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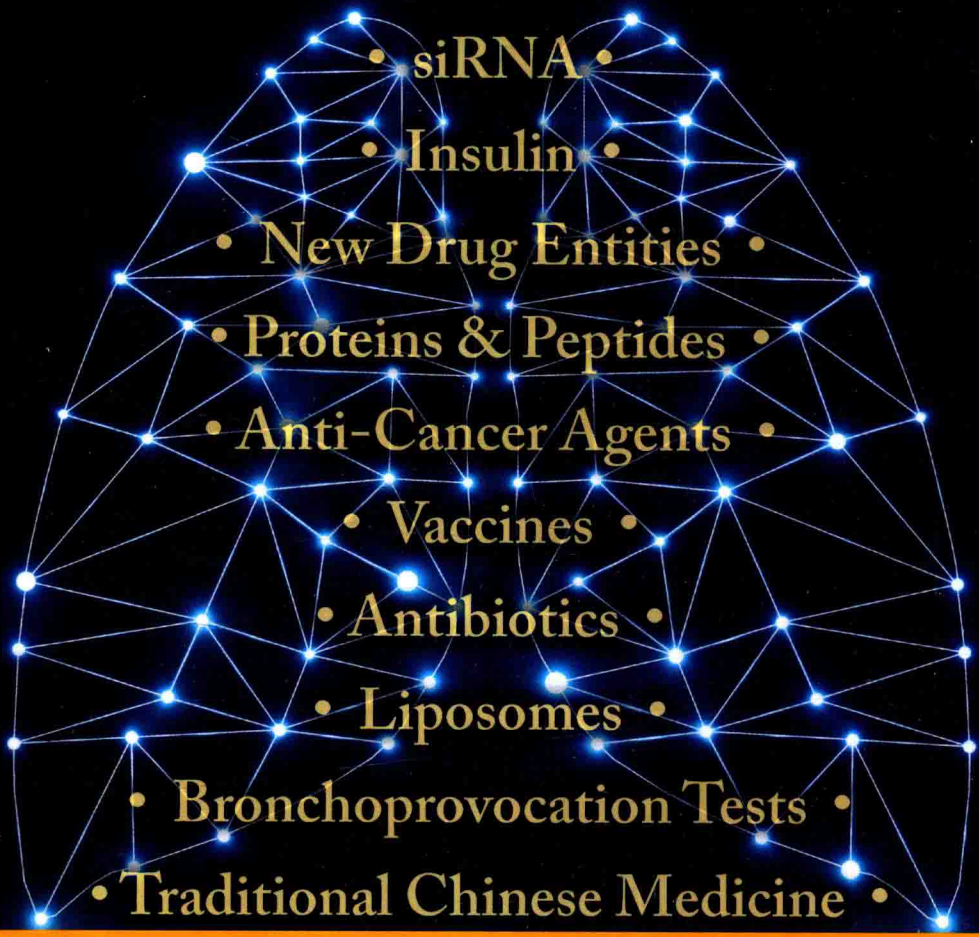
PHILIP CHI LIP KWOK • HAK-KIM CHAN

ADVANCES IN PULMONARY DRUG DELIVERY



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ADVANCES IN PULMONARY DRUG DELIVERY

Dedication

To my students

Hak-Kim Chan

To my family

Philip Chi Lip Kwok

Advances in Pulmonary Drug Delivery

The respiratory tract has been used to deliver biologically active chemicals into the human body for centuries. Inhaled volatile oils and herbal smokes have been employed by ancient civilizations for therapeutic and recreational purposes [1]. This demonstrates the efficiency of the lungs to absorb drugs into the systemic circulation, due to its large surface area and profuse blood supply. Inhalation is also the most effective route of administration for local diseases such as asthma, chronic obstructive pulmonary disease (COPD), and lung infections. The inhaled drug particles can target the site of action rapidly with a relatively low dose, thus reducing systemic adverse effects. However, the lungs are complex in their anatomy and physiology, which poses challenges to drug delivery. Therefore, inhaled formulations are generally more sophisticated than those for oral and parenteral administration. This book highlights the latest developments in this field.

Most classical inhaled drugs are small molecules (e.g., beta-2 agonists, anticholinergics, corticosteroids) for pulmonary diseases, but increasingly more biopharmaceuticals (e.g., proteins, peptides, nucleic acids) have been investigated or marketed for inhalation for both local and systemic delivery. This is a reflection of the global trend in the increasing use of biopharmaceutical formulations via other routes of administration. Wolff [2] provides an overview on various inhaled proteins and peptides such as insulin, alpha-1 antitrypsin, cyclosporine, human growth hormone, measles vaccine, and anti-IgE antibody for a variety of diseases. Of these agents, insulin and alpha-1 antitrypsin are further discussed by Patton and Miller [3]. The challenges of siRNA delivery and the use of nonviral vectors to enhance transfection efficiency are covered by Cun and Yang [4]. Advances in the understanding of the pathophysiology of asthma and COPD have led to the development of novel anti-inflammatory molecules (e.g., phosphoinositide-3-kinase-delta, dual phosphodiesterase-3 and -4, p38 mitogen-activated protein kinase, humanized antibodies, and chemokine receptor antagonists) [5]. Likewise, novel biopharmaceutical anticancer agents involving immunological and genetic agents are actively being investigated for lung cancer treatment [6]. Inhalation delivery is ideal for lung cancers to achieve local drug targeting and reduce potential systemic toxicity. The same rationale also applies to inhaled vaccines and antibiotics for pulmonary infections [7,8]. Liposomes have been used to effectively modify the release, and enhance the pharmacokinetics and pharmacodynamics, of inhaled antibiotics [9]. A novel trend for treating pulmonary diseases in China is the inhalation of traditional Chinese herbal formulae, which are usually administered orally or parenterally [10]. Although some effectiveness of inhaled traditional Chinese medicine has been demonstrated, more rigorously controlled clinical trials are required. Besides inhaling drugs to treat particular diseases, chemical trigger agents (e.g., mannitol, adenosine monophosphate, allergens) can also

be delivered into the airways for asthma diagnosis and antiasthma drug evaluation in bronchoprovocation tests [11].

Pulmonary drug delivery is a specialized field because of its many unique issues and challenges. Rapid progress is being made and novel solutions are being offered to existing treatment problems. The major direction in which this field is headed is the use of biologically active macromolecules. These new drugs are generally more fragile and potentially more immunogenic than traditional small molecular drugs. Therefore, active research on their stability, safety, and efficacy is anticipated in the near future. Despite the setback caused by the withdrawal of Exubera® in 2007, the approval in 2014 of yet another dry powder inhaled insulin (Afrezza® by MannKind Corporation) [12] is likely to kindle more interests in the field.

Philip Chi Lip Kwok
Hak-Kim Chan

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Hak-Kim Chan, professor in pharmaceutics, is leading the Advanced Drug Delivery Group and Respiratory Disease Theme at the Faculty of Pharmacy, University of Sydney. He graduated from the NDMC in Taiwan (BPharm) and the University of Sydney (PhD, DSc) and did his postdoc training at the University of Minnesota. He worked as a scientist at Genentech Inc. His research focuses on inhalation drug delivery, ranging from powder production by novel processes, particle engineering and aerosol formulation to scintigraphic imaging of lung deposition and clinical outcomes. He has over 300 scientific publications on pharmaceutical formulation and drug delivery (with over 9500 citations) and holds seven patents in these areas. He is an executive editor of Advanced Drug Delivery Reviews and on the editorial advisory boards of various pharmaceutical journals, including *Pharmaceutical Research* and *International Journal of Pharmaceutics*. He is a fellow of the American Association of Pharmaceutical Scientists and of the Royal Australian Chemical Institute (RACI) and was chair of the NSW Pharmaceutical Science Group of the RACI and vice president of the Asian Federation for Pharmaceutical Sciences.

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1 Inhaled Proteins and Peptides

Ron K. Wolff

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INTRODUCTION

Inhaled proteins and peptides have undergone a great deal of study in the last two decades. However, at present, there is only one approved inhaled protein, Pulmozyme (DNase), for the treatment of cystic fibrosis. Exubera (inhaled insulin) was approved but was withdrawn from the market primarily due to a lack of commercial success, not due to a lack of efficacy. Pulmozyme is an example of using inhalation for local lung treatment, while Exubera is an example of using lung delivery for systemic administration. A number of nonclinical and clinical studies for either local treatment or systemic delivery have shown varying degrees of success for a range of proteins and peptides. Summary information is provided for inhalation programs with DNase, insulin, growth hormone, cyclosporine, alpha-1-antitrypsin (AAT), anti-IgE, and

vaccines. Examination of the features of these various programs provides insights for future activities. This chapter will provide high-level information related to inhaled proteins and peptides. More details are available in recent review papers [1–6].

MECHANISMS OF ABSORPTION AND CLEARANCE

The major mechanisms for clearance of proteins from the lung have been reviewed by Patton and Byron [3] and Wolff [4]. Proteins that deposit on ciliated epithelium are not absorbed to a significant extent and are primarily cleared by mucociliary transport up the airways and then eliminated *via* the gastrointestinal tract where they are degraded and eliminated. Proteins that deposit in the alveolar region are cleared from the lung primarily *via* four routes: (1) phagocytosis by alveolar macrophages, (2) paracellular diffusion through tight junctions, (3) vesicular endocytosis or pinocytosis, and (4) receptor-mediated transcytosis.

Phagocytosis by alveolar macrophages does not appear to be as important a clearance mechanism as absorption. This may occur because phagocytosis is most efficient for uptake of relatively insoluble particles. Therefore, this clearance pathway is likely to be of most importance if there is degradation of proteins to insoluble forms. It appears that soluble proteins effectively dissolve in lung fluids and distribute themselves in the surfactant and mucus layers of the lung.

For soluble proteins, clearance of protein from the lung closely parallels absorption into blood. The inverse dependence of absorption versus protein molecular weight, as shown in Figure 1.1, has been used to suggest that diffusion across alveolar epithelial membranes through tight junctions is a major absorption mechanism [3]. The available data also support the view that absorption of high-molecular-weight proteins the size of albumin (68 kDa) or greater is not likely to be extensive or rapid because they are generally too large to be absorbed *via* tight junctions. Antibodies that are frequently in the >150 kDa also show little absorption from the lung into blood.

These data (Figure 1.1) indicate for proteins less than or equal to the MW of human growth hormone (hGH; 22 kDa) that absorption is adequate enough that systemic delivery can be considered. However, the aerosol delivery system needs to be considered to maximize the overall efficiency. For larger proteins, certainly those greater than 68 kDa, absorption into the blood is low, half-life in the lung is relatively long, and so potential for utility in local lung treatment is enhanced.

More research is needed in this area, however, and it is clear that at present, the absorption and disposition of each protein being considered for therapeutic use must be studied individually, because there is not sufficient knowledge for accurate predictions with currently available data [4].

Immunogenicity

In the case of a biopharmaceutical, antidrug antibodies (ADAs) may be raised against the biomolecule. If the ADA response is sufficiently robust, this may result in immune complex formation and subsequent toxicity. Typically immune complexes

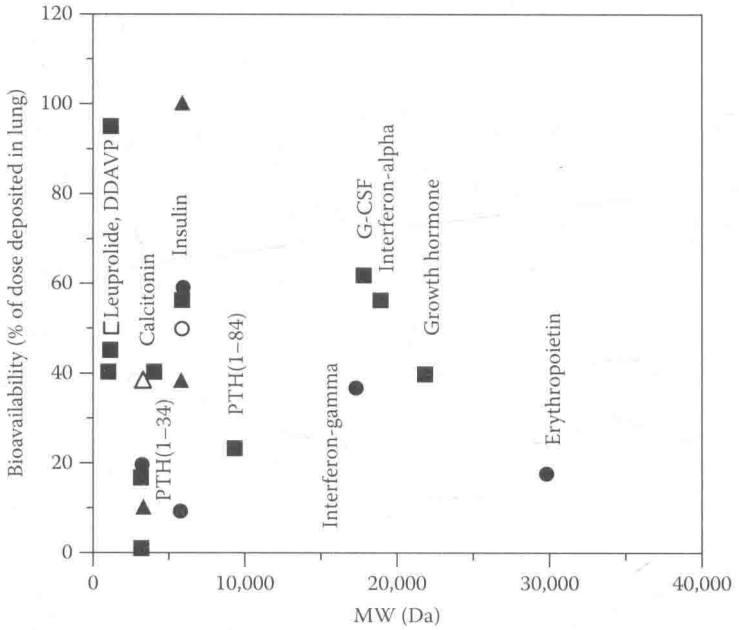

FIGURE 1.1 Bioavailability from the lung as a function of molecular weight calculated on the basis of the percentage of protein that is deposited in the lung and absorbed into the circulation.

are bound by red blood cells and then degraded in the spleen. However, immune complexes may result in inflammation and tissue damage following kidney deposition. ADA can have a direct effect on potency by preventing the antibody from binding to its ligand as well as a reduction in half-life *via* the clearance mechanisms involving neutralizing antibodies.

Assays for immunogenicity are able to measure the formation of ADA. However, these assays are typically unable to determine the impact of ADA on the potency of the drug; that is, they cannot differentiate between ADA and neutralizing antibody. For demonstration of neutralizing antibody, a cell-based potency assay may be necessary to clearly demonstrate a loss of drug potency in the presence of neutralizing antibody.

Nonclinical immunogenicity is not necessarily predictive of clinical immunogenicity but there are frequent parallels in relative immunogenicity between nonhuman primates (NHPs) and humans, particularly if there is close homology between these species. It is important to assess the pharmacokinetic/pharmacodynamic (PK/PD) exposure in terms of observed toxicities [7]. In practical terms, if the PK/PD profile of the biopharmaceutical is different between the first and last dose and/or there is evidence of immune-mediated toxicity, then ADA assessments can aid in the interpretation of the data. A dramatic decrease of C_{max} and AUC values after repeated doses is a major red flag that there may be antibody effects reducing the levels of the circulating protein. In this instance ADA measurements are definitely recommended. However,

if the PK/PD profile is unchanged, then ADA measurements are not necessarily warranted. However, because it is not possible to predict events in advance, it is good practice to collect plasma samples that may be used for later analysis if needed.

For vaccine products, immunogenicity is the intended pharmacology and immune responses are an important part of the nonclinical toxicity studies. Because vaccine dosing is general single doses or a few repeat doses at infrequent intervals, it is often difficult to measure TK. Therefore, assessment of the vaccine-induced immune antibody response can also serve as a measure of the exposure of the animals to the test material.

Nonclinical Development Considerations

Nonclinical studies to support human dosing should be conducted according to Good Laboratory Practices (GLP) and must reflect the intended clinical use (e.g., dose level and frequency of administration) of the biopharmaceutical. However, studies for individual proteins or peptides are developed on a case-by-case approach to generate the appropriate data for clinical development. Consultations with regulatory agencies are recommended.

The ICH S6 guidelines [8] define a relevant species as "one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies)." Unlike small molecules, a single species may be sufficient if a second species is not suitable to predict risk in humans. For example, for many biopharmaceuticals, the human therapeutic target is expressed in the NHP and not in other species or target binding and pharmacology is only present within the NHP. However, where two pharmacologically relevant species exist (one rodent and one nonrodent), both should be used for initial safety assessment studies. The ICHS6 R1 addendum allows chronic studies to proceed only in rodents rather than nonrodents where adequate long-term exposure and pharmacology can be maintained.

The ICH S6 guidelines recommend that dose levels should be selected to provide information on a dose response that includes a toxic dose and a no observed adverse effect level (NOAEL). However, for some types of biologics where toxicity is a result of exaggerated pharmacology, the pharmacologically active dose, or PAD, may be a better indicator of observations of potential toxicity than NOAEL [9]. The high dose level in toxicity studies should ideally include a safety margin of at least 10× the maximum anticipated clinical dose; however, sometimes exaggerated pharmacological responses preclude dosing this high.

In repeat dose nonclinical safety studies, dosing frequency should be similar to that of proposed clinical trial. However, more frequent dosing may be required if the test item is more rapidly cleared in animals than humans [9]. Repeat dose studies should include toxicokinetics and a recovery phase (to detect regression of any pathological change and/or detect potential delayed toxic effects). Repeat dose toxicity studies of 6-month duration in rodents and nonrodents have been shown to be generally sufficient to predict potential human risks [10].

Pharmacodynamic endpoints: Since the toxicity of most biopharmaceuticals is related to their mechanism of action, then high doses may be associated with

exaggerated pharmacology. Therefore, pharmacodynamic endpoints should be measured on studies to better understand adverse effects, such as glucose monitoring following insulin administration (discussed later).

EXAMPLES OF DEVELOPMENT CANDIDATES

Inhaled rhDNase (Pulmozyme)

There is currently only one marketed inhaled protein, recombinant human(rh) DNase (Pulmozyme), developed for the treatment of cystic fibrosis. Inhalation toxicology studies were conducted in both rats and monkeys for durations of 28 days and 6 months [11]. The exposure concentrations and daily exposure durations spanned inhaled doses of 1.3–69 times the expected daily clinical inhaled dose. In the 28-day rat study, mild to moderate alveolitis was observed in the high-dose group. This lesion was not evident after 4 weeks of recovery. Bronchiolitis was observed in one of eight high-dose monkeys after 4 weeks of exposure, but, again, there were no lesions observed after 4 weeks of recovery. In the 6-month rat study, bronchiolitis was observed at the end of the treatment period but at a somewhat lower incidence than in the 4-week study. These data suggest that the mild lesion was not progressive in rats. In the 6-month monkey study, respiratory rates measured during aerosol exposure to monitor for anaphylactic or irritant responses were unchanged compared to preexposure values. Positive serum antibody titers to rhDNase were observed beginning at week 4 and persisting through the treatment period. Serum concentrations of rhDNase at 24 h postdose indicated that there was no accumulation of rhDNase throughout the 6-month treatment period. Histopathologically, there was increased perivascular lymphocytic cuffing, peribronchial lymphoid hyperplasia, terminal airway-related bronchiolitis/alveolitis with eosinophilic infiltrates, and increased siderophages. There appeared to be a close relationship between the severity of the pulmonary lesions and the antibody titer to rhDNase measured in serum. The lesions were consistent with an allergic or hypersensitivity (type I) response to a foreign protein. This is not unexpected, because there are considerable differences between animal and human DNases. There is only an 80% homology between the rat and human DNases; monkey DNase, although it has not been completely sequenced, also appears to be highly dissimilar to the human form (S. Shak, personal communication, 1997).

Some of the most persuasive data related to challenges of assessing immune reactions to inhaled therapeutic proteins in humans versus animals come from the development of rhDNase (Pulmozyme) for the treatment of cystic fibrosis. Pulmozyme involves the inhalation of a relatively large amount of rhDNase, which is an enzyme that cleaves DNA. The clinical summary portion of the Summary Basis of Approval for Pulmozyme (FDA, 1993) provides information related to immune responses. It was concluded that antibodies to rhDNase were of little consequence to the safety profile of rhDNase on patients because levels were generally low and there was no correlation between antibody levels and clinical responses. As noted previously, there were immune responses found in the rat and monkey inhalation toxicology studies in which the animals were exposed to a highly heterologous