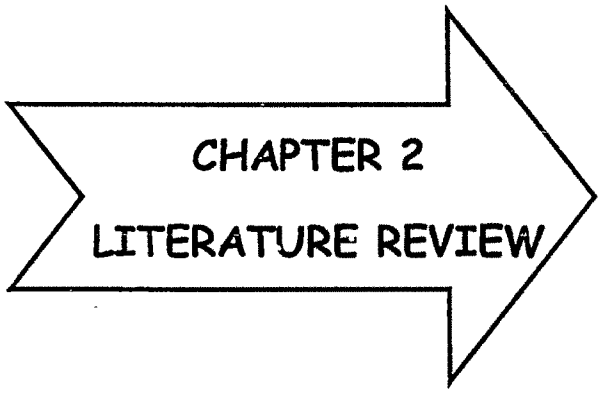

CHAPTER 2
LITERATURE REVIEW

A thorough literature is necessary to initiate and carry out the work on delivering the peptide drugs through the lung for systemic absorption. The pharmacology of the lung, barriers to peptide drug permeation through the alveolar membrane, formulation factors and their mechanisms to improve the pulmonary drug absorption and devices involved in delivering the drugs need to be reviewed well for successful delivery the drug candidates.

2.1 PULMONARY DRUG DELIVERY

During the past few years there has been an exponential rise in the number of reports describing absorption of peptides and proteins following administration to the lung [1]. Results from these studies have clearly demonstrated the feasibility of systemic peptide and protein delivery via this route. At present, development of leuprolide is in stage III clinical trials and will probably be the first peptide developed for systemic delivery following administration via the pulmonary route [2,3,4,5-7].

Studies designed to evaluate absorption from different regions of the lung as well as surface area considerations suggest that the alveolar region is the site of delivery required for maximal absorption from the lung [8]. Transport and absorption of SK&F 110679 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH2, GHRP), a hexapeptide with potent growth hormone releasing activity in animals was evaluated employing both *in vitro* and *in vivo* methodologies [9,10,11] to investigate the mechanisms involved in absorption of peptides from the lung. From animal studies, it was shown that SK&F 110679 absorption via the lung results in ~43% bioavailability compared to less than 1% following intraduodenal administration [8,12]. The greater bioavailability of SK&F 110679 following intratracheal administration vs. intraduodenal administration is not explained by the differences in relative membrane permeabilities of the lung and intestinal tissues (~4 –fold greater permeability in the lung). Therefore, higher absorption following administration via the lung most likely results from the simultaneous exposure of the airway epithelium to the undiluted peptide solution compared to the sequential exposure and concomitant dilution by intestinal secretions that occur following oral administration.

The intrapulmonary route of administration represents an alternative to oral administration. The pulmonary route is promising for delivering peptide and proteins. Presently, however, little systemic information is available on the absorption of proteins and peptides via the lung, even though the extracellular peptidase activity is much lower in the lung than the gastrointestinal tract and the surface area available for absorption is large. Studies demonstrate that the lung has both active and passive absorption pathways, similar to the intestine.

2.2 LUNG MORPHOLOGY AND PHSIOLOGY

The human lung is often conceptually divided into conducting and respiratory zones. The conducting zone serves as the relatively long channel for inhaled air and consists of trachea, bronchus, bronchioles, and terminal bronchioles (Table 2.1).

AIR WAY EPITHELIA

The respiratory epithelia consist of two primary divisions: the proximal conducting airways and the distal respiratory portion responsible for gas exchange. In addition, the nasal turbinates are lined by respiratory epithelia that are morphologically and functionally similar to the proximal conducting airways [13]. The conducting airways extend proximally from the trachea (~2 cm diameter) branching initially into the mainstem bronchi, then dichotomously ~16-20 times ending in the terminal bronchioles (~0.2 mm diameter). The epithelium lining the proximal (cartilaginous) conducting airways is pseudostratified columnar and consists of three major cell types: basal cells, ciliated cells, and goblet cells. Although the exact function of basal cells is not fully understood, they appears to serve either as a scaffolding cell or alternatively as a pluripotential cell. Ciliated cells are highly polarized columnar cells with cilia, microvilli, and a prominent glycocalyx located on the apical surface. Goblet cells, prominent in the trachea and proximal bronchi, progressively decrease in frequency with airway division in the normal lung until they disappear from epithelia of the more distal airway regions. These cells are primarily secretory and contain large numbers of periodic acid-Schiff (PAS)-

stained granules, the contents of which can be released onto the apical surface of the epithelium. In the smaller airways, the Clara cell or non-ciliated bronchiolar cell becomes one of the two dominant cell types, the other being the ciliated cell which becomes much more cuboidal with decreasing airway diameter. The Clara

INFLATED HUMAN LUNG MORPHOLOGY

Z	Name	Diameter (cm)	Cross Section (cm^2)	Number 2^2	Total Cross-sectional Area	Mean Air Velocity Cm/sec
0	Trachea	2	3	1	3.0	330
1	Mainstem	1.3	1.35	2	2.7	370
2	Lobar	0.9	0.7	3	2.8	360
3	Segmental	0.7	0.38	8	3.0	330
4	Subsegmental	0.5	0.20	16	3.2	310
...						
12	Bronchiole	0.05	0.0021	4,096	8.6	125
13	Bronchiole	0.04	0.0012	8,192	9.8	100
...						
16	TB	0.018	0.00024	65,536	16	66
17	RB1	0.015	0.00015	131,072	20	50
18	RB2	0.012	0.00011	262,144	30	35
19	RB3	0.011	0.00010	524,288	50	20
20	AD1	0.010	0.00008	1,048,576	80	12
21	AD2	0.010	0.00008	2,097,152	160	6
22	AD3	0.010	0.00008	4,194,304	-	-
23	AS	0.005	0.00002	8,388,608	-	-

Average dimensions for human lung inflated to three-fourths of total lung capacity; z is the generation number.

TABLE 2.1

cell is a secretory cell with the ability to secrete apolipoprotein, phospholipids, high-molecular-weight glycoconjugates, and a 10-kDa protein homologous to uteroglobin [14,15]. Accordingly it has been implicated as a potential progenitor

cell for the globat cell wall as a progenitor cell for the type II pneumocyte [16] and ciliated cell.

An interdigitated lateral intercellular spaces and gap junction at the lateral surfaces and tight junctions at the apical surface connect superficial airway epithelial cells. The tight junctions function at the cellular level to separate the apical and basolateral surfaces and at the epithelial level as a molecular sieve restricting the flow of large polar solutes across the airway mucosa. The goblet cell, ciliated cell, and type II pneumocyte have all been implicated as cell types involved in the transcellular transport of proteins and peptides across respiratory epithelia [17-20].

Proximal airways also have submucosal glands, which arise from invaginations of the surface epithelium into the submucosa during fetal development and consist of two principal cell types, serous cells and mucous cells. Both cell types secrete high-molecular-weight glycoconjugates and mucous. In addition, serous cells secrete lysozyme, lactoferrin, antileukoprotease, and possibly albumin [21,22]. Submucosal glands have also been shown to participate in IgA secretion in human bronchial epithelium [23]. The ability of these glands to absorb proteins and peptides has not been studied. Similar to goblet cells, submucosal glands are not present in the distal airway regions.

The airway epithelium is lined by an airway surface liquid (ASL) ~10 μm in depth and is thought to consist of two layer: (1) a mucous or gel layer positioned atop the cilia presumably composed of mucus glycoproteins secreted from goblet and glandular cells, and (2) a serous periciliary fluid (sol) layer in which the cilia beat synchronously to propel the mucus containing trapped inhaled particles, cellular debris, or infectious agents toward the major bronchi, trachea, and then the pharynx where it can be either swallowed or expectorated. The composition and some of the protein constituents of the periciliary fluid have been identified and include albumin, immunoglobulins, and a variety of enzymes [24, 25]. The regulation of the volume and contents of this periciliary fluid is only partially understood and is reviewed elsewhere [26,27].

ALVEOLAR EPITHELIA

The gas exchange portion of the lung in humans consists of two to four generations of respiratory bronchioles (0.15-0.2 μm diameter), alveolar ducts, and alveolar sacs, and alveoli. While the major conducting airways have a surface area of only ~2 μm, the estimated 300 million alveoli present a surface area for gas exchange of ~143 μm [16]. The major cell types in the respiratory portion of the lung are the type I pneumocyte or squamous alveolar cell, the type II pneumocyte, the type III pneumocyte or alveolar brush cell, and the alveolar macrophage. These cell types are all located within the alveolar ducts, alveolar sacs, and alveoli, and Clara cells particularly in the region of the respiratory bronchioles. With the exception of the alveolar macrophage, all of these cells rest on a basement membrane forming a complex epithelium for the intimate contact of air and blood over a large surface area.

The type I cell apposes the endothelial cells at the fusion of the two basement membranes to form an air-blood gas exchange barrier measuring 0.2-0.5 μm thick, separating the alveolar wall into the septal and alveolar surfaces. This cell, known for its extensive surface area and branching, also has limited phagocytic abilities to ingest particulates, e.g., chrysotile asbestos [16]. The type II pneumocyte, the predominant cell type in the respiratory region of the lung, is a cuboidal cell characterized by numerous cytoplasmic lamellar bodies representing the storage granules for surfactant. This organelle-rich secretory cell replaces the type I cell in the event of injury, becoming attenuated and losing its organelles as it migrates down the basement membrane. This cell is rich in a basolateral Na^+, K^+-ATPase and rich in cytochrome P-450 isozymes. The rate type III pneumocyte or alveolar brush cell is a cuboidal cell present in the region of the first alveolar duct bifurcation and is essentially absent in the more distal alveoli. This type III cell may play a role in detoxification of inhaled pollutants given their location in a region that is often the primary site of initial injury evoked by these agents.

As observed in airway epithelia, the alveolar cell types possess the ability to form tight junctions and generate spontaneous transepithelial potential differences both *in vivo* and *in vitro*. In fact, alveolar tight junctions may even be tighter than airway tight junctions as evidenced by freeze-fracture studies [28,29], increased

transepithelial resistance in culture [30,31], and the increased transepithelial resistance of excised bullfrog alveolar epithelia relative to airway tight junctions [32,33]. Alveoli, like the airways, are covered by a complex epithelial lining fluid (ELF) consisting of (1) an epiphase or surfactant layer comprised of a surface-active phospholipid layer at the air-liquid interface, and (2) a hypophase beneath this layer comprised of tubular myelin, apoprotein, IgG, albumin, phospholipid, and carbohydrates. Unlike the conducting airways, no cilia are located in this region to facilitate clearance. Moreover, no glands or goblet cells are present.
Macrophages are also located in the respiratory portion of the lung. The alveolar macrophage is a postmitotic cell with a half-life of several days residing in the alveolar lumen that may increase in number in response to inhaled pollutants. The septal macrophage, which retains the ability to divide, putatively resides in the alveolar septum for years, and is the immediate precursor cell for the alveolar macrophage. Both macrophages are obviously derived from peripheral blood monocytes. Although these cells are not of epithelial origin, they serve important lung defense functions and may potentially have an impact on the stability of proteins in the epithelial lining fluid.

Insoluble particles deposited in this conducting zone are propelled upward by means of cilia action within the mucus layer residing on the surfaces of the conducting zone. Eventually the insoluble particles reach the pharynx, where they are swallowed. The clearance of insoluble particles from the conducting airways by mucociliary activity is usually complete in 12-24 h [34-37]. Respiratory bronchioles are located beyond the terminal bronchioles, followed by alveolar ducts and alveolar sacs; together they comprise the respiratory zone, where primary gas exchange occurs between the air spaces and the blood. Insoluble particles deposited in this zone are quickly engulfed by alveolar macrophages, whose ability to chemically break down engulfed material makes them the nemesis of inhaled drugs.

The average alveolar surface area in human lungs [38] is 102 ± 21 m^2, corresponding to 95% of the total surface area in the lungs. The total surface area of alveoli in contact with blood capillaries is approximately 75 m^2 (roughly 40 times greater than the external body surface area) [39]. This broad area facilitates

the lung's function as the primary gas exchange organ (the lung receives all the blood circulated through each of the peripheral organs and tissues, whereas the abdominal organs receive about 24% of body blood [40]. The epithelial lining of the alveolar spaces, the most significant absorption barrier separating blood from the alveolar lumen [41], has an average thickness of only 0.2 μm - approximately 34 times smaller than the diameter of an average red blood cell [41]. The thinness of this barrier and its extensive surface are permits a high accessibility of inhaled drugs to the blood stream. This makes the alveolar region a natural target for inhaled drugs destined for the systemic circulation.

2.3 POTENTIAL ADVANTAGES OF PULMONARY DRUG DELIVERY

These include improved biological dosimetry (i.e., limiting metabolism) and potential reduction of drug-dose, as well as other factors such as those discussed below:

Physiological factors

A relatively larger surface area for drug absorption
Rapid drug absorption
Comparatively lower enzymatic drug degradation
Avoidance of hepatic first-pass effect
Drug targeting

Patient-related factors

Non-invasive drug delivery
Reduced risk of infection
Patient compliance due to self-medication
Compact packaging
Tamper-proof construction
Higher bioavailability as compared to oral route.

Commercial factors

Specialization and exclusivity

Cost effectiveness

These advantages have led scientists to investigate inhalation for the delivery of macromolecules which otherwise require parenteral administration due to proteolysis and hepatic metabolism after oral ingestion.

2.4 CRITICAL ISSUES IN LUNG DELIVERY OF THERAPEUTIC PEPTIDES AND PROTEINS

Formulation and biophysical factors may influence successful development and future commercial application of peptidic compounds for lung delivery. Some of these include developmental pharmaceutics and drug chemistry issues a few of that are generically described in Table 2.2.

Main issue	Factors affecting Product performance	Factors resolving problem
Systemic permeation	Alveolar tight junction	Physical and chemical promoters
Degradation of protein	Alveolar membrane of enzymes	Inhibit the proteolytic enzymes (Proteases)
Dispersion quality	Surface energetic (charge and molecular size); surfactant Compatibility With Propellant or carrier	Micronization; protective colloids; ion-pairing agents; elimination of moisture

Lung deposition efficiency	Drug dose; particle size distribution, morphology, shape, density; Pathology; and disease	Micronization of drug; device geometry; proper breathing maneuver
Physical and chemical stability	Aggregation, hydrolytic absorption to device surfaces, decreased respirable fraction	Molecular modification; lower moisture content and product storage temperature; protective colloids; ion-pairing agents
Patient compliance, Correlation of pharmacokinetic and pharmacodynamic data	Product taste and gag reflex; stability to lung metabolizing enzymes; lung function, pathology	Micronization; drug solubility; proper breathing and through-life dose uniformity
Formulation issues and environmental impact of MDI propellants	Dispersion quality; compatibility of propellant with surfactant systems; aggregation; device extractable.	Cosolvents; protective colloids; lower moisture content
Alternate and specialized lung delivery systems	Dry powder inhalers and powder flow problems; liposomal delivery systems And dispersion stability Questions; microspheres and Biophysical limitations (upper Airway deposition); Piezo-electric devices and Dose uniformity problems	Change-transfer devices; protective colloids; lower moisture content; molecular modification; ion-pairing agents; improved solubility

TABLE 2.2

2.5 REGULATION OF PULMONARY ABSORPTION

The rate of diffusion through the paracellular path may be regulable. A variety of physical factors have been shown to affect the paracellular permeability of airway epithelia. Lowering the pH of the luminal bathing fluid to 2.8 has been shown to increase paracellular permeability in isotopic tracer studies in airway epithelia [42], possibly by displacing calcium from the tight junctions. Increased osmolality of the luminal fluid has been reported to increase mannitol permeability across canine trachea. Transalveolar pressures greater than 30cm H_2O, which are known to occur during barotrauma, increase apparent equivalent pore radii, making the barrier less effective [43,44]. Finally, metabolic inhibition of airway epithelia, with either hypoxia or sodium cyanide, has been shown to increase paracellular permeability [45]. Hence, a variety of factors may affect paracellular permeability to increase access of inhaled macromolecules to the submucosa or interstitium of respiratory epithelia. Similarly, these factors may facilitate increased transudation of serum proteins or locally produced proteins into the ASL or ELF.

Adjuvants or promoters that enhance paracellular permeability might be used to increase delivery of peptides and proteins across respiratory epithelia. Current research has largely focused on the nasal epithelia where a number of peptides are already available for intranasal delivery clinically in the United States and Europe. Among the list of approved peptides are oxytocin, desmopressin, luteinizing hormone-releasing hormone analogues and salmon calcitonin. The first three of these are small peptides of less than 10 amino acids that easily cross the epithelia presumably through pores. Larger peptides contain greater than 30 amino acids traverse the nasal epithelia very poorly. Some of these that have been more actively investigated include insulin, glucagon, growth hormone-releasing hormone, and corticotrophin-releasing hormone, and calcitonin.

A variety of promoters have been used in an attempt to enhance intranasal absorption of these peptides and include chelating agents, surfactants, fatty acids, and bile salts [46-50]. The mechanisms of action of these promoters have not been clearly delineated but focus mainly on changes in the paracellular path and the

formation of hydrophilic pores or channels for transepithelial flow. Chelating agents such as disodium ethylenediamine tetraacetate (Na_2-EDTA), despite increasing the permeability of the tight junctions as consequences of removal of luminal Ca^{2+}, cause only a minimal increase in intranasal absorption of insulin [48]. Simply dissolving insulin in dilute acid medium (pH 3.1) to increase paracellular permeability also enhances intranasal insulin absorption [51]. In contrast, surfactants act to enhance intranasal absorption of peptides by a different mechanism. These agents have been reported to bind to hydrophobic regions of membranes and tight junctions to form pores or hydrophilic channels for the transfer of peptides from the nasal lumen down a concentration gradient into the extracellular space [52]. The most frequently studied surfactant, Laureth-9, has been shown to significantly enhance intranasal absorption of insulin in both animals and humans sufficiently to achieve desired hypoglycemic effects [46,48]. However, intranasal insulin remains only about 30% as efficacious as intramuscular insulin and may be associated with local toxicity at concentrations above 0.25% laureth-9 [46,48]. Like surfactants, bile salts such as sodium glycocholate and its derivatives may also enhance intranasal absorption of peptides by reverse micellar binding with subsequent formation of hydrophilic pores or channels in either cell membranes or tight junctions [47, 52]. Biles salts may also enhance absorption by binding $Ca2^+$ [53] to increase paracellular permeability [54] and by inhibiting intranasal proteases to increase drug availability for absorption. Enhancement of intranasal delivery using bile salts as a promoter has been reported for insulin, calcitonin, corticotropin-releasing hormone, and growth hormone-releasing hormone [47,49,50].

Hence, transudation of peptides and proteins across respiratory epithelia can be regulated using a variety of physical factors and promoters. The efficiency of delivery can perhaps be further increased through the use of protease inhibitors such as p-chloromercuriphenysulfonic acid (PCPMS) and aprotinin [48]. However, in the absence of physical factors or promoters, the contribution of protease inhibitors to enhancement of peptide absorption may be small.

2.6 DRY POWDER INHALER DELIVERY TO LUNGS

Inhalation drug delivery has been used for many years for the delivery of pharmacologically active agents to treat respiratory disease. Traditional asthma therapy with bronchodilators, steroids, mast cell stabilizers, and anticholinergic drugs has primarily used the pressurized metered-dose inhaler (MDI). However, this delivery system is now under increasing threat because of the environmental concerns regarding chlorotluorocarbon (CFC) propellants. A range of alternative devices, such as dry powder inhalers, which do not contain propellants are being evaluated and developed. This article covers the development of dry powder inhalers, including the design of the formulation, metering system and flow path.

Powder inhalers are versatile delivery systems that may require some degree of dexterity to operate, although one of the objectives of recent developments has been to simplify their operation. Typically, they dispense a metered quantity of powder in a stream of air drawn through the device by the patients own inspiration. In the design of a new powder inhaler consideration must be given to optimizing the formulation of the powder containing the drug substance to ensure a chemically stable and consistent dose; design of the metering system within the inhaler to provide consistent doses over a range of inhalation conditions; and design of the powder inhaler itself to produce a convenient device that is comfortable and easy for the patient to use.

In as much as these devices do not require CFC propellants to disperse the drug, they can be regarded as ozone-friendly delivery systems. However, concerns that they will not be able to totally replace MDIs due to limitations of dose delivered and flow rates achieved through the devices for severely diseased patients are probably valid [55], based on the capabilities of currently available powder inhalers. Vidgren et al. [56] have shown different deposition patterns in healthy volunteers from the same formulation in four single-dose DPIs. Newman et al. [57] have also shown different in-vivo deposition patterns in healthy volunteers using Turbuhaler inhalers operated at optimal and sub-optimal peak inspiratory flow rates. Clearly, some current designs of DPIs are subject to variations in

performance due to differences in inhalation flow rates. Future designs may require that the dispersion of the powder dose be independent of patient inhalation.

DRY POWDER INHALERS

Dry powder inhalers offer a unique opportunity for the delivery of drugs to the lung as aerosols. These devices combine powder technology with device design in order to disperse drug particles as an aerosol in the patient's inspiratory airflow [58]. The therapeutic drug is manufactured in powder form as small particles a few micrometers in diameter. In many DPIs the drug is mixed with much larger sugar crystals, such as lactose, and the smaller drug particles attaches to these excipients particles, improving entrainment of the drug upon inhalation.

All DPIs have four basic features

1) A dose metering mechanism
2) An aerosolization mechanism
3) A deaggregation mechanism, and
4) An adapter to direct the aerosol into a patient's mouth.

The major components of a dry powder inhaler are the drug powder, and other powdered exicipients where necessary, a drug reservoir or premiered individual doses, the body of device, and a cover to prevent ingress of dust or moisture. To introduce drug particles into the lung, they must be $\leq$ 5 μm in aerodynamic diameter [59,60]. Small particles are notoriously difficult to disperse [61]. The lactose particles are intended to act as carrier particles for the drug and as such they are in a much larger particle size range, 60-90 μm [62,63]. Drug particles are theoretically stripped from the surface of the lactose particles, to which they are loosely attached, during the generation process [64,66]. This is illustrated schematically in Figure 2.1.

DPIs can be divided into two major types on the basis of how the medication is contained Reservoir and Discrete. Reservoir devices have several disadvantages not found with discrete dose device. Reservoir devices are susceptible to humidity

MECHANISM OF GENERATION OF FINE PARTICLES

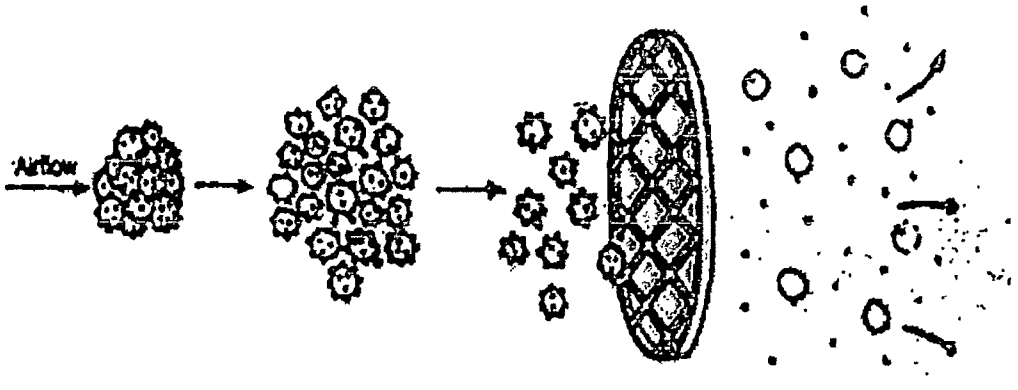


FIGURE 2.1

and moisture. In addition, they can only be checked for quality control by patient use and thus have dose-to-dose variation [65]. If a reservoir device gets wet or is dropped, the drug consistency for the entire inhaler may be adversely affected, unlike the discrete does device in which only one dose is lost. In some systems, the powder was placed in capsules or blisters containing one dose, therefore referred as single – dose systems. Other devices containing a powder reservoir, known as a multi-dose systems.

Addition of fine lactose to the coarse in carrier systems has been proposed as a superior performance modifier than the single coarse carrier. The mixing sequence also plays an important role.

The different grades of lactose were tried as a carrier for aerosolized salbutamol sulphate. The control of particle morphology such as surface rougosity and particle size distributions of the carrier may be crucial in determining the reproducibility and efficiency of drug delivery to the respiratory tract from dry powder inhaler formulations. [67].

The influence of carrier surface on the carrier surface on the characteristics of respirable powder aerosols. The carrier lactose with low rougosity facilitates more effective redispersion of drug particles even at low air speeds. Therefore generation of a deeply inspirable cloud is possible with a minimum inspiratory effort.

A number of previous studies have reported improvements which can be made to the amount of respirable drug delivered from a DPI by means of manipulating the powder formulations and these includes smoothing the carrier surface, reducing the particle size of the carrier and the use of ternary materials in the powder formulations.

The addition of fine lactose as ternary components to interactive mixtures has been investigated to improve the efficiency of lung deposition from DPI formulations. Ternary mixtures produced higher *in vitro* aerosol deposition for salbutamol and also the generation of respirable clouds from coarse powder aggregates was studied [68,69]. The protein deposition from dry powder inhaler was studied using fine particles as multiplet's as performance modifier using spray dried bovine serum albumin [70].

The influence of lactose carrier on the content homogeneity and dispersibility of beclomethasone dipropionate from dry powder aerosols were studied and its found that the addition of fine lactose of fine lactose does not affect the content uniformity and the presence of fine lactose in powder formulations was shown to increase the dispersion and deaggregation of drug in an air stream. [71]. Increasing concentration of fine lactose concentration the fine particle fraction also increases proportionally by using micronized lactose.

The use of fine carrier particles to improve drug delivery is likely to be preferred as ternary material, since the latter will require toxicological testing.

The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulfate in an air stream *in vitro* was studied and it shows that adding lactose fine particles to the dry powder aerosol formulations appears to reduce the drug –

carrier interaction by occupying possible drug binding sites on the large lactose particles leading to improvement in the respirable fraction of drug [72].

Protein deposition from dry powder aerosol was studied using fine particle multiplet's as performance modifiers. The study suggest that the use of fine particle carrier for the improvement of the bioavailability of dry powder aerosols [73].

The effect of particle size & adding sequencing of fine lactose on the deposition of salbutamol sulfate from a dry powder formulation were also been studied and the study showed that the fine particle lactose was improving deposition markedly and the adding sequence plays an important role in deposition of drug. [74]

The saturation of active sites on the carrier particles has been proposed as the mechanism for increased drug deposition, where by the adhesion of ternary components onto active (high adhesion) sites leave passive (low adhesion) sites for drug adhesion. The reduced adhesion between drug and carrier particles was suggested to increase the drug attachment [74].

The influence of relative humidity on particulate interactions in carrier-based dry powder inhaler formulations was also been studied and it shows that storage of formulations at higher humidity also affects the deposition of aerosolized drug.

FORMULATION DESIGN

Of critical importance in the development of powder inhalation products is the optimization and control of flow and dispersion (deaggregation) characteristics of the formulation. These properties are a function of the principal adhesive forces between particles, including Van der Waals forces, electrostatic forces and the surfaces tension of absorbed liquid layers [75]. The forces are influenced by several fundamental physiocochemical properties, including particle density and size distribution, particle morphology (shape, habit, surface texture) and surface composition (including absorbed moisture) [76].

Interparticle forces that influences flow and dispersion properties are particularly dominant in the micronized or microcrystalline powders that are required for inhalation therapy (particles smaller than 5 μm). Bulk drug modifications, both chemical and physical, have been attempted in order to enhance respirable dose performance. In one study [77], spray-dried salbutamol sulfate was seen to perform as well as micronised material. In the case of sodium cromoglycate, several approaches have been successfully employed to improve flow and dispersion characteristics, including controlled aggregation of the undiluted drug to form loosely adherent flocs [78,79]. This approach takes advantage of the inherent cohesiveness of the particles.

To minimize hygroscopic growth, lipophillic coating materials have been investigated using disodium cromoglycate [86]. In addition, crystals of the parent acid and the effects of aspect ratios (longest and shortest dimensions) have been studied [80]. Other techniques for modifying drug alone characteristics have been discussed, such as recrystallization from supercritical fluid [81,82].

A DPI formulation may consist of drug alone, or of drug blended with a carrier material (which is usually lactose). Blending the drug with a carrier has a number of potential advantages, such as increasing the bulk of the formulation. This allows easier metering of small quantities (typically < 100 g) of potent drugs, either at the manufacturing stage (if the doses are pre-metered) or within the device itself for a reservoir device. Provided that content uniformity of the blend is well controlled, this approach can improve the subsequent dosing consistency of the inhaler. The presence of the material, in separating the very fine drug particles, can also improve processing (e.g. flow characteristics) of the formulation. The carrier properties (particle size distribution, particle surface characteristics) can be used to influence / control fine particle mass.

An additional benefit that may be gained from the use of a carrier such as lactose is the taste / sensation on inhaling, which can assure the patient that a dose has been taken. Clearly, the influence of the carrier material on product stability must be carefully assessed, and the range of materials available for use as carriers in inhaled products is limited for toxicological reasons. Lactose and other sugars have

been studied and used, and modification optimization. Modifications to the lactose surface have been proposed that would improve the surface characteristics (reduce the rugosity) of the material. Ganderton [83] claims that reducing the rugosity increase the percentage of respirable particles in conventional powder inhalers.

MECHANISM OF PARTICLE DEPOSITION IN THE AIRWAYS

Three principle mechanisms operate within the lower respiratory tract.

a. Inertial impaction
b. Sedimentation
c. Brownian diffusion

a) INERTIAL IMPACTION

This is dominant deposition mechanism for particles > 1 μm in the upper tracheobronchial regions, impaction usually occurs near the bifurcation. The probability of inertial impaction will be dependent upon particle momentum, thus particles with larger diameters or higher densities of those traveling in air streams of higher velocity will show greater impaction.

b) SEDIMENTATION

This is particle deposition resulting from settling under gravity. It becomes increasingly important for particles that reach airways where the air stream velocity is relatively low, e.g. the bronchioles and alveolar region. The fraction of particles depositing by this mechanism will be dependent upon the time the particles spend in these regions.

C) BROWNIAN DIFFUSION

This is of little significance for particles > 1 μm. The probability of particle deposition by diffusion increases as the particle size decreases. Brownian diffusion is also more prevalent in region where airflow is very lower or absent, e.g. in the alveoli.

PHYSIOLOGICAL FACTORS AFFECTING PARTICLE DEPOSITION IN THE AIRWAYS

A basic understanding of pharmacokinetics of drug delivered to the lung is essential in the design and evaluation of formulation with anticipated efficacy. Drug absorption and bioavailability is critical for drugs including for systemic effect. Plasma as well as urine analysis can be used to estimate pharmacokinetic parameters of the pulmonary delivered drugs [84]. Some of the issues are summarized in the following section.

DEPOSITION ASSESSMENT

Absorption rates for lung-delivered compound are generally lowered after intratracheal instillation than after aerosol delivery.

This may be due to the higher fraction deposition in the peripheral zone of the lung following aerosolization compared to instillation. The large surface area coupled with a greatly reduced thickness of the absorption barrier at the alveoli regions facilitates drug diffusion into the systemic circulation at a relatively faster rate than drug absorption from the tracheobronchial region.

LUNG FUNCTION PARAMETERS

Forced vital capacity (FEV), inspiratory flow rates (IFR) and tidal volumes are some of key lung function parameters used to measure the extent of lung disease. These lung function parameters are also crucial factors that can affect the particle deposition characteristics *in vivo* studies. Lung deposition studies of aerosolized drug should be carefully designed so that effects of model dependent variables, i.e. lung physiology and respiration rate are kept at minimum.

ORAL *vs* NASAL BREATHING

During normal breathing the majority of inhaled environmental particles deposited in the nose and pharynx. Deposition by diffusion increases as the particle size

decreases. Brownian diffusion is also more prevalent in region where airflow is very lower or absent, e.g. in the alveoli. Hence for pulmonary drug delivery the aerosols are inhaled via the mouth.

PHARMACEUTICAL FACTORS AFFECTING AEROSOL DEPOSITION

The pharmaceutical factors like particle size, shape, density, physical stability, moisture content, particle velocity and respirable fraction, which are discussed below.

a) PARTICLE SIZE

Data published by the task Group of the International Radiological Protection Commission confirmed the three areas of particle deposition within the respiratory system. The study identified particles of 8 μm or greater are deposited in nasal region of airway axis, whereas particles less than 3 μm are deposited in the pulmonary zones of the lung. Particles between 3 μm and 8 μm are largely deposited in the nasal and tracheobronchial zone *In vitro* measurement of particle size is therefore crucial in characterizing aerosol formulations with a subsequent goal of enabling the prediction of lung deposition [85].

b) SHAPE

Particles, which are non-spherical, will have at least one physical dimension, which is greater than the aerodynamic diameter. Environmental fibers 50 μm in length can reach the alveoli region because they align with the inspired airflow. Such materials then impact in the airways by a process of interception with the airway walls.

c) DENSITY

Particles with densities less than 1 g cm $^{-3}$ (unit density) will have a mean physical diameter greater than the aerodynamic parameter. Most micronised drugs for inhalations will have particle density of 1 g cm $^{-3}$, although materials produced by

freeze – drying or spray – drying methods are likely to be significantly dense. Large porous particles with physical diameters of 20 μm and densities of 0.4 g cm^{-3} are efficiently deposited in the lungs.

d) PHYSICAL STABILITY

Therapeutic aerosols are often inherently physically unstable since they have a high concentration of particles and their close proximity may lead to mutual repulsion or other inter-particulate reactions. Aerosol particles generated by DPIs may be hygroscopic and during their passage through the high humidity environments of the airways may increase in size and thus have a greater chance of being prematurely deposited.

e) MOISTURE CONTENT

Pharmaceutical dry powder formulations having physical mixture of drug and carrier generally lactose. The formulations may during storage, transportation might be directly or indirectly get exposed to the atmosphere having high humidity, leading to absorption of moisture by ingredients leading to formation of aggregates and ultimately affecting the flow properties. The increase in moisture uptake that facilitates upper airway deposition thus increases drug removal by mucocillary clearance with a subsequent decrease in the pulmonary bioavailability of the drug [86].

f) PARTICLE VELOCITY

Velocity of aerosolized drug particles often decreases dramatically as a result of inertial forces and drag by the humid air in the lung. This facilitates upper airway deposition and subsequent mucociliary clearance of the aerosolized drug. Particle velocity in aerosols is proportional to droplet or particle size. Increasing particle size causes decreasing in lower airway deposition and also decreases pulmonary absorption of the aerosolized drug.

g) RESPIRABLE FRACTION

Particle in the size range of respirable fraction (≤ 5 μm dia) are capable to deposit in the lungs. There is good relationship between % RF and bioavailability of inhaled drugs in humans [87].

BIOPHYSICAL ISSUES

There is significantly asymmetry in branching within the lungs of animal species when compared to human beings. Where animal model are used to estimate formulation performance *in vivo*, efforts are made to ensure that drug deposition characteristics in such an animal models are consistent and can be quantitatively extrapolated to human airway geometry.

Several morphologic models exist that describe the geometry of human lung. The most widely used model for describing the morphologic structure within the lung was initially given by Weibel [88,89]. Using the scheme based on that proposed by Weibal, the branched airways of the lung have been characterized fall into three distinct compartments. These are conducting zones, Transitional zone and Respiratory Zone. Guidelines have also been given by NCRP (National Council of Radiation Protection).

2.7 PROFILE OF INSULIN (PORCINE)

Insulin, a well-known polypeptide drugs used for diabetes mellitus, which is ranked 7^{th} amongst the killer diseases. People with type I diabetes take this subcutaneous injection of insulin one to four times (depending on blood glucose level and individual requirements) for their whole life period. Porcine insulin is the natural antidiabetic principle obtained from pork and purified.

DESCRIPTION

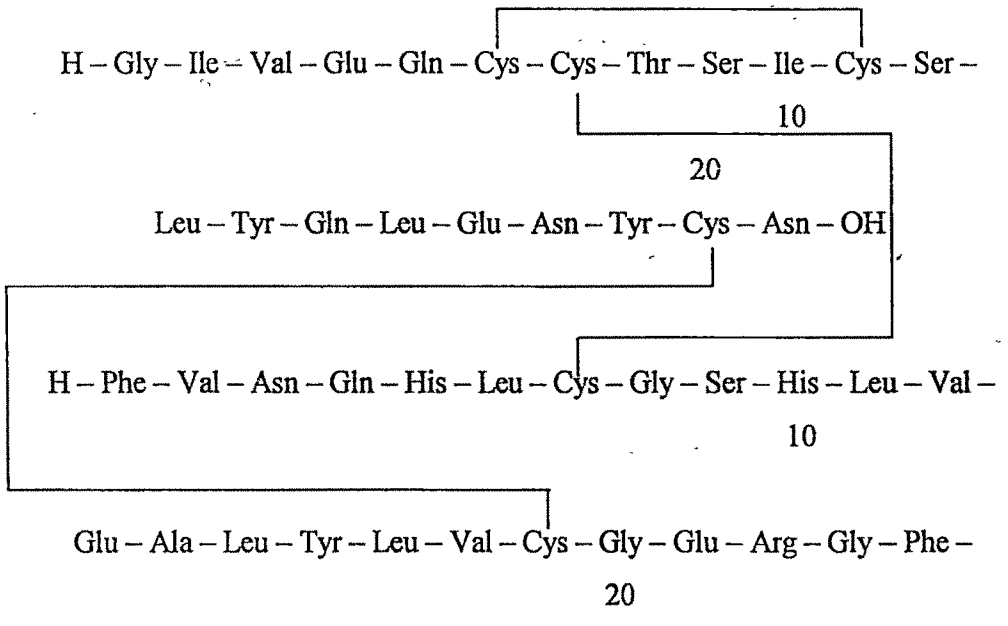
White or almost white powder.

Insulin consists of 2 chains of amino acids, the A and B chains connected by the disulfide bridges. Insulin produced by different sequences of amino acids in the chains.

Porcine insulin differs from human insulin in only one amino acid in the B chain whereas bovine insulin differs from human insulin not only in the same amino acid in the B chain but also in 2 amino acids in the A chain. Practically insoluble in

water and in ethanol. It dissolves in dilute mineral acids and with decomposition in dilute solutions of alkali hydroxides.

STRUCTURE



$C_{256}H_{381}N_{65}O_{76}S_6$ **MW: 5778**

STABILITY AND STORAGE

Insulin in powder from should be stored in airtight containers and protected from light. Storage at a low temperature is recommended. The BP advises storage at a temperature not exceeding-20°C while the USP require storage at –10°C to – 20°C.

It is stressed that this temperature is for the powder and not for the preparations; preparations should not be subjected to storage conditions that lead to freezing. Both the BP and USP recommend that insulin preparations be stored in a refrigerator at 2°C to 8°C, protected form light, and not allowed to freeze.

DOSE

By subcutaneous, intramuscular or intra venous injection, or intravenous injection, in accordance with the needs of the patient.

MECHANISM OF ACTION

Insulin's anabolic action includes the stimulation of intracellular utilization and storage of cellular nutrients. Insulin's anabolic actions include the stimulation of intracellular utilization and storage of glucose, amino acids and fatty acids, while it inhibits catabolic processes, such as the breakdown of glycogen, fat and protein.

PHARMACOKINETICS

The half-life of insulin in plasma is about 5 to 6 minutes in normal subjects and patients with uncomplicated diabetes. Degradation of insulin occurs primarily in liver, kidney and muscles. Insulin has no hypoglycemic effect when administered by mouth since it is inactivated in the gastrointestinal tract. It is fairly rapidly absorbed from subcutaneous tissue following injection and although the half life in blood is very short (only a matter of minutes). The absorption of insulin after intramuscular administration is more rapid than that following subcutaneous administration.

INDICATIONS

Human, porcine, bovine or mixed porcine-bovine insulin is given to patients with insulin-dependent diabetes mellitus to control their blood-glucose concentrations. It may also be necessary in some non-insulin dependent diabetics. Insulin is also an essential part of the emergency management of diabetic ketoacidosis.

ADVERSE EFFECTS

The most frequent complication of insulin therapy is hypoglycemia, the speed and meet and duration of which may vary according to the type of preparation used and

the route of administration. It is usually associated with an excess dosage of insulin.

CLINICAL NEED FOR MORE FREQUENT USE OF INSULIN

Diabetes mellitus (DM) is a disease characterized by the inability of the body to produce insulin or use insulin effectively. Insulin is a hormone produced in the pancreas. It is required for the transport of glucose, the body's main energy source, from the blood stream to the body's cells. Complete lack of insulin causes the blood glucose levels to become dangerously high resulting eventually in death. Inadequate levels of insulin cause blood glucose levels that are above normal levels. If this persists for long periods of time, the complications of diabetes, nephropathy, retinopathy, neuropathy and other serious microvascular and macrovascular complications, develop. The consequences of poorly managed diabetes are dire. Forty percent of deaths in people with diabetes are due to heart disease. By 15 years after diagnosis, 97% of people with diabetes who use insulin have retinopathy. Twelve percent of all new cases of blindness and over one-third of new cases of end stage renal disease are due to diabetes. Over 385,000 people with diabetes die per year, and nearly half of these deaths are directly related to diabetes or the complications of diabetes [90]. Approximately 6% of the U.S. population, 16 million Americans [90], has diabetes mellitus, which can generally be divided into two categories: Type I (juvenile onset or IDDM) or Type II (adult onset or NIDDM).

Type I DM is characterized by the autoimmune destruction of the pancreatic beta cells resulting in little or no production of insulin [91]. Peak incidence is from 10 to 12 years of age in girls and from 12 to 14 in boys. There are approximately 0.7 million people with type I DM in the U.S. It is one of the most prevalent chronic diseases of children in the U.S. with ~0.12 million cases in the under 19 year age group. To put this in perspective, 30,000 children develop type I DM per year. This is more than 14 times that seen for childhood AIDS [92].

Type II DM is a heterogeneous disease characterized by insulin resistance and impaired insulin secretion [93]. Ninety percent of people with DM have the type II form of the disease, which has a strong genetic basis and increases in prevalence with obesity and age. Fifty to 90% of these patients are obese. There are approximately 99 million people worldwide [93] with type II DM with the greatest prevalence being in North America (4.7 % of total).

The economic impact of DM is substantial in terms of both direct medical costs and loss of productivity. The direct costs in 1992 in the U.S. for hospitalization due to DM have been estimated to be as high as $65 billion per year [93]. Greater than 2.2 million-hospital days were attributed to providing care to persons with diabetes related renal, ophthalmologic, neurologic, and cardiovascular complications in 1987 [94]. The total estimated cost of DM in the U.S. exceeds $90 billion per year [90]. A treatment that can provide diabetic patients with improved glucose control and/or a more convenient treatment could have a huge economic impact on health care costs.

Blood glucose levels normally are reduced by the action of insulin, which is released from the pancreas into the blood as a pulse during meal times (peak at 20-30 min after starting a meal) and at low levels during the rest of the day.

Physicians have suspected that the tight control of blood glucose through intensive insulin therapy could reduce the frequency and severity of diabetes complications, but until recently, hard evidence supporting this suspicion was lacking. The publication of the results of the 9-year, 1441 Type I patient, Diabetes Control and Complications Trial an enhanced rate of systemic absorption occurs after SC injection. The manufacturer recommends that (DCCT) [95] has eliminated any doubt. The trial demonstrated that intensive therapy (defined as three or more insulin injections/day) as compared with conventional therapy (two injections/day) reduced the risk for retinopathy by 50.76%, nephropathy by 35.56% and neuropathy by 60%. The major adverse event associated with the intensive therapy was a two- to three-fold increase in severe hypoglycemia. Although the DCCT study was conducted with Type I diabetes, Type II diabetes suffer the same side effects of poor glucose control. A non-injectable form of regular insulin would

make it easier for patients to take insulin more frequently and thereby lower their risk of long-term diabetes complications.

2.8 PROFILE OF CALCITONIN (SALMON)

Calcitonin is a 32 amino acid polypeptide mainly secreted by the thyroid C-cells which originate from the ultimobranchial body in the embryo. Molecules of both calcitonin and its precursors are found in these cells; calcitonin and the calcitonin gene-related peptide (CGRP) coexist in the C-cells of various species [96]. Its main biological effect is to inhibit osteoclastic bone resorption. This property has led to its pharmacological use in processes involving an increases in bone resorption. Thus, parenteral and intranasal formulations of this hormone are used in the treatment of Paget's disease, osteoporosis and tumoral hypocalcaemia [97]. The secretion of calcitonin is acutely regulated by other factors such as sex and possibly also age. Calcitonin is metabolized in the liver and the Kidney. Moreover, this peptide is the marker for medullary thyroid carcinoma and for type 2 multiple endocrine neoplasia [98].

Since calcitonin was discovered by Mac-Intyre's group in 1963 [99], this hormone has been the subject of extensive research, both in animals and in humans. Nevertheless, the findings have not always been consistent and the precise role of the hormone calcitonin in human metabolism has not been satisfactorily established. Its physiological importance has been questioned due to the fact that no significant or lasting biochemical alteration arising from the excess or deficiency of this hormone has been found, and the fact that no disease involving either raised or nonexistent titers of calcitonin shows any clinical alterations that can be attributed to its action [100].

DESCRIPTION

SYNONYM -Salcatonin

A white or almost white, light powder. Freely soluble in water, very slightly soluble in alcohol, soluble 1 in 10 of methyl alcohol; soluble in glacial acetic acid.

STRUCTURE

Cys – Ser – Asn – Leu – Ser – Thr – Cys – Val – Leu – Gly – Lys – Leu –

Ser – Gln – Gly – Leu – His – Lys – Leu – Gln – Thr – Tyr – Pro – Arg –

Thr – Asn – Thr – Gly – Ser – Gly – Thr – Pro – NH_2

$C_{145}H_{240}N_{44}O_{48}S_2$ MW: 3432

BIOCHEMISTRY

Calcitonin is a peptide hormone with a molecular weight of approximately 3,500. The structure of at least 12 types of calcitonin, including human calcitonin has been determined. The common characteristics include a disulfide bond between positions 1 and 7, forming a ring of seven amino-terminal amino acids, glycine in position 28 and a proline amide group at the carboxyterminal end. The greatest divergences are found in the 27 central amino acids [101].

Calcitonin can be divided into three groups according to their primary structure: i) artiodactyl, which includes porcine, ovine and bovine calcitonin; ii) primate/rodent, including human and rat calcitonin; and iii) teleost/avian, including salmon, eel and chicken calcitonin. In various biological trials, the order of potency of calcitonin is as follows: teleost > artiodactyl > human. However, the absolute potency of each one depends on the species in which it is studied [102]. Fish and bird calcitonin are produced in the ultimobranchial glands, which in these species are independent organs; in mammals, these structures migrate early on in the development of the thyroid, constituting a second hormone system between the follicles of the thyroid gland, known as C-cells.

Studies on substitution, elimination and other types of modification of the calcitonin molecule have provided considerable information concerning the relationship between the structure and the activity of this hormone. Thus, although the ring structure appears to protect and stabilize the molecule, linear analogs of salmon calcitonin retain their hypocalcemic activity and their ability to activate adenylate cyclase [103]. Accordingly, position 1-7 aminosuberic eel calcitonin increases its biological stability and its potency. The proline amide group located at the carboxyterminal end is essential, but numerous changes are tolerated in positions 8-12.The general conclusion is that maintaining the tertiary structure is more important for biological potency than the length of the chain per se.

MECHANISM OF ACTION

The study of calcitonin's mechanism of action has led to a greater understanding of the metabolism of both calcium and bone, as well as a better appreciation of the bone's contribution to the homeostasis of extracellular calcium. The original observation that the hypocalcemic effect of calcitonin persists after the removal of the kidneys and the gastrointestinal tract showed that this was due to its action on the bone [104]. Thus, studies of organs in culture have confirmed the direct inhibitory effect of calcitonin on bone resorption, and various *in vivo* techniques have identified the same activity [105]. However, there is no convincing evidence, either from *in vitro* or *in vivo* studies, that calcitonin increase the bone's calcium uptake.

The main biological effect of calcitonin is to inhibit bone resorption through the mediation of osteoclasts, which have a large number of receptors for this hormone [106]. Thus, autoradiograph imaging of this has become an essential criterion in the identification of osteoclasts in mixed cultures. Inhibition of bone resorption is dependent on calcitonin binding with its receptors in the osteoclasts, which results from a marked decrease in the activity and recruitment of these cells [107]. The morphology of the osteoclasts changes rapidly after exposure to picomolar concentrations of calcitonin. These multinucleate cells decrease in size and their ruffled borders, which are considered to be essential to bone resorption, recede

from the resorptive surface [108]. This characteristics and complex phenomenon is accompanied by an increased production of cyclic adenosine 3',5'-cyclic monophosphate (cAMP) and an increase in cytosolic calcium in the osteoclast [109]. The specificity and sensitivity of these responses strongly suggest that the effect is physiological. In addition to the direct effect of calcitonin on the motility, morphology and activity of mature osteoclasts, there is also some evidence to show that it inhibits the formation of osteoclasts by inhibiting the fusion of mononucleate cells to form multinucleate osteoclasts [110]. The continued presence of calcitonin leads to an "escape phenomenon" from its inhibitory action; this is not due to the metabolism of the hormone and can be prevented by means of combined treatment with glucocorticoids or radiation [111]. It is thought that this escape phenomenon is due to a down-regulation of the calcitonin receptors or to the predominance of a population of cells, which are resistant to this substance, although the molecular mechanisms involved have not been explained [112].

An increase in calciuria, phosphaturia and changes in the gastrointestinal calcium flux have been described, but these effects occur at supraphysiological concentrations of calcitonin [113]. However, it must be kept in mind that the concentrations of this hormone in the places where it is synthesized may be sufficiently high to explain some extra skeletal actions of calcitonin through a paracrine mechanism. Thus, calcitonin may exert physiological effects on the pituitary gland or the central nervous system (CNS). Moreover, the observation of calcitonin and its receptors for intracraneal localizations may qualify this hormone as a neurotransmitter [114]. Calcitonin may regulate and be regulated by the other calciotropic hormones, and there is some evidence to suggest a self-regulatory effect. However, the importance of these actions has not been fully established.

PHARMACOKINETIC PROPERTIES

Calcitonin is rapidly inactivated when administered orally. After injection they are quickly metabolized, mainly in the kidney and the peripheral tissues. The peripheral metabolites and a small amount of unaltered drug are excreted in the urine. Calcitonin is absorbed through the nasal and rectal mucosa. Following

subcutaneous administration of salmon calcitonin, it is rapidly absorbed, with an absorption half-life of 23.4 min. Maximum plasma concentrations are soon reached and then elimination is relatively fast, with an elimination is relatively fast, with an elimination half-life of 87 min. Intranasal administration of salmon calcitonin produces maximum plasma concentrations in 31-39 min, slightly longer than in the case of subcutaneous or intramuscular administration [115]. Generally, the maximum plasma concentrations of intranasally administered salmon calcitonin are lower, but also more sustained than those with parenteral administration. An evaluation of the acute biochemical responses of salmon calcitonin shows that intranasal administration provided approximately 25-50% of the biological activity compared with parenteral administration.

ADVERSE EFFECTS

The appearance of adverse effects and need for repeated subcutaneous or intramuscular injections are the main reasons for discontinuing calcitonin treatment. Various symptoms have been reported in 40-60% of cases, although the rates of discontinuation of treatment vary from 5-15%. The most frequently reported symptoms are rubefaction, nausea and irritation at the injection site. These symptoms are more common with human calcitonin than in salmon calcitonin [116].

The tolerability profile for intranasal administration of calcitonin is well established. Relatively fewer and less frequent adverse effects are found with intranasal administration of salmon calcitonin than with parenteral administration. Thus, patients who do not tolerate parenteral administration of calcitonin show few if any adverse effects when intranasal administration is used, which suggests different absorption efficiency levels and tissue distribution patterns for the two administration routes. Preliminary results of long-term studies do not indicate any increase in adverse effects in the treatment groups using intranasal salmon calcitonin as compared to placebo [117]. The most frequently described symptoms are facial redness, nasal congestion and irritation, and rhinitis. More rarely, isolated incidents of nosebleeds, nausea and itchy palms have been reported. Although the

pathogenesis of these adverse effects is not fully understood, most of the vasomotor effects can be related to interaction with the CGRP receptors since they have important vasoactive properties [118]. On the whole, less than 5% of patients treated with intranasal calcitonin find it necessary to discontinue treatment due to the appearance of adverse effects.

DOSES AND ADMINISTRATION ROUTES

In the case of parenteral calcitonin, doses of 100IU/day were initially suggested for the treatment of established osteoporosis. However, the difficulty in maintaining compliance with therapy involving daily injections, and evidence that lower doses are potentially effective, has led to a progressive decrease in the doses of parenteral calcitonin in clinical practice. Dosing regimens of 100 IU on alternate days, or even less (50 IU on alternate days) in cases where there are significant adverse effects, are currently recommended [116].

In the case of intranasal formulations of calcitonin used in the prevention and treatment of osteoporosis, different regimens and doses ranging from 50-400 IU/day have been used in clinical trials [115]. However, from the available information and the preliminary conclusions of the Prevention of the Reappearance of Osteoporotic Fractures (PROOF) study (a 5-year clinical trial involving 1,255 postmenopausal women) [119], it may be deduced that the correct dose to achieve effects on bone mass and the reduction of fractures in women with postmenopausal or involutional osteoporosis is 200 UI/day salmon calcitonin administered intranasally, which is, moreover, the dose approved by the US Federal Drug Administration. Its efficacy is prolonged and independent of major pretreatment risks factors, such as low bone density or high bone remodeling activity [120].

STORAGE

Storage at 2°C and protect from light. Under these conditions it may be expected to retain its potency for not less than 2 years.

INDICATIONS & PREPARATIONS

- ❖ Postmenopausal osteoporosis
- ❖ Osteitis deformans (Paget's disease of bone)
- ❖ Hypercalcemia from a variety of causes including vitamin D intoxication, neoplastic disease and
- ❖ Hyperparathyroidism.

- Calcitonin (Pork) Injection (B.P.)
- Salcatonin Injection (B.P.)
- Clacitare (Armous, U.K.) (Contains Calcitonin (Pork) in vials of 160 IU)
- Calsynar (Armour UK)
- Miacalcin Nasal Spray (Novartis)

2.9 AN OVERVIEW ON ADVANCES IN PULMONARY DELIVERY OF DRUGS

The literature related to advances in pulmonary delivery are reviewed below:

Kawashima, Y., et.al [121] prepared insulin loaded PLGA nanosperes having weight mean diameters of 400 nm by the modified emulsion solvent diffusion method in water. The aqueous dispersions (6 mg/ml) of PLGA nanospheres were nebulized by a sieve type ultrasonic nebulizer to discrete droplets of 5 approximately 7 μm in mean diameters, 75% of which were successfully delivered into the alveolar fraction in a cascade impactor inhaled at 28.3 l/min. The nebulized PLGA nanospheres were administered via a spacer by using a constant volume respirator into the trachea of the fasted guinea pig for 20 min. After the administration of 3.9 I.U./Kg insulin with the PLGA nanospheres, the blood glucose level was reduced significantly and the hypoglycemia was prolonged over 48 h, compared to the nebulized aqueous solution of insulin as a reference (6 h). This result could be attributed to the sustained releasing of insulin from the nanospheres deposited widely on to the whole of lung.

Inoue, K., Yoshioka, K. [122] studied pulmonary absorption of insulin administered in the form of an aerosol.

Koshkina, N.V., et.al [123] studied the distribution of camptothecin after delivery as a liposome aerosol or following intramuscular injection in mice. The plant alkaloid camptothecin (CPT) has shown significant antitumor activity against a wide variety of human xenografted in nude mice. The purpose of this study was to analyze the pharmacokinetics and tissue distribution of inhaled CPT formulated in DLPC liposomes. After 30 min inhalation of CPT liposome aerosol, drug was deposited in the lungs (310ng/g) and was followed promptly by the appearance of high concentrations in the liver (192 ng/g) and with lesser amounts appearing in other organs. Drug concentration in the brain was 61 ng/g. After intramuscular injection of CPT dissolved in DMSO, drug was released from the site of injection very slowly and accumulated mainly in the liver (136 ng/g). Only trace amounts appeared in the lungs (2-4 ng/g). These results demonstrate a prompt pulmonary and later systemic distribution of CPT following liposome aerosol administration. The substantial concentrations of CPT in lungs and other organs following inhalation of liposome aerosol suggest the possible benefit of it and of its more active derivative, 9-NC, in the treatment of lung, liver, kidney and brain cancer in humans.

Cass, L.M., et al [124] evaluated lung deposition of inhaled zanamivir in healthy volunteers by pharmacoscintigraphy. The sites of zanamivir deposition in the respiratory tract and the pharmacokinetics of zanamivir after oral inhalation from the Diskhaler device and from a prototype of novel breath-activated device was determined. Participants were given dry powder zanamivir 10 mg formulated with 99mTc from the Diskhaler or the protoytpe device on separate days. Scintigraphic images of the chest and oropharynx were recorded. Blood samples for determination of serum zanamivir and urine for excretion studies were taken up to 8 hours after drug administration. Safety was evaluated by monitoring lung function tests, adverse events and laboratory parameters. Orally inhaled zanamivir was well tolerated, as demonstrated by lung function tests. A mean of 13.2% (n=11) of the 10 mg does from the Diskhaler was deposited in the bronchi and lungs. The deposition pattern varied between individuals, showing a preferentially

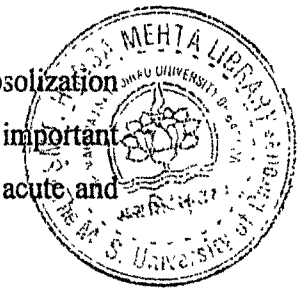
central deposition pattern in some and a uniform distribution pattern in others. The major deposition site was the oropharynx (mean 77.6%), with a mean of 1.2% deposited on the trachea and a mean of 3.2% retained in the blister. Similar data were obtained with the prototype device. Inhalation of zanamivir gave a broad peak of systemic absorption with mean maximum serum concentrations of approximately 30 to 40 micrograms/L after 1.5 hours. The rate and extent of absorption were similar irrespective of inhalation device. Less than 5% of drug was excreted unchanged in urine within 8 hours of inhalation, conforming the low bioavailability of zanamivir after pulmonary delivery. A significant correlation existed between systemic exposure and peripheral lung deposition. The local concentrations of zanamivir that result from oral inhalation via the Diskhaler are estimated to be > 10 mumol/L throughout the respiratory tract, well in excess of the concentrations observed to inhibit influenza virus neuraminidase by 50% (0.64 to 7.9 nmol/L). Similar deposition data were obtained with the diskhaler and the prototype device, which was consequently not developed further. Pharmacoscintigraphy was confirmed as being a reliable technique for measuring zanamivir deposition in the respiratory tract.

Morimoto, K., et.al [125] examined the permeability of model hydrophilic compounds with different molecular weights and model dipeptides to characterize the tracheal epithelial barrier *in vitro* experiments using excised rabbit trachea. 6-Carboxyfluorecein (6-CF; 376 Da) and fluorescein isothiocyanate (FITC)-dextrans (FDs) with varying molecular weights (4 to 70 kDa) were used as model hydrophilic and macromolecular compounds, and glycyl-D-phenylalanine (Gly-D-Phe) and glycyl-L-phenylalanine (Gly-L-Phe) were used as model dipeptides in this experiment. The apparent permeability coefficients (Papp) of 6-CF and FDs with Mw 376 Da to 70 kDa ranged from 2.35*10(-7) to 4.05*10(-10)cm/s and exhibited a good inverse correlation with their molecular weights. The tracheal permeability of 6-CF, FD-4 (4 kDa) and FD-10 (10 kDa) were increased over three fold by 10 mM glycocholate, which is an absorption enhancer. The Papp of Gly-D-Phe was 1.03*10(-6)cm/s and there was no metabolism during tracheal permeation. Gly-L-Phe was immediately degraded in the mucosal fluid and its intact form was not detected in serosal fluid during the 150-min experimental period. These results suggest that absorption of some peptide drugs via the respiratory tract may

contribute to their systemic delivery following pulmonary administration by intratracheal insufflation and instillation.

LiCalsi, C., et.al [126] proved dry powder inhalation as a potential delivery method for vaccines. Measles vaccine is administered to millions of children annually via a percutaneous injection. There are, however, compelling reasons to search for alternative routes of administration, especially in mass vaccination campaigns. Two key factors are (1)-decreased stability of the vaccine upon reconstitution and, (2) the potential risks of contamination associated with needles. Dura has developed a unique inhaler that can deliver a powder dose via the pulmonary route for local or systemic action. The breath-actuated Spiros inhaler uses electromechanical energy to aerosolize and deliver a consistent dose over a wide range of inspiratory flow rates. To achieve alveolar (deep lung) deposition for subsequent systemic absorption, dry-powder vaccine is size reduced to a mass median diameter between 1 and 5 microns. Small vaccine particles are blended with an inert carrier to improve dispersion. Measles vaccine formulated as a powder blend may be more thermostable than existing reconstituted formulations. The Spiros technology is available in three powder storage platforms. Two of these formats are designed specifically for moisture and/or light sensitive compounds and may be particularly suitable for delivery of measles vaccines in mass campaigns because their design (1) eliminates the need for powder reconstitution, and (2) reduces the risk of contamination.

Langenback, E.G., et.al [127] improved the pulmonary distribution of recombinant human Cu/Zn superoxide dismutase, using a modified ultrasonic nebulizer. Instillation of small volumes of rhSOD intratracheallly would not be expected to result in uniform pulmonary distribution. Aerosolization is a technique that may improve pulmonary distribution of drugs, but is limited by the poor efficiency of most nebulizers. A newly modified ultrasonic nebulizer was tested to assess pulmonary distribution of rhSOD compared to that achieved by intratracheal instillation. rhSOD was dual-labeled with technetium-99m (99m Tc) and a fluorescent analog (permitting quantitative and qualitative assessments of pulmonary distribution), and administered to neonatal piglets by intratracheal instillation or by aerosolization. Intratracheal instillation of rhSOD in small



volumes results in non-uniform pulmonary distribution, while aerosolization enhances rhSOD distribution and alveolar deposition. This has important implications for ongoing clinical trials of rhSOD for the prevention of acute and chronic lung injury in premature neonates.

Parthasarathy, R., et.al [128] optimized the delivery of all-trans-retinoic acid (ATRA) to lung tissue by determining the potential of vehiculating the drug in liposomes (L-ATRA) and delivering it via aerosol. Liposomes may provide a means to prevent local irritation of lung tissue and reduce pulmonary toxicity, prolong therapeutic levels and generate high drug concentrations at the tumor sites. Cumulatively, this would result in reduced systemic toxicity and enhanced drug efficacy. The drug was effectively delivered at high concentrations (10 +/- 2 μg/g of tissue) to the lungs of mice and was retained for at least up to 96 h after a single exposure to L-ATRA aerosol. No appreciable levels of ATRA were detected in the blood or the liver of treated mice. The aerosol-delivered ATRA was biologically active as demonstrated by its ability to include the expression of tissue-type transglutaminase. Aerosol delivery of L-ATRA offers an effective way to deliver high levels of ATRA to the lung without apparent pulmonary toxic effects.

Suntres, Z.E., Shek, P.N. [129] studied the pulmonary glucocorticoid delivery using liposomes. The beneficial effects of glucocorticoids in treating pulmonary inflammatory disorders are complicated by systemic adverse effects. Thus, a possible reduction in dosage and dosing frequency would be advantageous, particularly for patients requiring high doses of the drug. We believe that this can be achieved by developing formulations that increase the retention of glucocorticoids in the lung and a liposome-based drug delivery system may be useful. Male adult rats were intratracheally instilled with free [3H] dexamethasone (DEX) or [14C] liposome-entrapped [3H] dexamethasone (L-DEX) (800 μg DEX kg body weight) and animals were killed at different times within a 72-h treatment period. Pulmonary retention of [3H] DEX in animals instilled with free DEX was found to be approximately 1.5% of the administered dose 4h post-instillation, with no radioactivity detectable 24h post-instillation. Liposome encapsulation of the drug altered the pulmonary retention of DEX with about 34% and 8% of radioactivity remaining in the lung at 4 and 24h post-instillation, respectively. The

intratracheal instillation of free DEX or L-DEX reduced the number of leukocytes in peripheral blood to a similar extent (50% of control values) at 4h. However, unlike free-DEX-treated animals whose leukocyte counts returned to control levels by 24h, the circulating leukocyte counts of L-DEX-treated animals remained depressed in the same period. Furthermore, DEX-induced changes in ACTH levels were less evident in animals treated with the liposomal formulation than those treated with free DEX. The data suggest that the administration of liposome-entrapped DEX has the distinct advantage of enhancing the anti-inflammatory activity of the drug and therefore, possibly reducing its need for frequent administration.

Mather, L.E., et.al [130] administered fentanyl by aerosolization and analyzed pharmacokinetics of systemic delivery. They determined whether a novel breath-actuated, microprocessor-controlled metered dose oral inhaler (SmartMist, Aradigm Corporation) could deliver fentanyl in a way suitable for control of severe pain. Aerosolised pulmonary fentanyl base 100-300 μg was administered to healthy volunteers using SmartMist and the resultant plasma concentration-time data were compared with those from the same doses administered by intravenous injection in the same subjects. Plasma concentrations from mist were similar to those from i.v. Injection. Time-averaged bioavailability based upon nominal doses averaged approximately 100%, and was > 50% within 5 min of delivery. Fentanyl systemic pharmacokinetics were similar to those previously reported with no trends to dose-dependence from either route. Side effects (e.g. sedation, lightheadedness) were the same from both routes. Fentanyl delivery using small mist can provide analytically relevant plasma drug concentrations. This, combined with its ease of noninvasive use and transportability, suggests a strong potential for field and domiciliary use, and for patient controlled analgesia without the need for i.v. cannulae.

Edwards, D.A., et.al [131] engineered large, porous particles for inhalation. For most of the therapy by inhalation, aerosols are designed to comprise small spherical droplets or particles of mass density near 1 g/cm3 and mean geometric diameter between approximately 1 and 3 micron, suitable for particle penetration into the airways or lung periphery. Studies performed primarily with liquid

aerosols have shown that these characteristics of inhaled aerosols lead to optimal therapeutic effect, both for local and systemic therapeutic delivery. Inefficient drug delivery can still arise, owing to excessive particle aggregation in an inhaler, deposition in the mouth and throat, and overly rapid particle removal from the lungs by mucocilliary or phagocytic clearance mechanisms. To address these problems, particle surface chemistry and surface roughness are traditionally manipulated. Recent data indicate that major improvements in aerosol particle performance may also be achieved by lowering particle mass density and increasing particle size, since large, porous particles display less tendency to agglomerate than (conventional) small and nonporous particles. Also, large, porous particles inhaled into the lungs can potentially release therapeutic substances for long periods of time by escaping phagocytic clearance from the lung periphery, thus enabling therapeutic action for periods ranging from hours to many days.

Saari, S.M., et.al [132] studied the regional lung deposition and clearance of 99mTc-labeled beclomethasone-DLPC liposomes in mild and severe asthma. To compare the distribution and clearance of inhaled beclomethasone dipropionate (Bec)-dilauroylphosphatidylcholine (DLPC) liposomes in-patients with mild and severe asthma. A 99mTc-labeled Bec-DLPC suspension was delivered via a nebulizer (Aerotech II). Immediately after inhalation, anterior and posterior views of the lungs and an anterior view of the oropharynx were measured by a large field gamma camera with the patient in a supine position. To evaluate the mucociliary clearance of the inhaled liposomes, anterior and posterior lung scans were repeated 1,2,4, and 24h after the aerosol delivery. Ten patients with mild asthma (FEVI > 80% of the predicted) and 10 patients with severe asthma (FEVI<60% of the predicted) were included in an open, parallel group study. Clearance is more rapid among patients with severe asthma ($p<0.0001$). At the 4-h measurement, a mean of 82% (SD, 5.9) of the total pulmonary dose was detected in the lungs of patients with mild asthma while in those with severe asthma the figure was 69% (SD, 10.9). The ratio between central and peripheral deposition was significantly higher for patients with severe asthma than for those having a mild form of these disease; 1.07 (SD, 0.29) and 0.76 (SD, 0.07), respectively ($p=0.008$). Inhaled Bec-DLPC liposomes were deposited more centrally in the lower airways of patients with severe asthma than those having a milder form of the disease. The clearance of

Bec-DLPC liposomes is strikingly slow in both groups of asthmatic patients. However, due to the more peripheral penetration of inhaled liposomes in-patients with mild asthma, the clearance rate in this group was slower than in those with severe asthma.

Ward, M.E, et.al [133] studied morphine pharmacokinetics after pulmonary administration from a novel aerosol delivery system. Successful pharmacotherapy of pain often depends on the mode of drug delivery. A novel, unit dose, aqueous aerosol delivery system (AERx Pulmonary Drug Delivery System) was used to examine the feasibility of the pulmonary route for the noninvasive systemic administration of morphine. The study had two parts: (1) a dose-ranging study in four subjects with three consecutive aerosolized doses of 2.2,4.4, and 8.8 mg (nominal) morphine sulfate pentahydrate at 40-minute intervals, and (2) a crossover study, on separate days, in six subjects with 4.4mg(nominal) aerosolized morphine sulfate administered over 2.1 minutes on three occasions and intravenous infusions of 2 and 4mg over 3 minutes. Subjects were healthy volunteers from 19 to 34 years old. Arterial blood was sampled for a total of 6 hours and plasma morphine concentrations were measured by gas chromatography-mass spectrometry. In part 1, plasma morphine concentrations were proportional to dose. In part 2, the mean ± SD peak plasma concentration (C_{max}) occurred at 2.7 ± 0.8 minutes after the aerosol dose, with mean values for C_{max} of 109 ± 85, 165 ± 22, and 273 ± 114 ng/ml for the aerosol and 2 and 4 mg intravenous doses, respectively. The bioavailability [AUC (0-360 min)] of aerosol-delivered morphine was approximately 100% relative to intravenous infusion, with similar intersubject variability in AUC for both routes (coefficient of variation < 30%). The time courses of plasma morphine concentrations after pulmonary delivery by the AERx system and by intravenous infusions were similar. This shows the utility of the pulmonary route in providing a noninvasive method for the rapid and reproducible systemic administration of morphine if an appropriate aerosol drug delivery system is used.

Khanna, C., et.al [134] nebulized interleukin 2 liposomes and studied aerosol characteristics and biodistribution. Although interleukin 2 (IL-2) has been associated with modest anti-tumour responses in man, treatment-related toxicity

has limited its widespread use. The local delivery of liposomal formulations of interleukin 2 to the lung as aerosols has been demonstrated to be non-toxic, biologically active, and associated with regression of spontaneous pulmonary metastases in dogs. This study was undertaken to evaluate the physical and biological characteristics of nebulized interleukin 2 liposomes. The aerosol droplet size distribution and the physical stability of interleukin 2 liposomes were examined in-vitro using an Andersen cascade impactor and studies of liposome entrapment of interleukin 2 before and after nebulization. The biological stability of interleukin 2 liposomes after nebulization was demonstrated using the CTLL-2 bioassay for interleukin 2. In-vivo studies of pulmonary biodistribution and clearance of inhaled technetium (99mTc)-labeled interleukin 2 liposomes were undertaken in a normal dog. Aerosols of free interleukin 2 and of interleukin 2 liposomes were compared in both in-vitro and in-vivo experiments. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of interleukin 2 liposomes were 1.98 microns and 2.02, respectively. Independent analysis of aerosol particle-size distribution using the constitutive compounds of the interleukin 2 liposomes (interleukin 2:lipid:HAS) demonstrated a close correlation of size distributions ($r=0.9445$; $P < 0.001$). The entrapment of interleukin 2 in liposomes was 93 ± 4.3% before nebulization and 90 ± 8.9% after. After delivery to an anaesthetized dog, interleukin 2 liposome aerosols were deposited evenly throughout the lung (mean ± s.d. central lung-to-peripheral lung deposition was 1.12 ± 0.03). After approximately 24-h inhalation, interleukin 2 liposomes were retained within the lung and were taken up in part by the spleen. The results of this study are indicative of the stability of this interleukin 2 liposome formulations to nebulization. Such nebulization might be an attractive immunotherapeutic strategy for treatment of pulmonary metastases and primary lung cancers.

Taljanski, W., et.al [135] studied Pulmonary delivery of intratracheally instilled and aerosolized cyclosporine A in young and adult rats. The delivery and pharmacokinetics of cyclosporine A (CyA) given locally to the airways or iv was evaluated in young and adult rats. After interatracheal (i.t.) instillation of saline suspended CyA to adult rats, the CyA plasma levels peaked at 30 min with a bioavailability of 78.1 ± 6.9%. After the i.t. instillation of CyA with micelles

forming surfactant, Cremophor EL, in adult and young rats, the plasma levels peaked at 5 min with a bioavailability of 77.5 ± 7.2% and 66.3 ± 4.5%, respectively. The bioavailability of aerosolized CyA was 80.1 ± 4.1% in adults. Thus, CyA is absorbed by the lungs into the systemic circulation of the rat in high amounts, independent of age and type of delivery system. Long-term treatment with i.t. instillation did not affect body weight gain in young and adult rats, and no histopathological changes were found in the lungs. It is important to emphasize that CyA plasma clearance in young rats was lower and elimination half-life longer than in adults. The slow elimination of CyA in young rats indicated profound pharmacokinetic age differences for this species.

Edwards, D.A., et.al [136] studied Large porous for pulmonary drug delivery. A new type of inhalation aerosol, characterized by particles of small mass density and large size, permitted the highly efficient delivery of inhaled therapeutics into the systemic circulation. Particles with mass densities less than 0.4 gram per cubic centimeter and mean diameters exceeding 5 micrometers were inspired deep into the lungs and escaped the lungs' natural clearance mechanisms until the inhaled particles delivered their therapeutic payload. Inhalation of large porous insulin particles resulted in elevated systemic levels of insulin and suppressed systemic glucose levels for 96 hours, whereas small nonporous particles had this effect for only 4 hours. High systemic bioavailability of testosterone was also achieved by inhalation delivery of porous particles with a mean diameter (20 micrometers) approximately 10 times that of conventional inhaled therapeutic particles.

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