BIOEQUIVALENCE OF INHALED CORTICOSTEROIDS – A PHARMACOKINETIC APPROACH

By

NAVINKUMAR S. GOYAL

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

2009
To my mom
ACKNOWLEDGMENTS

I would like to acknowledge Dr. Guenther Hochhaus, my supervisor, for the opportunity to pursue my graduate studies in his lab. I am grateful for the help, guidance and support I have received from him all these years. He has been a great mentor.

I am thankful to Dr. Jeffrey Hughes, Dr. Hartmut Derendorf and Dr. Andre Mauderli for being a part of my graduate committee. I am also grateful to my labmates and other graduate students in the department for all their support. I would also like to thank Dr. Sreedharan Sabarinath, Dr. Varun Goel and Dr. Jian Xu for the great technical arguments and discussions we have had through my project work.

I cannot thank enough my friends Dr. Preeti Yadava, Vineet Miharia, Hemal Vyas and Naresh Pai who made have been a great company all these years during my graduate studies and provided a home away from home.

Finally, I would like to thank my family, especially my sister Dr. Meena Goyal for their love and encouragement without which I would have never ever been able to accomplish any of this. I am thankful to my grandfather Ramkumar for his everlasting support and blessings that have helped me through all the tough times.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>AIRWAYS AND ASTHMA</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Airways and Lung Function</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Mucociliary Clearance</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Asthma Pathophysiology</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>ASTHMA THERAPY</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Inhaled Corticosteroids</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Fate of Inhaled Corticosteroids</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Inhalers</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Issues Involved in Bioequivalence Testing</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>POTENTIAL TOOLS</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Potential tools</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Pharmacodynamic Studies</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Pharmacokinetic (PK) Studies</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Scintigraphic Studies</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Correlation of Lung Deposition Studies</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>In vitro Studies</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Summary</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>REGULATORY RECOMMENDATIONS</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Health Canada</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>European Medicines Agency</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>United Kingdom</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>United States Food and Drug Administration</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Regulatory Approach Comparisons</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Summary</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Conclusion</td>
<td>54</td>
</tr>
</tbody>
</table>
5 HOW MUCH DRUG IS AVAILABLE? .............................................................60
   Introduction: .............................................................................................................60
   Methods ......................................................................................................................62
   Simulations .................................................................................................................63
   Results .........................................................................................................................64
   Conclusion .................................................................................................................66
   Discussion .................................................................................................................66

6 WHERE DOES THE DRUG DEPOSIT? ..........................................................75
   Introduction .................................................................................................................75
   Absorption and Muco-ciliary Clearance .................................................................76
   Methods ......................................................................................................................79
   Simulations ...............................................................................................................80
   Results .......................................................................................................................81
   Special Scenario .......................................................................................................82
   Conclusion ...............................................................................................................84
   Discussion ...............................................................................................................85

7 HOW LONG DOES DRUG STAY IN LUNG? ..................................................92
   Introduction ...............................................................................................................92
   Methods and Simulations .......................................................................................94
   Results .......................................................................................................................95
   Conclusion ...............................................................................................................96
   Discussion ...............................................................................................................96

8 STUDY DESIGN .................................................................................................100
   Introduction .............................................................................................................100
   Bioequivalence study design .............................................................................101

APPENDIX
A NONMEM CODE FOR PK MODEL OF FP AFTER INHALATION ......................106
B R CODE FOR AUC AND CMAX CALCULATION ........................................108
LIST OF REFERENCES .........................................................................................110
BIOGRAPHICAL SKETCH ...................................................................................128
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Pharmacokinetic properties of inhaled corticosteroids</td>
<td>25</td>
</tr>
<tr>
<td>3-1</td>
<td>Summary of few studies with different dose levels</td>
<td>44</td>
</tr>
<tr>
<td>4-1</td>
<td>Clinical models to determine relative potency of ICS</td>
<td>56</td>
</tr>
<tr>
<td>5-1</td>
<td>Parameters and their associated variability</td>
<td>69</td>
</tr>
<tr>
<td>5-2</td>
<td>Results from simulations</td>
<td>70</td>
</tr>
<tr>
<td>6-1</td>
<td>Variability on various parameters in the simulated POPPK model</td>
<td>86</td>
</tr>
<tr>
<td>6-2</td>
<td>Simulation results with Kmuc~0.5/hr Same dose available through reference and generic inhaler.</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Slowly dissolving drugs such as FP</td>
<td></td>
</tr>
<tr>
<td>6-3</td>
<td>Simulation results with Kmuc~0.2/hr Same dose available through reference and generic inhaler.</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Slowly dissolving drugs such as FP</td>
<td></td>
</tr>
<tr>
<td>6-4</td>
<td>Simulation results for special scenario. Change in the drug dose and regional deposition pattern.</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Slowly dissolving drugs such as FP</td>
<td></td>
</tr>
<tr>
<td>7-1</td>
<td>Simulation results for inhalers with different absorption profiles. Slowly dissolving drugs such as FP</td>
<td>98</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>The bronchial tree (Weibel model)</td>
<td>17</td>
</tr>
<tr>
<td>1-2</td>
<td>Different processes of dissolution, absorption and muco-ciliary clearance occurring on particles deposited in lung post inhalation</td>
<td>17</td>
</tr>
<tr>
<td>2-1</td>
<td>Activation of anti-inflammatory gene expression by corticosteroids (Barnes [30])</td>
<td>26</td>
</tr>
<tr>
<td>2-2</td>
<td>Fate of inhaled corticosteroid</td>
<td>26</td>
</tr>
<tr>
<td>3-1</td>
<td>Ahrens design of crossover clinical study for BE of ICS</td>
<td>45</td>
</tr>
<tr>
<td>4-2</td>
<td>Health Canada Draft Guidance - Requirements for ICS Bioequivalence</td>
<td>58</td>
</tr>
<tr>
<td>4-3</td>
<td>EMEA guideline scheme for bioequivalence</td>
<td>59</td>
</tr>
<tr>
<td>5-1</td>
<td>Flow chart for modeling and simulation of trials</td>
<td>71</td>
</tr>
<tr>
<td>5-2</td>
<td>Goodness of fit plots. The open circles are the observed values and the lines are model fits</td>
<td>72</td>
</tr>
<tr>
<td>5-3</td>
<td>Individual and population fits from the model</td>
<td>73</td>
</tr>
<tr>
<td>5-4</td>
<td>Spaghetti plots of plasma concentrations from simulations</td>
<td>74</td>
</tr>
<tr>
<td>6-1</td>
<td>Different central and peripheral deposition in healthy and asthmatics</td>
<td>90</td>
</tr>
<tr>
<td>6-2</td>
<td>Compartmental model for inhaled fluticasone propionate used for simulation</td>
<td>91</td>
</tr>
<tr>
<td>7-1</td>
<td>Compartmental model scheme for inhaled and intravenous fluticasone propionate used for simulation</td>
<td>99</td>
</tr>
</tbody>
</table>
Inhaled drug products are the treatment of choice for respiratory conditions like asthma and chronic obstructive pulmonary disease. Since the patents for several inhaled corticosteroids (ICS) expired, less expensive generic inhalers are possible. Inhalers are intended to provide drug for local lung delivery and hence pose special considerations while conducting bioequivalence (BE) studies. The generic inhaler needs to demonstrate equivalent efficacy and safety as that of the original inhaler. Lack of consensus exists between industry, academia and regulatory authorities over criteria to establish BE of inhaled drugs.

The US Food and Drug Administration recommends use of \textit{in vitro} studies for drug product performance, a clinical efficacy (Pharmacodynamic) study to ensure equivalence of local effects and a systemic exposure (Pharmacokinetic) study to ensure similar safety profiles. However, pharmacodynamic studies have proven to be an inefficient tool to evaluate bioequivalence due to the poor dose-response relationship of ICS. Any alternative approach to the current FDA strategy must address the following three key questions to ensure BE of inhalation drugs.
> How much drug is available in the lung? How much is absorbed orally (only relevant for drugs with significant oral bioavailability)?
> Where is the drug deposited (central vs. peripheral lung)?
> How fast is the drug absorbed?

We hypothesize that a pharmacokinetic (PK) approach that utilizes PK endpoints such as area under the curve (AUC) and $C_{\text{max}}$, could answer these questions. Population pharmacokinetic simulations were used to test this hypothesis. Simulations demonstrated that for ICS with negligible oral bioavailability, the AUC will be able to detect a difference (>20%) in the amount of ICS available to the lung when 30-50 subjects are used. For slowly dissolving drugs, the AUC is also sensitive to differences in the regional lung deposition (central to peripheral (C/P) deposition ratio). Similar AUCs may result from a lower lung dose with more peripheral deposition and higher dose with more central deposition for slowly dissolving drugs. To ensure that a similar AUC is not due to the simultaneous difference in these two parameters, one may use in vitro data from cascade impactor studies or conduct PK studies in asthmatics to support the BE decisions in such scenarios. The PK endpoint - $C_{\text{max}}$ can detect differences in the absorption rate of the drug deposited from two inhalers. To summarize, the PK approach provides a cost and time effective tool to evaluate the bioequivalence of inhaled corticosteroids.
CHAPTER 1
AIRWAYS AND ASTHMA

Introduction

The airways are an essential organ system that allows air to come in close contact of the blood. They also present a unique system for systemic as well as local drug delivery. This chapter explains the structural details of the lung. We discuss pulmonary processes such as mucociliary clearance that occur in the lung and its significance in drug delivery. Asthma and its pathophysiology are also discussed briefly to get a better insight into the disease process.

Airways and Lung Function

The airways originate at trachea, branch into smaller and smaller airway passages and terminate in the alveolar sacs that could be described as a pulmonary tree. The structure can be well explained with the help of figure 1-1. The tree trunk is represented by the trachea which bifurcates into the two main bronchi. The bronchi further subdivide into smaller bronchi, bronchioles and terminal bronchioles. This is followed by the respiratory bronchioles, alveolar ducts and ultimately the alveolar sacs. The Weibel model categorizes the airway into 24 distinct generations with trachea as the 0th generation and the alveolar sacs as 23rd generation [1]. As one moves from the upper airways or trachea and bronchi towards the lower airways, the airway caliber decreases. There are functional differences with the upper airways acting as conducting airways and the lower airways as respiratory airways where actual gas exchange occurs. Apart from gas exchange, the lung performs other important functions such as maintaining the acid base balance and consequently blood pH. Certain pulmonary cells secrete substances such as histamine, prostaglandins and leukotrienes that exert local and/or systemic action. The enzymes produced by the pulmonary epithelium are involved in metabolism of selective substances that may be of exogenous or endogenous nature [2].
The lung epithelium is the cell sheet lining the airway lumen. It separates the internal environment of the lung from the external environment and is exposed to substances such as gases, aerosols along with any particulate matter that may be inhaled. The different types of cells that line the airway epithelium confer various functions to the airway epithelium. These functions depend on the area where these cell types may be located. Some of these cell types include ciliated columnar cells, mucous secreting goblet cells, basal cells, dendrite cells, alveolar type I and II cells, basal and serous cells. The ciliated cells extend from the trachea to the terminal bronchioles. They bathe in the fluid bilayer secreted by the goblet cells. The fluid bilayer contains a sol or watery phase adjacent to the epithelium and a gel or mucus phase adjacent to the lumen. Together they constitute what is called the mucociliary clearance processes which provide an important defensive mechanism of the lung.

**Mucociliary Clearance**

The fluid bilayer performs 4 important functions of:

- particle entrapment
- humidifying the inhaled air
- protecting the epithelium from being dehydrated and
- housing various antibacterial enzymes and proteins that prevent microbial colonization of the airways [3].

The ciliary processes beat rapidly in coordination and push the mucus layer towards the pharynx out of the lung. This mucus may be expectorated or swallowed. Thus the mucociliary processes act as a defense mechanism by entrapping all xenobiotics. The mucus is propelled at different rates in different regions of the lung. The rate decreases towards the peripheral airways. The particle entrapment is of importance for asthma treatment since any drug particles that may be delivered to the lung will also be trapped in this mucus layer. The drug particle has to dissolve in the lung fluids before it can interact with receptor targets in the epithelium and be absorbed. This process will have to compete with the mucociliary clearance mechanism.
The ciliated and mucus producing airway structures start disappearing as the bronchioles branch down further into terminal bronchioles and alveolar regions that are non-ciliated [4]. Consequently, the inhaled particles may be retained in the alveolar region for long periods of time (in days). The rate of absorption in the upper or central airways is small as compared to that in the lower or peripheral regions. At the same time the absorption from the alveolar regions is very high. Studies have shown that the absorption rate from the peripheral compartment is twice as high as that from the central region [5, 6]. The inhaled drug particles (which are soluble in nature) will be absorbed before any significant clearance through mucociliary mechanism occurs in these peripheral regions of the lung. The rates of dissolution, absorption and muco-ciliary clearance thus govern the amount of drug in the lung. This is well explained with figure 1-2 that has been adapted from Edsbacker et al. [7] It can be seen that some particles in the ciliated airways in the central region of the lung dissolve in the lung fluids (mucus layer) and are absorbed, while some particles are cleared by the ciliated cells before they are absorbed.

The mucociliary clearance rates differ depending on the lung region [8]. Coughing may increase the mucus propulsion rate, whereas some other clinical conditions may slow down this process. The mucociliary process may also be affected (slower) in subjects with lung diseases [9]. For example in cystic fibrosis or bronchitis, there is hyper secretion of the mucus and ciliary dysfunction which leads to congestion. It has been rightly mentioned that determination of mucociliary clearance is difficult to quantify [10] and depends upon nature of particles deposited- soluble or insoluble particles. Currently prescribed inhalation drugs can be considered to behave as soluble particles. The inhaled corticosteroids act at the receptor level at intra or extracellular locations in the lung. Alternatively, the ICS may also be retained in interstitial fluid before being ultimately absorbed into systemic circulation or be cleared by mucociliary
mechanism [11]. The ciliary processes are predominantly active in the tracheo-bronchial region and such a phenomenon is absent in the peripheral region.

Studies in the past have attempted to estimate the mucociliary clearance rates using radio-labeled particles such as liposomes, ferrous oxide particles, carbon particles, Teflon particles as well as drug molecules. These studies have found that the tracer particles have a half life ranging from 0.5 hours to 24 hours depending on whether they are deposited centrally or peripherally within the lung [12-16]. It may take even days in some cases for the drug deposited in deep areas of the lung to be cleared. However, since the ICS are soluble and absorbable drugs, the drug deposited in the peripheral area of the lung will be absorbed before being cleared over such a long period of time. Hence, only the rapid muco-ciliary clearance which occurs from the central region of the lung would predominantly affect the amount of drug available for systemic absorption. Since some drug fraction may be cleared by mucociliary transport before it is able to elicit its effect, this might reduce the dose available to the lung. Any condition that changes the mucus secretion or clearance will affect the amount of drug available. Apart from this the drug characteristics such as lipophilicity may also influence the amount of drug available to the lung. For highly lipophilic and slowly dissolving ICS like fluticasone, the mucociliary clearance will affect the lung dose. This may not be the case for the more hydrophilic drug budesonide. We will discuss in the later chapters how the regional differences in the absorption rates and mucociliary clearances can be taken advantage of to understand and estimate the regional drug deposition in the lung.

The lung function can be evaluated by measuring various pulmonary volumes and airway caliber using spirometry that measures the volume of air entering and exiting the airways. These volumes and airway caliber change due to disease conditions. Various lung volumes include tidal
volume, inspiratory reserve volume, expiratory reserve volume, residual volume and total lung capacity. The peak expiratory flow rate, forced expiratory flow measures are noninvasive measures of airway caliber. Some of the important and commonly used measurements include the FEV1 or the volume expired in first second and FVC or the forced vital capacity. The FEV can be normalized for various physiological factors such as sex, age and body weight. This enables comparison of lung function values to normal or expected estimates. For example the ratio of FEV1 and FVC in individuals with normal lung function is around 0.8 and in subjects with airway obstruction is lower than 0.8 [2]. Other highly specialized equipments as well as invasive techniques are also available to measure airway resistance and dynamic lung compliance. This includes whole-body plethysmograph and pneumotachygraph.

**Asthma Pathophysiology**

Asthma and chronic obstructive pulmonary disorder (COPD) are chronic inflammatory diseases affecting the airways. There is no cure for these airway disorders, although they can be controlled so as to have fewer symptoms. Asthma is characterized by variable airflow limitation and airway hyper responsiveness [17, 18]. The asthma symptoms or clinical features include wheezing (a whistling sound when one breathes), coughing, chest tightness and troubled breathing, especially late night and early morning [19]. COPD constitutes obstruction to airflow resulting in interference with breathing. COPD refers to bronchitis and emphysema, conditions that frequently coexist. [19]. Asthma results in occlusions of airway lumen by tenacious mucus secretions, infiltration of eosinophils and lymphocytes in the airway wall as well as shedding of airway epithelia [20]. There is also bronchial smooth muscle enlargement and thickening of reticular basement membrane in asthma [21]. The complex pathophysiology of asthma can be accounted for by a number of inflammatory cells which include mast cell, macrophages, dendritic cells, eosinophils and T lymphocytes. There is lack of sufficient evidence about the role
of neutrophils in asthma. The airway structural cells such as the epithelial cells, fibroblast and smooth muscle cells are important source of inflammatory mediators such as cytokines [22]. The epithelial cells are also attributed to play an important role in the airway inflammatory response by interacting with the inhaled environmental signals. Consequently they are a major target for the therapy with the inhaled corticosteroids [23]. A number of inflammatory mediators have been implicated in asthma. These include cytokines, histamine, prostaglandins, leukotrienes, bradykinins, nitric oxide, endothelins, growth factors. These mediators bring about airway smooth muscle contraction, increased mucus secretion. Every mediator has several effects and thus estimation of contribution by any single mediator is difficult.

It was initially thought that asthma was a disease of the central airways. Bronchoscopic studies have clearly shown that asthma is associated with infiltration of the major airways with chronic inflammatory cells, particularly lymphocytes and eosinophils [24]. There also exists upregulation of cytokine profiles. Postmortem systemic studies have confirmed the presence of inflammation throughout the airways [25, 26]. Advances in immunohistochemical techniques have also provided evidence that the peripheral airways are significant sites of inflammation in asthma [24, 27]. Comparison between several markers of inflammation in mucosal biopsy specimens and BAL fluid from healthy control subjects, patients with intermittent asthma, and patients with mild-to moderate persistent asthma found significant increase in the alveolar macrophage activation in patients with asthma compared with control patient [28]. All this has led to an increased acceptance that asthma is an inflammatory disease of lower and upper airways. Consequently any asthma therapy should be targeted towards the entire lung rather than just the central airways.
Figure 1-1. The bronchial tree (Weibel model)

Figure 1-2. Different processes of dissolution, absorption and muco-ciliary clearance occurring on particles deposited in lung post inhalation
CHAPTER 2
ASTHMA THERAPY

The rationale for asthma therapy has evolved with better understanding of the underlying inflammatory processes. Rather than solely relying on relieving symptoms there has been increased use of agents that control underlying inflammation. Asthma therapy could be classified into mainly anti-inflammatory or controller agents (corticosteroids and cromolyn sodium) and bronchodilators ($\beta_2$ agonists) or relievers. There has also been some degree of success reported with the anti-leukotriene drugs. This is especially attributed to the fact that corticosteroids have been unable to effectively block the leukotriene synthesis.

**Inhaled Corticosteroids**

Inhaled corticosteroids (ICS) represent one of the most effective anti-inflammatory treatment options by acting on a variety of targets such as eosinophils, macrophages, T lymphocytes, dendritic cells, mast cells as well as the structural epithelial cells. Corticosteroids reduce the recruitment of inflammatory cells into the airway. ICS do so by suppressing the production of chemotactic mediators and adhesion molecules. ICS also inhibit the survival of eosinophils, T lymphocytes and mast cells in the airways [29]. This potentially blocks a major source of inflammatory mediators in asthma.

The process begins with the ICS binding to the glucocorticoids receptors in the cytoplasm of target cells. Binding of corticosteroids to GR results in a conformational change which allows the ICS-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, specific nucleotide palindromic sequences termed “glucocorticoid response elements” (GRE). Then the transcription of specific genes can be increased (transactivation) or decreased (transrepression). This depends on whether the GRE is positive or negative. Two GR molecules bind together as a
homodimer and bind to GRE. This brings about increase in gene transcription, also known as transactivation. The binding of GR homodimer to the negative GRE results in cis-repression leading to gene suppression. It is estimated that between 10 and 100 genes per cell are directly regulated by the ICS. Many genes are regulated indirectly through the interaction between corticosteroids and other transcription factors and coactivators. CREB (cyclic adenosine monophosphate response element- binding protein) (CBP), p300/CBP –associated factor (PCAF) or steroid receptor coactivator-1 (SRC-1) are such coactivator molecules. In particular, transactivation by corticosteroids increases the expression of anti-inflammatory proteins such as annexin-1 (lipocortin-1), interleukin-1 receptor antagonist (IL-1ra), interleukin-10 (IL-10), secretory leukocyte inhibitory protein (SLPI), neutral endopeptidase and the inhibitory protein (IκB) of nuclear factor-κB (NF-κB). Corticosteroids switch on the synthesis of two proteins that affect the signal transduction pathways, glucocorticoid – induced leucine zipper protein (GILZ) and MAP kinase phosphatase-1 (MKP-1) [30]. Corticosteroids also inhibit the synthesis of many inflammatory proteins through suppression of genes that encode them. In spite of lacking GRE in their promoter regions, many inflammatory genes activated in asthma are repressed by corticosteroids. The effects of pro-inflammatory transcription factors such as AP-1 and NF-κB are inhibited by corticosteroids [31, 32]. Corticosteroids are also able to enhance the transcription of the gene encoding the β2-adrenergic receptor. This is postulated to result in reversing and/or preventing the down-regulation of the β2 receptors possibly induced by long-term treatments with β2-agonist bronchodilators [33]. Thus, corticosteroids reduce airway inflammation and hyperresponsiveness by altering the production of inflammatory mediators. Figure 2-2 explains this complex mechanism of action. The decrease in inflammation can be clinically correlated to improved asthma symptoms.
The negative GRE examples include genes that regulate HPA axis (pro-opiomelanocortin and corticotrophin releasing factor), bone metabolism (osteocalcin) and skin structure (keratins) [29]. The exact molecular mechanisms of corticosteroid induced side effects is not clear [29]. It has been thought that gene activation may be the cause. Few systemic side effects with ICS are osteoporosis, reduced growth velocity in children, skin thinning, cataracts and glaucoma.

Exogenous corticosteroids bring about HPA axis suppression by downregulating the adrenocorticotropic hormone (ACTH) production by the same feedback inhibition loops that control endogenous glucocorticoid production. This leads to adrenal suppression and decreased cortisol levels. ICS dose, duration of treatment and time of drug administration are factors shown to affect the degree of adrenal suppression [34].

**Fate of Inhaled Corticosteroids**

Considerable development has taken place on the drug as well as the inhaler device development fronts. This has had significant effects on pulmonary drug targeting, since pulmonary targeting is a function of drug as well as inhaler device. Before proceeding to discuss the various inhaler devices lets understand the drug delivery process through inhalers. The fate of the inhaled drug can be explained well by the figure 2-3. A major fraction (40-90%) of the dose from the inhaler gets deposited in the upper respiratory tract while a part of it (10-60%) reaches the lungs. The fraction of the drug deposited in the oropharyngeal region gets swallowed and enters the systemic circulation via GI absorption [35]. Hence, the total systemic bioavailability of an inhaled glucocorticoid is the sum of the oral and pulmonary bioavailable faction. The free fraction of ICS in the systemic circulation binds to the systemic glucocorticoids receptors to produce the systemic side effects. The drug is eventually eliminated from the systemic circulation mainly by hepatic clearance mechanisms. An ideal ICS is a compound with high pulmonary activity which is inactivated rapidly and efficiently after absorption. Such an agent would have high pulmonary
activity but minor or no systemic side effects when administered in the desired therapeutic dose range [36]. At the same time the oral bioavailability should be negligible so that the dose that enters the GI tract does not enter the systemic circulation. FP and BUD are two such ICS with favorable pulmonary effects and minimal systemic side effects. The newer ICS such as fluticasone propionate, mometasone furoate and ciclesonide have negligible oral bioavailability (<1%) as compared to the older ones such as beclomethasone and budesonide (10-30%) [37]. These drugs also have high protein binding and high systemic clearance. This reduces the systemic side effects of the drug that is absorbed via lung. However, an ICS with high plasma protein binding will also possess high tissue binding. This will result in lower desired effects due to high lung tissue binding. The pharmacokinetic properties of various ICS are presented in table 2-1. After deposition in the lung, the drug dissolves in the lung fluids, interacts with the receptors and is finally absorbed into the systemic circulation. A fraction of the undissolved drug particles may be cleared out of the lung by the mucociliary transport processes as discussed in the preceding section. In a pharmacokinetic study, the drug levels are measured in plasma which is downstream from where the drug is deposited. There is a general view that the systemic drug levels do not contain sufficient information about drug fate in the lung. We hypothesize that the systemic drug levels carry sufficient information about what goes on with the drug in the lung. The use of these systemic drug levels in evaluating bioequivalence will be discussed later.

**Inhalers**

Devices available for delivery of the ICS include pressurized metered dose inhaler (pMDI), dry powder inhaler (DPI) and nebulizers. Inhaler development, necessary to switch from the CFC propellants to the HFA propellants also resulted in an improved design. The newer inhaler designs have allowed the formation of smaller droplet sizes and increased pulmonary deposition. The HFA propellants provide with an eco friendly option as well as smaller particle size.
distribution (PSD) of the aerosol generated. The smaller PSD leads to greater overall deposition (up to 60%) in addition to more peripheral deposition. There are numerous studies comparing the efficiency of these devices in terms of amount of lung deposition. However, the difficulties in optimally handling pMDIs are well documented [38, 39]. These require sufficient coordination on part of the subject during actuation and inhalation. The problems in co-coordinating actuation and inhalation from a pMDI can be minimized by placing a spacer device between the actuator and mouth. At the same time there is also greater pulmonary drug deposition with a spacer. There are studies describing the lung deposition of ICS from pMDIs with such spacer devices [40].

DPIs, on the other hand are gaining popularity and have been successfully shown to be better or at least equal in performance when compared to pMDIs [41-43]. The drug is released when the patient inhales. Hence less coordination is required by the patient while using a DPI. Some DPIs provide drug alone or in combination with a carrier substance usually lactose. The drug may be available in pre filled blisters or capsules or as a reservoir system. They are available as single dose inhalers or multiple dose inhalers as well. However, the DPIs are inspiratory flow driven and hence require the subject to have a forceful deep inhalation. This allows the breaking up of micron sized drug and diluent particle aggregates into smaller respirable particles in the oropharynx and the larger airways which can then enter the lung. The higher the inspiratory flow, the smaller will be the particles generated resulting in an increased lung deposition. Inhaler device performance is routinely evaluated at inspiratory flow rates of 30-120 L/min with 60L/min being considered as an optimal flow rate. It should be ensured that subjects with varying severity of asthma should be able to get enough pulmonary dose by generating sufficient inspiratory airflow rate [18]. In one study, the lung dose decreased from
27.7% to 14.8% when the inspiratory flow rate decreased from 58 L/min to 36 L/min indicating the influence of flow rate on lung deposition [44].

The commonly used jet nebulizer is based on constant output design and is run by compressed air or oxygen. Supplemental air is drawn across the top of the nebulizer. The drug may be present in the form of suspension or solution. Latest developments in the nebulizers have led to the introduction of devices based on electro-hydrodynamic principles. The underlying process utilizes electrostatic energy to create fine aerosols from the drug formulation. This leads to an increased pulmonary drug deposition. Among various types of nebulizers, the Mystic® from Batelle provided pulmonary deposition as high as 80%. Another nebulizer device called Respimat® from Boehringer Ingelheim utilizes a high pressure micro-spray system of nozzles to slowly release a metered dose to the patient. This system also results in spraying a high concentration of respirable particles.

With better devices, one can achieve greater pulmonary targeting by controlling different factors, the most important of which is PSD. In case of ICS which have considerable oral bioavailability, this may prove beneficial as smaller dose may be required with greater fraction of dose deposited in the lung. Apart from this, the side effects that one may experience from the high oropharyngeal deposition can be minimized with better pulmonary targeting. In case of newer corticosteroids with <1% oral bioavailability, the dose swallowed may not be of consequence as the systemic exposure is dependent only on the lung dose [45]. The delivery devices are patent protected and these patents are often valid for longer periods of time than the patent for the drug ingredient. This makes it difficult to have a device very similar to the innovator inhaler.
Issues Involved in Bioequivalence Testing

The reference or brand formulation is generally the original manufacturer’s product. Bioequivalence indicates similar rate and extent of drug absorption from a test and reference formulation at the site of action and is aimed to ensure that two different products when administered in equal doses by same route and dosage form yield same pharmacodynamic (PD) effect [46]. There are standardized methods for drugs administered orally and parenterally. However, this is not the case for bioequivalence of inhalers. Bioequivalence of inhalers would require demonstrating similar rate and extent of drug absorption in the biophase-lung. The main aim of the bioequivalence study for inhalation drugs is to ensure that the brand and the generic inhalers deliver similar amounts of drug, with similar regional deposition and similar lung residence times. The regional deposition is of importance since we saw in the above discussion that asthma is a disease of the lower and upper airways. Any study designed to compare inhaled products should be able to discriminate between two the inhalers with respect to these criteria. Needless to mention is the fact that the generic inhaler should demonstrate a safety profile similar to the brand inhaler. It will be evident from the following chapters how difficult it is to evaluate bioequivalence of inhaled corticosteroids.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Oral Bioavailability (F) %</th>
<th>Protein Binding %</th>
<th>Clearance L/hr</th>
<th>Vd L</th>
<th>Half Life hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclomethasone dipropionate</td>
<td>15</td>
<td>87</td>
<td>230</td>
<td>20</td>
<td>0.1</td>
</tr>
<tr>
<td>Beclomethasone monopropionate</td>
<td>26</td>
<td>NA</td>
<td>120#</td>
<td>424#</td>
<td>2.7</td>
</tr>
<tr>
<td>Budesonide</td>
<td>11</td>
<td>88</td>
<td>84</td>
<td>183</td>
<td>2.8</td>
</tr>
<tr>
<td>Ciclesonide</td>
<td>&lt;1</td>
<td>99</td>
<td>152</td>
<td>207</td>
<td>0.4</td>
</tr>
<tr>
<td>des Ciclesonide</td>
<td>&lt;1</td>
<td>99</td>
<td>396#</td>
<td>1190#</td>
<td>3.6-5.1</td>
</tr>
<tr>
<td>Flunisolide</td>
<td>7</td>
<td>80</td>
<td>58</td>
<td>96</td>
<td>1.6</td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>&lt;1</td>
<td>90</td>
<td>69</td>
<td>318</td>
<td>14.4</td>
</tr>
<tr>
<td>Mometasone furoate</td>
<td>&lt;1</td>
<td>98</td>
<td>54</td>
<td>332</td>
<td>4.5</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>23</td>
<td>71</td>
<td>37</td>
<td>103</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table 2-1. Pharmacokinetic properties of inhaled corticosteroids
NA – Not Available, Vd – Volume of Distribution, # - apparent maximum approximation based on complete conversion of the parent compound into metabolite
Figure 2-1. Activation of anti-inflammatory gene expression by corticosteroids (Barnes [30])

Figure 2-2. Fate of inhaled corticosteroid
CHAPTER 3
POTENTIAL TOOLS

Introduction

Factors relevant for evaluating bioequivalence (BE) of inhaled corticosteroids (ICS) are pulmonary drug deposition, pulmonary residence time and the central to peripheral deposition (C/P) ratio in lung. Ahrens et al. have proposed to test the BE of inhalation products by measuring clinically relevant pharmacodynamic (PD) endpoints such as FEV₁, PEF and FEF in asthmatics. However, with corticosteroids, the high variability and quantitative insensitivity of such PD endpoints might necessitate large asthmatic population to establish BE. The regulatory authorities from different countries have different views as to what constitutes sufficient conditions to demonstrate BE of ICS. Though there has been a change in the approach by these authorities, some regulatory agencies such as the US FDA are still in the process of formulating them. What needs to be remembered is that the requirements should be rational and feasible. As no single approach suffices the need of time and cost effective technique to establish BE, a combination of in vivo and in vitro techniques may prove to be useful. This chapter reviews guidelines by regulatory authorities from different countries and discusses in detail options available.

Potential tools

A number of methods are available and being developed to evaluate the bioequivalence of inhalation drugs. The primary characteristic needed with any evaluation method is that the tests should be able to distinguish between different inhalers based on amount and rate of drug being available in the lung. These tools involve

- pharmacodynamic (PD) or clinical efficacy trials
- pharmacokinetic (PK) studies
- scintigraphy or imaging techniques
- in vitro formulation characterization
This chapter reviews in detail various tools and discusses them in light of the latest developments. The PD or clinical efficacy studies aim to compare two inhalers to ensure that they have similar efficacy. The PK studies use the plasma concentration time profile data to answer BE questions. One of the important in vitro tests include studies using cascade impactor to compare the respirable drug dose. Cascade impactor tests can also be used to get a more detailed analysis of the particle size distribution (PSD) of the aerosol generates by the inhaler. The PSD directly affects the regional drug deposition within the lung. The smaller the particle size, the deeper it will be able to travel within the lung airways that narrow as they branch into smaller and smaller airways. We will also discuss the attempts to analyze the cascade impactor study data to answer certain questions involved in bioequivalence studies.

**Pharmacodynamic Studies**

The pharmacodynamic (PD) studies are *in vivo* clinical efficacy studies that measure and compare clinical endpoints after administering drug with innovator and generic inhalers [46]. The PD endpoints such as forced expiratory volume in one second (FEV₁), peak expiratory flow rate (PEFR), forced vital capacity (FVC), and forced expiratory flow (FEF) are measured in asthmatics using spirometry. Subjective measurements may be recorded by the patients using diary cards. In this diary card, the patients make a note of the events such as coughing, sleep disturbances due to asthma attack, chest tightness score, how often rescue medication (bronchodilator) was used. There are other tests to detect the drug induced changes in nonspecific airway hyper responsiveness (AHR). AHR can be measured following the inhalation of direct bronchoconstrictors (*e.g.* histamine or methacholine) or indirect agents such as adenosine-5′-monophosphate (AMP). Directly acting agents activate cholinergic receptors in bronchial smooth muscle and cause airway narrowing. Bronchoconstriction by the indirect
agents is a result of degranulation of mast cells which results in release of proinflammatory mediators [47-49]. This test, also known as PC$_{20}$ or PD$_{20}$ measures the concentration or dose of these bronchoconstrictors that bring about a 20% drop in the FEV$_1$. High reproducibility has been established with this test [50]. ICS have shown to attenuate the AHR provoked with these molecules. Thus, these endpoints have been used as clinical outcomes with ICS therapy [51, 52]. A large number of studies have used these endpoints to evaluate the performance of anti asthmatic drugs in mild to moderate asthmatic subjects. The information obtained from such bronchoprovocation studies has similar clinical relevance as that obtained from bronchodilation studies. Airway hyperresponsiveness induced by AMP is regarded as a more reliable model for the evaluation of anti-asthmatic effects of ICS rather than the direct bronchoconstrictors [53]. Budesonide, fluticasone propionate and ciclesonide have been shown to improve AHR induced by AMP [54-56]. However, the duration of action of these drugs in this model has been rarely studied [56]. Also the changes in drug induced AHR induced by corticosteroids is modest [57].

Spirometry tests as well as other categorical PD endpoints such as sleep disturbance scores, wheezing scores, shortness of breath, and frequency of $\beta_2$ agonists’ usage to maintain asthma stability are also evaluated when comparing the ICS. Asthmatic subjects also show increased levels of exhaled nitric oxide (eNO) [57, 58]. Treatment with anti inflammatory agents like glucocorticoids resulted in decrease in the eNO levels [59]. It has been suggested to use NO levels as a biomarker to monitor the underlying inflammation in asthma symptoms [60]. However, the use of eNO to assess or modify asthma therapy with inhaled glucocorticoids has not resulted in any significant success [61, 62]. More information is needed on use of eNO in evaluating glucocorticoids to treat underlying inflammation in asthma.
Any PD test employed to compare glucocorticoids (or any other drug) must be able to differentiate the responses between two clinically relevant ICS dose levels. In the absence of this property, the test may demonstrate that two inhalers to be equivalent even if they delivered different doses. All of the above discussed PD endpoints fail to efficiently differentiate between different dose levels of ICS. There exists a lack of well defined and immediate pharmacodynamic response with ICS. More than 3-4 weeks of dosing period is required to see any significant effects of ICS in improvement in asthma or maintaining the stability of an existing asthma condition. Such lengthy treatment duration makes the parallel study design a viable option. With parallel study designs, BE clinical trials between corticosteroid inhalers would necessitate study populations in excess of hundreds of patients with at least around 3-4 weeks of ICS dosing [63, 64]. The sensitivity and variability of the selected pharmacodynamic end points toward the ICS play a deciding role in whether a dose-response can be shown or not [65].

In the past, a large multi-center trials have been conducted for periods of about two to four weeks (some even up to twelve weeks and greater) to elucidate the dose-response for ICS. These trials have used parallel study designs. Only a few studies were able to demonstrate statistically significant differences between different dose levels [66]. Table 3-1 highlights some studies where absence of dose-response can be seen at various dose levels. In the study by Pearlmen and coworkers, 327 subjects with moderate asthma were given either placebo, 50, 100 or 250 µg of fluticasone propionate for a period of 12 weeks. FEV1 and FEF were the PD endpoints measure. The study could detect no differences between any of these dose levels [67]. In another study by Singh et al. more than 300 subjects received 400 and 800 µg of budesonide over 12 weeks. There was no difference in the PEFR measured [68]. In another PD study, Peden et al. compared 50
and 100 µg of fluticasone propionate in more than 400 subjects with chronic asthma. The study failed to differentiate the two dose levels with the FEV1 and PEFR tests [69]. It can be seen in these studies summarized in table 4-2 that even up to 4 fold dose differences cannot be detected with the PD studies. This can be attributed to the low sensitivity of the lung function tests to assess the differences between different dose levels with inhaled corticosteroids. Difference in individual responses to inhaled corticosteroids is illustrated as one of the reason of the flat dose-response curve. Some patients respond to very small doses of ICS achieving maximum benefit whereas some of them are steroid resistant. A study with a mix of steroid sensitive and steroid resistant population can confound the results of such studies. Use of steroid naïve population is recommended in studies comparing inhaled corticosteroids to avoid such issues [33].

The PD studies represent dose response studies where the reference and test drugs are given at different dose levels to human. At least one formulation is given at more than one dose level. This will help establish a dose response curve. The other formulation can then be compared against this curve. Hence, in essence the PD studies depict the human studies to estimate relative potency rather than clinical efficacy studies [70]. However, this approach has met with only limited success. The ratio of the dose response slope and the variability of the responses, (variability/slope) determines the power to estimate the relative potency between two formulations. A small ratio will mean low response variability and high dose response slope and will provide greater power to estimate relative potency [71]. In case of ICS, the shallow dose-response curve and high variability in responses makes it difficult to use such a PD approach. Crossover designs may help to reduce the variability in the responses seen in parallel study designs. Also, the use of steroid naïve patients may overcome some limitations of steroid resistance [66, 71]. This may result in a smaller variability/slope ratio and consequently greater
power in estimating the potency ratio of inhalers as discussed above. The crossover study design gives rise to the issue of carryover from previous study arm. This is due to the fact that the effect of ICS in maintaining asthma stability continues for sufficient time post discontinuation of the ICS.

Ahrens et al. proposed a cross over design to study the clinically relevant pharmacodynamic responses to evaluate the BE of the generic inhalers. The authors initially employed the study design to compare the efficacy of inhaled β-2 agonist inhalers [72-75]. These studies employed two dose levels of each of the generic and innovator inhalers being compared. The β2 agonist mediated inhibition of bronchospasm induced by methacholine or histamine was used as the pharmacodynamic endpoint. The Finney 2X2 bioassay statistical procedures were used to estimate the relative potency of the generic inhaler relative to the innovator inhaler [76]. This method estimated the relative number of actuations of the innovator (standard) inhaler to yield approximately similar effect as one actuation of the generic (test) inhaler. The approach was utilized to demonstrate the BE of an albuterol generic inhaler to the innovator inhaler Ventolin® [77]. The authors applied the same approach to estimate relative potency on glucocorticoids inhalers (hence indirectly evaluate BE of the inhalers) – Beclamethasone dipropionate (BDP) [78]. Ahrens et al. proposed a different approach to avoid this carry over effect. The study scheme is depicted in figure 3-1. Briefly, asthmatic subjects were screened and entered into the trial. There was a 5-14 day run in period to check compliance with data recording. During this period the subjects used their regular ICS. After this the subjects were pre dosed for 4-7 days with 40 mg twice daily dose of prednisone. This was done to bring the patients to maximum possible corticosteroid response in spirometry tests. The FEV1 was checked and then the subjects started their treatment with either the innovator or test (generic)
inhaler for three weeks. At the end of three weeks, a histamine challenge was performed along with other spirometry tests. After this, the subject entered the next study arm which started by pre-dosing with prednisone for 4-7 days. This was done to avoid the carry over effect in cross-over design [71]. The outcomes were then analyzed to determine the dose response curve. The authors suggested that the test and reference drugs be dosed for more than three weeks. The endpoint was maintenance of asthma stability rather than improvement in the asthma condition. The authors assert that using their proposed study design, a BE of glucocorticoids inhalers could be evaluated using fewer (30-50 subjects). A complete study evaluating innovator and generic inhaler using this approach is yet to be conducted. Additionally, such a cross over design with multiple dose levels of each inhaler would lengthen the study period over several months. This could result in poor study compliance.

There is lack of consensus with regards to optimal study design. There are issues with parallel and cross over study designs as we saw in the preceding sections. Finding sufficient number of steroid naïve patients with a predefined level of asthma (generally mild to moderate) is difficult. The PD approach though enticing and intuitive fails to establish BE of inhaled drugs in a time and cost-effective manner. After a more clear review of literature studies, it is obvious that such an emphasis by the regulatory authorities is unfounded and unfeasible. Such PD studies take patients in excess of hundreds, lengthy trial periods and still fail to distinguish between dose levels of ICS. To summarize, the pharmacodynamic approach with ICS is difficult and has been attributed to their shallow dose-response relationship, poor selection of dose ranges, high variability of the responses, lack of well defined site of action for ICS in asthma as well as to the limitations of the study design employed [79, 80].
Pharmacokinetic (PK) Studies

Pharmacokinetic studies involve measuring plasma drug concentrations after inhalation. Basic PK parameters such as area under the curve (AUC) and maximum plasma concentration (Cmax) are used to evaluate systemic drug exposure. While AUC is a measure of the cumulative drug exposure to the body, Cmax is a measure of the rate of drug absorption into systemic circulation. In case of inhalation drugs, PK studies have been viewed to be of limited use in the past. This was due to the limitations of the analytical techniques to detect the low plasma drug levels that one encounters after drug delivery via inhalation. However, PK of most inhaled glucocorticoids can be assessed with assay techniques like LC-MS/MS and tandem mass spectrometry. The ICS drug levels in plasma can now be measured in the low picogram levels [81-83]. Hence, plasma levels after inhalation don’t seem to be a limiting factor as reasoned out in the past.

Absorption of the swallowed drug fraction may occur from the GI tract as discussed in chapter 2. Charcoal block is utilized to prevent the oral absorption of the fraction of the drug that is deposited in the oropharyngeal region and may be swallowed [84, 85]. Essentially, one conducts a PK study with and without charcoal administration. The charcoal suspension adsorbs most of the ICS that enters the GI tract and prevents it from being absorbed in the systemic circulation. The newer ICS like fluticasone propionate, ciclesonide and mometasone have negligible (< 1%) oral bioavailability and any plasma concentration would be reflective of only pulmonary deposition [86-89]. Hence, charcoal block is unnecessary with such ICS. Plasma level for these drugs with negligible oral bioavailability reliably reflects the amount of drug absorbed from the lung only. Thus, the plasma levels of ICS after inhalation are indicative of the amount deposited in the lung. Attempts have been made to compare the lung deposition of ICS based on plasma levels after inhalation. A study demonstrated equivalent lung deposition after inhalation.
of single dose of budesonide from test and reference DPIs using the PK data [90]. Studies have compared inhalers by employing more than one tool such as PD study, PK data, imaging techniques and in vitro tests. In the ensuing review, we will see examples where PK data detected differences in drug exposure when PD approach failed to do so due to the shallow dose response relationship.

Singh et al. compared the equivalence of budesonide in healthy and asthmatics, children and adults using PK as well as PD measures [68]. The study demonstrated equivalence between two pMDIs (based on CFC and HFA propellants) using PK data. The study compared single and multiple doses with 3 dose levels as well as varying number of inhaler actuations. There was no difference in the plasma drug levels between the CFC and HFA pMDIs with single or multiple doses. Dose proportionality in AUCs was observed with all the dose levels selected (400 µg - 1600 µg). Interestingly there were no statistically significant differences in pulmonary function endpoints, asthma symptoms, sleep disturbance or use of rescue medication between the two inhalers at two dose levels (400 µg and 800 µg). Thus, from the PK data (AUC) we see that there was different drug exposure between 400 and 800 µg dose levels. Hence, the 400 µg and 800 µg dose levels were not bioequivalent. If only a PD endpoint was to be considered, the inhalers would have been wrongly qualified as equivalent.

Another study by Daley-Yates et al. compared two in-house inhalers, each delivering a combination of 50 µg of salmeterol and 250 µg of fluticasone propionate [91]. One inhaler was a multiple dose dry powder inhaler DISKUS® while the other was a reservoir powder inhalation device (RPID). PD, PK and in vitro comparisons were made. The difference in the mean change from baseline morning peak expiratory flow rate (PEF) was used as the PD end point. The study was conducted for 12 weeks and 270 subjects completed the study. There was no statistically
significant difference observed in this efficacy endpoint. *In vitro* particle size distribution (PSD) comparison was made using 8 stage Anderson Cascade Impactor. The PSD profiles were comparable for the two inhalers. Interestingly, the PK data revealed that there was more than 2 fold systemic exposure to FP with RPID as compared to DISKUS. The estimated AUC ratio for FP exposure was 2.00 with 90% CI 1.56-2.55. This shows that the PK approach has greater sensitivity in detecting the differences between inhalers with regards to dose delivered to the lung.

One of the biggest advantages of using the PK approach is that it can be performed in healthy volunteers, obviating the necessity of asthmatics. With ICS like budesonide the *in vivo* PK in healthy volunteers is similar to that in asthmatics [92, 93]. There are some references which demonstrate lower plasma levels for drugs like fluticasone propionate in asthmatics. This has been attributed to the high lipophilicity of fluticasone propionate and in such drug specific cases further studies might be necessary [92, 94]. With ICS exhibiting slow absorption, other PK parameters such as mean residence time (MRT) and mean absorption time (MAT) could be estimated and compared. We hypothesize that differences in regional drug distribution within the lung can also be estimated with PK studies for such drugs. PK studies have been consequential in explaining pulmonary drug deposition and understanding the therapeutic equivalence [95-97]. The safety profile of the drug is directly correlated to the drug exposure that can be estimated from the plasma levels. Hence, a PK study would be sufficient to estimate the drug exposure (and hence efficacy) and safety profile as well.

The preceding discussion highlights the importance of PK studies to measure the pulmonary drug deposition. Such a PK approach can be used to estimate the pulmonary as well as the total body exposure. The number of days and subjects required for such an approach will
be greatly reduced in comparison to a clinical efficacy trial. A PK study can be completed over few days in a cost effective manner. PK approach under appropriate conditions exhibits immense potential in the design of BE studies for inhalers.

**Scintigraphic Studies**

Drug deposition in the central and peripheral airways is of critical importance in asthma. The site of action of anti asthmatics is poorly defined. The inflammatory process in asthma occurs throughout the lung and hence the deposition of corticosteroids (anti-inflammatory) is essential throughout the bronchopulmonary regions [98]. There have been arguments that regional drug deposition cannot be appreciated with PK data. We hypothesize that this is true only for fast dissolving drugs. Hence, differential drug deposition in the central and peripheral regions within the lung for such fast dissolving drugs may be required to elucidate maximal response or efficacy. Scintigraphic studies for inhaled medications can accomplish this task. The use of drug attached with a radioactive tracer has been traditionally employed to determine the spatial or regional drug distribution within the lung after inhalation [99]. It involves radio labeling the formulation with radionuclide such as technetium $^{99m}$Tc or other gamma emitting isotope which is then inhaled by the subject. Occasionally, inert carrier particles, such as Teflon particles have also been used instead of the drug in such deposition studies. Two-dimensional planar gamma images are taken and compared after subtracting for background levels. The central, intermediate and peripheral pulmonary deposition is compared between the test and reference product. The total lung deposition can also be calculated from the scintigraphic images. This technique suffers from that fact that it is often not possible to radiolabel the drug molecules as such. Change in the radio-labeled particle behavior as compared to the original particle has also been argued. Validation of the *in vitro* radio-labeling experiments are needed to demonstrate that the aerodynamic PSD of radio-labeled product is similar to original product [100, 101].
There also may be overlap of regions due to the two dimensional nature of the image. Variability between different observers is also unavoidable. Recent developments in imaging techniques like single photon emission computed tomography (SPECT) and positron emission tomography (PET) have led to techniques in which three dimensional images can be obtained [102, 103]. Currently these imaging methods are under development to overcome some shortcomings. With increasing popularity, they may prove of critical importance in comparison of deposition profiles in the future.

**Correlation of Lung Deposition Studies**

An obvious question is whether there exists a correlation between lung deposition and efficacy or response of inhaled drugs. Lung deposition serves as a measure of local drug bioavailability in the lung. Hence, with drug delivery to the site of action, improved lung deposition should result in an increased efficacy or response [63, 101]. There are reviews of studies discussing the positive correlation between lung deposition and clinical effect [18, 104]. These studies are classified into two broad categories. One type of study is where an equivalent control of asthma with a lower dose of drug from a device can be directly related to the improved pulmonary drug delivery from that particular device. The other types of studies demonstrate direct correlation between the efficacies of inhaled drug with its lung deposition for two or more treatment regimens. It must be emphasized that studies with concomitant assessment of lung deposition and pharmacodynamic effect provide strongest evidence. However, such studies are scarce [101].

A clinical trial with two dose levels, 400 µg/day and 1600 µg/day of budesonide from a pMDI and a pMDI attached with a spacer (Nebuhaler) in 35 asthmatics concluded that a given level of antiasthmatic response could be achieved at half the dose when Nebuhaler was used. This was attributed to the higher pulmonary deposition resulting from the efficient delivery from
With improved lung deposition there was reduction in oropharyngeal drug deposition and consequently reduced occurrence of oral candidiasis [107, 108]. Numerous studies have been published demonstrating the higher efficiency (almost twice) in terms of lung deposition of drugs from a DPI (Turbuhaler) as compared to a pMDI. One such study with Turbuhaler showed that 400 µg by pMDI and 200 µg by Turbuhaler were equipotent, and similarly 100 µg by pMDI and 50 µg by Turbuhaler were equipotent. The study concluded that half the dose of salbutamol from a Turbuhaler produced a similar bronchodilator effect when compared with pMDI. The improved efficiency of drug delivery to the lung thus correlated to lung function improvement [109]. There exists a paucity of such studies correlating lung deposition and clinical efficacy with the ICS with most of them involving β₂ agonists [18].

Another PK study demonstrated a direct correlation between lung deposition and the pharmacodynamic effect with terbutaline, a β₂ agonist [110].

Along with the absolute values for lung deposition, the variability associated with it is also of particular interest. The variability in lung deposition can be explained by the variability in throat deposition and vice versa [111]. The variability is a measure of the range of lung deposition of ICS that can be expected with daily use in individuals. This information will be of great importance whilst assessing the treatment regimens [111]. The variability in dose deposition to the lung could be assessed by in vitro as well as in vivo experiments. A few factors to be mentioned are device performance, device handling, patient co-ordination of actuation and inhalation, inhalation flow, patient throat anatomy etc [112]. A number of studies have attempted to account for the absolute values of lung deposition as well as the inter-individual and intra-individual variability associated with the amount deposited [85, 112, 113]. Studies have also
compared the lung deposition and variability associated with it using different inhalers like DPI and the pMDI.

The aforementioned studies provide sufficient evidence to suggest that lung deposition data could be used as surrogate marker for clinical response data under appropriate considerations. Consequently, PK studies in conjunction with the \textit{in vitro} or scintigraphy studies, could be a useful tool to establish the BE of inhaled drugs. This data could be used to demonstrate the effectiveness of inhaled drug products to the regulatory agencies [101]. The number of patients as well as duration of study will be greatly reduced by using such a surrogate marker [114]. Significant time and financial resources could thus be conserved during the drug development and approval process using this approach. This approach could substitute the necessity of the inconsequential clinical efficacy trials. Two way cross-over PK study over single days could be sufficient for BE of ICS as against 4 weeks or more of parallel or months of crossover study designs in large patient population with poor or confounding outcome [101]. This approach could also be useful in special cases like regulatory approvals after change in the manufacturing site [115]. The outcome of this approach in cases where the delivery characteristics from the inhaler product are dissimilar needs to be carefully monitored.

\textit{In vitro Studies}

The preceding sections discussed various \textit{in vivo} techniques. Particle size distribution (PSD) is one \textit{in vitro} measurement that yields information to compare two inhalers. Spray pattern and plume geometry are \textit{in vitro} tests used to characterize the performance of the valve and actuator. Plume geometry can be measured using laser diffraction and time of flight aerodynamic particle size analysis. Microscopy is also conducted to study the particles and their aggregates. One can get information about parameters such as mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle mass (FPM) and fine particle
fraction (FPF) from the PSD. All these parameters are used to describe the particle size of the aerosol generated by the inhaler. Cascade Impactors (CI) and multistage liquid impingers (MSLI) are used to study the PSD. The FPF represents the fraction of the so called ‘respirable fraction’ (representing bronchopulmonary deposition) of dose and usually are particles or droplets with MMAD of 1-5 um [116, 117]. It has been demonstrated that lung deposition efficiency is correlated to the MMAD and GSD [118, 119]. Improved efficacy of the inhalation therapy can be achieved by selective deposition of the particles or droplets generated by the inhaler.

The Anderson eight stage cascade impactor is extensively used to assess PSD, MMAD and GSD and is the device of choice [120]. The US FDA does not consider it adequate to compare PSD of two inhalers in terms of MMAD, GSD or the FPF but encourages the individual plate comparisons of particles in all size ranges in the cascade impactor [121]. There exist some limitations in precision to compare particle masses on a plate by plate basis. This has been explained and related to the manufacturing tolerances in the plate pore cut-off points [122]. Consequently the plate by plate comparison is highly variable and impractical. Plate grouping analysis and use of model fitting software (to fit bimodal or more complex PSD) is suggested to overcome some of these limitations [122]. The selection of induction port influences the outcome of PSD measured by cascade impactor [123, 124]. Using the same cascade impactor to measure the test and reference inhaler (cross-over design) helps to reduce the variability of PSD. As of now, the in vitro tests are considered to be more of quality control significance by the US FDA and are not sufficient to demonstrate BE of OINDP by themselves. They are important for product characterization and performance. This may be attributed to the fact that the in vivo
inhalation process may be more complex. A simple metal port inductor used in cascade impactor may not be a good replication. This is well explained by the study discussed above.

In the above mentioned study by Daley-Yates et al, two in-house inhalers, each delivering a combination of 50 µg of salmeterol and 250 µg of fluticasone propionate [91] were compared. PD, PK and in vitro comparisons were made. The difference in the mean change from baseline morning peak expiratory flow rate (PEF) was used as the PD end point. The study was conducted for 12 weeks and 270 subjects completed the study. There was no statistically significant difference observed in this efficacy endpoint. The in vitro particle size distribution (PSD) was compared using a 8 stage Anderson Cascade Impactor. Interestingly, the PK data revealed that there was more than a 2 fold systemic exposure to FP with RPID as compared to DISKUS. The PK based parameter such as AUC ratio for FP exposure was 2.00 with 90% CI 1.56-2.55. Thus similar PSD profiles as determined by cascade impactor may not lead to similar lung deposition in vivo.

Summary

Most of the spirometry tests as well as other clinical end points (PD markers) have high variability. The issue of steroid sensitivity within study subjects further complicates the study design. With the newer drugs that have negligible oral bioavailability, the plasma levels are a reliable measure of the dose deposited in lung. Advances in the analytical techniques make it easy to accurately estimate the low plasma drug levels. For ICS with significant oral bioavailability, alternative techniques like charcoal block, can help differentiate between systemic drug levels resulting from lung or GI tract. This aids the reliable estimation of pulmonary drug deposition after inhalation. The in vitro comparison such as similar PSD may not necessarily translate in vivo similarity. Imaging techniques are still under development phase. In such situations, PK studies are better suited as compared to the PD studies to detect
differences between ICS. Not only is this approach cost-effective but also saves time. It is better suited to detect differences between two inhalers than the PD method. Consequently, the PK approach provides a tool that can effectively be used to evaluate whether a generic inhaler will provide similar efficacy and safety as that of the brand inhaler before such switch is made.
Table 3-1. Summary of few studies with different dose levels

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug &amp; Doses</th>
<th>Design and Duration</th>
<th>Population/ Sample size (total)</th>
<th>Spirometry PD endpoints measured</th>
<th>Dose-response observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearlmen et al</td>
<td>FP 50, 100 &amp; 250 ug</td>
<td>Placebo controlled, parallel, 12 week</td>
<td>Moderate asthma/ 327 subjects</td>
<td>FEV1 FEF (am/pm)</td>
<td>No</td>
</tr>
<tr>
<td>Singh et al</td>
<td>BUD 400 &amp; 800 ug HFA and CFC pMDIs</td>
<td>Parallel, 12 week</td>
<td>Adult and adolescent asthma patients/ 321 subjects</td>
<td>PEFR (am)</td>
<td>No</td>
</tr>
<tr>
<td>Peden et al</td>
<td>FP 50 &amp; 100 ug by Diskus and Diskhaler</td>
<td>Placebo controlled, parallel, 12 week</td>
<td>Children (4-11 yrs) with chronic asthma/ 437 subjects</td>
<td>FEV1 PEFR (am)</td>
<td>No</td>
</tr>
</tbody>
</table>
Figure 3-1. Ahrens design of crossover clinical study for BE of ICS
CHAPTER 4
REGULATORY RECOMMENDATIONS

There still exists a lack of consensus between industry, academia and regulatory authorities over criteria to establish bioequivalence (BE) of inhalation drug products. Regulatory agencies are in the process of finalizing a definitive guidance document about inhaler bioequivalence. Currently, two organizations, the European Medicines Agency (EMEA) and Health Canada have published guidance documents for establishing the bioequivalence of inhalation drugs. In this chapter we discuss these guidance documents. The US FDA viewpoint will also be discussed.

Health Canada

The Canadian Health Ministry released a draft guidance for a generic inhaled corticosteroid product in 2007 [125]. This document resulted from a long ongoing series of discussions through a series of symposia over more than a decade. The Canadian Thoracic Society with the support of Health Canada held the following three symposia that comprised international experts:

- Toronto, ON, Canada- December 1995
- Toronto, ON, Canada- May 2000

The discussions from the first two symposia were published in 1998 [126]. The publication [126] favored a pharmacodynamic approach over the direct pharmacokinetic measurement. The efficacy study represents a relative potency study that could be accomplished with clinical studies conducted in a number of ways. This publication elaborately discussed the various aspects including pharmaceutical factors that should be included in the guidance document in such comparative studies. The discussions from the third symposia were published in 2003 [127]. It summarized some of the possible approaches with their advantages and drawbacks. The various clinical study designs discussed (Table 4-1) were essentially human
relative potency tests to determine the relative potency of the ICS. The two main study design approaches used either a controlled or uncontrolled asthma model. There was a consensus in all the three symposia that there exists a need for a validated method to compare relative potency of ICS.

Following the three symposia, a guidance document was published by Health Canada that states the requirements for BE studies with ICS [125]. As per the document, two trials are necessary for establishing bioequivalence for the generic inhalers. The first is a well controlled, double blinded, randomize study with three parallel arms: reference product, generic inhaler and placebo. This resembles a Phase III efficacy study. The use of steroid naïve mild asthmatic subjects with the lowest possible dose is recommended. Minimum of three weeks of trial duration is recommended in a parallel study design. The absence of clinically significant differences in the comparison of an inflammatory marker and pre-bronchodilator FEV1 is required. The guidance document clearly states the criteria required when comparing the two inhalers:

- A difference in the mean sputum eosinophil count (expressed as a percentage of the total count) of at least 50% between the active treatment (pre-treatment minus post-treatment, for both test and reference) and the placebo treatment (pre-treatment minus post treatment) will be considered clinically significant.
- A difference in the mean FEV1 (expressed as percentage of predicted) of at least 10% between the active treatments (post-treatment minus pre-treatment, for both test and reference) and the placebo treatment (post-treatment minus pre-treatment) will be considered clinically significant.

The second trial required is a single dose PK study at upper dose limit to measure systemic exposure as a surrogate for long term systemic effects. In the absence of reliable analytic techniques in case of low blood levels, the draft guidance allows for evaluation of systemic exposure by measuring the hypothalamus-pituitary-axis (HPA) function. This entails measuring
the serum cortisol over 24 hours with either a single or multiple dose study design. The summary of these requirements from the draft guidance are enlisted in figure 4-2. The draft guidance document lists other details that are required to be submitted for generic drug approval. However, it may be difficult to evaluate the BE of ICS with a single dose efficacy study. With difficulties in differentiating between different dose levels of ICS based on PD endpoints, it seems a daunting task to detect differences between inhalers based on a single dose level. The difficulties with using the PD approach have been reviewed in the previous chapter 3.

European Medicines Agency

The committee on medicinal products for human use of the European Medicines Agency (EMEA) has published a draft guideline on bioequivalence requirements for orally inhaled products in 2007 and 2009 [128, 129]. The suggested approach from this guideline can be summarized as in figure 4-3. The guideline requires only an in vitro equivalence study to be substantiated with regards to drug, excipients and container performance criteria. Such an in vitro test can be conducted with use of multistage impactor. The following criteria for the in vitro tests needs to be fulfilled. The criteria include that the generic product contains the same active substance in an identical dosage form. This means that a pMDI should be compared to a pMDI and a DPI to a DPI. There should be similar instructions for the use of the two inhalers. The differences in the excipients (if any) should not influence the product performance, aerosol particle behavior or the inhalation behavior of the patient. Similarities between device performances such as resistance to air flow, dose delivered are required as well. The guidance requires that either the individual stages are directly compared or the stages are divided into at least four groups. Comparison should be made for the stages that represent fine particles, as well as the upper stages of the impactor. In the event of failure to establish the in vitro equivalence, an in vivo study is required to substantiate the equivalence of ICS.
Deposition studies comparing the extent and pattern of pulmonary deposition could be done by imaging or PK studies. The imaging study could be done either by using two or three dimension scintigraphy. The regional pulmonary deposition can be compared by measuring the radioactivity in the different segments of the lung. A PK study is still required as a measure of systemic safety. A PK study may be used alone to assess the pulmonary deposition. The study design for such a PK study should be able to exclude drug absorption from the GI tract. In the event of this approach failing too, a Phase III PD efficacy trial will be required to investigate the therapeutic equivalence of the inhaled drug products as per these guidelines. The clinical endpoint should be a pulmonary function measure and preferably FEV1 although other endpoints such as expired nitric oxide (NO) PC20, PD20 and sputum eosinophils. The choice of efficacy endpoint needs to be justified based on its sensitivity to detect differences between adjacent doses. A minimum of eight weeks of dosing is recommended with a parallel study design as concerns are expressed over the carry over effect in cross over study design. There is emphasis on the selection of homogenous study population to minimize the variability in response which would thereby increase the power in detecting dose response relationship and differences in formulations if any.

The EMEA published an updated guideline document recently 2009 [128]. The most important update in the latest document was in the BE test requirements of ICS for pediatric population. The document quotes that

“the pulmonary deposition studies(PK and imaging) are not appropriate in children. Pharmacokinetic studies as a surrogate for efficacy only imply efficacy, they increase the burden on the child and have insufficient advantages over pharmacodynamic and/or clinical studies in...
the assessment of therapeutic equivalence in children to warrant their use. Imaging studies in children are also not appropriate.”

With children aged 6 years and older, the clinical efficacy endpoint for the pharmacodynamic study should be a pulmonary function measure such as FEV1. PEF may be used with appropriate justification. In children, the systemic safety can be compared with a PK test if sufficient information about effect of reference drug on child HPA axis is available. In the absence of this information a pharmacodynamic safety test is required. This includes measuring the effect of drug on the HPA axis and on the lower leg bone growth rate as a surrogate marker for growth.

The EMEA presents a rather over-optimistic approach to evaluate BE of inhaled drug products. In vitro particle size distribution comparison by itself may be inadequate. There are instances where similar PSD in vitro may not necessarily translate into similar drug amounts in vivo. The study by Daley-Yates et al was discussed above where such a situation arises [91]. The two dimensional imaging techniques have limited advantage to differentiate between regional depositions. The three dimensional imaging techniques are still in development stage and posses cost constraints. In our view, one needs a PK approach supported by in vitro tests for example using cascade impactor to answer specific questions that arise with regards to slowly and fast dissolving glucocorticoids is better suited as compared to the rather ambitious concept of establishing BE of inhalation drugs on in vitro tests only.

United Kingdom

A workshop report from the British Association for Lung Research (BALR) has been published in 1994 [120]. The overall recommendations are outlined in figure 4-1. This workshop was aimed by the British Agency to formulate guidance for BE of inhalation drugs. With the current EMEA guidance in effect, the UK document is obsolete. A combination of clinical
efficacy studies as well as in vitro tests was recommended though not specified explicitly to demonstrate bioequivalence of inhaled medications. The BALR workshop recommended that pharmacodynamic studies be conducted to compare the clinical efficacy of the inhalers in a controlled population.

With ICS, studies for at least one month of drug dosing are recommended. The recommended PD endpoints include peak expiratory flow rate (PEFR - am and pm), reduction in response to bronchoprovocation (PC20), improvement in the frequency of bronchodilator use, sleep disturbances and fewer exacerbations. The workshop considered PK studies of limited value and inessential in determining inhaler bioequivalence due to low plasma levels of ICS. Spacer devices and their combinations complicate the assessment and PD studies with and without spacer are recommended for their comparison.

United States Food and Drug Administration

Currently, there is no guidance from the United States, Food and Drug Administration (US FDA) for BE of inhalation drugs. This is unlike for the nasal products where the US FDA has published a draft guidance in 2003 [130]. The US FDA is actively seeking to establish BE a guidance for inhaled products. There have been ongoing discussions by the Orally Inhaled and Nasal Drug Products (OINDP) subcommittee about this issue. The discussions encompass issues with BE of nasal sprays, inhalers that include bronchodilators, corticosteroids and as well as combination products. The subcommittee convened a meeting in 2000 (Rockville, Maryland) with the aim of devising guidelines for BE studies [131]. However, no consensus was reached about what appropriate tests were required.

The committee agreed that the overall approach should be to establish BE of inhaled drug products by demonstrating equivalence based on in vitro tests, clinical efficacy study and systemic exposure safety study. The various PD study designs discussed included the study
design proposed by Ahrens et al [71]. The design has been discussed in detail in the previous chapter. The study design was used by FDA to evaluate the BE of the inhaled bronchodilator, albuterol. The relative bioavailability is estimated in terms of the dose of the generic inhaler required to produce the same effect as that of the reference inhaler. The FDA has funded the follow up study with ICS using Ahrens’ approach at University of Iowa. The committee also expressed interest in exploring the use of exhaled nitric oxide as a surrogate marker for treatment with ICS. The FDA has also funded a study to be conducted at National Jewish Medical and Research Center in Denver, Colorado.

The International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) and the scientists of the Inhalation Technology Focus Group (ITFG) of the American Association of Pharmaceutical Scientists (AAPS) have been collaborating to address specific issues for the OINDP. The Product Quality Research Institute’s (PQRI) Working Group (WG) has published the results (2007) of the chi-square test statistic used to compare the aerodynamic particle size distribution (APSD) from cascade impactor data [132-134]. Summarizing their findings in the final paper, the WG pointed out the limitations of using the chi-square test statistic as well as its combination with the supplemental population based equivalence (PBE) in comparing the APSD cascade impactor profiles [134].

**Regulatory Approach Comparisons**

The regulatory authorities in different countries have made recommendations to demonstrate inhaler bioequivalence [120, 121, 126, 135]. A detailed study of the recommendations from these regulatory agencies highlights the lack of consensus and varied approaches taken by regulatory authorities in different countries. While the Canadian Regulatory requirements find the *in vitro* tests inconsequential, the EMEA is ready to authorize generic inhalers that pass the *in vitro* tests alone. The UK, US FDA and Health Canada stress the need
for a clinical efficacy study and tend to minimize the relevance of pharmacokinetic studies. This emphasis on conducting a clinical efficacy study sounds intuitive but upon further reasoning seems unfeasible. These reasons with examples have been discussed above in detail. This has been mainly attributed to the poor dose-response curves for the inhaled corticosteroids. The required duration for such efficacy trial differs from country to country ranging from three to eight weeks minimum. The clinical endpoints that need to be compared also differ between various regulatory agencies. Although, all allow the crossover study design, a parallel design is preferred to prevent carry over effects. The selection of low dose and steroid naïve patients is something which is not easily met. The requirement of single dose study comparison as per Health Canada seems irrational when one considers the flat dose response curve with ICS. The two dose level requirement with EMEA guidelines may not be required if the inhalers pass in vitro tests. There are cases when in vitro similarity does not necessarily translate into in vivo equivalence [91]. There still lacks a definitive uniform guideline from these agencies that could be used by the pharmaceutical manufacturers to get their products approved. All in all, the relevance of conducting a PK study is assigned to evaluate the safety or systemic exposure of the ICS. The requirements range from being too daring as with EMEA to no guidance as seen with the US FDA.

**Summary**

There are ongoing efforts everywhere to provide a robust and reliable method to establish BE of inhaled products. These efforts are active at all levels including the academia, industry as well as the regulatory authorities. The regulatory authorities recommend clinical efficacy studies, safety studies in all the drug classes for inhaled drugs. There exists some contention over what constitutes a sound clinical study design in terms of clinical end points, patient selection criteria, study duration, clinical and statistical significance of the elucidated response for BE of inhaled
products. However, finding such a universal time and cost-effective study design to establish BE of inhaled drugs has so far resembled the search of ‘Holy Grail’ with every attempt turning futile. The drug class- bronchodilators or ICS differ in their mechanism of action and need to be targeted in different regions in the lung. It might be possible to perform clinical efficacy trial with the bronchodilators, although even these drugs easily tend to reach the dose-response curve plateau. There exist inherent difficulties in clinical trials to compare the ICS due to the flat dose-response curves. Most of the spirometry tests as well as other clinical end points (PD markers) have high variability. The issue of steroid sensitivity within study subjects further complicates the study design. With the newer drugs that have negligible oral bioavailability, the plasma levels are a measure of the dose deposited in lung. The lung deposition studies can be used as a bridge between in vitro and clinical studies in drug development. Advances in the analytical techniques and using alternative techniques like charcoal block, make it easy to accurately estimate the plasma profiles which reflect lung deposition of the drug. This aids the reliable estimation of pulmonary drug deposition after inhalation. In vitro PSD comparisons as well as lung deposition have a great potential to be used as a surrogate marker for clinical response studies. In such situations, PK studies have an upper edge over the PD studies. Not only is this approach cost-effective but also saves time. It is better suited to detect differences between two inhalers than the PD method. Consequently, PK approach provides a tool that can effectively be used to evaluate whether a generic inhaler will provide similar efficacy and safety as that of the brand inhaler before such switch is made.

**Conclusion**

It can be concluded that each of the PK, PD, scintigraphy or in vitro approaches has its own advantages and disadvantages. Any approach cannot be used as standalone to establish the BE of inhaled medications. A combination of these methods can be employed and their
importance is directly related to the drug as well as the drug product. While it may seem possible to perform the clinical study with some drugs like \( \beta_2 \) agonists, this approach is fraught with difficulties and has been inefficient in case of ICS. This has been proved by the numerous failed efficacy trials that can be studied from the past. We hypothesize that the study design with PK method in conjunction with appropriate \textit{in vitro} tests may be one of the solutions to unravel the BE conundrum with inhaled drug products. Any approach should be devised with the intent to provide quality drug products to the patient in a cost-effective way and timely manner. The PK approach poses the potential to fulfill this requirement.

\textbf{Hypothesis}

We hypothesize that combination of \textit{in vitro} and PK studies are sufficient to evaluate the BE of ICS. Such an approach requires few healthy subjects and provides a time and cost effective solution for BE studies of such inhalation drugs. All the three questions of how much drug is available, where does it get deposited and how fast the drug is absorbed can be answered successfully with this approach.
Table 4-1. Clinical models to determine relative potency of ICS

<table>
<thead>
<tr>
<th>Clinical efficacy model</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergen provocation</td>
<td>Measure airway response- late asthmatic response (LAR) due to allergen provocation</td>
<td>Possible use at lower dose level (give references)</td>
<td>Expertise needed to perform the provocation tests</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shallow dose-response</td>
</tr>
<tr>
<td>Exercise/ methacholine/ AMP provocation</td>
<td>Airway hyper-responsiveness to bronchial provocation</td>
<td>Bronchial provocation by AMP more sensitive</td>
<td>Results not reproducible</td>
</tr>
<tr>
<td>Uncontrolled Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>-subjects with moderate to severe asthma subjected to treatment -subjects with mild asthma stabilized with high prednisone dose and treated to maintain asthma stability (Ahrens)</td>
<td>-simple and relatively easy -Crossover design possible to reduce variability</td>
<td>Large sample size due to shallow dose response</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shallow dose response and lengthy trial periods</td>
</tr>
<tr>
<td>Steroid reduction</td>
<td>Subjects with stable asthma on ICS have steroid dose reduction by 50% at definite intervals until asthma is not under control</td>
<td>-model good for comparing different ICS</td>
<td>50% dose reduction occurs - long duration - confounding by natural exacerbation possible</td>
</tr>
</tbody>
</table>
Figure 4-1. Recommendations for Bioequivalence of Inhaled Medications by BALR
### Trials Requirements for Bioequivalence of Inhaled Corticosteroids

<table>
<thead>
<tr>
<th>Therapeutic equivalence</th>
<th>Systemic exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Efficacy study)</td>
<td>(Safety profile study)</td>
</tr>
<tr>
<td>➢ Single (lowest possible) dose</td>
<td>➢ Single dose (upper dose limit)</td>
</tr>
<tr>
<td>➢ Placebo controlled</td>
<td>➢ Compare AUCn, AUCi, Cmax, Tmax, t1/2 and Kel</td>
</tr>
<tr>
<td>➢ Parallel design (preferred)</td>
<td>➢ (if plasma levels too low for quantification then)</td>
</tr>
<tr>
<td>➢ FEV1 and Eosinophil counts – PD markers</td>
<td>➢ PD study for serum cortisol</td>
</tr>
<tr>
<td>➢ Steroid naïve and mild asthmatics</td>
<td>➢ Single or multiple dose study</td>
</tr>
</tbody>
</table>

Figure 4-2. Health Canada Draft Guidance - Requirements for ICS Bioequivalence
Figure 4-3. EMEA guideline scheme for bioequivalence
CHAPTER 5
HOW MUCH DRUG IS AVAILABLE?

Introduction:

The ICS are intended to deliver the drugs in the lung where they exhibit their efficacy before being absorbed into the systemic circulation where they may exert their side effects. It would be very easy to demonstrate BE if one could obtain lung fluid samples (e.g., bronchial lavage) and measure the drug concentrations in the lung. Another approach would be to demonstrate that both inhalers have similar efficacy and safety when given at equal doses, which is the ultimate aim of demonstrating BE. The PD approach has difficulties as discussed in chapter 3 which make it an inefficient tool to evaluate BE. The shallow dose response curve with ICS is one of the main reasons for the problems with the PD approach. We hypothesize that the pharmacokinetic (PK) approach with suitable in vitro technique can be used to demonstrate the bioequivalence of ICS. The PK approach involves using the plasma concentrations as well some information from the in vitro tests to answer the bioequivalence question.

The questions that one needs to answer to demonstrate bioequivalence are – how much drug enters the lung, where is it deposited within the lung and how fast is it absorbed. The presence of mucociliary clearance as well as systemic absorption from lung necessitates studying how long the drug stays in the lung before being absorbed. In other words, the residence time of the drug from the two inhalers also needs to be compared. The question of how much goes into the lung arises because the dose delivered by the inhaler in one actuation is not the dose deposited in the lung. Some terms that are generally used while describing dose from inhalers are discussed below. Nominal dose is the dose delivered by the inhaler in one actuation as per the manufacturer’s claim. The ex-actuator dose is the dose which actually leaves the actuator in a single puff. There may be some drug loss in the spacer (if used). Most of the drug which enters
the mouth is impacted on the throat (oral cavity and oropharynx) and is swallowed (drug enters the GI tract). Only a small fraction of inhaled drug enters into the lung. This fraction of drug which is the effective dose deposited in lung is termed as respirable dose and can be expressed as a percentage of the nominal dose. This value is highly variable and generally ranges from 5-40% of the nominal dose. A very small fraction of this inhaled drug will be exhaled as well. The inhaler type, particle size generated from the inhaler, inspiratory flow rate, lung function, throat geometry, inhalation technique, use of spacer, inhaler priming are some factors that affect this fraction reaching the lung [112]. All the above factors related to the drug, inhaler and individual explain why the final amount reaching the lung could be very low at times as well as highly variable. We will discuss in sufficient detail in the following chapter about mucociliary transport process that exists in the upper or the central airways. This process can clear some solid drug particles out of the lung before they are dissolved in lung fluids and undergo absorption into systemic circulation. This is a critical phenomenon for slowly dissolving drugs. Latest developments in inhaler technology (eg. dry powder inhalers) have successfully attempted to increase the amount of drug reaching the lung. At the same time these inhalers have also decreased the effort in terms of coordination required on part of the patient during inhalation.

The plasma levels of ICS reflect the drug deposited and absorbed from the lung. This is due to the negligible (<1%) oral bioavailability of most of the ICS [86-89]. For drugs that display significant oral bioavailability one could use the charcoal block technique to estimate the drug absorption from the GI tract after being swallowed [84, 85]. We hypothesize that the plasma levels obtained by a PK study after inhalation could be used to successfully answer all the questions pertaining to establishing the BE of inhalation drug products. We use modeling and simulation technique to test our hypothesis. This would be a better way to test the feasibility of
conducting a PK study by simulating it first \textit{in silico} to get better understanding of the outcomes from such a study. The modeling and simulations were done with reference to fluticasone propionate. In this chapter, only AUC is used to answer this specific question of how much drug is available to the lung.

**Methods**

The data to develop a PK model following administration of ICS was obtained from a previously published study comparing the single dose and steady state pharmacokinetics and pharmacodynamics of inhaled FP and BUD in healthy volunteers [136]. The study was conducted in accordance with the revised Declaration of Helsinki and in compliance with good clinical practice guidelines. 14 healthy male volunteers completed this double-blind, double-dummy, randomized, placebo-controlled, 5-way crossover study. The subjects had a mean age of 26.4 years (range, 22-32), average weight of 72.7 kg (range, 62-85 kg), and average height of 179.2 cm (range, 172-187 cm). The five treatments consisted of 200 μg FP and 500 μg FP (both doses delivered via the Diskus® inhaler, 400 μg BUD and 1000 μg BUD (both delivered via the Pulmicort Turbohaler®, AstraZeneca), and placebo delivered via Diskus® and Turbohaler. The subjects were administered single doses of drugs at 8.00 AM on day 1 and then twice daily from days 2-5. Blood samples were collected frequently on day 1 and day 5. On the other days, days 2-4 only the trough samples were collected i.e. samples collected at 8.00 AM and 8.00 PM. The samples were analysed for drug, cortisol and lymphocytes. The details of this study can be found elsewhere [137]. Only the FP data for first 24 hours was used to develop the model.

**Population Pharmacokinetic (POPPK) Model:**

The population pharmacokinetic model was built using a step wise model building approach. The model was developed using the nonlinear mixed effects modeling software NONMEM VI (Globomax LLC, Hanover MD). The data was plotted to see any outliers as well
Model fits with one and two compartment body models with first order absorption were evaluated. The individual fits, populations fits, minimum objective function value, residual plots were used to determine the best fit model. The inter-individual variability (also known as inter-subject variability or between subject variability [BSV]) was modeled as exponential error model as equation 5-1

\[ P_{ij} = \theta_i \exp(\eta_i) \]  

(5-1)

where \( P_{ij} \) is the \( i \)th parameter for the \( j \)th subject. \( \theta_i \) is the \( i \)th population mean parameter and \( \eta_i \) is the random intersubject variability with mean \( \theta \) and variance \( \omega^2 \).

The residual error (also known as intra-subject variability or within subject variability [WSV]) was modeled as proportional error model as equation 5-2

\[ Y_{ij} = Y_{pij} \times (1 + \varepsilon_{ij}) \]  

(5-2)

In the above equation, \( Y_{ij} \) and \( Y_{pij} \) are the \( i \)th individual’s \( j \)th observed and model predicted concentration respectively. \( \varepsilon_{ij} \) is the random residual error with mean 0 and variance \( \sigma^2 \). First order conditional estimation (FOCE) was used for parameter estimation. Individual parameter estimates were estimated post hoc. The final model was bootstrapped using Wings for NONMEM developed by Dr Nick Holford to perform the internal validation of the model. The criterion to include a parameter (theta or eta) in the model was greater than 6.61 points reduction in the minimum objective function value within the hierarchal model. The likelihood ratio follows a \( \chi^2 \) distribution and hence with df=1, and \( P<0.01 \) level of significance a drop of 6.61 points would make the added parameter significant to be retained in the model.

Simulations

The above described POPPK model was used to simulate plasma profiles of FP following various scenarios of drug deposition in the lung. The different scenarios tested were combinations of different amount of drug being deposited in the lung as well as differing
variabilities associated with this amount. The pulmonary dose lies anywhere between 5-40 % of
the nominal dose (in some cases it maybe up to 60%). However, this amount is also accompanied
by huge variability of around 15-45% (in some cases it could be even higher) [111]. We
simulated various scenarios by assigning different amount of dose deposited by two inhalers
representing the innovator (brand or reference) and generic inhaler. The plasma profiles were
generated from these simulations. The area under the curve (AUC) for every subject is calculated
using the trapezoidal rule from these plasma profiles. The AUC is a measure of cumulative drug
exposure to the subject. The AUCs are log transformed and then the AUC ratios are calculated.
The 90% confidence interval (CI) is then calculated and compared as done in traditional BE
studies [138, 139]. The AUCs are said to be equivalent if the 90% CI of the ratio of the log
transformed AUCs is between 0.8-1.25 (or 80-125% when expressed as percentage). There exists
variability for all the parameters such as drug clearance, apparent volume of distribution, amount
deposited as well as drug absorption. We incorporated all the parameter variabilities as listed in
Table 5-1 while testing the different scenarios with this model. To ensure that we have enough
sampling for these variabilities, we simulated 200 runs for every scenario and then summarized
the BE results for these 200 runs. The process was repeated for every scenario. The entire
modeling and simulation process is depicted stepwise with a flow chart in fig 5-1. The trial was
simulated using NONMEM while the huge amount of data generated from these simulations was
analyzed by using the statistical software R.

Results

A two compartment body model was used to describe the plasma concentrations of FP
after inhalation. Literature references have demonstrated that FP exhibits flip-flop kinetics [140].
This phenomenon is observed when the absorption is the rate limiting step in drug disposition.
The prolonged absorption of FP is also of interest to study the residence time of FP and other
ICS which exhibit similar absorption profiles [141]. The flip-flop behavior of FP was incorporated into the model and fitted the data well. The residual plots and population and individual fits were observed to assess the goodness of fits. It can be seen from the plots in figures 5-2 and 5-3 that there is no significant bias in the data. The predicted population as well as the predicted individual concentration time plots fit the observed data very well. This model was further used to perform simulations under various scenarios. It must be remembered that the model assumed that the plasma levels reflected drug absorbed from lung only. This is a valid assumption since FP has less than 1% oral bioavailability [142, 143]. Consequently any drug swallowed during or post inhalation (due to mucociliary clearance) will not be reflected in plasma.

The spaghetti plots of simulated plasma profiles are presented in figure 5-4. The results from the simulations are present in Table 5-2. Let’s discuss first scenario where both inhalers deliver equal amount of drug (500 µg) in the lung with 30% variability. It can be seen from run #1 that with 24 subjects, 60% of the trials could establish equivalence. The equivalence is established if the 90% CI of the ratios of the AUCs of the generic and brand inhaler are within 80-125%. The considerable variabilities on various parameters necessitated more subjects in the study. Hence the same scenario was simulated with greater number of subjects. From run numbers 2-5 it can be seen that with increased subjects, the percentage of trials that could demonstrate equivalence improved and gave greater confidence in the outcome. With 40-50 subjects, equivalence could be established in more than 90% of trials. PK trials with scenarios that had greater variabilities on dose deposited in the lung were also simulated. As is evident from runs 6-9 with equal dose delivered to lung equivalence could be established in most cases with sufficient number of subjects in the study.
Another scenario where the generic inhaler delivered a different dose as compared to the brand inhaler to the lung was simulated. In this case, the generic inhaler delivered lower dose than the reference inhaler. Such a scenario could be attributed to a lot of factors such as larger aerosol particles, poor performance by the inhaler device. In this particular case, the generic inhaler was simulated to deliver 20% lower dose as compared to the reference inhaler. Runs 10-11 demonstrate that with 30-40 subjects majority of the trials fail to establish equivalence. In cases where there were actual significant differences between the doses deposited in the lung, increasing the number of study subjects would still result in failure to establish bioequivalence. In fact the difference would be easier to capture with an increased number of study subjects. This will result in greater confidence in the trial outcome.

**Conclusion**

From the above simulations we prove our hypothesis that AUC is a sensitive parameter to answer the question as to how much drug is available to the lung. This parameter can thus be used to compare the inhalers with respect to the amount delivered to the lung. There exists a theoretical exception to this for slowly dissolving drugs such as fluticasone propionate. One may argue that the regional deposition pattern may be adjusted with a lower or higher dose from generic inhaler and yet have comparable AUCs. Although highly unlikely, theroretically it may be possible to have such a scenario. This represents a special case and is discussed in detail in the next chapter and appropriate measures that could be employed in such scenario are proposed.

**Discussion**

ICS are the best way to deliver the corticosteroids to the site of action. Establishing bioequivalence with inhaled corticosteroids requires demonstrating equivalent dose deposition, comparable regional deposition and similar residence times with the innovator and generic inhaler. Each part of the above question represents a different challenge. The pharmacokinetic
approach seems to present a potential solution to this issue. In this chapter we discussed the PK approach to answer the first part about how much drug enters the lung. The simulations demonstrated that this answer could be successfully answered by using the plasma concentration time profiles of the drug from a PK study.

Such a pharmacokinetic approach utilizes the plasma levels which are not as invasive or uncomfortable as obtaining lung fluid samples. The assumption that the plasma levels reflects only drug deposited in lung is true for newer ICS like ciclesonide and mometasone as they have less than 1% oral bioavailability [144, 145]. ICS like beclomethasone and budesonide exhibit some degree of oral bioavailability (10-30%) [146, 147]. Concomitant oral administration of activated charcoal to prevent GI absorption is one approach to account for the swallowed fraction of dose. [85]. In this method, a charcoal suspension is administered to the subject. The drug that enters the GI tract is then adsorbed into charcoal and does not enter the systemic circulation. Another approach involves estimation of lung deposition after inhalation by correcting for an assumed oral availability. By simulating different scenarios, it can be seen that the question of how much drug goes into the lung can be successfully answered by this approach. The high variability may warrant a few more subjects as long as the amount of drug from two inhalers does not vary significantly. In cases with significant dose differences with high variability, the model identified correctly the bioequivalence to fail. The above observation is intuitively correct that if the same amount of drug is dispensed from two inhalers and only variability is high then one needs higher number of subjects to correctly identify whether differences exist.

The approach is similar to the traditional BE studies with oral dosage forms and can be completed over few days including the washout periods between crossover arms. There may be minor adjustments that one might make depending on the ICS drug characteristics (eg. charcoal
block). A big advantage with such approach is the use of healthy volunteers to demonstrate the BE unlike the pharmacodynamic approach where one cannot use healthy volunteers. Also the number of subjects required for such an approach is very small (30~50). The PD approach would require 100s and 100s of patients (depending on the study design), to be dosed over extended periods and yet fail to achieve any significant conclusion. We already discussed this in the 2nd chapter.

Research and studies have shown that there may be differences in the amount of drug reaching the lung in healthy volunteers and asthmatics. It seems obvious that in asthmatics the pulmonary obstruction will cause such a difference. Due to narrowed airways resulting from pulmonary congestion, most of the inhaled drug will be deposited in the central airways and not reach the peripheral region within the lung. Another reason suggested for such an observation is the drug characteristics. For example, such a difference in regional deposition is more marked and been reported for slowly dissolving drugs such as fluticasone propionate. The high lipophilicity of FP results in slow absorption from the lung. The mucociliary action which is predominantly active in the central airways clears the drug out of the lung which is either expectorated or swallowed. This results in an overall reduction in drug dose available in the lung. In the next chapter we discuss this issue and see how PK approach can be used to answer this question. A theoretically probable scenario for slowly dissolving drugs where AUCs may be inconclusive to answer the BE questions is also discussed in that chapter. Finally we will also discuss how PK approach could be used to answer the question of comparing the residence time of drug in the lung. Any approach should be devised with intent to provide quality drugs to the patients in a cost effective and timely manner.
Table 5-1. Parameters and their associated variability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>10%</td>
</tr>
<tr>
<td>Vd</td>
<td>10%</td>
</tr>
<tr>
<td>ka</td>
<td>30%</td>
</tr>
<tr>
<td>Flung</td>
<td>30%</td>
</tr>
<tr>
<td>Residual</td>
<td>20%</td>
</tr>
</tbody>
</table>
Table 5-2. Results from simulations

<table>
<thead>
<tr>
<th>run #</th>
<th># of subjects</th>
<th>% Variability on dose</th>
<th>% Dose-Generic</th>
<th>% BE Trials successful</th>
<th>% Dose-Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>30</td>
<td>500</td>
<td>60</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>30</td>
<td>500</td>
<td>78</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>30</td>
<td>500</td>
<td>90</td>
<td>500</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>30</td>
<td>500</td>
<td>95</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>30</td>
<td>500</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>35</td>
<td>500</td>
<td>87</td>
<td>500</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>35</td>
<td>500</td>
<td>99</td>
<td>500</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>40</td>
<td>500</td>
<td>74</td>
<td>500</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>40</td>
<td>500</td>
<td>94</td>
<td>500</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>30</td>
<td>400</td>
<td>4</td>
<td>500</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>30</td>
<td>400</td>
<td>1</td>
<td>500</td>
</tr>
</tbody>
</table>
Figure 5-1. Flow chart for modeling and simulation of trials

1. Develop PK model & validate it
2. Select scenario & # of subjects
3. Simulate 1 clinical trial
4. Estimate AUCs, C\text{\text{\text{max}}}, \text{MAT}, \text{MRT}
5. Compare reference & generic (80-125%)
6. BE - Yes / NO ?
7. Repeat trial 200 times (variability on parameters)
8. Summarize results

NONMEM
POP PKPD software

R
Statistical software
Figure 5-2. Goodness of fit plots. The open circles are the observed values and the lines are model fits.
Figure 5-3. Individual and population fits from the model
Figure 5-4. Spaghetti plots of plasma concentrations from simulations
CHAPTER 6
WHERE DOES THE DRUG DEPOSIT?

Introduction

The questions to be answered to demonstrate the BE of ICS are how much drug enters lung, where does it get deposited, how long does it stay there before being absorbed systemically. The previous chapter discussed how pharmacokinetic (PK) studies can assess how much drug is available to the lung. We also hypothesized that the PK approach can be used to answer the remaining questions. We discuss in this chapter the use of PK approach to answer where does the drug get deposited within the lung - central to peripheral (C/P) lung deposition ratio. Post inhalation, the ICS is deposited in the lung where it exhibits its efficacy. Eventually, the inhaled corticosteroids will either be absorbed into systemic circulation or will be cleared by mucociliary transport process [148, 149]. Attempts to capture and model such phenomenon are available in literature [10, 150]. A slow absorption process (consequently greater residence time) leads to prolonged exposure and may translate into better efficacy. Prior to its activity at the receptor level, there may be additional processes occurring such as drug release from the formulation and dissolution in the lung fluids prior absorption [151]. It has been known that there exists difference in the absorption rates of drugs from different regions in the lung. The absorption rate is higher in the alveolar region (AL) or the peripheral region as compared to the tracheo-bronchial (TB) region or the central region [5, 152]. Some other processes such as drug metabolism may also occur in the lung [153]. The drug may also be cleared by mucociliary transport from the lung after being deposited. These processes contribute collectively towards the duration of activity of ICS making it a complex phenomenon [8, 10, 151].
**Absorption and Muco-ciliary Clearance**

The mucociliary clearance and drug absorption are the two processes which predominantly affect the drug presence within the lung. These two processes thereby characterize how long the drug stays in the lung before being cleared from the lung or absorbed into the systemic circulation. The absorption of ICS occurs after the inhaled particles have undergone dissolution in the lung fluids. The mucociliary clearance is very slow in the peripheral region. This is well explained by the anatomy of the lung airways. In the central airways, the tracheal region along with the bronchial airways are ciliated and heavily lined by mucus producing goblet cells. The ciliary structures bathe and beat rapidly in a blanket of mucus layer. The epithelia in this region are covered by mucus, lipids, glycoproteins, inorganic salts and water [154]. Airway mucus humidifies the inhaled air. It also entraps particulate matter, bacteria, viruses, gaseous particles, and aerosols. The entrapped material is expectorated by the ciliary action thereby providing the lung a defense mechanism against foreign particulate matter. The TB regions have considerable mucociliary clearance that can clear the particles in few hours. However, such clearance mechanism may be affected by clinical conditions such as asthma, bronchitis and cystic fibrosis [155, 156]. While the drug is being cleared by mucociliary mechanism, drug absorption into systemic circulation also occurs simultaneously. The rate of absorption in the upper or central airways is small as compared to that in the lower or peripheral regions. The ciliated and mucus producing airway structures start disappearing as the bronchioles branch down further into terminal bronchioles and alveolar regions that are non-ciliated [4]. Consequently, the inhaled particles may be retained in the alveolar region for long periods of time (in days). At the same time the absorption from the alveolar regions is very high. The absorption rate from the peripheral compartment is twice as high as that from the central region [5, 6]. Hence, the inhaled drug particles (which are soluble in nature) will be absorbed before any significant clearance.
through mucociliary mechanism occurs in these peripheral regions of the lung. The rates of
dissolution, absorption and muco-ciliary clearance thus govern the amount of drug in the lung.
This is well explained with figure 4-1 that has been adapted from Edsbacker et al [7]. It can be
seen that some particles in the ciliated airways in the central region of the lung dissolve in the
lung fluids (mucus layer) and are absorbed, while some particles are cleared by the ciliated cells
before they are absorbed. Such a phenomenon is absent in the peripheral region.

The mucociliary clearance rates as well as absorption rates differ depending on the lung
region [8]. The mucociliary process may also be affected (slower) in subjects with lung diseases
[9]. It has been rightly mentioned that determination of mucociliary clearance is difficult to
quantify [10] and depends upon nature of particles deposited- soluble or insoluble particles.
Currently prescribed inhalation drugs can be considered to behave as soluble particles. This can
be construed such that the inhaled corticosteroids act at the receptor level at intra or extracellular
locations in the lung, or may also be retained in interstitial fluid before being ultimately absorbed
into systemic circulation. At the same time some drug may be cleared by mucociliary mechanism
[11]. The ciliary processes are predominantly active in the tracheo-bronchial region or the central
region of the lung. Studies in the past have attempted to estimate the mucociliary clearance rates
using radio-labeled particles such as liposomes, ferrous oxide particles, carbon particles, Teflon
particles as well as drug molecules. These studies have found that the tracer particles have a half
life ranging from 0.5 hours to 24 hours depending on whether they are deposited centrally or
peripherally within the lung [12-16]. It may take even days in some cases for the drug deposited
in deep areas of the lung to be cleared. However, since the ICS are soluble and absorbable drugs,
the drug deposited in the peripheral area of the lung will be absorbed before being cleared over
such a long period of time. Hence only rapid muco-ciliary clearance which occurs from the
central region of the lung would predominantly affect the amount of drug available for systemic absorption.

Models of varying complexity have been used to study such drug deposition profiles. For simplicity of modeling, the lung could be considered to be made up of two regions, the central and the peripheral regions. The deposition pattern depends upon airway caliber, particle size distribution of aerosol generated and the lung function. The larger particles tend to be deposited in the upper airways while the smaller particles can escape to deeper or peripheral regions within the lung. The suboptimal lung function in asthmatic population leads to more proximal drug deposition. The narrowed airways cause more of the drug to be deposited in the central airways than in peripheral airways. This has been explained graphically by Edsbacker et al in figure 6-1 [7]. Consequently the C/P ratio, which is the ratio of drug deposited centrally Vs peripherally, could vary in healthy and asthmatics. Ideally one would want to deliver uniform amount of drug throughout the airways since asthma is said to be disease of upper and lower airways. With more central deposition in asthmatics there is more drug available for mucociliary clearance than in healthy volunteers for the same given lung dose. Added to this if the drug was slowly dissolving drug such as fluticasone propionate then there could be noticeable drug removal by such mucociliary processes. Hence less amount of drug actually is available systemically in asthmatics which could reflect as lower AUCs. Such a difference in AUCs between healthy and asthmatics has been reported in literature for slowly dissolving drugs like fluticasone [157-159]. We hypothesize that pharmacokinetic approach can be used to study the differences in regional deposition of drugs as well as the residence time of the drug in lung.

As mentioned above, studies have also shown that there are differences in the drug distribution within the lung in healthy and asthmatic subjects. This is attributed to reduced
airway caliber in asthmatics. At the same time, the mucociliary clearance is decreased in the asthmatic population. The mucus viscosity increases and the ciliary processes are inhibited thereby lowering the drug clearance [155, 156]. Other factors such as the particle size distribution, device, nature of aerosol generated and individual inhalation technique also influence the regional drug distribution. The regional distribution of drugs is measured in terms of the C/P ratio or the central to peripheral ratio. The C/P ratio is calculated by dividing the amount of drug deposited in the central region by the amount of drug deposited in the peripheral region. Several studies have used radio-labeling techniques to determine the C/P ratio using various inhalers in healthy and asthmatic subjects with different drug molecules. These ratios range from 0.7 to 2 (or more) depending on the clinical condition of the subject, the device used, particle size distribution and method used in the study [12, 160-162]. One would want comparable deposition in the central and peripheral regions of the lung with preferably slightly higher deposition in peripheral airways. Such a deposition pattern would give a C/P ratio of 1 or less.

We hypothesized that the PK approach can be used to detect differences in the regional distribution of slowly dissolving ICS within the lung. Using population pharmacokinetic modeling techniques we test this hypothesis by employing PK trial simulations as demonstrated in previous chapter.

Methods

A population PK model was developed to describe the time profile of inhaled drug with the lung being divided into two distinct regions – central and peripheral. Data from 30 asthmatic subjects was used to build the model. The study details can be found elsewhere [163]. The primary purpose was to build a model that could be used for simulation purposes. Our model incorporates the drug clearance from TB region with no clearance occurring from peripheral
regions. The absorption rates in the central and peripheral regions were constrained to have two fold differences for reasons mentioned above.

The population model was developed and this model was used to simulate different trial scenarios by varying parameters such as mucociliary clearance rates, actual dose deposited, variability on dose deposited, and C/P ratios. Details about the different scenarios are mentioned later. Every trial was simulated 200 times to get enough sampling for the given parameter variabilities. The AUC and Cmax ratios were estimated for each simulation. The success or failure of every single trial was determined based on the AUC ratios. The population model was developed using NONMEM VI. NONMEM was also used for simulations of PK trials. The data obtained from simulations was processed using the statistical software R.

**Simulations**

To study the effect of different regional drug deposition patterns as well as changes in the mucociliary clearance values, we simulated trials by keeping one parameter such as mucociliary clearance constant at a time and then varying the regional deposition patterns and vice versa. Every trial was simulated 200 times to allow enough sampling for the given variability on all parameters. The results were also compared by simulating 500 times to see if that resulted in different outcome. It was observed that having 200 runs was a good number with negligible changes in the outcome with 500 runs except for increased computing and post processing times. The BE success or failure is calculated for every trial and then the number of runs that demonstrate BE are reported as a percentage of total number of trial runs.

The mucociliary clearance rates are discussed in the preceding section. Mucociliary clearance values of $K_{muc}$ of 0.5 /hr and 0.2 /hr were used for the simulations. With each of these mucociliary clearance values various deposition scenarios were simulated. These scenarios varied from more peripheral deposition to more central deposition. The changes in either
direction were in increments of 50 percent. When a majority of trials for a situation failed or passed, we checked this effect by increasing the number of subjects. The starting number of subjects for most of the cases was 30. This number was increased to 40 and eventually 50. In some cases, trials were simulated with 75 subjects to check how the results changed with additional subjects. Table 6-1 mentions the variability associated with various parameters in the model.

**Results**

The compartmental model is depicted in figure 6-2. There is negligible oral bioavailability for FP (< 1%), hence the plasma levels reflect the drug absorbed from the lung. Hence, the GI compartment was not included. Drug is available for inhalation from the central and the peripheral regions of the lung. Part of the drug deposited in the central region is cleared by mucociliary mechanisms while the remaining amount is absorbed into systemic circulation. Once absorbed, FP follows a two compartment model with rapid distribution into peripheral tissues. Ultimately the systemically available drug is cleared from the central compartment. These systemic levels are used to generate the plasma concentration time profiles for the reference and the generic inhalers.

Initially, the $K_{muc}$ of 0.5/hr and 30 subjects were used for the simulations. The C/P ratio was 0.82 which meant that about 45% of the drug entering the lung was deposited centrally while the remaining 55% of the drug was deposited in the peripheral regions of the lung. The C/P ratios for the generic inhaler were then altered to give either a more central or more peripheral deposition as compared to brand inhaler. The dose delivered by the two inhalers was kept constant. The regional deposition from the brand inhaler was also altered to test different scenarios. As we saw in the previous chapter that the high variabilities necessitated study with
more than 30 subjects, we also simulated the current study with 30 and 50 subjects. The results from these simulations are presented in table 6-2.

Few scenarios from these simulations are discussed. It can be seen from table 6-2, run #1 and 2, the brand inhaler deposited 45% drug in the central and 55% drug in the peripheral part of the lung. In the same run we have a generic inhaler that has similar deposition pattern. With 30 subjects 82% of trials could establish BE and with 50 subjects 98% trials could establish BE successfully. In run # 3 and 4 we have a generic inhaler that has predominant drug deposition in the central region of the lung. In such scenario, majority of the trials fail to establish BE. Intuitively this makes sense, since for the same total lung dose a greater portion is in the central part of the lung. Hence, the amount being cleared by mucociliary mechanism is greater as compared to brand inhaler and less drug is actually available for the lung. Hence inhalers should fail to demonstrate BE. Similarly when we have a generic inhaler that deposits more drug peripherally (run # 11 and 12), there is less drug available that can be cleared by mucociliary process as compared to the reference inhaler. In such cases we see that majority of the trials fail to establish BE. Similar observations were made with other C/P ratios with the brand inhaler. This confirms that for slowly dissolving drugs, AUC can detect differences in regional distribution pattern. The same process was then repeated with the mucociliary clearance rate of 0.2/hr. The results from those simulations are presented in table 6-3. Similar findings are observed with this mucociliary clearance rate. However, the sensitivity of differences in regional deposition decreases at lower mucociliary clearance rates. Greater than two fold differences in the regional deposition can be picked up from the AUCs.

Special Scenario

From the above simulation results we see that the AUCs are affected by the dose available and the regional deposition as well. A situation may arise where the dose deposited and the
regional deposition may be altered simultaneously. Such a scenario is theoretically possible but is highly unlikely practically. We simulated such scenarios and demonstrated that inhalers with lower respirable dose and more peripheral deposition have similar or comparable AUCs to inhalers with more respirable dose and more central deposition. The difference in dose is adjusted by differences in regional drug deposition. The respirable dose is the dose that reaches the lung post-inhalation.

The simulations for such special scenario are summarized in table 6-4. Run # 41 describes a generic inhaler that has more peripheral deposition (70%) as compared to the brand inhaler with only 50% peripheral deposition. Consequently with the same respirable dose, dose ratio of 1, the AUC with generic inhaler will be higher as seen (AUC generic/brand ratio -1.25). This is due to the fact that the brand inhaler has more drug deposited centrally that can be cleared and hence not available. We see that in run # 42, the dose ratio is 0.8 with the generic inhaler delivering lower dose with more peripheral deposition as compared to the brand inhaler. This demonstrates that the doses could be adjusted with different regional drug distribution to give similar AUCs. There are two solutions to this scenario. One way is to conduct a PK study in asthmatics. Since the asthmatics have predominant central deposition, the two inhalers when compared in asthmatics could help identify these differences. We simulated various trials to see what C/P ratios would give similar AUCs with different doses in healthy volunteers and asthmatics. The assumption for the simulation was that the asthmatics have predominant central deposition. We see from run # 48 that the generic inhaler would need to almost revert the deposition pattern to have comparable AUCs as brand inhaler in asthmatics. This seems highly unlikely that for the same generic inhaler the deposition patterns would be totally opposite. In
fact even in most cases with greater deposition in upper airways AUCs would not be similar as seen from runs 45 through 51.

Another way to overcome this scenario would be to do an *in vitro* test such as using cascade impactor and compare the respirable dose. This will let us know whether the dose available is different and one may not need to do the study in asthmatics. For slowly dissolving drugs, only the comparison of respirable dose should suffice. This may not be the case with fast dissolving drugs such as budesonide. For fast dissolving drugs the regional deposition may still need to be answered by a more detailed comparison of cascade impactor data. The primary reason being that the drug is absorbed fast before significant amount of drug is cleared through mucociliary process. However, most of the glucocorticoids including the new ones are slowly dissolving.

**Conclusion**

The PK approach could be used effectively to answer the question of where does the drug deposit in the lung, especially for the slowly dissolving drugs. In some scenarios where the dose and deposition pattern might be altered simultaneously appropriate tests could be conducted to tackle those situations. However such scenario though theoretically possible is highly unlikely practically. For fast dissolving drugs one needs to do a more detailed analysis of cascade impactor data. This is to compare the particle size distribution (PSD) of aerosol generated by the brand and generic inhaler. This is because we know that the PSD governs the regional deposition of the drug within the lung. Such an approach requires not more than 30-50 subjects. Regional drug distribution has been one of the main concerns about employing PK approach to evaluate the BE of inhalation drugs. PK data successfully addresses this concern for slowly dissolving drugs.
Discussion

The regional distribution for ICS is of importance since asthma involves inflammation of upper as well as lower airways. The aim with these drugs is not only to deliver drug to the lung but also achieve deposition throughout the lung. If the generic inhaler can demonstrate that it delivers comparable drug dose with similar regional deposition and that the drug stays in the lung for same period of time, one can confidently assume that the generic inhaler will demonstrate similar efficacy as the reference inhaler. Evaluation of differences in the regional deposition seems difficult by using the PK data. However, the differences in absorption rates, the slowly dissolving nature of most glucocorticoids and the presence of mucociliary clearance in upper airways can be effectively used to answer this question. Appropriate tests and studies can be used to supplement such PK study. The aim of such supplemental tests is to avoid remote possibilities of approving substandard product. The sensitivity of the PK approach is better than the clinical efficacy trial. The PK approach provides an effective tool that can better compare the brand and generic inhalers to reach a correct decision with regards to their bioequivalence in a timely and cost effective manner.
Table 6-1. Variability on various parameters in the simulated POPPK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>10%</td>
</tr>
<tr>
<td>V_d</td>
<td>10%</td>
</tr>
<tr>
<td>K_a</td>
<td>30%</td>
</tr>
<tr>
<td>C/P ratio</td>
<td>40%</td>
</tr>
<tr>
<td>K_muc</td>
<td>20%</td>
</tr>
<tr>
<td>F_lung</td>
<td>30%</td>
</tr>
<tr>
<td>Residual</td>
<td>18%</td>
</tr>
</tbody>
</table>
Table 6-2. Simulation results with Kmuc=0.5/hr. Same dose available through reference and generic inhaler. Slowly dissolving drugs such as FP

<table>
<thead>
<tr>
<th>run #</th>
<th># of subjects</th>
<th>Generic inhaler</th>
<th>% BE Trials successful</th>
<th>Brand inhaler</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central</td>
<td>Peripheral</td>
<td>C/P ratio</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>45</td>
<td>55</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>45</td>
<td>55</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>63</td>
<td>37</td>
<td>1.70</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>63</td>
<td>37</td>
<td>1.70</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>70</td>
<td>30</td>
<td>2.33</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>75</td>
<td>25</td>
<td>3.00</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>75</td>
<td>25</td>
<td>3.00</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>75</td>
<td>25</td>
<td>3.00</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>30</td>
<td>70</td>
<td>0.43</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>30</td>
<td>70</td>
<td>0.43</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>22</td>
<td>78</td>
<td>0.28</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>22</td>
<td>78</td>
<td>0.28</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>33</td>
<td>67</td>
<td>0.49</td>
</tr>
<tr>
<td>14</td>
<td>50</td>
<td>33</td>
<td>67</td>
<td>0.49</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>25</td>
<td>75</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Table 6-3. Simulation results with K_{muc}~0.2/hr. Same dose available through reference and generic inhaler. Slowly dissolving drugs such as FP.

<table>
<thead>
<tr>
<th>run #</th>
<th># of subjects</th>
<th>Generic inhaler</th>
<th>Brand inhaler</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central</td>
<td>Peripheral</td>
<td>C/P ratio</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>18</td>
<td>30</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td>19</td>
<td>50</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>21</td>
<td>30</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>22</td>
<td>50</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>23</td>
<td>30</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>24</td>
<td>50</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td>26</td>
<td>50</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td>27</td>
<td>30</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>28</td>
<td>50</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>29</td>
<td>30</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
Table 6-4. Simulation results for special scenario. Change in the drug dose and regional deposition pattern. Slowly dissolving drugs such as FP.

<table>
<thead>
<tr>
<th>run #</th>
<th>Generic Inhaler</th>
<th>Brand Inhaler</th>
<th>Dose Ratio</th>
<th>AUC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central</td>
<td>Peri</td>
<td>C/P Ratio</td>
<td>% BE trials success</td>
</tr>
<tr>
<td>41</td>
<td>30</td>
<td>70</td>
<td>0.43</td>
<td>7</td>
</tr>
<tr>
<td>42</td>
<td>30</td>
<td>70</td>
<td>0.43</td>
<td>84</td>
</tr>
<tr>
<td>43</td>
<td>80</td>
<td>20</td>
<td>4.00</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>90</td>
<td>10</td>
<td>9.00</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>60</td>
<td>40</td>
<td>1.50</td>
<td>0</td>
</tr>
<tr>
<td>46</td>
<td>65</td>
<td>35</td>
<td>1.86</td>
<td>1</td>
</tr>
<tr>
<td>47</td>
<td>70</td>
<td>30</td>
<td>2.33</td>
<td>22</td>
</tr>
<tr>
<td>48</td>
<td>75</td>
<td>25</td>
<td>3.00</td>
<td>80</td>
</tr>
<tr>
<td>49</td>
<td>80</td>
<td>20</td>
<td>4.00</td>
<td>76</td>
</tr>
<tr>
<td>50</td>
<td>87</td>
<td>13</td>
<td>6.69</td>
<td>16</td>
</tr>
<tr>
<td>51</td>
<td>90</td>
<td>10</td>
<td>9.00</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 6-1. Different central and peripheral deposition in healthy and asthmatics
Figure 6-2. Compartmental model for inhaled fluticasone propionate used for simulation
CHAPTER 7
HOW LONG DOES DRUG STAY IN LUNG?

Introduction

In the previous chapters, pharmacokinetic (PK) approach was used to evaluate how much drug is available to the lungs and where does the drug deposit in the lung. The final question is how long does the drug stay in the lung before being absorbed into the systemic circulation. This is important since the goal of asthma inhalers is local lung delivery of the glucocorticoids. With all other factors being held constant, the longer the drug stays in the lung the greater will be the efficacy. However, this would also mean that the drug might be cleared out of the lung by the mucociliary action before interacting with the receptors [11]. Once the drug molecule has dissolved and interacted with receptors, it will be absorbed into systemic circulation. Drug dissolution into lung fluids from deposited aerosol is a rate limiting step before systemic absorption occurs. This process of drug dissolution may be a characteristic of the drug. It may also be influenced by a slow release formulation. The dissolution of drug molecule from the formulation blend in the generated aerosol is directly influenced by the formulation excipients. With the mucociliary mechanism in lung, part of the drug may be cleared before being dissolved in the lung fluid. Consequently, there may be less drug and hence change in efficacy as compared to the reference product. Hence, one may argue that even formulation differences may affect the drug profile in terms of the residence times in the lung. While one may measure the amount of drug available in lung by using a metric such as the AUC as we saw in previous chapters, one also needs to show that the rate at which drug is available and the duration for which it stays in lung is same between products to establish BE. This relates not only to the efficacy but also the drug safety. Ultimately, one needs to evaluate if there are differences between the times that the drug stays in the lungs when delivered by brand or generic inhalers.
There are certain PK parameters that characterize the duration of time a drug resides in the body. The mean residence time (MRT) is such a PK parameter that describes the average amount of time a drug molecule resides in the body. MRT is thus influenced by the drug absorption and clearance. Mean absorption time (MAT) is another PK parameter that could be used to answer residence times for drug in the body. It describes the average time required for the drug to be absorbed into systemic circulation. To be more specific, MAT refers to the time a drug molecule spends at the site of absorption before being absorbed into kinetic space. MAT thus gives an estimate about the time the drug stays in the lung before being systemically available. For example, one may measure MAT after oral or inhalation route. MAT by any route can be calculated as difference of MRT of a drug following administration by that route and intravenous route as explained in equation 7-1.

\[ \text{MAT}_{\text{inhal}} = \text{MRT}_{\text{inhal}} - \text{MRT}_{\text{i.v.}} \]  

(7-1)

In the traditional bioequivalence (BE) studies for oral generics, one can find maximum plasma concentration \( C_{\text{max}} \) being compared among formulations. This can be understood well when one reads BE definition which requires equal rate and extent of absorption. The \( C_{\text{max}} \) term reflects the rate of absorption. This term could be used with inhalation drugs as well since for drugs with same dose, similar \( C_{\text{max}} \) would mean similar rate of drug being available for absorption from lung site. With inhalers that have similar respirable dose, similar \( C_{\text{max}} \) could demonstrate that the residence times of the drug in the lung are same. We wanted to test which of these terms would be better suited to evaluate the lung residence time of the drug in the lung. In other words, we wanted to test the sensitivity of these various parameters to answer the final question in evaluating the inhaler BE.
Methods and Simulations

The model used for in chapter 5 was used in this case as well. The details for model building can be found in the methods section of chapter 5. With the inhalation route the dose is delivered into the lung from where it is systemically absorbed. The absorption process involves the dissolution step before the drug interacts with the receptor and gets systemically absorbed. Hence the absorption rate constant is a hybrid constant that reflects the processes of dissolution as well as absorption. Once absorbed, the drug is explained by a two compartment body model. The model was simulated to generate plasma concentration time profiles in various trial scenarios. The simulated scenarios involved brand and generic inhalers with different absorption rates. As discussed above, the different absorption rates incorporate differences in dissolution rates as well. The population PK software NONMEM was used to run the trial simulations. Sufficient variabilities, as one might expect in a clinical setting were added to the model parameters. Table 5-1 lists the variabilities used for the simulations. Every scenario simulation was run 200 times to have sufficient sampling for these variabilities. The plasma profiles were processed using R software. Statistical moment analysis was used to calculate PK MRT. These MRT calculations can be explained by equations 7-2 to 7-4

\[
AUC = \int_{0}^{t} (Conc) \, dt \quad (7-2)
\]

\[
AUMC = \int_{0}^{t} (Conc \times \text{time}) \, dt \quad (7-3)
\]

\[
MRT = \frac{AUMC}{AUC} \quad (7-4)
\]

We see from equation 7-1 that to calculate MAT we need MRT data after intravenous drug administration as well. MAT is then the difference between the MRTs. Hence the model had three arms where the subjects were given drug by inhalation route, generic and brand, and by IV
bolus injection in the third arm. The MAT and MRT were compared similarly as the AUC and $C_{\text{max}}$ parameters. Essentially, MAT and MRT were log transformed and similar comparison made based on the limits of 0.8-1.25

**Results**

The model that was setup can be explained by figure 5-1. We see that there is an intravenous dose arm in addition to the inhaled dose arms. The results from these simulations are presented in table 7-1. The respirable dose available is same for both these inhalers. They have the same variability on all parameters as well. The only parameter that was altered was the absorption rate constant. As discussed above, changes in absorption rates may be seen due to changes in dissolution profile of the formulation as well. We see that for run # 52, with 30 subjects and similar absorption rates, 76% of the trials have equivalent AUCs. For the same scenario, more than 72% of the trials have equivalent $C_{\text{max}}$ values. 100% of the trials were equivalent with respect to MAT and MRT comparisons. These results are expected since the inhalers have identical properties with respect to dose and absorption rates. A bigger study size with 36 and 50 subjects was simulated in runs 53 and 54 respectively by keeping everything else same as in run # 52. We now have a higher percentage of trials having AUCs and $C_{\text{max}}$ equivalent. Subsequent study scenarios were simulated with 36 subjects as we determined from run #2 that they had sufficient power. However for this particular question we were more interested in evaluating the $C_{\text{max}}$, MAT and MRT and hence we now focus only on these parameters in this chapter.

In run # 55, the absorption rate ratio is 2 and we see that none of the trials had equivalent Cmax or MAT. Around 40% of trials still show similar MRT. In runs 56 and 57, MRT is equivalent in all the trials despite different absorption rates for the two inhalers. The Cmax and MAT on the other end have most of the trials failing. The sensitivity of MRT to detect
differences was poor. Small changes in absorption rates do not affect MRT significantly as MRT is a parameter that is influenced by the drug absorption and drug clearance. Consequently with drug clearance remaining unchanged, MRT is not affected significantly with small changes in MRT. MAT was also not reliable in cases where absorption rates differed. These parameters do not give us any better comparison than Cmax which could still be used successfully as it correctly failed to show any equivalence when the absorption rates differed. Estimation of MAT requires an intravenous drug dosing arm in the study. This results in increased time and cost of study with no added benefit to the outcome.

**Conclusion**

The residence time of the drug in the lung (and the rate of absorption) could be compared using the traditional PK parameter $C_{\text{max}}$. Parameters such as MAT and MRT are not reliable in evaluating inhalers to answer the question of how long does the drug stay in lung (how soon is it available). $C_{\text{max}}$ can be used successfully to answer that question. Around 30-50 subjects are sufficient to compare this parameter. One needs no intravenous study to estimate the $C_{\text{max}}$ values. With all questions being answered, we propose a BE study design for inhaled glucocorticoids in the following chapter.

**Discussion**

The residence time of drug in the lung is influential factor in the drug efficacy. The longer a drug stays in the lung, the greater will be the efficacy. A slower release will lead to better lung targeting. One needs to remember that the mucociliary transport that exists in the upper airways could affect the amount of drug available if the release is very slow by removing the drug before it dissolves and interacts with the receptors. The drug lipophilicity is also of consequence since post release from formulation, the drug needs to dissolve in the lung fluids. For immediate release systems, the formulation blend will affect the drug release. Lactose is a commonly used
excipient. However, differences in the manufacturing process could affect the drug release profile. The results from simulations in this chapter suggest that $C_{\text{max}}$ is a better parameter to compare inhalers for the residence times. $C_{\text{max}}$ also is important in terms of drug safety. This brings up an entire discussion of the limits needed for $C_{\text{max}}$. While $C_{\text{max}}$ may be of critical importance for certain drugs, the equivalence limits could be widened for drugs that have broad therapeutic index. Such wider limits could be employed for inhaled corticosteroids. This is especially true in case of highly variable drug products.
Table 7-1. Simulation results for inhalers with different absorption profiles. Slowly dissolving drugs such as FP

<table>
<thead>
<tr>
<th>run #</th>
<th># of subjects</th>
<th>$K_a$-Generic</th>
<th>$K_a$-Brand</th>
<th>$K_a$ ratio</th>
<th>Parameters with their % equivalent trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>30</td>
<td>0.21</td>
<td>0.21</td>
<td>1.0</td>
<td>AUC</td>
</tr>
<tr>
<td>53</td>
<td>36</td>
<td>0.21</td>
<td>0.21</td>
<td>1.0</td>
<td>C_{max}</td>
</tr>
<tr>
<td>54</td>
<td>50</td>
<td>0.21</td>
<td>0.21</td>
<td>1.0</td>
<td>MAT</td>
</tr>
<tr>
<td>55</td>
<td>36</td>
<td>0.21</td>
<td>0.11</td>
<td>2.0</td>
<td>MRT</td>
</tr>
<tr>
<td>56</td>
<td>36</td>
<td>0.21</td>
<td>0.15</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>36</td>
<td>0.21</td>
<td>0.28</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>36</td>
<td>0.21</td>
<td>0.42</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>50</td>
<td>0.11</td>
<td>0.21</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>0.31</td>
<td>0.21</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>50</td>
<td>0.42</td>
<td>0.21</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>
Figure 7-1. Compartmental model scheme for inhaled and intravenous fluticasone propionate used for simulation
CHAPTER 8
STUDY DESIGN

Introduction

The aim of conducting a bioequivalence study is to determine whether the generic formulation could be switched for the reference or branded formulation. This is to ensure that the generic formulation has similar efficacy and safety as that of brand product. If the generic and brand formulation have similar rate and extent of drug availability at the site of action. We saw that this may not be always possible (feasible) for example in case of inhaled medications.

Alternatively one may argue since they need to be equally effective, one can conduct an efficacy comparison study. While this may be possible with certain class of inhaled medications like bronchodilators, it is not so for inhaled glucocorticoids. Shallow dose response curve and individual corticosteroid sensitivity are the main reasons that disable use of such efficacy studies.

Consequently one needs to look for other tools. We realize that the main questions that need to be answered are as follows

- How much is deposited in the lung? How much is absorbed orally (only relevant for drugs with significant oral bioavailability)?
- Where is it deposited (central vs. peripheral lung)?
- How fast is it absorbed?

If two inhalers deliver similar amounts of drug with similar regional distribution as well as similar drug dissolution and absorption profiles, we could say that these inhalers are equivalent. Of course one needs to ensure that the inhalers have similar safety profiles as well. We saw in the previous chapters that the pharmacokinetic (PK) tools have the ability to answer these questions relevant to establishing BE of inhaled corticosteroids (ICS). The systemic exposure of the drug could be used to estimate systemic safety of the inhaled drug. We also saw that in vitro tests such as using cascade impactor data may be needed to answer certain special scenarios (that
may be theoretically possible) or for some drugs which are rapidly absorbed into systemic circulation (for example budesonide).

**Bioequivalence study design**

With all questions being answered we propose a study design that could be employed to evaluate inhalers for BE. The flow chart is presented in figure 8-1 which details the path one could follow to evaluate generic inhalers. To begin with, one needs to perform a PK study with brand and generic inhalers in healthy volunteers. For drugs with significant oral bioavailability (e.g. budesonide or beclomethasone), one could employ the charcoal block method. The details of using charcoal block method have been discussed and can also be found in literature [85]. As charcoal adsorbs the drug and prevents its absorption from GI tract, one can estimate the oral bioavailability of the drug by administering the drug with and without charcoal. However most of the newer corticosteroids have negligible oral bioavailability (<1%) and hence don’t need charcoal block [86-89]. With crossover design, sufficient washout period should be included between various study arms.

The AUCs and Cmax should be compared between the two inhalers. If any of these parameters differ, this means that the inhalers are not equivalent. If they are equivalent then one moves down the decision line to perform further tests. The further tests depend on the type of ICS being compared. Following discussions from previous chapters and the figure 8-1 we demonstrated that if the drug is slowly dissolving such as fluticasone propionate, similar AUCs would mean similar dose and regional drug deposition within the lung. We also saw that one may argue that (although practically unlikely) theoretically this could also be seen with different regional distribution pattern by adjusting the drug dose from the inhaler. In such specific scenario, one needs to conduct an *in vitro* study and compare the respirable dose delivered by the two inhalers. If the respirable doses are not the same, then these inhalers are not equivalent. With
the respirable dose being the same, it can be said with sufficient confidence that the dose available and the regional distribution is the same for the two inhalers. This would mean that the two inhalers are bioequivalent.

There is another approach that one could use in this special scenario. A PK study to compare inhalers in asthmatics can be conducted. We discussed previously in chapter 4 that the drug aerosol deposition is more central in asthmatics. The lung congestion causes most of the drug to be deposited in the upper airways. If we had a generic inhaler with more peripheral deposition as compared to brand inhaler then we from run # 42 in table 6-4 we saw that a lower dose (approx 80% of dose compared to the brand inhaler) from generic could give similar AUC as brand inhaler. This scenario had 70% of drug deposited in peripheral areas with generic inhaler. One could only expect such high peripheral deposition with large fraction of particles in 1-3 um range and only in healthy volunteers with good airway function. If the same inhalers were then administered to asthmatics we would see a deposition pattern similar to runs # 50-52 (add simulation). With most of the aerosol being deposited in upper airways with both inhalers, a lower dose from generic inhaler would cause most studies to fail when the AUCs are compared. The simulations showed that more than 90% of trials fail to show equivalence. Also it is difficult to see such a huge turnaround of regional deposition with the same inhaler in healthy and asthmatic subjects. Hence one might compare the ratio of AUCs in healthy and asthmatics of generic inhaler with that of brand inhaler. If they are similar then there are negligible chances that the inhalers differ in the dose delivered and/or regional drug deposition within the lung.

If the AUCs and Cmax are same in healthy volunteers and the drug is fast dissolving, one can only say that the amount available to the entire lung is the same and the rate at which it is absorbed is same. One still needs to compare the regional drug deposition within the lung. The
respirable dose data from cascade impactor will not be of much help in this case. The drug aerosol particle size determines where exactly will it deposit within the lung. Smaller particles will tend to have deep (or peripheral) deposition with larger particles being deposited more centrally. The very large particles (>6-10 um) will not enter the lung and be impacted on the throat and swallowed. One needs more detailed analysis of the cascade impactor data to have a better comparison of the particle size distribution (PSD) profiles for the two inhalers. If the cascade impactor data suggest differences then, the inhalers are to be considered not equivalent. In case of similar PSD profiles, one can conclude that the inhalers are equivalent.

We have presented a detailed study design (figure 8-1) that could be employed for bioequivalence studies with inhaled corticosteroids. This design takes into consideration the lipophilicity of the drug. It also has appropriate tests recommended whether in vitro or in vivo in asthmatic population. We see that the PK study needs a single dose and then measuring plasma levels over 24 hours. This is to be followed by a washout period and then administering the other inhaler. In certain cases, a charcoal block arm may be included in the study. The safety profile of these inhalers can be estimated directly from the systemic exposure.

This modeling and simulation approach has helped us test the feasibility of using PK tools to evaluate inhaler BE. Such an approach helps understand the trial outcomes before actually conducting the trial. Various scenarios can be simulated in silico by using pharmaco-statistical models. These models include all relevant information from pre-clinical and clinical studies. Such models utilize information such as plasma concentration, drug effects and side effects, patient characteristics and other information which may lead to better prediction of the study outcome. Better understanding of these outcomes through such models can help improve clinical trial designs. Data from well designed trials could in turn help develop models with greater
predictive power. This learn and confirm cycle has been described in literature [164]. This kind of model based drug development has been gaining popularity in the industry [165, 166]. The probability of success of a trial can be estimated using these pharmacometric simulations and help in making go/no-go decisions in drug development programs. The approach saves time and resources by avoiding unnecessary trials and offers better chances of success by better trial design. This approach has also been supported by the FDA through the Critical Path Initiative [167].

We demonstrated that the PK approach in conjunction with appropriate in vitro tests can be effectively used to establish the BE of ICS. Such an approach needs few healthy subjects (30-50) and can be completed over a period of days. It is better suited to detect differences in the inhalers as compared to the lengthy and inconclusive efficacy trials. The ultimate aim of any bioequivalence test is to ensure that when a switch is made between the generic and the brand product, the patient receives the similar efficacy and safety from use of either product. For this to be possible the test must be able to distinguish between two products. In case of inhaled corticosteroids, the PK approach possesses the required potential.
Figure 8-1. Study design to conduct bioequivalence trials for inhaled corticosteroids
APPENDIX A
NONMEM CODE FOR PK MODEL OF FP AFTER INHALATION

$PROBLEM FP PK
$INPUT ID ACTI TIME AMT MDV DV EVID CMT
$DATA fpdata.csv IGNORE=C
$SUBROUTINE ADVAN6 TRANS1 TOL=3
$MODEL
COMP=(DEPOT,DEFDOSE);LUNGS
COMP=(CENTRAL);PLASMA
COMP=(PERIPH);PERIPHERAL

$PK
TVF1=THETA(1)
F1=TVF1*EXP(ETA(1))
TVCL=THETA(2)
CL=TVCL*EXP(ETA(2))
TVVC=THETA(3)
VC=TVVC*EXP(ETA(3))
K20=TVCL/TVVC
K12=THETA(4)*EXP(ETA(4));
K32=THETA(5)*EXP(ETA(5))
TVK23=(K12+THETA(6));
K23=TVK23*EXP(ETA(6))
SC=VC; OUTPUT IN ng/ml

$ERROR
IPRED=F
IRES=DV-IPRED
DEL=0
IF (IPRED.EQ.0) DEL=1
IWRE=(1-DEL)*IRES/(IPRED+DEL)
Y=F+F*ERR(1);

$DES
DADT(1)=-K12*A(1)
DADT(2)=K12*A(1)-K20*A(2)-K23*A(2)+K32*A(3)
DADT(3)=K23*A(2)-K32*A(3)

$THETA
(0.05,0.3,0.6);Pulmonary deposition
(70 FIXED);CL
(25 FIXED);VC
(0.01,0.1,2);K12
(0.01,0.1,10);K32
(0.01,0.1,10);K23
$\text{OMEGA} \\
(0.01);\text{INH} \\
(0.01);\text{CL} \\
(0.01);\text{VC} \\
(0.01);\text{K12} \\
(0.01);\text{K32} \\
(0.01);\text{K23} \\
$

$\text{SIGMA} \\
(0.04);$

$\text{ESTIMATION METHOD}=1 \text{ SIGDIGITS}=3 \text{ INTERACTION MAXEVAL}=9999 \text{ PRINT}=0 \text{ POSTHOC} \\
\text{TABLE ID TIME EVID CMT IPRED IWRE IRES CL VC K20 F1 NOPRINT ONEHEADER FILE}=\text{sdtabfp1}$
APPENDIX B
R CODE FOR AUC AND CMAX CALCULATION

area<-read.table("sdtaprob10",header=F,sep=' ')
names(area)<-c("ISIM", "ID", "TIME", "EVID", "CMT", "OCC", "IPRED", "DV")
head(area)
area$NEW<-(area$ISIM*1000+area$ID)
area$PROD<-(area$DV*area$TIME)
area0<-area[area$OCC==0 & area$CMT==3 & area$EVID==0,]
head(area0)
isim0<-area0[!duplicated(area0$NEW),]
tail(isim0)

area1<-area[area$OCC==1 & area$CMT==3 & area$EVID==0,]
head(area1)
isim1<-area1[!duplicated(area1$NEW),]
tail(isim1)

AUC <-function (data, time = "TIME", id = "ID", dv = "DV")
auc0<-AUC(data=area0,id="NEW",time="TIME",dv="DV")
auc0$loggen0<-log10(auc0$AUC*10)
head(auc0)
tail(auc0)
auc1<-AUC(data=area1, id="NEW",time="TIME",dv="DV")
auc1$loggen1<-log10(auc1$AUC*10)
head(auc1)
tail(auc1)
aucmerge<-merge(auc0,auc1, by="NEW")
aucmerge$aucdiff<-(aucmerge$loggen0-aucmerge$loggen1)
head(aucmerge)
tail(aucmerge)

Tmax<-function (data, id = "ID", dv = "DV", time = "TIME")
Cmax<-function (data, id = "ID", dv = "DV", time = "TIME", isim="ISIM", new="NEW")
tmax0<-Tmax(data=area0, id="NEW",time="TIME",dv="DV")
tmax1<-Tmax(data=area1, id="NEW",time="TIME",dv="DV")
cmax0<-Cmax(data=area0, id="NEW",time="TIME",dv="DV", isim="ISIM")
head(cmax0)
tail(cmax0)
cmax1<-Cmax(data=area1, id="NEW",time="TIME",dv="DV", isim="ISIM")

108
head(cmax1)
tail(cmax1)
cmerge<-merge(cmax0,cmax1,by="NEW")
head(cmerge)
tail(cmerge)
cmerge$cratio<- (log10(cmerge$DV.x)-log10(cmerge$DV.y))
head(cmerge)
tail(cmerge)

AUMC <-function (data, time = "TIME", id = "ID", dv = "PROD")
aumc0<-AUMC(data=area0,id="NEW",time="TIME",dv="PROD")
aumc1<-AUMC(data=area1, id="NEW",time="TIME",dv="PROD")
aumcmerge<-merge(aumc0,aumc1,by="NEW")
bigdata<-merge(aumcmerge,aucmerge, by="NEW")
set<-merge(bigdata,cmerge, by="NEW")

set$mrt0<-(set$AUMC.x/set$AUC.x)
set$mrt1<-(set$AUMC.y/set$AUC.y)
Myfunc3 <- function(x) {10^(mean(x)-0.3052*sd(x))}
Myfunc4 <- function(x) {10^(mean(x)+0.3052*sd(x))}
LIST OF REFERENCES


27. Hamid Q, Song Y, Kotsimbos TC, Minshall E, Bai TR, Hegele RG and Hogg JC (1997) Inflammation of small airways in asthma. Journal of Allergy and Clinical Immunology 100: 44-51


35. Thorsson L E. S., Conradson TB (1994) Lung deposition of budesonide from Turbuhaler is twice that from a pressurized metered-dose inhaler P-MDI. The European Respiratory Journal 7: 1839-1844


42. Langdon CG and Thompson J (1994) A multicentre study to compare the efficacy and safety of inhaled fluticasone propionate and budesonide via metered-dose inhalers in adults with mild-to-moderate asthma. British journal of Clinical Research 5: 73-84


52. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP and Sterk PJ (1999) Clinical control and histopathologic outcome of asthma when using airway...


75. Harris JB A. R., Milavetz G, et al. (1986) Relative potencies and rates of decline in effect of inhaled albuterol (A) and terbutaline (T). Journal of Allergy and Clinical Immunology 77 (Suppl): 147


85. Thorsson L, Edsbacker S and Conradson TB (1994) Lung deposition of budesonide from Turbuhaler is twice that from a pressurized metered-dose inhaler P-MDI. The European Respiratory Journal 7: 1839-1844


98. Howarth PH (1997) What is the nature of asthma and where are the therapeutic targets? Respiratory Medicine 91: 2-8


121


129. EMEA (2007) Guideline on the requirements for clinical documentation for Orally inhaled products (OIP) including the requirements for Demonstration of therapeutic equivalence between two inhaled Products for use in the treatment of asthma and chronic Obstructive pulmonary disease (COPD). CPMP/EWP/4151/00 Rev 1:


BIOGRAPHICAL SKETCH

Navin Goyal was born in 1980 in Pune, India. He completed his Bachelors in Pharmacy from Maharashtra Institute of Pharmacy in Pune in 2002. He worked for Torrent Pharmaceuticals for a year. He then joined Nicholas Piramal India Ltd, another pharmaceutical company where he worked for a year before joining the graduate program in pharmaceutics in August 2004 at University of Florida.