model. First-order (FO) method was used for parameter estimation. Individual subject PD parameters were obtained as empirical Bayes estimates using the POSTHOC option of NONMEM. The lymphocyte data were modeled to estimate $K_{in}$, $K_{out}$, $EC_{50}^{Cort\rightarrow Lym}$ and $EC_{50}^{ECS\rightarrow Lym}$ with all the other PK and PD parameters fixed.

Inter-occasion variability was explored for all PD parameters. The significance level required to keep inter-occasion variability on a parameter in a hierarchical model was set to $p < 0.01$, which corresponds to a drop in the objective function by 6.64. The final model was validated by prediction check: the blood lymphocyte counts were simulated 100 times, then 24-hour CLS at steady state and observed 24-hour CLS at steady state were compared using Kruskal-Wallis test followed by Dunn’s multiple comparison. The 24-hour CLS is quantified as follows [127]:

$$
\%CLS = \frac{AUC^{Supp, 24}_{\text{AUC}_{\text{Base}, 24}}} = \frac{AUC^{Supp, 24}}{AUC^{Therapy, 24}} - \frac{AUC^{Therapy, 24}}{AUC^{Base, 24}}
$$

where $AUC^{Supp, 24}$ is the difference between the area under the lymphocytes plasma number of counts time curve at baseline ($AUC^{base, 24}$) and during therapy ($AUC^{therapy, 24}$) over 24hr.

**Population PK/PD Simulation to Evaluate Administration Time Effect on CCS and CLS for Once-daily Dose of BUD and FP**

Using the developed population PK/PD model, we performed simulations to evaluate the effect of the administration time effect on 24-hour CCS and 24-hour CLS at steady state for 4 clinically relevant dosing regimens: 1mg once-daily BUD, 2mg once-daily BUD, 0.5mg once-daily FP and 1mg once-daily FP. For each dosing regimen,
simulation was performed at 24-hourly distributed administration time points throughout the day. NONEME was used for execution of the simulation. 75 hypothetic subjects are included in the simulation. The 75 subjects’ parameters were assumed to follow the same distribution as the 14 subjects’ whose population means and variances were estimated in above analysis. Each subject’s parameters were randomly sampled from the estimated populations respectively, and kept the same throughout the simulation. For those parameters whose inter-subject variability was not estimated, the values of population mean, either estimated or fixed in above analysis, were applied to each subject in the simulation. The whole simulation for the four dosing regimens was repeated 8 times with different seed numbers assigned in NONMEM, which were to generate different groups of 75 hypothetic subjects in each simulation. For each simulation, the 75 subjects’ 24-hour CCS at steady state resulted from different administration time were calculated and then compared, respectively for each dosing regimen, using Friedman test followed by Dunn’s multiple comparison. Similarly, the 75 subjects’ 24-hour CLS at steady state resulted from different administration time were calculated and then compared, respectively for each dosing regimen in each simulation, using Friedman test followed by Dunn’s multiple comparison.

Results

Population Pharmacokinetic Model

Budesonide

A one-compartment model was chosen as structure model for BUD since two-compartment model was over-parameterized and one-compartment model was sufficient to describe the data (Appendix A for NONMEM code). No trend was observed in the diagnostic plot of weighted residuals versus time, indicating no bias in the model.
prediction. The relationship between population predicted, individual predicted and observed plasma concentrations as a function of time is shown in Figure 5-1. There was no significant inter-occasion variability found for any parameter (Table 5-1) based on likelihood ratio test ($P<0.01$, $\chi^2_{0.05} = 6.64$, $df = 1$). The parameter estimates were in good agreement with the mean parameter estimates of the bootstrap replicates (Table 5-1), indicating stability of the model [143]. The value for the absorption rate ($K_a$) was fixed because BUD’s fast absorption and plasma sampling time scheme did not allow estimation of this parameter.

Figure 5-1. Relationship between population predicted (open triangle) and observed BUD (open circle) concentrations, individual predicted (open square) and observed BUD (open circle) concentrations as function of time.
Table 5-1. Summary of BUD pharmacokinetic model parameter estimates

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Original dataset estimate (RSE %)</th>
<th>200 bootstrap replicates mean (RSE %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \theta_{K_a} ), h(^{-1} )</td>
<td>10 (Fixed)</td>
<td>10 (Fixed)</td>
</tr>
<tr>
<td>( \theta_{K_{10}} ), h(^{-1} )</td>
<td>0.21 (5.5)</td>
<td>0.21 (5.3)</td>
</tr>
<tr>
<td>( \theta_V ), L</td>
<td>1320 (9.8)</td>
<td>1336 (9.2)</td>
</tr>
<tr>
<td>( \omega_{K_a} ), %CV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \omega_{K_{10}} ), %CV</td>
<td>15.5 (85.4)</td>
<td>14.2 (46.5)</td>
</tr>
<tr>
<td>( \omega_V ), %CV</td>
<td>31.2 (37.2)</td>
<td>30.0 (20.6)</td>
</tr>
<tr>
<td>( \sigma ), ng/ml</td>
<td>0.54 (17.5)</td>
<td>0.54 (8.7)</td>
</tr>
</tbody>
</table>

RES, relative standard error; CV, coefficient of variation; Ka, absorption rate; K10, elimination rate constant; V, volume of distribution central compartment; - = not calculated.

**Fluticasone propionate**

A two-compartment model was chosen as structure model for FP since one-compartment model did not fit data well (Appendix B for NONMEM code). No trend was observed in the diagnostic plot of weighted residuals versus time. The relationship between population predicted, individual predicted and observed plasma concentrations as a function of time is shown in Figure 5-2. There was no significant inter-occasion variability found on any parameter (Table 5-2) based on likelihood ratio test (P<0.01, \( \chi^2_{0.05} = 6.64, df = 1 \)). The parameter estimates were in good agreement with the mean parameter estimates of the bootstrap replicates (Table 5-2), indicating stability of the model.

**Population Pharmacokinetic/Pharmacodynamic Model**

The population PK/PD model provided reasonable fits for the cortisol and lymphocyte data (Appendix C and D for NONMEM code). No trend was observed in the diagnostic plot of weighted residuals versus time. The relationship between population predicted, individual predicted and observed plasma concentrations as a function of time is shown in Figure 5-3, and 5-4. The parameter estimates are listed in Table 5-3. A
significant inter-occasion variability was found for Rmax for both BUD and FP. None of the 24-hour CCS and CLS at steady state calculated from simulated cortisol concentrations and lymphocyte counts time-profile was different from observed CCS and CLS in the original study [112], respectively (p < 0.05), indicating stability of the model.

![Graph](image)

Figure 5-2. Relationship between population predicted (open triangle) and observed FP (open circle) concentrations, individual predicted (open square) and observed FP (open circle) concentrations as function of time.

**Population Pharmacokinetic/Pharmacodynamic Simulation**

The relationship between administration time and corresponding mean CCS and CLS of the 75 subjects from each simulation is shown in Figure 5-5 and 5-6 (Appendix E and F for NONMEM code). For once-daily of BUD, maximum CCS was observed when BUD was given in 2am-5am, while minimum CCS was observed when BUD was given
in 3pm-6pm. Similarly, for once-daily of FP, maximum CCS was observed when FP was given in 4am-8am, and minimum CCS was observed when FP was given in 3pm-8pm.

On the contrary to what we observed for CCS, maximum CLS was observed when BUD was given in 3pm-8pm and when FP was given in 3pm-7pm, while minimum CLS was observed when BUD or FP was given in 3am-9am. The maximum and minimum mean CCS and CLS of the subjects and the corresponding administration time points of the 8 simulations are summarized in Table 5-4 and 5-5. The difference between the maximum and minimum mean suppression of the subjects are also summarized in Table 5-4 and 5-5. The maximum mean CCS of the subjects is significantly different from minimum mean CCS of the subjects, respectively for each dosing regimen of each simulation (p < 0.001).

In addition, the maximum mean CLS of the subjects is significantly different from minimum mean CLS of the subjects, respectively for each dosing regimen of each simulation (p < 0.001).

**Table 5-2. Summary of FP pharmacokinetic model parameter estimates**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Original dataset estimate (RSE %)</th>
<th>200 bootstrap replicates mean (RSE %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_{K_a}$, h^{-1}</td>
<td>2.79 (13.7)</td>
<td>3.3 (39.9)</td>
</tr>
<tr>
<td>$\theta_{K_{12}}$, h^{-1}</td>
<td>0.21 (13.0)</td>
<td>0.24 (56.7)</td>
</tr>
<tr>
<td>$\theta_{K_{21}}$, h^{-1}</td>
<td>0.16 (8.0)</td>
<td>0.18 (26.6)</td>
</tr>
<tr>
<td>$\theta_{K_{10}}$, h^{-1}</td>
<td>0.14 (6.9)</td>
<td>0.13 (11.7)</td>
</tr>
<tr>
<td>$\theta_V$, L</td>
<td>4130 (8.0)</td>
<td>4454 (18.2)</td>
</tr>
<tr>
<td>$\omega_{K_{10}}$, %CV</td>
<td>13.7 (95.2)</td>
<td>11.4 (60.8)</td>
</tr>
<tr>
<td>$\omega_{K_{12}}$, %CV</td>
<td>16.1 (100.8)</td>
<td>18.6 (72.4)</td>
</tr>
<tr>
<td>$\omega_{K_{21}}$, %CV</td>
<td>30.1 (67.1)</td>
<td>28.5 (77.9)</td>
</tr>
<tr>
<td>$\omega_{K_{10}}$, %CV</td>
<td>14.5 (70.3)</td>
<td>14.4 (96.3)</td>
</tr>
<tr>
<td>$\omega_{V}$, %CV</td>
<td>10.7 (80.6)</td>
<td>9.2 (49.6)</td>
</tr>
<tr>
<td>$\sigma$, ng/ml</td>
<td>0.27 (10.0)</td>
<td>0.26 (5.5)</td>
</tr>
</tbody>
</table>

RES, relative standard error; CV, coefficient of variation; $K_a$, absorption rate; $K_{12}$, transfer rate constant from central compartment to peripheral compartment; $K_{21}$, transfer rate constant from peripheral compartment to central compartment; $K_{10}$, elimination rate constant; $V$, volume of distribution central compartment; $-$ = not calculated.
Figure 5-3. Diagnostic plots of BUD PK/PD model. (a) Relationship between individual predicted (open square) and observed (open circle) cortisol concentrations as function of time for BUD PK/PD model. (b) Relationship between individual predicted (open square) and observed (open circle) lymphocyte counts as function of time for BUD PK/PD model.
Figure 5-4. Diagnostic plots of FP PK/PD model. (a) Relationship between individual predicted (open square) and observed (open circle) cortisol concentrations as function of time for FP PK/PD model. (b) Relationship between individual predicted (open square) and observed (open circle) lymphocyte counts as function of time for FP PK/PD model.
Table 5-3. Summary of FP and BUD pharmacokinetic/pharmacodynamic model parameter estimates

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BUD</th>
<th>FP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (RSE %)</td>
<td>Mean (RSE %)</td>
<td></td>
</tr>
<tr>
<td>$\theta_{T_{\text{max}}}$, h</td>
<td>22.7 (2.5)</td>
<td>22.7 (2.5)</td>
</tr>
<tr>
<td>$\theta_{T_{\text{min}}}$, h</td>
<td>17.5 (0.7)</td>
<td>17.5 (0.7)</td>
</tr>
<tr>
<td>$\theta_{V_d}$, L</td>
<td>33.7 (Fixed)</td>
<td>33.7 (Fixed)</td>
</tr>
<tr>
<td>$\theta_{K_e}$, h$^{-1}$</td>
<td>0.56 (Fixed)</td>
<td>0.56 (Fixed)</td>
</tr>
<tr>
<td>$\theta_{R_{\text{max}}}$, ng/h</td>
<td>3270 (4.1)</td>
<td>2490 (7.6)</td>
</tr>
<tr>
<td>$\theta_{t_{\text{EC50}}}$, ng/ml</td>
<td>0.02 (20.9)</td>
<td>0.008 (37.7)</td>
</tr>
<tr>
<td>$\theta_{K_{\text{Kin}}}$, %/h</td>
<td>21.7 (25.3)</td>
<td>14.4 (60.8)</td>
</tr>
<tr>
<td>$\theta_{K_{\text{Out}}}$, %/h</td>
<td>0.19 (28.4)</td>
<td>0.13 (77.5)</td>
</tr>
<tr>
<td>$\theta_{t_{\text{EC50}}}$, ng/ml</td>
<td>30.8 (15.6)</td>
<td>26.8 (31.8)</td>
</tr>
<tr>
<td>$\omega_{t_{\text{max}}}$, %CV</td>
<td>7.0 (54.1)</td>
<td>7.0 (54.1)</td>
</tr>
<tr>
<td>$\omega_{t_{\text{min}}}$, %CV</td>
<td>4.0 (33.9)</td>
<td>4.0 (33.9)</td>
</tr>
<tr>
<td>$\omega_{V_d}$, %CV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\omega_{K_e}$, %CV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\omega_{R_{\text{max}}}$, %CV</td>
<td>9.4 (42.9)</td>
<td>21.8 (54.9)</td>
</tr>
<tr>
<td>$\omega_{R_{\text{max}}}$, %CV</td>
<td>17.6 (44.7)</td>
<td>15.4 (59.3)</td>
</tr>
<tr>
<td>$\omega_{t_{\text{EC50}}}$, %CV</td>
<td>60.0 (47.1)</td>
<td>46.0 (158.0)</td>
</tr>
<tr>
<td>$\omega_{K_{\text{Kin}}}$, %CV</td>
<td>n/c</td>
<td>n/c</td>
</tr>
<tr>
<td>$\omega_{K_{\text{Out}}}$, %CV</td>
<td>7.8 (162.6)</td>
<td>6.3 (127.9)</td>
</tr>
<tr>
<td>$\omega_{t_{\text{EC50}}}$, %CV</td>
<td>35.6 (69.0)</td>
<td>71.7 (80.4)</td>
</tr>
<tr>
<td>$\omega_{t_{\text{EC50}}}$, %CV</td>
<td>80.4 (36.6)</td>
<td>88.7 (133.3)</td>
</tr>
<tr>
<td>$\sigma_{\text{cortisol}}$, ng/ml</td>
<td>0.54 (8.7)</td>
<td>0.54 (10.0)</td>
</tr>
<tr>
<td>$\sigma_{\text{lymphocytes}}$, %</td>
<td>0.17 (12.2)</td>
<td>0.17 (15.2)</td>
</tr>
</tbody>
</table>
Figure 5-5. Relationship between administration time and corresponding mean (+ SE) cumulative suppression. a) CCS of the 75 subjects after once-daily 1mg of BUD. b) CCS of the 75 subjects after once-daily 2mg of BUD. c) CLS of the 75 subjects after once-daily 1mg of BUD. d) CLS of the 75 subjects after once-daily 2mg of BUD. Empty circle: simulation 1; Filled circle: simulation 2; Empty triangle: simulation 3; Filled triangle: simulation 4; Empty Square: simulation 5; Filled Square: simulation 6; Empty heart: simulation 7; Filled heart: simulation 8.
Figure 5-6. Relationship between administration time and corresponding mean (+ SE) cumulative suppression. a) CCS of the 75 subjects after once-daily 0.5mg of FP. b) CCS of the 75 subjects after once-daily 1mg of FP. c) CLS of the 75 subjects after once-daily 0.5mg of FP. d) CLS of the 75 subjects after once-daily 1mg of FP. Empty circle: simulation 1; Filled circle: simulation 2; Empty triangle: simulation 3; Filled triangle: simulation 4; Empty Square: simulation 5; Filled Square: simulation 6; Empty heart: simulation 7; Filled heart: simulation 8.
Table 5-4. Summary of the mean cumulative cortisol suppression of the 75 subjects of the 8 simulations

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Maximum ± SD (%)</th>
<th>Administration time point</th>
<th>Minimum ± SD (%)</th>
<th>Administration time point</th>
<th>Fluctuation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUD 1 mg</td>
<td>27.5 ± 0.9</td>
<td>2am – 5am</td>
<td>-3.5 ± 1.9</td>
<td>3pm – 6pm</td>
<td>31.0 ± 2.5</td>
</tr>
<tr>
<td>2 mg</td>
<td>44.0 ± 1.6</td>
<td>3am – 5am</td>
<td>12.5 ± 1.2</td>
<td>4pm – 5pm</td>
<td>31.5 ± 2.0</td>
</tr>
<tr>
<td>FP 0.5 mg</td>
<td>12.9 ± 2.6</td>
<td>4am – 8am</td>
<td>-4.4 ± 2.4</td>
<td>5pm – 8pm</td>
<td>17.3 ± 3.3</td>
</tr>
<tr>
<td>1 mg</td>
<td>22.1 ± 1.3</td>
<td>4am – 5am</td>
<td>-0.2 ± 1.4</td>
<td>3pm – 6pm</td>
<td>22.3 ± 3.1</td>
</tr>
</tbody>
</table>

a. The mean and standard deviation of the maximum mean CCS of the 75 subjects of the 8 simulations.
b. The range of administration time points which corresponds to the maximum mean CCS of the 75 subjects of the 8 simulations.
c. The mean and standard deviation of the minimum mean CCS of the 75 subjects of the 8 simulations.
d. The range of administration time points which corresponds to the minimum mean CCS of the 75 subjects of the 8 simulations.
e. The mean and standard deviation of the difference between maximum mean CCS and the minimum mean CCS of the 75 subjects of the 8 simulations.

Table 5-5. Summary of the mean cumulative lymphocytes suppression of the 75 subjects of the 8 simulations

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Maximum ± SD (%)</th>
<th>Administration time point</th>
<th>Minimum ± SD (%)</th>
<th>Administration time point</th>
<th>Fluctuation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUD 1 mg</td>
<td>9.6 ± 0.5</td>
<td>4pm – 8pm</td>
<td>4.7 ± 0.6</td>
<td>5am – 9am</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td>2 mg</td>
<td>15.8 ± 0.7</td>
<td>3pm – 8pm</td>
<td>9.8 ± 1.0</td>
<td>3am – 5am</td>
<td>6.0 ± 1.5</td>
</tr>
<tr>
<td>FP 0.5 mg</td>
<td>6.8 ± 0.3</td>
<td>4pm – 7pm</td>
<td>2.5 ± 0.4</td>
<td>3am – 8am</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>1 mg</td>
<td>9.6 ± 0.4</td>
<td>3pm – 6pm</td>
<td>4.5 ± 0.4</td>
<td>5am – 9am</td>
<td>5.2 ± 0.4</td>
</tr>
</tbody>
</table>

a. The mean and standard deviation of the maximum mean CLS of the 75 subjects of the 8 simulations.
b. The range of administration time points which corresponds to the maximum mean CLS of the 75 subjects of the 8 simulations.
c. The mean and standard deviation of the minimum mean CLS of the 75 subjects of the 8 simulations.
d. The range of administration time points which corresponds to the minimum mean CLS of the 75 subjects of the 8 simulations.
e. The mean and standard deviation of the difference between maximum mean CLS and the minimum mean CLS of the 75 subjects of the 8 simulations.

Discussion

Without exogenous stimuli, both endogenous cortisol concentrations and lymphocyte counts undergo a circadian rhythm. Moreover, the circadian rhythm of lymphocyte counts is inversely correlated with the circadian rhythm of endogenous
cortisol [121, 144]. One therefore should expect that administration time of an ICS has an effect on not only the cortisol but also lymphocytes suppression. In the current study we employed a population PK/PD simulation approach to evaluate the effect of the administration time on cumulative cortisol suppression and cumulative lymphocytes suppression simultaneously, and to identify an optimum administration time point for once-a-day dosing regimen in terms of minimum CCS and CLS, respectively.

A population PK/PD model was developed in this study to describe the disposition of BUD and FP after inhalation, the effect of BUD and FP on cortisol, and the combined effect of BUD or FP and cortisol on lymphocytes in healthy subjects. The structure (PK/PD) model was a mechanism-based model and previous study has demonstrated the validity of the model via standard two-stage analysis [121]. The current study successfully applied the model to non-linear mixed effects modeling analysis and estimated the inter and intra-subject and inter-occasion variability of model. After repeated inhalation, BUD plasma concentrations time-profiles were well described by one-compartment model with first-order absorption, while FP plasma concentrations time-profile was well described by two-compartment model with first-order absorption. The extra disposition of FP to a peripheral compartment compared with BUD could result from the more sensitive assay, about 5 folds more sensitive than assay for BUD, and its higher lipophilicity than BUD’s. The PK parameters of BUD and FP derived from this study are in broad agreement with previously reported values [145]. The steroid-independent pharmacodynamic parameters (Tmax, Tmin, Rmax, Kin and Kout), EC50ICS→cort of BUD and FP, EC50ICS→lym of BUD and EC50Cort→lym observed with the non-linear mixed effect modeling approach are comparable to results of previous
A linear relationship between EC50 for cortisol suppression for various corticosteroids and their relative receptor affinity (RRA), estimated in vitro, has been established in previous studies [147]. Hence, a linear relationship between EC50 for lymphocytes suppression for various corticosteroids and their RRA is reasonably expected. If we plot the 1/RRA versus EC50 of BUD, cortisol and FP for suppression on lymphocytes obtained in this study, a good linear curve (R2 = 0.95) could be established and should prove useful in future prediction of EC50 of other steroids (in vivo effects) based on their in vitro RRA (Figure 5-7).

![Figure 5-7](image_url)

Figure 5-7. Relationship between EC50, free of steroids for lymphocytes suppression and their reciprocal of the relative receptor affinity.
For both drugs, the maximum CCS was observed when ICS was administered in the early morning and the minimum CCS was observed when ICS was administered in the afternoon, and this observation is in agreement with previous report [127]. This circadian rhythm results from the interaction between the systemic activity of exogenous corticosteroids and endogenous cortisol release: CCS is maximized when the period of high systemic activity of exogenous corticosteroids (concentrations higher than EC50) covers the period of high cortisol release in the early morning; CCS is minimized when the period of high systemic activity covers the period of low cortisol release in the late night. On the contrary, the maximum CLS was observed when ICS was given in the afternoon while the minimum CLS was observed when ICS was given in the early morning. This circadian rhythm is the result of combined modulation of natural circadian rhythm of the lymphocytes release by systemic activity of exogenous corticosteroids and endogenous cortisol, whose release is suppressed by the same systemic exogenous corticosteroids. Hence, the CLS reaches the maximum when the period of high systemic activity of the net corticosteroids, the total of the suppressed endogenous cortisol and exogenous corticosteroids, covers the period of high lymphocytes release in the late night. And it reaches the minimum when the period of high systemic activity of the net corticosteroids covers the period of low lymphocytes release in the early morning. So for once-a-day dosing regimen, the optimum administration time is in the afternoon in terms of minimized CCS, however, the optimum administration time is in the morning in terms of minimized CLS.

In our simulations, the administration time for which maximum suppression of cortisol and blood lymphocytes was observed differed. The advantage to use cortisol
suppression as biomarker is that cortisol is more sensitive to the presence of exogenous corticosteroids since the EC50 values for the effect of ICS on lymphocytes was about 1 - 8 times higher than that for the effect on cortisol as demonstrated in previous and this study [146, 148]. It is, however, disputed whether cortisol suppression is a good marker for systemic effects or side effects, as the mechanism of cortisol suppression is likely to not reflect anti-inflammatory events or effects on growth that is affected by both endogenous and exogenous glucocorticoids.

While the lymphocytes suppression is somewhat less sensitive than the serum cortisol suppression, the immunological character of lymphocytopenia, has the advantage of representing a more relevant pharmacodynamic endpoint, that is affected by both endogenous and exogenous glucocorticoids. Lymphocytopenia is a systemic effect, and should represent a side effect for inhalation therapy. According to this definition delivery of ICS in the evening hours would induce the highest degree of side effects. Similar, administration of a systemic glucocorticoid (e.g. oral delivery) would induce the maximum effects when given in the evening. On the other hand, it is also likely that the desired pulmonary effects of inhaled glucocorticoids are the sum of endogenous and exogenous glucocorticoids. Consequently, one would also predict maximum antiasthmatic effects for an evening administration. The differences observed in the simulations between the two biomarkers supports the note that these biomarkers are not interchangeable on a qualitative and quantitative level.

The terminal half-life of FP after inhalation is much longer than that of BUD (14.3 hours vs. 3.3 hours as estimated from this study). It has been recognized that for a receptor mediated response, the duration and decline of this response depends partly on
the elimination rate of the drug [149, 150]. For FP, a longer terminal life leads to less fluctuated concentration during the dosing interval fluctuate less. This resulted in our “dosing time” simulations in a smaller difference between maximum and minimum cumulative cortisol suppression for 500 and 1000 µg FP than for 1,000 and 2,000 µg BUD (Table 5-4). Thus, the dosing time is less important for the CCS of FP. Interestingly, the difference between maximum and minimum CLS after once-daily administration of 1mg or 2mg BUD and after once-daily of 0.5 or 1mg FP were rather similar (Table 5-4), which is likely be related to the rather small overall effect on blood lymphocytes and the “buffering” capacity of cortisol.

Once-a-day dosing has been successfully evaluated for some of the newer inhaled glucocorticoids (budesonide, ciclesonide) because of the easy compliance [48]. Our results indicate that the administration time may be important for once-a-day dosing regimen and that a late afternoon or evening administration is likely to result in maximum effects. Clinical studies evaluating the effect of time of dosing on ICS efficacy and side effects are limited. Some clinical studies suggested that afternoon or early evening dosing tends to be superior in efficacy to a morning dosing especially for patients with nocturnal asthma [151, 152]. This is in agreement with our simulation results when data on systemic lymphocytopenia are applied to the local pulmonary effects.

Conclusion

The population PK/PD modeling/simulation presented in current study is a step further into predicting outcome of clinical study based on previous developed PK/PD model. The population approach taken in our study incorporates both inter-subject and intra-subject variability, and allows probing for significant differences. Previous non-population PK/PD simulation approaches have already proven high predictive power for
CCS [153], our population PK/PD simulation should be even more informative with respect to the statistical evaluation of the simulated events. Employing this approach, we demonstrated the administration time is an important factor to minimize the systemic side effects and maximize the benefit/risk ratio for once-daily regimen of ICS. However, further clinical studies are needed to verify the prediction by our simulation.
Pulmonary targeting is a desired characteristic for successful inhaled corticosteroids. The overall goal of this study was to evaluate certain aspects of factors affecting pulmonary targeting of current ICSs, hence to gain more understanding of those factors and contribute to the journey of developing “ideal” ICS for asthmatic patients.

Our first study investigated how different valved holding chambers (VHCs) affect fluticasone propionate aerosols delivered from a metered dose inhaler (MDI). It was found that all three VHCs tested, AeroChamber-Plus™, OptiChamber® and OptiChamber®-Advantage significantly reduced the in vitro oropharyngeal deposition of fluticasone propionate. It was also found that the respirable dose from the MDI attached to AeroChamber™-Plus or OptiChamber® was not significantly different from the respirable dose from fluticasone propionate MDI alone, while the respirable dose of fluticasone from OptiChamber®-Advantage was 40-45% less than from the MDI alone. This result suggests that AeroChamber™-Plus or OptiChamber® could be successfully used with fluticasone MDI since both VHCs not only could reduce the risk of topical adverse effects induced by fluticasone, but also deliver the same amount of respirable dose of fluticasone, while OptiChamber®-Advantage would not be a good choice for patients on fluticasone MDI since much less of respirable dose of fluticasone was delivered by it. This study shows that choice of VHC is an important factor for determination of pulmonary targeting of fluticasone propionate.
Our second study showed that the systemic absorption of beclometasone monopropionate, budesonide, fluticasone propionate and mometasone furoate appears to be greater in patients with a higher FEV$_1$ % predicted although other factors, including age and gender affect this relationship. In the third study, using a methacholine challenge we were able to study the effect of change in lung function within an individual on plasma concentrations of fluticasone and budesonide under controlled conditions. A 33 % reduction in FEV$_1$ in our study caused a 60 % reduction in AUC$_{0-5h}$ values for fluticasone and 29% reduction in AUC$_{0-5}$ values for budesonide, suggesting a 60 % reduction and a 29% in systemic exposure for fluticasone and budesonide respectively. Results from these two studies indicate that the systemic exposure, hence the risk adverse systemic effects from ICSs is correlated with asthmatic patient’s lung function, and extent of the correlation varies for different ICSs. We suggest that a difference in lipophilicity among ICSs probably is the reason for the difference in the extent of correlation among various ICSs. Further study is necessary to explore this. Our findings re-enforce the importance of reviewing the need for higher doses of inhaled corticosteroids and particularly fluticasone, as lung function improves and in patients with relatively normal lung function, to improve the benefit to risk ratio of ICSs.

Use of biomarker, such as cortisol suppression and lymphocytes facilitates the evaluation of systemic effects of different ICS regimens. Population PK/PD modeling and simulation provides us a great tool to describe the relationship between drug concentrations and biomarker concentrations, and to predict and assess statistical significance of the outcomes of future studies. Hence, we developed a population PK/PD model for inhaled budesonide and fluticasone, respectively to describe the relationship
between drug concentrations, cortisol concentrations and lymphocyte counts. Further, we used this model to evaluate the administration time effect on the cumulative cortisol suppression (CCS) and lymphocytes suppression (CLS) for once-daily inhaled BUD and FP, respectively. Our results indicate that the optimal time for administration of BUD or FP, in terms of minimized systemic side effects, depends on the choice of the biomarker: the minimum CCS is achieved when ICS is given in the afternoon, while minimum CLS is achieved when ICS is given in the morning. This administration time effect is the result of interaction between natural circadian rhythm of endogenous cortisol concentration and lymphocyte concentrations, and the effect of PK/PD properties of ICSs on these two rhythms. Our study also showed that both the difference between maximum CCS and minimum CCS, and the difference between maximum CLS and minimum CLS were significant (P < 0.001). The extent of difference between maximum and minimum CCS suppression differs between BUD and FP because their different PK properties. However, whether the difference between maximum and minimum suppression is clinically significant needs further clinical studies to evaluate.
APPENDIX A
NONMEM CODE FOR PK MODEL OF BUD AFTER INHALATION

$PROBLEM BUD PK MODEL
$INPUT ID TIME AMT DV CMT EVID MDV OCC
$DATA .LNPK1.CSV IGNORE=#
$SUBROUTINES ADVAN2 TRANS1
$PK
TVKA=THETA(1);ABSORPTION
TVK=THETA(2)
TVVC=THETA(3)
KA=TVKA
K=TVK*EXP(ETA(1))
VC=TVVC*EXP(ETA(2))
CL=K*VC
S2=VC
$ERROR
IPRED=0
IF(F.GT.0) IPRED=LOG(F)
Y=IPRED+ERR(1)
$THETA
(10 FIXED)
(0 0.22)
(0 1080)
$OMEGA 0.1 0.1
$SIGMA 0.04
$ESTIMATION METHOD=1 NOABORT MAXEVAL=9999 PRINT=5 POSTHOC
$COV PRINT=E
$TABLE ID TIME DV IPRED KA K VC CL ETA(1) ETA(2)
NOPRINT ONEHEADER FILE=BUDPK.FIT
APPENDIX B
NONMEM CODE FOR PK MODEL OF FP AFTER INHALATION

$PROBLEM FP PK MODEL
$INPUT ID TIME AMT DV CMT EVID MDV OCC
$DATA LNFPPK.CSV IGNORE=_
$SUBROUTINES ADVAN4 TRANS1
$PK
TVKA=THETA(1) ;ABSORPTION
TVK=THETA(2)
TVK23=THETA(3)
TVK32=THETA(4)
TVVC=THETA(5)
KA=TVKA*EXP(ETA(1))
K=TVK*EXP(ETA(2))
K23=TVK23*EXP(ETA(3))
K32=TVK32*EXP(ETA(4))
VC=TVVC*EXP(ETA(5))
CL=K*VC
S2=VC
$ERROR
IPRED=0
IF(F.GT.0) IPRED=LOG(F)
Y=IPRED+ERR(1)
$THETA
(0 4)
(0 0.1)
(0 0.159)
(0 0.1)
(0 2000)
$OMEGA 0.1 0.15 0.1 0.1 0.1
$SIGMA 0.1
$ESTIMATION METHOD=0 NOABORT MAXEVAL=9999 PRINT=5 POSTHOC
$COV PRINT=E
$TABLE ID TIME DV IPRED KA K K23 K32 VC CL ETA(1) ETA(2)
ETA(3) ETA(4) ETA(5) NOPRINT ONEHEADER FILE=FPPK.FIT
APPENDIX C
NONMEM CODE FOR PK/PD MODEL OF BUD

$PROBLEM CORTISOL BASELINE PD MODEL
$INPUT ID TIME AMT DV EVID CMT MDV
$DATA lncortbase.CSV IGNORE=#
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(CORTISOL);1
$ABBREVIATED DERIV2=NO
$PK
TVTMAX=THETA(1); TIME OF MAX RELEASE
TVTMIN=THETA(2); TIME OF MINIMUM RELEASE
TVRMAX=THETA(3); MAXIMUM RELEASE RATE
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
TMAX=TVTMAX*EXP(ETA(1))
TMIN=TVTMIN*EXP(ETA(2))
RMAX=TVRMAX*EXP(ETA(3))
S1=VD
Rc1=RMAX/(TMAX-TMIN-24)*TIME-RMAX*TMIN/(TMAX-TMIN-24)
Rc2=RMAX/(TMAX-TMIN)*TIME-RMAX*TMIN/(TMAX-TMIN)
Rc3=RMAX/(TMAX-TMIN-24)*(TIME-24)-RMAX*TMIN/(TMAX-TMIN-24)
Rc4=RMAX/(TMAX-TMIN)*(TIME-24)-RMAX*TMIN/(TMAX-TMIN)
Rc5=RMAX/(TMAX-TMIN-24)*(TIME-48)-RMAX*TMIN/(TMAX-TMIN-24)
Rc6=RMAX/(TMAX-TMIN)*(TIME-48)-RMAX*TMIN/(TMAX-TMIN)
Rc7=RMAX/(TMAX-TMIN-24)*(TIME-72)-RMAX*TMIN/(TMAX-TMIN-24)
Rc8=RMAX/(TMAX-TMIN)*(TIME-72)-RMAX*TMIN/(TMAX-TMIN)
Rc9=RMAX/(TMAX-TMIN-24)*(TIME-96)-RMAX*TMIN/(TMAX-TMIN-24)
Rc10=RMAX/(TMAX-TMIN)*(TIME-96)-RMAX*TMIN/(TMAX-TMIN)
Rc11=RMAX/(TMAX-TMIN-24)*(TIME-120)-RMAX*TMIN/(TMAX-TMIN-24)
Rc12=RMAX/(TMAX-TMIN)*(TIME-120)-RMAX*TMIN/(TMAX-TMIN)
Rc13=RMAX/(TMAX-TMIN-24)*(TIME-144)-RMAX*TMIN/(TMAX-TMIN-24)
Rc14=RMAX/(TMAX-TMIN)*(TIME-144)-RMAX*TMIN/(TMAX-TMIN)
M1=0
IF (TMIN.GT.TIME) M1=1
M2=0
IF (TIME.GT.TMAX) M2=1
M3=0
IF (TMIN+24.GT.TIME) M3=1
M4=0
IF (TIME.GT.TMAX+24) M4=1
M5=0
IF (TMIN+48.GT.TIME) M5=1
M6=0
IF (TIME.GT.TMAX+48) M6=1
M7=0
IF (TMIN+72.GT.TIME) M7=1
M8=0
IF (TIME.GT.TMAX+72) M8=1
M9=0
IF (TMIN+96.GT.TIME) M9=1
M10=0
IF (TIME.GT.TMAX+96) M10=1
M11=0
IF (TMIN+120.GT.TIME) M11=1
M12=0
IF (TIME.GT.TMAX+120) M12=1
M13=0
IF (TMIN+144.GT.TIME) M13=1
M14=0
IF (TIME.GT.TMAX+144) M14=1
Rcort1=Rc1*M1+Rc2*(1-M1)*(1-M2)+Rc3*M2*M3+Rc4*(1-M3)*(1-M4)
Rcort2=Rc5*M4*M5+Rc6*(1-M5)*(1-M6)+Rc7*M6*M7+Rc8*(1-M7)*(1-M8)
Rcort4=Rc13*M12*M13+Rc14*(1-M13)*(1-M14)
Rcort=Rcort1+Rcort2+Rcort3+Rcort4
$DES
DADT(1)=Rcort-KE*A(1)
$ERROR
IPRED=0
IF(F.GT.0) IPRED=LOG(F)
Y=IPRED+ERR(1)
$THETA
(0 22 24)
(0 18 24)
(0 5000 20000)
$OMEGA
(0.2)
(0.2)
(0.2)
$SIGMA
(0.1)
$ESTIMATION METHOD=0 NOABORT MAXEVAL=9999 PRINT=5 POSTHOC
$COV PRINT=E
$TABLE ID TIME DV IPRED TMAX TMIN RMAX ETA(1) ETA(2)
ETA(3) NOPRINT ONEHEADER FILE=CORTBASE.FIT
$PROBLEM BUD CORTISOL PK/PD MODEL
$INPUT id time amt dv evid cmt mdv occ ika ikv itma itmi irma
$DATA Lncort.CSV IGNORE=#
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(DEPOT, DEFDOS) ;1
COMP=(CENTRAL) ;2
COMP=(CORTISOL) ;3
$ABBREVIATED DERIV2=NO
$PK
KA=IKA
K=IK
VC=IVC
S2=VC
TMAX=ITMA; TIME OF MAX RELEASE
TMN=ITMI; TIME OF MINIMUM RELEASE
BOV1=ETA(2)
IF (OCC. EQ. 2) THEN
BOV1=ETA(3)
ENDIF
BSV1=ETA(1)
BOV2=ETA(5)
IF (OCC. EQ. 2) THEN
BOV2=ETA(6)
ENDIF
BSV2=ETA(4)
RMAX=THETA(1)*EXP(BSV1+BOV1); MAXIMUM RELEASE RATE
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
S3=VD
EC50=THETA(2)*EXP(BSV2+BOV2)
Rc1=RMAX/(TMAX-TMIN-24)*TIME-RMAX*TMIN/(TMAX-TMIN-24)
Rc2=RMAX/(TMAX-TMIN)*TIME-RMAX*TMIN/(TMAX-TMIN)
Rc3=RMAX/(TMAX-TMIN-24)*(TIME-24)-RMAX*TMIN/(TMAX-TMIN-24)
Rc4=RMAX/(TMAX-TMIN)*(TIME-24)-RMAX*TMIN/(TMAX-TMIN)
Rc5=RMAX/(TMAX-TMIN-24)*(TIME-48)-RMAX*TMIN/(TMAX-TMIN-24)
Rc6=RMAX/(TMAX-TMIN)*(TIME-48)-RMAX*TMIN/(TMAX-TMIN)
Rc7=RMAX/(TMAX-TMIN-24)*(TIME-72)-RMAX*TMIN/(TMAX-TMIN-24)
Rc8=RMAX/(TMAX-TMIN)*(TIME-72)-RMAX*TMIN/(TMAX-TMIN)
Rc9=RMAX/(TMAX-TMIN-24)*(TIME-96)-RMAX*TMIN/(TMAX-TMIN-24)
Rc10=RMAX/(TMAX-TMIN)*(TIME-96)-RMAX*TMIN/(TMAX-TMIN)
Rc11=RMAX/(TMAX-TMIN-24)*(TIME-120)-RMAX*TMIN/(TMAX-TMIN-24)
Rc12=RMAX/(TMAX-TMIN)*(TIME-120)-RMAX*TMIN/(TMAX-TMIN)
Rc13=RMAX/(TMAX-TMIN-24)*(TIME-144)-RMAX*TMIN/(TMAX-TMIN-24)
Rc14=RMAX/(TMAX-TMIN)*(TIME-144)-RMAX*TMIN/(TMAX-TMIN)
M1=0
IF (TMIN, GT, TIME) M1=1
M2=0
IF (TIME, GT, TMAX) M2=1
M3=0
IF (TMIN+24, GT, TIME) M3=1
M4=0
IF (TIME, GT, TMAX+24) M4=1
M5=0
IF (TMIN+48, GT, TIME) M5=1
M6=0
IF (TIME, GT, TMAX+48) M6=1
M7=0
IF (TMIN+72, GT, TIME) M7=1
M8=0
IF (TIME, GT, TMAX+72) M8=1
M9=0
IF (TMIN+96, GT, TIME) M9=1
M10=0
IF (TIME, GT, TMAX+96) M10=1
M11=0
IF (TMIN+120, GT, TIME) M11=1
M12=0
IF (TIME, GT, TMAX+120) M12=1
M13=0
IF (TMIN+144, GT, TIME) M13=1
M14=0
IF (TIME, GT, TMAX+144) M14=1
Rcort1=Rc1*M1+Rc2*(1-M1)*(1-M2)+Rc3*M2*M3+Rc4*(1-M3)*(1-M4)
Rcort2=Rc5*M4*M5+Rc6*(1-M5)*(1-M6)+Rc7*M6*M7+Rc8*(1-M7)*(1-M8)
Rcort4=Rc13*M12*M13+Rc14*(1-M12)*(1-M14)
Rcort=Rcort1+Rcort2+Rcort3+Rcort4
$DES
DADT(1)=-KA*A(1)
DADT(2)=KA*A(1)-K*A(2)
CP=A(2)/S2
EFF=1-CP/(EC50+CP)
DADT(3)=Rcort*EFF-KE*A(3)
CORT=A(3)/S3
$ERROR
IPRED=0
IF (F, GT, 0) IPRED=LOG(F)
Y=IPRED+ERR(1)
$THETA
(0 3100 20000)
(0 0.2)
$OMEGA 0.1
$OMEGA BLOCK(1) 0.1
$OMEGA BLOCK(1) SAME
$OMEGA 0.2
$OMEGA BLOCK(1) 0.1
$OMEGA BLOCK(1) SAME
$SIGMA
(0, 2)
$ESTIMATION METHOD=0 NOABORT MAXEVAL=9999 PRINT=5 POSTHOC
$COV PRINT=E
$TABLE ID TIME DV IPRED TMAX TMIN RMAX EC50 ETA(1) ETA(2) ETA(3) ETA(4)
ETA(5) ETA(6) NOPRINT ONEHEADER FILE=BUDCORT.FIT

$PROBLEM BUD LYMPHOCYTES PK/PD MODEL
$INPUT id time amt dv evid cmv mdv occ ika ik ivc itma itmi irma iecc
$DATA Lnlum2.CSV IGNORE=#
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(DEPOT, DEFOSE) ;1
COMP=(CENTRAL) ;2
COMP=(CORTISOL) ;3
COM=(LYM) ;4
$ABBREVIATED DERIV2=NO
$PK
KA=IKA
K=IK
VC=IVC
KIN=THETA(1) * EXP(ETA(1));
KOUT=THETA(2) * EXP(ETA(2))
EC50BL=THETA(3) * EXP(ETA(3))
EC50CL=THETA(4) * EXP(ETA(4))
TMAX=ITMA
TMIN=ITMI
RMAX=IRMA
EC50=IECC
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
S2=VC
S3=VD
Rc1=RMAX/(TMAX–TMIN–24)*TIME–RMAX*TMIN/(TMAX–TMIN–24)
Rc2=RMAX/(TMAX–TMIN)*TIME–RMAX*TMIN/(TMAX–TMIN)
Rc3=RMAX/(TMAX–TMIN–24)*(TIME–24)–RMAX*TMIN/(TMAX–TMIN–24)
Rc4=RMAX/(TMAX–TMIN)*(TIME–24)–RMAX*TMIN/(TMAX–TMIN)
Rc5=RMAX/(TMAX–TMIN–24)*(TIME–48)–RMAX*TMIN/(TMAX–TMIN–24)
Rc6=RMAX/(TMAX–TMIN)*(TIME–48)–RMAX*TMIN/(TMAX–TMIN)
Rc7=RMAX/(TMAX–TMIN–24)*(TIME–72)–RMAX*TMIN/(TMAX–TMIN–24)
Rc8=RMAX/(TMAX–TMIN)*(TIME–72)–RMAX*TMIN/(TMAX–TMIN)
\[ Rc_9 = \frac{R_{\text{MAX}}}{(T_{\text{MAX}} - T_{\text{MIN}} - 24)} \times (T_{\text{TIME}} - 96) - R_{\text{MAX}} \times T_{\text{MIN}} \] \[ Rc_{10} = \frac{R_{\text{MAX}}}{(T_{\text{MAX}} - T_{\text{MIN}})} \times (T_{\text{TIME}} - 96) - R_{\text{MAX}} \times T_{\text{MIN}} \] \[ Rc_{11} = \frac{R_{\text{MAX}}}{(T_{\text{MAX}} - T_{\text{MIN}} - 24)} \times (T_{\text{TIME}} - 120) - R_{\text{MAX}} \times T_{\text{MIN}} \] \[ Rc_{12} = \frac{R_{\text{MAX}}}{(T_{\text{MAX}} - T_{\text{MIN}})} \times (T_{\text{TIME}} - 120) - R_{\text{MAX}} \times T_{\text{MIN}} \] \[ Rc_{13} = \frac{R_{\text{MAX}}}{(T_{\text{MAX}} - T_{\text{MIN}} - 24)} \times (T_{\text{TIME}} - 144) - R_{\text{MAX}} \times T_{\text{MIN}} \] \[ Rc_{14} = \frac{R_{\text{MAX}}}{(T_{\text{MAX}} - T_{\text{MIN}})} \times (T_{\text{TIME}} - 144) - R_{\text{MAX}} \times T_{\text{MIN}} \]

\[
M_1 = 0 \\
\text{IF} \ (T_{\text{MIN}} > T_{\text{TIME}}) \ M_1 = 1 \\
M_2 = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}}) \ M_2 = 1 \\
M_3 = 0 \\
\text{IF} \ (T_{\text{MIN}} + 24 > T_{\text{TIME}}) \ M_3 = 1 \\
M_4 = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}} + 24) \ M_4 = 1 \\
M_5 = 0 \\
\text{IF} \ (T_{\text{MIN}} + 48 > T_{\text{TIME}}) \ M_5 = 1 \\
M_6 = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}} + 48) \ M_6 = 1 \\
M_7 = 0 \\
\text{IF} \ (T_{\text{MIN}} + 72 > T_{\text{TIME}}) \ M_7 = 1 \\
M_8 = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}} + 72) \ M_8 = 1 \\
M_9 = 0 \\
\text{IF} \ (T_{\text{MIN}} + 96 > T_{\text{TIME}}) \ M_9 = 1 \\
M_{10} = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}} + 96) \ M_{10} = 1 \\
M_{11} = 0 \\
\text{IF} \ (T_{\text{MIN}} + 120 > T_{\text{TIME}}) \ M_{11} = 1 \\
M_{12} = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}} + 120) \ M_{12} = 1 \\
M_{13} = 0 \\
\text{IF} \ (T_{\text{MIN}} + 144 > T_{\text{TIME}}) \ M_{13} = 1 \\
M_{14} = 0 \\
\text{IF} \ (T_{\text{TIME}} > T_{\text{MAX}} + 144) \ M_{14} = 1 \\
R_{\text{cort1}} = Rc_1 \times M_1 + Rc_2 \times (1 - M_1) \times (1 - M_2) + Rc_3 \times M_2 + Rc_4 \times (1 - M_3) \times (1 - M_4) \\
R_{\text{cort2}} = Rc_5 \times M_4 \times M_5 + Rc_6 \times (1 - M_5) \times (1 - M_6) + Rc_7 \times M_6 \times M_7 + Rc_8 \times (1 - M_7) \times (1 - M_8) \\
R_{\text{cort3}} = Rc_9 \times M_8 \times M_9 + Rc_{10} \times (1 - M_9) \times (1 - M_{10}) + Rc_{11} \times M_{10} \times M_{11} + Rc_{12} \times (1 - M_{11}) \times (1 - M_{12}) \\
R_{\text{cort4}} = Rc_{13} \times M_{12} \times M_{13} + Rc_{14} \times (1 - M_{13}) \times (1 - M_{14}) \\
R_{\text{cort}} = R_{\text{cort1}} + R_{\text{cort2}} + R_{\text{cort3}} + R_{\text{cort4}} \\
\text{IF} \ (\text{NEWIND} \leq 1) \ \text{THEN} \ ; \ this \ is \ only \ executed \ once \ per \ subject \\
KTC = 300000000 \\
KALB = 5000 \\
QTC = 0.0000007 \\
QALB = 0.00055 \\
MW = 362.47 \times (10^{**6}) \\
ENDIF \\
$DES
DADT(1) = -KA*A(1)  
DADT(2) = KA*A(1) - K*A(2)  
CP = A(2)/S2  
CPF = CP*0.12  
EFFC = 1 - CP/(EC50 + CP)  
DADT(3) = RCORT*EFFC - KE*A(3)  
Cort = A(3)/S3  
CortM = Cort/MW  
B1 = KTC*QTC + KALB*QALB + 1 - CortM*KTC  
A1 = KALB*QALB*KTC + KTC  
CORTFM = (-B1 + SQRT(B1**2 + 4*A1*CortM))/(2*A1)  
Cortf = CORTFM*MW  
UPP = CPF + Cortf*EC50BL/EC50CL  
DOW = EC50BL + CPF + Cortf*EC50BL/EC50CL  
EFFL = 1 - UPP/DOW  
DADT(4) = KIN*EFFL - KOUT*A(4)  
$ERROR  
IPRED = 0  
IF (F.GT.0) IPRED = LOG(F)  
Y = IPRED + ERR(1)  
$THETA  
(0 22)  
(0 0.2)  
(0 0.2)  
(0 20)  
$OMEGA  
(0.01)  
(0.01)  
(0.1)  
(0.1)  
$SIGMA  
(0.2)  
$ESTIMATION METHOD = 0 NOABORT MAXEVAL = 9999 PRINT = 5 POSTHOC  
$COV PRINT = E  
$TABLE ID TIME DV IPRED KIN KOUT EC50BL EC50CL ETA(1) ETA(2) ETA(3) NOPRINT ONEHEADER FILE = BUDLYM.FIT
APPENDIX D
NONMEM CODE FOR PK/PD MODEL OF FP

$PROBLEM FP CORTISOL PK/PD MODEL
$INPUT id time amt dv evid cmt mdv occ ika ik23 ik32 ivc itma itmi irma
$DATA Lnfpcort1.CSV IGNORE=_
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(DEPOT, DEFDOSCE) ;1
COMP=(CENTRAL) ;2
COMP=(PERIH);3
COMP=(CORTISOL);4
$ABBREVIATED DERIV2=NO
$PK
KA=IKA
K=IK
K23=IK23
K32=IK32
VC=IVC
S2=VC
TMAX=ITMA; TIME OF MAX RELEASE
TMIN=ITMI; TIME OF MINIMUM RELEASE
BOV1=ETA(1)
IF (OCC.EQ.2) BOV1=ETA(2)
BSV1=ETA(3)
RMAX=THETA(1)*EXP(BOV1+BSV1); MAXIMUM RELEASE RATE
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
S4=VD
BOV2=ETA(4)
IF (OCC.EQ.2) BOV2=ETA(5)
BSV2=ETA(6)
EC50=THETA(2)*EXP(BOV2+BSV2)
Rc1=RMAX/(TMAX-TMIN-24)*TIME-RMAX*TMIN/(TMAX-TMIN-24)
Rc2=RMAX/(TMAX-TMIN)*TIME-RMAX*TMIN/(TMAX-TMIN)
Rc3=RMAX/(TMAX-TMIN-24)*(TIME-24)-RMAX*TMIN/(TMAX-TMIN-24)
Rc4=RMAX/(TMAX-TMIN)*(TIME-24)-RMAX*TMIN/(TMAX-TMIN)
Rc5=RMAX/(TMAX-TMIN-24)*(TIME-48)-RMAX*TMIN/(TMAX-TMIN-24)
Rc6=RMAX/(TMAX-TMIN)*(TIME-48)-RMAX*TMIN/(TMAX-TMIN)
Rc7=RMAX/(TMAX-TMIN-24)*(TIME-72)-RMAX*TMIN/(TMAX-TMIN-24)
Rc8=RMAX/(TMAX-TMIN)*(TIME-72)-RMAX*TMIN/(TMAX-TMIN)
Rc9=RMAX/(TMAX-TMIN-24)*(TIME-96)-RMAX*TMIN/(TMAX-TMIN-24)
Rc10=RMAX/(TMAX-TMIN)*(TIME-96)-RMAX*TMIN/(TMAX-TMIN)
Rc11=RMAX/(TMAX-TMIN-24)*(TIME-120)-RMAX*TMIN/(TMAX-TMIN-24)
Rc12=RMAX/(TMAX-TMIN)*(TIME-120)-RMAX*TMIN/(TMAX-TMIN)
Rc13=RMAX/(TMAX-TMIN-24)*(TIME-144)-RMAX*TMIN/(TMAX-TMIN-24)
Rc14=RMAX/(TMAX-TMIN)*(TIME-144)-RMAX*TMIN/(TMAX-TMIN)

M1=0
IF (TMIN.GT.TIME) M1=1
M2=0
IF (TIME.GT.TMAX) M2=1
M3=0
IF (TMIN+24.GT.TIME) M3=1
M4=0
IF (TIME.GT.TMAX+24) M4=1
M5=0
IF (TMIN+48.GT.TIME) M5=1
M6=0
IF (TIME.GT.TMAX+48) M6=1
M7=0
IF (TMIN+72.GT.TIME) M7=1
M8=0
IF (TIME.GT.TMAX+72) M8=1
M9=0
IF (TMIN+96.GT.TIME) M9=1
M10=0
IF (TIME.GT.TMAX+96) M10=1
M11=0
IF (TMIN+120.GT.TIME) M11=1
M12=0
IF (TIME.GT.TMAX+120) M12=1
M13=0
IF (TMIN+144.GT.TIME) M13=1
M14=0
IF (TIME.GT.TMAX+144) M14=1
Rcort1=Rc1*M1+Rc2*(1-M1)*(1-M2)+Rc3*M2*M3+Rc4*(1-M3)*(1-M4)
Rcort2=Rc5*M4*M5+Rc6*(1-M5)*(1-M6)+Rc7*M6*M7+Rc8*(1-M7)*(1-M8)
Rcort4=Rc13*M12*M13+Rc14*(1-M13)*(1-M14)
Rcort=Rcort1+Rcort2+Rcort3+Rcort4

$DES
DADT(1)=-KA*A(1)
DADT(2)=KA*A(1)-K*A(2)-K23*A(2)+K32*(3)
CP=A(2)/S2*0.1
DADT(3)=K23*A(2)-K32*A(3)
EFF=1-CP/(EC50+CP)
DADT(4)=Rcort*EFF-KE*A(4)
CORT=A(4)/S4
$ERROR
IPRED=0
IF (F. GT. 0) IPRED=LOG(F)
Y=IPRED+ERR(1)
$THETA
(0 3000 20000)
(0 0.01)
$OMEGA BLOCK(1) 0.02
$OMEGA BLOCK(1) SAME
$OMEGA
(0.1)
$OMEGA BLOCK(1) 0.02
$OMEGA BLOCK(1) SAME
$OMEGA
(0.1)
$SIGMA
(0.02)
$ESTIMATION METHOD=0 NOABORT MAXEVAL=9999 PRINT=5 POSTHOC
$COV PRINT=E
$TABLE ID TIME DV IPRED RMAX EC50 IKA IK23 IK32 IVC
itma itmi OCC ETA(1) ETA(2) NOPRINT ONEHEADER FILE=FPCORT.FIT

$PROBLEM FP LYMPHOCYTES PK/PD MODEL
$INPUT id time amt dv evid mdv cmt occ IKA IK IK23 IK32 ITMA ITMI IVC IRMA IEC
$DATA LNlym1.CSV IGNORE=_
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(DEPOT, DEFDOSER) ;1
COMP=(CENTRAL) ;2
COMP=(PERIH) ;3
COMP=(CORTISOL) ;4
COM=(LYM) ;5
$ABBREVIATED DERIV2=NO
$PK
KA=IKA
K=IK
K23=IK23
K32=IK32
VC=IVC
S2=VC
TMAX=ITMA; TIME OF MAX RELEASE
TMIN=ITMI; TIME OF MINIMUM RELEASE
RMAX=IRMA
EC50=IEC
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
BOV1 = ETA(3)
IF (OCC. EQ. 2) BOV1 = ETA(4)
IF (OCC. EQ. 3) BOV1 = ETA(5)

BSV1 = ETA(1)
KIN = THETA(1) * EXP (BOV1 + BSV1);

BOV2 = ETA(6)
IF (OCC. EQ. 2) BOV2 = ETA(7)
IF (OCC. EQ. 3) BOV2 = ETA(8)

BSV2 = ETA(2)
KOUT = THETA(2) * EXP (BOV2 + BSV2)

EC50BL = THETA(3) * EXP (ETA(9))
EC50CL = THETA(4) * EXP (ETA(10))

S2 = VC
S4 = VD

Rc1 = RMAX / (TMAX - TMIN - 24) * TIME - RMAX * TMIN / (TMAX - TMIN - 24)
Rc2 = RMAX / (TMAX - TMIN) * TIME - RMAX * TMIN / (TMAX - TMIN)
Rc3 = RMAX / (TMAX - TMIN - 24) * (TIME - 24) - RMAX * TMIN / (TMAX - TMIN - 24)
Rc4 = RMAX / (TMAX - TMIN) * (TIME - 24) - RMAX * TMIN / (TMAX - TMIN)
Rc5 = RMAX / (TMAX - TMIN - 24) * (TIME - 48) - RMAX * TMIN / (TMAX - TMIN - 24)
Rc6 = RMAX / (TMAX - TMIN) * (TIME - 48) - RMAX * TMIN / (TMAX - TMIN)
Rc7 = RMAX / (TMAX - TMIN - 24) * (TIME - 72) - RMAX * TMIN / (TMAX - TMIN - 24)
Rc8 = RMAX / (TMAX - TMIN) * (TIME - 72) - RMAX * TMIN / (TMAX - TMIN)
Rc9 = RMAX / (TMAX - TMIN - 24) * (TIME - 96) - RMAX * TMIN / (TMAX - TMIN - 24)
Rc10 = RMAX / (TMAX - TMIN) * (TIME - 96) - RMAX * TMIN / (TMAX - TMIN)
Rc11 = RMAX / (TMAX - TMIN - 24) * (TIME - 120) - RMAX * TMIN / (TMAX - TMIN - 24)
Rc12 = RMAX / (TMAX - TMIN) * (TIME - 120) - RMAX * TMIN / (TMAX - TMIN)
Rc13 = RMAX / (TMAX - TMIN - 24) * (TIME - 144) - RMAX * TMIN / (TMAX - TMIN - 24)
Rc14 = RMAX / (TMAX - TMIN) * (TIME - 144) - RMAX * TMIN / (TMAX - TMIN)

M1 = 0
IF (TMIN, GT, TIME) M1 = 1
M2 = 0
IF (TIME, GT, TMAX) M2 = 1
M3 = 0
IF (TMIN + 24, GT, TIME) M3 = 1
M4 = 0
IF (TIME, GT, TMAX + 24) M4 = 1
M5 = 0
IF (TMIN + 48, GT, TIME) M5 = 1
M6 = 0
IF (TIME, GT, TMAX + 48) M6 = 1
M7 = 0
IF (TMIN + 72, GT, TIME) M7 = 1
M8 = 0
IF (TIME, GT, TMAX + 72) M8 = 1
M9 = 0
IF (TMIN + 96, GT, TIME) M9 = 1
M10 = 0
IF (TIME.GT.TMAX+96) M10=1
M11=0
IF (TMIN+120.GT.TIME) M11=1
M12=0
IF (TIME.GT.TMAX+120) M12=1
M13=0
IF (TMIN+144.GT.TIME) M13=1
M14=0
IF (TIME.GT.TMAX+144) M14=1
Rcort1=Rc1*M1+Rc2*(1-M1)*(1-M2)+Rc3*M2*M3+Rc4*(1-M3)*(1-M4)
Rcort2=Rc5*M4*M5+Rc6*(1-M5)*(1-M6)+Rc7*M6*M7+Rc8*(1-M7)*(1-M8)
Rcort4=Rc13*M12*M13+Rc14*(1-M13)*(1-M14)
Rcort=Rcort1+Rcort2+Rcort3+Rcort4
IF (NEWIND.LE.1) THEN ; this is only executed once per subject
KTC=30000000
KALB=5000
QTC=0.0000007
QALB=0.00055
MW=362.47*(10**6)
ENDIF
$DES
DADT(1)=-KA*A(1)
DADT(2)=KA*A(1)-K*A(2)-K23*A(2)+K32*(3)
CPF=A(2)/S2*0.1
DADT(3)=K23*A(2)-K32*A(3)
EFF=1-CPF/(EC50+CPF)
DADT(4)=Rcort*EFF-KE*A(4)
CORT=A(4)/S4
CortM=Cort/MW
B1=KTC*QTC+KALB*QALB+1-CortM*KTC
A1=KALB*QALB*KTC+KTC
CORTFM=(-B1+SQRT(B1**2+4*A1*CortM))/(2*A1)
Cortf=CORTFM*MW
UPP=CPF+Cortf*EC50BL/EC50CL
DOW=EC50BL+CPF+Cortf*EC50BL/EC50CL
EFFL=1-UPP/DOW
DADT(5)=KIN*EFFL-KOUT*A(5)
$ERROR
IPRED=0
IF (F.GT.0) IPRED=LOG(F)
Y=IPRED+ERR(1)
$THETA
(0 22)
(0 0.2)
(0 0.08)
(0 30)
$\Omega$ BLOCK(2) 0.02 0.018 0.02
$\Omega$ BLOCK(1) 0.04
$\Omega$ BLOCK(1) SAME
$\Omega$ BLOCK(1) SAME
$\Omega$ BLOCK(1) 0.04
$\Omega$ BLOCK(1) SAME
$\Omega$ BLOCK(1) SAME
$\Omega$ BLOCK(1) 0.04
$\Omega$ BLOCK(1) SAME
$\Omega$ BLOCK(1) SAME
$\Omega$ 0.1 0.1
$\Sigma$
(0.05)
$\$ESTIMATION METHOD=0 NOABORT MAXEVAL=9999 PRINT=5 POSTHOC
MSFO=run1.msf
$\$COV PRINT=E
$\$TABLE ID TIME DV IPRED KIN KOUT EC50BL EC50CL ETA(1) ETA(2)
ETA(3) NOPRINT ONEHEADER FILE=FPLYM.FIT
APPENDIX E
NONMEM CODE FOR BUD PK/PD SIMULATION MODEL

$PROBLEM BUD PK/PD SIMULATION MODEL
$INPUT ID TIME AMT DV EVID CMT OCC TM
$DATA combinela.CSV IGNORE=#
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(DEPOT, DEFDOS) ;1
COMP=(CENTRAL) ;2
COMP=(CORTISOL) ;3
COMP=(LYM) ;4
$ABBREVIATED DERIV2=NO
$PK
KA=THETA(1)
K=THETA(2)*EXP(ETA(1))
VC=THETA(3)*EXP(ETA(2))
CL=K*VC
S2=VC
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
TMAX=THETA(4)*EXP(ETA(3))
TMIN=THETA(5)*EXP(ETA(4))
KIN=THETA(6)*EXP(ETA(5));
KOUT=THETA(7)*EXP(ETA(6))
EC50CL=THETA(8)*EXP(ETA(7))
EC50BL=THETA(9)*EXP(ETA(8))
IF (OCC.EQ.3) EC50BL=EC50CL
BOV1=ETA(9)
BOV2=ETA(11)
IF (OCC.EQ.2) THEN
BOV1=ETA(10)
BOV2=ETA(12)
ENDIF
BSV1=ETA(13)
BSV2=ETA(14)
RMAX=THETA(10)*EXP(BSV1+BOV1)
IF (OCC. EQ. 3) RMAX=THETA(12)*EXP(ETA(15))
EC50=THETA(11)*EXP(BSV2+BOV2)
S3=VD
AD=0
IF (TM. EQ. 1) AD=1
IF (TM.EQ.2) AD=2
IF (TM.EQ.3) AD=3
IF (TM.EQ.4) AD=4
IF (TM.EQ.5) AD=5
IF (TM.EQ.6) AD=6
IF (TM.EQ.7) AD=7
IF (TM.EQ.8) AD=8
IF (TM.EQ.9) AD=9
IF (TM.EQ.10) AD=10
IF (TM.EQ.11) AD=11
IF (TM.EQ.12) AD=12
IF (TM.EQ.13) AD=13
IF (TM.EQ.14) AD=14
IF (TM.EQ.15) AD=15
IF (TM.EQ.16) AD=16
IF (TM.EQ.17) AD=17
IF (TM.EQ.18) AD=18
IF (TM.EQ.19) AD=19
IF (TM.EQ.20) AD=20
IF (TM.EQ.21) AD=21
IF (TM.EQ.22) AD=22
IF (TM.EQ.23) AD=23
RC1=RMAX/(TMAX−TMIN−24)∗(TIME+AD)−RMAX*TMIN/(TMAX−TMIN−24)
RC2=RMAX/(TMAX−TMIN)∗(TIME+AD)−RMAX*TMIN/(TMAX−TMIN)
RC3=RMAX/(TMAX−TMIN−24)∗(TIME+AD−24)−RMAX*TMIN/(TMAX−TMIN−24)
RC4=RMAX/(TMAX−TMIN)∗(TIME+AD−24)−RMAX*TMIN/(TMAX−TMIN)
RC5=RMAX/(TMAX−TMIN−24)∗(TIME+AD−48)−RMAX*TMIN/(TMAX−TMIN−24)
RC6=RMAX/(TMAX−TMIN)∗(TIME+AD−48)−RMAX*TMIN/(TMAX−TMIN)
RC7=RMAX/(TMAX−TMIN−24)∗(TIME+AD−72)−RMAX*TMIN/(TMAX−TMIN−24)
RC8=RMAX/(TMAX−TMIN)∗(TIME+AD−72)−RMAX*TMIN/(TMAX−TMIN)
RC9=RMAX/(TMAX−TMIN−24)∗(TIME+AD−96)−RMAX*TMIN/(TMAX−TMIN−24)
RC10=RMAX/(TMAX−TMIN)∗(TIME+AD−96)−RMAX*TMIN/(TMAX−TMIN)
RC11=RMAX/(TMAX−TMIN−24)∗(TIME+AD−120)−RMAX*TMIN/(TMAX−TMIN−24)
RC12=RMAX/(TMAX−TMIN)∗(TIME+AD−120)−RMAX*TMIN/(TMAX−TMIN)
RC13=RMAX/(TMAX−TMIN−24)∗(TIME+AD−144)−RMAX*TMIN/(TMAX−TMIN−24)
RC14=RMAX/(TMAX−TMIN)∗(TIME+AD−144)−RMAX*TMIN/(TMAX−TMIN)
RC15=RMAX/(TMAX−TMIN−24)∗(TIME+AD−168)−RMAX*TMIN/(TMAX−TMIN−24)
RC16=RMAX/(TMAX−TMIN)∗(TIME+AD−168)−RMAX*TMIN/(TMAX−TMIN)
M1=0
IF (TMIN.GT.TIME+AD) M1=1
M2=0
IF (TIME+AD.GT.TMAX) M2=1
M3=0
IF (TMIN+24.GT.TIME+AD) M3=1
M4=0
IF (TIME+AD.GT.TMAX+24) M4=1
M5=0
IF (TMIN+48 .GT. TIME+AD) M5=1
M6=0
IF (TIME+AD .GT. TMAX+48) M6=1
M7=0
IF (TMIN+72 .GT. TIME+AD) M7=1
M8=0
IF (TIME+AD .GT. TMAX+72) M8=1
M9=0
IF (TMIN+96 .GT. TIME+AD) M9=1
M10=0
IF (TIME+AD .GT. TMAX+96) M10=1
M11=0
IF (TMIN+120 .GT. TIME+AD) M11=1
M12=0
IF (TIME+AD .GT. TMAX+120) M12=1
M13=0
IF (TMIN+144 .GT. TIME+AD) M13=1
M14=0
IF (TIME+AD .GT. TMAX+144) M14=1
M15=0
IF (TMIN+168 .GT. TIME+AD) M15=1
M16=0
IF (TIME+AD .GT. TMAX+168) M16=1
RCORT1=RC1*M1+RC2*(1-M1)*(1-M2)+RC3*M2*M3+RC4*(1-M3)*(1-M4)
RCORT2=RC5*M4*M5+RC6*(1-M5)*(1-M6)+RC7*M6*M7+RC8*(1-M7)*(1-M8)
RCORT=RCORT1+RCORT2+RCORT3+RCORT4
IF (NEWIND.LE.1) THEN ; this is only executed once per subject
KTC=30000000
KALB=5000
QTC=0.0000007
QALB=0.00055
MW=362.47*(10**6)
ENDIF
$DES
DADT(1)=-KA*A(1)
DADT(2)=KA*A(1)-K*A(2)
CP=A(2)/S2
CPF=CP*0.12
EFFC=1-CPF/(EC50+CPF)
DADT(3)=RCORT*EFFC-KE*A(3)
CORT=A(3)/S3
CORTM=CORT/MW
B1=KTC*QTC+KALB*QALB+1-CORTM*KTC
A1=KALB*QALB*KTC
Cортфм=(-B1+SQRT(B1**2+4*A1*Cортм))/2*A1
CORTF = CORTFM * MW
UPP = CPF + CORTF * EC50BL / EC50CL
DOW = EC50BL + CPF + CORTF * EC50BL / EC50CL
EFFL = 1 - UPP / DOW
DADT(4) = KIN * EFFL - KOUT * A(4)

$ERROR
IPRED = 0
IF (F, GT. 0) IPRED = LOG(F)
O1 = 0
IF (CMT, EQ. 3) O1 = 1
O2 = 0
IF (CMT, EQ. 4) O2 = 1
Y1 = IPRED + ERR(1)
Y2 = IPRED + ERR(2)
Y = O1 * Y1 + O2 * Y2
YT = EXP(Y)

$THETA
(58.1 FIXED)
(0.203 FIXED)
(1350 FIXED)
(22.7 FIXED)
(17.5 FIXED)
(21.7 FIXED)
(0.193 FIXED)
(30.8 FIXED)
(0.161 FIXED)
(3280 FIXED)
(0.019 FIXED)
(2510 FIXED)

$OMEGA
(0.025 FIXED)
(0.1 FIXED)
(0.0049 FIXED)
(0.0016 FIXED)
(0.0135 FIXED)
(0.00624 FIXED)
(0.13 FIXED)
(0.377 FIXED)

$OMEGA BLOCK(1)
(0.018 FIXED)

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1)
(0.157 FIXED)

$OMEGA BLOCK(1) SAME

$OMEGA
(0.0088 FIXED)
(0.213 FIXED)
$SIGMA
(0.064 FIXED)

(0.082 FIXED)

(0.0346 FIXED)

$SIMULATION (95846) SUBPROBLEMS=1 ONLYSIMULATION
$TABLE ID TIME YT IPRED OCC TM CMT KA K VC CL RMAX EC50 KIN KOUT
EC50CL EC50BL NOPRINT ONEHEADER FILE=combine1a.fit
APPENDIX F
NONMEM CODE FOR FP PK/PD SIMULATION MODEL

$PROBLEM FP PK/PD SIMULATION MODEL
$INPUT ID TIME AMT DV EVID CMT OCC TM
$DATA combine8a.CSV IGNORE=_
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
COMP=(DEPOT, DEPDOSE):1
COMP=(CENTRAL):2
COMP=(PERIH):3
COMP=(CORTISOL):4
COM=(LYM):5
$ABBREVIATED DERIV2=NO
$PK
KA=THETA(1)*EXP(ETA(1))
K=THETA(2)*EXP(ETA(2))
K23=THETA(3)*EXP(ETA(3))
K32=THETA(4)*EXP(ETA(4))
VC=THETA(5)*EXP(ETA(5))
TMAX=THETA(6)*EXP(ETA(6)); TIME OF MAX RELEASE
TMIN=THETA(7)*EXP(ETA(7)); TIME OF MINIMUM RELEASE
KIN=THETA(8)*EXP(ETA(8)));
KOUT=THETA(9)*EXP(ETA(9))
EC50BL=THETA(10)*EXP(ETA(10))
EC50CL=THETA(11)*EXP(ETA(11))
VD=33.7; DIST VOLUME OF CORTISOL
KE=0.56; ELIMINATION RATE OF CORTISOL
S2=VC
S4=VD
BOV1=ETA(12)
BOV2=ETA(14)
IF (OCC.EQ.2) THEN
BOV1=ETA(13)
BOV2=ETA(15)
ENDIF
BSV1=ETA(16)
BSV2=ETA(17)
RMAX=THETA(12)*EXP(BSV1+BOV1)
IF (OCC.EQ.3) RMAX=THETA(13)*EXP(ETA(18))
EC50=THETA(14)*EXP(BSV2+BOV2)
AD=0
IF (TM.EQ.1) AD=1
IF (TM.EQ.2) AD=2
IF (TM.EQ.3) AD=3
IF (TM.EQ.4) AD=4
IF (TM.EQ.5) AD=5
IF (TM.EQ.6) AD=6
IF (TM.EQ.7) AD=7
IF (TM.EQ.8) AD=8
IF (TM.EQ.9) AD=9
IF (TM.EQ.10) AD=10
IF (TM.EQ.11) AD=11
IF (TM.EQ.12) AD=12
IF (TM.EQ.13) AD=13
IF (TM.EQ.14) AD=14
IF (TM.EQ.15) AD=15
IF (TM.EQ.16) AD=16
IF (TM.EQ.17) AD=17
IF (TM.EQ.18) AD=18
IF (TM.EQ.19) AD=19
IF (TM.EQ.20) AD=20
IF (TM.EQ.21) AD=21
IF (TM.EQ.22) AD=22
IF (TM.EQ.23) AD=23
RC1=RMAX/(TMAX−TMIN−24)*(TIME+AD)−RMAX*TMIN/(TMAX−TMIN−24)
RC2=RMAX/(TMAX−TMIN)*(TIME+AD)−RMAX*TMIN/(TMAX−TMIN)
RC3=RMAX/(TMAX−TMIN−24)*(TIME+AD−24)−RMAX*TMIN/(TMAX−TMIN−24)
RC4=RMAX/(TMAX−TMIN)*(TIME+AD−24)−RMAX*TMIN/(TMAX−TMIN)
RC5=RMAX/(TMAX−TMIN−24)*(TIME+AD−48)−RMAX*TMIN/(TMAX−TMIN−24)
RC6=RMAX/(TMAX−TMIN)*(TIME+AD−48)−RMAX*TMIN/(TMAX−TMIN)
RC7=RMAX/(TMAX−TMIN−24)*(TIME+AD−72)−RMAX*TMIN/(TMAX−TMIN−24)
RC8=RMAX/(TMAX−TMIN)*(TIME+AD−72)−RMAX*TMIN/(TMAX−TMIN)
RC9=RMAX/(TMAX−TMIN−24)*(TIME+AD−96)−RMAX*TMIN/(TMAX−TMIN−24)
RC10=RMAX/(TMAX−TMIN)*(TIME+AD−96)−RMAX*TMIN/(TMAX−TMIN)
RC11=RMAX/(TMAX−TMIN−24)*(TIME+AD−120)−RMAX*TMIN/(TMAX−TMIN−24)
RC12=RMAX/(TMAX−TMIN)*(TIME+AD−120)−RMAX*TMIN/(TMAX−TMIN)
RC13=RMAX/(TMAX−TMIN−24)*(TIME+AD−144)−RMAX*TMIN/(TMAX−TMIN−24)
RC14=RMAX/(TMAX−TMIN)*(TIME+AD−144)−RMAX*TMIN/(TMAX−TMIN)
RC15=RMAX/(TMAX−TMIN−24)*(TIME+AD−168)−RMAX*TMIN/(TMAX−TMIN−24)
RC16=RMAX/(TMAX−TMIN)*(TIME+AD−168)−RMAX*TMIN/(TMAX−TMIN)
M1=0
IF (TM1.GT.TIME+AD) M1=1
M2=0
IF (TIME+AD.GT.TMAX) M2=1
M3=0
IF (TM1+24.GT.TIME+AD) M3=1
M4=0
IF (TIME+AD.GT.TMAX+24) M4=1
M5=0
IF (TMIN+48.GT.TIME+AD) M5=1
M6=0
IF (TIME+AD.GT.TMAX+48) M6=1
M7=0
IF (TMIN+72.GT.TIME+AD) M7=1
M8=0
IF (TIME+AD.GT.TMAX+72) M8=1
M9=0
IF (TMIN+96.GT.TIME+AD) M9=1
M10=0
IF (TIME+AD.GT.TMAX+96) M10=1
M11=0
IF (TMIN+120.GT.TIME+AD) M11=1
M12=0
IF (TIME+AD.GT.TMAX+120) M12=1
M13=0
IF (TMIN+144.GT.TIME+AD) M13=1
M14=0
IF (TIME+AD.GT.TMAX+144) M14=1
M15=0
IF (TMIN+168.GT.TIME+AD) M15=1
M16=0
IF (TIME+AD.GT.TMAX+168) M16=1
RCORT1=RC1*M1+RC2*(1-M1)*(1-M2)+RC3*M2*M3+RC4*(1-M3)*(1-M4)
RCORT2=RC5*M4*M5+RC6*(1-M5)*(1-M6)+RC7*M6*M7+RC8*(1-M7)*(1-M8)
RCORT=RCORT1+RCORT2+RCORT3+RCORT4
IF (NEWIND.LE.1) THEN ; this is only executed once per subject
KTC=30000000
KALB=5000
QTC=0.0000007
QALB=0.00055
MW=362.47*(10**6)
ENDIF
$DES
DADT(1)=-KA*A(1)
DADT(2)=KA*A(1)-K*A(2)-K23*A(2)+K32*(3)
CPF=A(2)/S2*0.1
DADT(3)=K23*A(2)-K32*A(3)
EFF=1-CPF/(EC50+CPF)
DADT(4)=RCORT*EFF-KE*A(4)
CORT=A(4)/S4
CORTM=CORT/MW
B1=KTC*QTC+KALB*QALB+1-CORTM*KTC
A1=KALB*QALB*KTC+KTC
CORTF = (-B1 + SQRT (B1**2 + 4 * A1 * CORTM)) / (2 * A1)
CORTF = CORTF * MW
UPP = CPF + CORTF * EC50BL / EC50CL
DOW = EC50BL + CPF + CORTF * EC50BL / EC50CL
EFFL = 1 - UPP / DOW
DADT(5) = KIN * EFFL - KOUT * A(5)
$ERROR
IPRED = 0
IF (F.GT.0) IPRED = LOG (F)
O1 = 0
IF (CMT.EQ.4) O1 = 1
O2 = 0
IF (CMT.EQ.5) O2 = 1
Y1 = IPRED + ERR(1)
Y2 = IPRED + ERR(2)
Y = O1 * Y1 + O2 * Y2
YT = EXP (Y)
$THETA
(2.77 FIXED)
(0.137 FIXED)
(0.215 FIXED)
(0.167 FIXED)
(4110 FIXED)
(22.7 FIXED)
(17.5 FIXED)
(14.4 FIXED)
(0.129 FIXED)
(0.0218 FIXED)
(30.2 FIXED)
(2500 FIXED)
(2510 FIXED)
(0.0082 FIXED)
$OMEGA
(0.032 FIXED)
(0.196 FIXED)
(0.015876 FIXED)
(0.074 FIXED)
(0.0139 FIXED)
(0.0049 FIXED)
(0.0016 FIXED)
(0.019321 FIXED)
(0.01062 FIXED)
(0.4998 FIXED)
(0.148 FIXED)
$OMEGA BLOCK(1)
(0.0222 FIXED)
$OMEGA BLOCK(1) SAME
$\Omega$ BLOCK(1)
(0.11 FIXED)
$\Omega$ BLOCK(1) SAME
$\Omega$
(0.0484 FIXED)
(0.154 FIXED)
(0.064 FIXED)
$\Sigma$
(0.0876 FIXED)
(0.031 FIXED)
$\Sigma$ IMULATION (1450) SUBPROBLEMS=1 ONLY SIMULATION
$\Sigma$ IMULATION ID TIME YT IPRED OCC TM CMT NOPRINT ONEHEADER FILE=combinela.fit
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Kai Wu was born on April 25, 1977, in Fujian, P. R. China. Kai got his bachelor’s degree in microbiology from Nankai University in July 1999. Kai entered the graduate school in Southern Illinois University in August 1999 and graduated with a master’s degree in molecular biology in December 2001. In August 2002, Kai entered the Ph.D. program at the Department of Pharmaceutics, College of Pharmacy, University of Florida, working under the supervision of Dr. Guenther Hochhaus. In August 2003, Kai entered the master’s program at the Department of Statistics, College of Liberal Arts and Sciences, working under the supervision of Dr. Rongling Wu. Kai received his Master of Statistics degree in December 2005 and Doctor of Philosophy degree in pharmaceutics in December 2006.