



CHAPTER 1

INTRODUCTION

Molecular biological research has contributed significantly to the identification of new therapeutic targets. This has resulted in an explosive growth in the number of peptide and protein drugs derived from both recombinant technology and conventional peptide drug design. By 2001, the United States Food and Drug Administration reported 417 biotechnology based dosage form in some stage of review or approval. Protein drug pharmaceutical sales currently reach \$15 billion annually. The predicted market will reach \$25 billion annually by 2005. Most of these new dosage forms are fragile protein molecules that require special formulations and processing to produce usable drug products with a marketable shelf life.

However, there are problems with protein drug dosage forms. The route of administration greatly affects whether a protein drug product will produce the desired therapeutic effect or not. Low membrane permeability, inadequate stability, potential safety issues and relatively short half-lives of many of these protein and peptide therapeutics limits their potential since they can only be administered by injection. Development of suitable non-injectable routes of administration (e.g. low cost, reproducible and safe) could significantly enhance patient compliance thereby increasing the benefit to be derived from this novel therapeutics. This has led to investigation of a variety of routes [1-5] and a multitude of approaches to 'package' peptides and proteins to circumvent potential delivery issues (e.g. stability, membrane permeability, clearance).

However, an inability to achieve adequate delivery of many of these peptides and proteins via conventional routes (e.g. oral, nasal, transdermal) even in the presence of agents designed to 'enhance' membrane permeability [6,7] together with the observations that many peptides and proteins are relatively well absorbed when delivered by the lung has provided the impetus for further evaluation of the airway as a route for systemic delivery [5, 8-22]. Successful development of approaches for delivery of protein and peptide therapeutics via the airways requires an understanding of the following:

- (1) The barriers to absorption presented by the respiratory tract (e.g. geometry, morphology of the cells comprising the air-blood interface, mucocilliary and enzymatic clearance mechanisms),
- (2) Methodological approaches for evaluating lung delivery and absorption and potential limitations of these models,
- (3) Advances in formulation development designed to address the inherent instability of these molecules, and
- (4) Progress in device design that will enhance reproducibility and efficiency of delivery to the lung thereby optimizing absorption and reducing variability.

The respiratory tract has several unique features which make it an attractive site for peptide and protein drug delivery including:

- (1) A large surface area which can be exposed to drug almost simultaneously as opposed to the intestine which has a similar total surface area but does not allow for simultaneous exposure.
- (2) A high blood flow which does not directly expose absorbed drug to the clearance mechanisms present in the liver, and
- (3) Relatively less metabolic activity. The upper respiratory tract including the trachea and large bronchi have a relatively limited surface area for absorption compared to the alveolar region which provides more than 95% of the surface area of the lung [23]. These results suggest that systemic absorption of peptides and proteins occurs in the alveolar region of the lung but the site of action with local effects may be in either the alveolar region or in the larger airways.

It is more necessary to understand the barriers involved in the efficient delivery of peptides and proteins to the lung, mechanisms involved in transport of peptides and proteins across the respiratory tract epithelium, examples of molecules which have been or are being developed for local or systemic delivery via the lung and a brief overview of future directions.

Barriers to efficient delivery and absorption from the respiratory tract:

There are a number of barriers to the efficient delivery of drugs to the respiratory tract including geometry of the airways. Morphology of the airway epithelial cells and clearance mechanisms, which are present in the respiratory tract (Figure 1.1). Successful development of therapeutics for administration by the respiratory route requires an understanding of these barriers to allow rational design of the aerosol formulation and device to achieve optimal delivery.

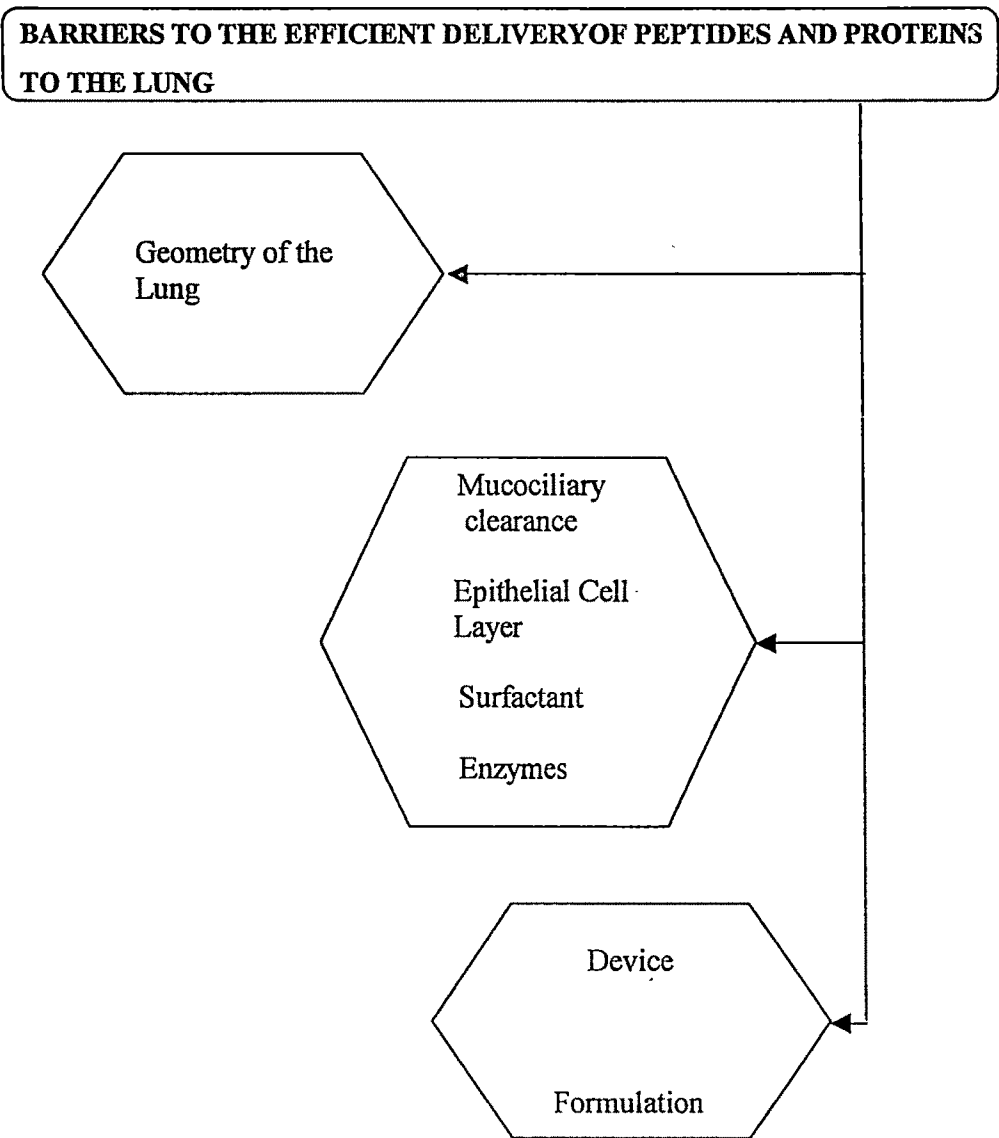


FIGURE 1.1

Aerosol can be delivered to the respiratory tract by either nasal or oral administration. However, the passages leading to the lower lung from the nasal region are narrower than the oral passages. This allows for much more efficient filtration and much less efficient delivery following nasal administration and has led to the development of a variety of devices that deliver aerosols via the mouth [24]. Inertial impaction, sedimentation and diffusion that occur following administration dictate efficient deposition of aerosol [25]. The greater the momentum a particle has, the greater the chance for deposition in the larger airways due to the inability of the particle to follow changes in airflow direction at bifurcation. Thus, particle size and particle velocity can be critical in the efficient delivery of aerosols. For particles greater than 8 μm , more than 50% will be deposited in the oropharynx and the bifurcation between the large central airways. Particles with diameters less than 3 μm are deposited in the smaller conducting airways and alveolar region by sedimentation, whereas particles with diameters of 0.5 μm or less do not sediment but are deposited by diffusion.

The respiratory tract is lined on its luminal surface by a layer of columnar epithelial cells that become progressively less columnar in the smaller airways and alveolar region. The surface of the airways epithelial cells in the larger conducting airways is covered with cilia, which aid in the clearance of material from the lung. Two types of epithelial cells are present in the alveolar region of the lung: the Type I and the Type II pneumocytes. Type I cells are flat cells with broad, thin extensions coverings up to 95% of the alveolar surface whereas Type II cells are cuboidal cells without extensions which can differentiate into Type I cells and participate in the repair of the epithelial cell surface following damage.

The alveolar epithelium is assumed to be the site of absorption of peptides and proteins delivered via the lung based on surface area considerations and the limited data available which suggests low permeability of the larger airways (e.g. trachea) to proteins [26-29]. Within the alveolar region, the epithelial cells are most likely the major barriers to absorption of peptides and proteins following aerosol administration. Schneeberger and coworkers have provided morphologic evidence that circulating proteins enter the interstitium to the level of the alveoli [30,31]. These data support the hypothesis that the epithelium is rate limiting for peptide

and protein absorption. Additional evidence for this hypothesis is provided by physiological studies which demonstrate that the movement of injected solutes into the interstitium occurs more rapidly than permeation across the alveolar epithelium and results indicating that the equivalent pore size of the junction between alveolar epithelial cells is between 0.6 and 1 nm, while the pore size between the underlying endothelial cells is between 4 and 6 nm [32]. Type I cells have been shown to have endocytic vesicles which have been postulated to be involved in the absorption of peptides and proteins.

Mucociliary clearance provides another barrier to the efficient delivery of therapeutic agents to the lung [33]. Mucociliary clearance is coordinated by the cilia beating to move the overlying mucus layer toward the mouth. Ciliated epithelial cells are most abundant in the tracheal region and decrease in the subsequent airway generations to approximately 15% in the fifth generation [34]. In the alveolar region, the epithelial surface is covered with a complex mixture of lipids and proteins produced by Type II pneumocytes, lung surfactant. Alveolar surfactant is stored in lamellar bodies and secreted into the alveolar lumen where it functions to reduce surface tension. Several studies have described the bi-directional flux of surfactant between the alveolar lumen and Type II pneumocytes [35-37] although the role of Type I cells, Clara cells and other parenchymal cells in the clearance of surfactant from the alveolar surface is not known [37-39]. Pulmonary surfactant is an important factor in the clearance mechanisms for inhaled particles. Particles, which escape the mucociliary clearance mechanisms in the lung, may also be efficiently removed by alveolar macrophages through phagocytosis.

For delivery of therapeutic proteins and peptides, it is also necessary to consider the potential for degradation by the proteases and peptidases which exist in the lung and which may dramatically reduce the efficiency of delivery following aerosol administration [40].

It is apparent that opportunity for systemic delivery as well as efficient local delivery of peptides, proteins and genes exist, from the preceding discussion. As our understanding of the barriers involved in efficient delivery/absorption of

molecules increases, the number of development candidates will increase. A major hurdle for continued progress with pulmonary delivery will be the successful development of a stable formulation/device combination that provides efficient delivery to the appropriate region of the lung at pharmacologically relevant doses. Following the appropriate pharmaceutical development, demonstration of safety and efficacy in man will then need to be evaluated. With the rapid progress which has been demonstrated thus far, it is apparent that development of peptide / protein delivery systems will continue to increase in the future.

The aim of this research work was to successfully deliver macromolecular drugs like insulin (5,800 Dalton) and calcitonin (3,400 Dalton) through the pulmonary route in the form of dry powder.

Insulin and calcitonin were selected as model polypeptide drugs, because of their large molecular size, well established method of analysis, great therapeutic utility and known pharmacokinetic and pharmacodynamic parameters.

RESEARCH ENVISAZED

Various classes of absorption promoters, which are known to be comparatively safer, shall be employed to promote their pulmonary absorption to obtain enhanced systemic bioavailability. It was hypothesized that one or more absorption promoter may be used for achieving the required flux of the selected drug through the alveolar membrane to transfer complete dose of the drug. Absorption promoters enhance the absorption by different mechanisms. Combination of promoters may enhance the drug absorption synergistically and the bioactivity of the drug may increase manifold leading to requirement of smaller concentrations of these promoters to achieve the same extent of bioavailability. It may reduce the required dose of drug by increasing the pulmonary bioavailability. Use of absorption promoters in small concentrations is expected to reduce or eliminate the toxicities associated with them at higher concentrations.

By formulating in the form of dry powder the stability of the selected protein drugs increased and it can be possible to deliver using inhaler devices for absorption via the pulmonary route.

By controlling the particle size of the drug and carrier of the dry powder formulation the deposition of the drug may be increased to avoid the drug losses during delivery. The deaggregation of the dry powder inhalers may be improved by altering the flow properties of the powder resulted into increased deposition of drug at the site of absorption in the lung. Higher in deposition will reduce the dose of drug and adverse effects.

Hence, plan of work was proposed

1. To review the available literature on the pulmonary route for the delivery of peptides and proteins.
2. To select suitable method for the estimation of insulin and calcitonin in formulations and biological fluids.
3. To prepare insulin and calcitonin formulations for intratracheal instillation using various absorption promoters.
4. To conduct in vivo pulmonary absorption studies in normal rats to understand the influence of absorption promoters and their mechanisms on pulmonary absorption of insulin and calcitonin.
5. To develop an in vitro method to study the transalveolar permeation of the selected drugs.
6. To establish correlation between in vitro – in vivo data.
7. To prepare dry powder inhaler formulations (DPIs) of insulin and calcitonin with suitable carriers.

8. To establish characterization parameters for optimum delivery of DPI of insulin and calcitonin.
9. To carry out the in vitro lung deposition studies of the developed DPI formulations using impactors.

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