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Review

Prospects of formulating proteins/peptides as aerosols for pulmonary drug delivery

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Abstract

Formulation of proteins/peptides for therapeutic uses has often posed some challenges to drug formulators. The main problem is the relatively weak forces involved in the native conformation of these proteins and so making them quite labile. Furthermore, their susceptibility to proteolytic enzymes in the gut makes oral administration quite challenging. While various routes like, ocular, transdermal, nasal and buccal have been tried, none of these routes has proved to be a potential alternative to the invasive injection. However, various studies have been performed on the formulation of these proteins as aerosols for pulmonary delivery and promising results have been obtained. This article looks at the prospects of inhaled proteins as a delivery route for systemic activity.

Keywords: Proteins and peptides; Aerosols; Metered dose inhalers; Dry powder inhalers; Nebulizers; Pulmonary

Contents

1. Introduction

Proteins are made up of amino acids chains. These form the primary structure of proteins and for these proteins to be biologically active, the amino acid sequence must form a well-defined three-dimensional structure (Crommelin et al., 2002). The

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secondary structure involves the α helices or β sheets which are locally identifiable discrete structures while the overall structure of the protein is the tertiary structure which is established by the proper positioning of different sub-units relative to each other (Manning et al., 1989). Sometimes, individual protein molecules interact and build a larger, well-defined structure called the quaternary structure (Manning et al., 1989).

The formation of the secondary, tertiary and quaternary structures is based on relatively weak physical interactions (e.g. electrostatic interactions, hydrogen bonding, Van der Waals forces and hydrophobic interactions) and not on the much stronger covalent bonding (Crommelin et al., 2002). Due to these weak interactions, proteins can easily undergo conformational changes, which can lead to a reduction of their biological activity. Since the native conformations of proteins seem only marginally stable (5–20 kcal/mol), their tertiary structure is very labile and so is easily disrupted (Quinn et al., 1999). Formulation of proteins for therapeutic use will thus depend on the physical and chemical stability of such molecules since the loss of the native conformation may result in reduction or complete loss of biological activity.

Furthermore, proteins are vulnerable to some gastrointestinal enzymes and first pass metabolism in the liver when administered orally. This makes oral administration of proteins quite challenging, although recent developments have given new hopes to oral delivery. For instance, the problem of reduced bioavailability has been addressed by various authors and techniques such as the use of absorption enhancers have been tried with some measure of success (Zheng and Fulu, 2006). Furthermore, Emisphere Technologies have developed Eligen™ technology for oral absorption of proteins and peptides using *N*-acylamino acids. This technology is effective for various peptides and proteins including PTH, calcitonin and interferon (Bagger et al., 2005). The nasal route is another promising route in which a lot of work has been performed on protein delivery. Hoegen (2001) described the use of synthetic biomimetic supra molecular biovector™ (SMBV), which has proven in preclinical and clinical evaluation to be a suitable candidate for the delivery of nasal vaccines. Macalcin™ is a nasal salmon calcitonin formulation developed by Novartis and has proved to be effective in the treatment of post-menopausal syndrome.

Other routes such as transdermal, buccal and ocular have been investigated (Cullander and Guy, 1992; Edman and Bjork, 1992; Harris et al., 1992; Ho et al., 1992; Smith et al., 1992). Although, these alternative routes showed a little success, no commercially viable non-injection delivery system of proteins has yet been developed. The main disadvantages of these other routes are variable bioavailability (Shen et al., 1992) and the safety of the enhancers used in the formulations (Hilsted et al., 1995).

However, inhalation offers potential possibilities for the delivery of proteins and peptides for systemic activity. Although the lungs and respiratory tract can metabolise some fraction of a delivered dose, the route offers an enormous absorptive surface area in the range 35–140 m (Hollinger, 1985). Furthermore, in most societies, oral inhalation is well accepted by the general population (Byron, 1990).

2. Pulmonary drug delivery

Pulmonary drug delivery involves the delivery of drugs to the respiratory tract either for the treatment or prophylaxis of airways diseases or systemic absorption for the treatment or prophylaxis of other diseases (Taylor, 2002).

Systemic delivery of macromolecules by inhalation has attracted considerable attention over the last decade because a number of peptides or proteins are more efficiently absorbed from the lungs compared to oral, nasal or transdermal routes (Coldrons et al., 2003). The efficiency of the lung for systemic absorption arises from the fact that the lung has large absorptive surface area, very thin diffusion path to the blood stream, elevated blood flow, relatively low metabolic activity as well as avoidance of first pass hepatic metabolism (Adjei and Gaupta, 1997).

For any drug to be delivered to the lungs by inhalation, it has to be formulated as an aerosol. Aerosol preparations are stable dispersions or suspensions of solid materials and liquid droplets in a gaseous medium. Drug delivered by aerosol is deposited in the airways by gravitational sedimentation, inertial impaction and diffusion. Mostly, larger particles are deposited by first two mechanisms in the airways, while the smaller particles reach the peripheral region of the lungs by diffusion (Purewal, 1998).

There are three commonly used pharmaceutical aerosols: jet or ultrasonic nebulizers, metered dose inhalers (MDIs) and dry powder inhalers (DPIs) (Byron, 1990).

2.1. Nebulizers

Nebulizers deliver relatively large volumes of drug solutions and suspensions for inhalation and are frequently used for drugs that cannot be easily formulated into MDI or DPI. They are used for drugs such as proteins where the therapeutic dose is too large for delivery by either MDI or DPI (Taylor and McCallion, 1997).

During nebulization, drug is inhaled during normal tidal breathing, through a mouthpiece or facemask and so nebulizers are useful for patients who experience difficulties when using MDIs and DPIs.

There are two types of nebulizers available commercially:

- Jet nebulizers are most commonly used clinically and utilise compressed gases to produce aerosol droplets in the respirable size range.
- Ultrasonic nebulizers use ultrasonic energy to convert liquid into a spray and have been less extensively studied and characterised than jet devices (Taylor and McCallion, 1997).

2.2. Metered dose inhalers

A pharmaceutical MDI may be defined as a pressurised dosage form designed to deliver therapeutic agent to human respiratory tract (Purewal, 1998). MDIs contain active substance, dissolved or suspended in a propellant system, which contains at least one liquefied gas in a pressurised container that is sealed with a metering valve. The actuation of the valve delivers a metered dose of the medicament in the form of an aerosol spray,

which is directed by a suitable adaptor/actuator for administration via oral or nasal inhalation.

Pressurised MDIs have been used to deliver discrete doses of aerosol medicament to the lungs since 1955 (Thiel, 1996), although the use of pressurised aerosols dates back to 1943, when the US Department of Agriculture developed a portable insecticide using propellant 12.

The advantages of MDIs are their portability, low cost and disposability. Many doses (up to 200) are stored in the small canister and dose delivery is reproducible (Taylor, 2002). The inert conditions created by the propellant vapour, together with the hermetically sealed container, protects drug from oxidative degradation and microbiological contamination. However, MDIs have some limitations, they are relatively inefficient at drug delivery. On actuation, the first propellant droplets exit at a high velocity, which may exceed 30 m/s (Taylor, 2002). Consequently, much of the drug is lost through impaction of these droplets in oropharyngeal areas. The introduction of HFA based formulations has, however, improved the lung deposition of drug up to about 53% especially when the formulation is a solution aerosol (Leach et al., 2000).

2.3. Dry powder inhalers

DPIs are aerosol systems in which drugs are inhaled as clouds of fine particles. The drug is either pre-loaded in an inhalation device or filled into hard capsules or foil blister discs which are loaded into a device prior to use (Taylor, 2002).

DPIs may have some advantages over nebulizers and MDIs especially for the administration of peptide and protein therapeutics to the lungs. DPIs are portable, easy to operate (breath actuated), inexpensive, propellant free (ozone friendly) and show improved stability of formulation as a result of the dry state (Timsina et al., 1994).

Furthermore, newer generation of DPI device, such as the Aspirair™ from Vectura and Inhace™ from Nektar has helped improved systemic delivery of DPI formulations (Tobyn et al., 2004; Dunkley et al., 2004).

The Aspirair™, which is currently under development at Vectura, is a novel dry powder inhaler drug delivery device, designed for use in systemic conditions and for conditions where the inspiratory power of the patient cannot be relied upon (Tobyn et al., 2004).

The Inhace™ device from Nektar makes use of compressed air to increase the disaggregation force available to the device. This helps in the deep lung delivery of drugs for systemic delivery (Dunkley et al., 2004).

However, DPI formulations are bedevilled by the problem of being hygroscopic even when successfully de-aggregated from micronized materials (Byron, 1990), although research is being done to combat this problem (Clark et al., 2001).

3. Use of nebulizers for pulmonary delivery of proteins/peptides

Nebulizers have been used for the pulmonary delivery of proteins in the last decade and have not proved 100% successful so far. Nebulization is seen as an easy method for producing aerosol mist from a protein solution considering the difficulties accompanied by its formulation as DPI or MDI (Steckel et al., 2003) but while taking advantage of nebulization as a method, it is important that the integrity and activity of proteins should not be affected or otherwise be influenced by nebulization (Ip et al., 1995).

Steckel et al. (2003) studied the effect of formulation variables on the stability of nebulized Aviscumine. Aviscumine is recombinant mistletoe lectin (ML) which is a dimeric 57-kDa protein produced in *E. coli* (Langer et al., 2002) and the stabilization of Aviscumine has been achieved in an aqueous solution using different excipients (Witthohn et al., 2002) as well as by lyophilization (Gloger et al., 2002). It had been successfully formulated for parenteral application for cancer therapy and new efforts are now being directed towards developing an alternative, non-invasive delivery systems (Steckel et al., 2003).

Steckel et al. successfully formulated Aviscumine for nebulization but discovered that it lost 50% of its activity after nebulization. The ultrasonic nebulizers destabililised Aviscumine to a higher extent than the air-jet nebulizers and the use of lyoprotectants like dextran T1 and hydroxyethyl starch only showed minor stabilizing effects of Aviscumine. They however found that the addition of surfactants to the reconstitution medium could reduce the Aviscumine denaturation as well as its adsorption onto the inner surfaces of the nebulizer.

In nebulizers, only a small fraction of the energy available in the nebulizer is used to generate the new surface necessary in the production of droplets. The excess energy is converted to heat causing the temperature of the liquid within the nebulizers to increase until the input energy balances the energy removed by evaporating solvent molecules and circulating air (Mercer et al., 1968). This causes the temperature of the drug solution in medical ultrasonic nebulizers to rise up to 20 °C above ambient temperature during use (Phipps and Gonda, 1990). Generated heat may cause chemical degradation of heat labile materials such as proteins (Cipolla et al., 1994) thereby limiting the application. For instance, ultrasonic nebulizers are specifically prohibited for the delivery of the enzyme, recombinant human deoxyribonuclease (rhDNase) for this reason (Taylor et al., 1992).

4. Use of DPIs for pulmonary delivery of proteins/peptides

Most literatures favour the use of DPIs for the pulmonary delivery of protein because of the fear of instability in MDIs and nebulizers. For instance, Timsina et al. (1994), Niven (1997) and Coldrons et al. (2003) believe DPIs present advantages over nebulizers and MDIs for the administration of peptides and proteins to the lungs. Their claims are that DPIs are portable, easy to operate (breath-actuated), inexpensive, propellant-free and show improved stability of the formulation as a result of the dry state. While these arguments are genuine, it is important to bear in mind that most carriers used in DPIs are often hygroscopic and agglomeration does occur in DPIs (Byron, 1990). Byron further observed that even when successfully de-aggregated from micronized materials, dry powders are often hygroscopic

resulting in changes in their particle size distribution in human environment.

Despite these problems, a lot of proteins have been successfully formulated as DPIs using different techniques.

Chan et al. (1997) were able to investigate the feasibility of developing a protein dry powder aerosol for inhalation delivery. They prepared powders of rhDNase alone and with sodium chloride (NaCl) by spray drying. It was observed that pure rhDNase was quite cohesive with a fine particle fraction of about 20% but when particles also contained NaCl, the powders were dispersed better to form aerosols. A linear relationship was observed between the NaCl content and fine particle fraction for a similar primary size (approximately 3 μm volume median diameter) of particles. A monolayer-like adhesion of the fine drug particles to the NaCl at drug content ≥50 wt% was responsible for the better aerosol properties of the drug powder. It was therefore concluded that the aerosol properties of spray-dried rhDNase powder could be controlled by incorporation of a suitable excipient such as NaCl.

Bovine serum albumin (BSA) was worked on by Lucas et al. (1998). The use of carrier-based dry powder aerosols for inhalation delivery of proteins was evaluated. They also examined the effect of fine particle excipients as potential formulation performance modifiers. It was observed that inhalation performance of binary ordered mixes prepared using BSA–maltodextrin and lactose (63–90 μm) using the Diskhaler^{TM} was increased from 31.7 ± 2.4 to 47.4 ± 2.2%. The easy release of the drug in the inhaled air stream was due to redistribution of protein particles from coarse carrier particles to the fine particle component in the ternary mix. Inclusion of fine particle lactose and micronized polyethylene glycol-6000 (PEG-6000) changed the bulk properties of inhalation powders and reduced powder flow but did not affect device emptying. It was therefore concluded that fine particle excipients can be used to improve the performance of carrier-based protein dry powder aerosols. Mechanistically, enhancement of performance was proposed to result from a redistribution of protein particles from coarse particles to the fine particle component in the ternary mix.

Different authors have also studied the effects of different powder engineering techniques on the efficiency of DPIs.

Maa et al. (1998) studied the effect of spray drying on residual moisture content and physical/biochemical stability of protein inhalation powders. They were able to establish that spray-dried powders with a moisture level of approximately 3% (equivalent to the freeze-dried material) could only be achieved using high-temperature spray drying conditions, which were not favourable to large-scale manufacturing or subsequent vacuum drying. It was also found out that the dry powders would equilibrate with the subsequent processing and storage environments regardless of the manufacturing condition. Aerosol performance (fine particle fraction) was maintained as long as the relative humidity of air during processing and storage was lower than 50%, but powders stored under drier conditions exhibited better long-term protein biochemical stability.

Maa et al. (1999) went on to compare both spray drying and spray freeze-drying techniques for preparing protein aerosol powders. The comparison was based on physical properties of protein powders and aerosol performance. Two recombinant therapeutic proteins used for treating respiratory tract related diseases: rhDNase and anti-IgE monoclonal antibody (anti-IgE MAb) were employed and formulated with different carbohydrate excipients. It was established that spray freeze-drying, produced protein particles with light and porous characteristics, which offered powder with superior aerosol performance due to favourable aerodynamic properties.

Human growth hormone (hGH) has been formulated for dry powder inhalation (Yang et al., 2002) and the chemical and physical stability were acceptable for all formulations including the neat hGH formulation. It was found that using trileucine as an excipient successfully reduced spray-drying induced aggregation for hGH at pH 3.6 and 7.8 and may be useful for other proteins. Trileucine affected formulation attributes in different ways:

- Improved shelf stability was obtained at pH 7.8.
- Improved aerosol characteristics (emitted dose) were obtained at pH 3.6.

If the aim were to formulate a chemically stable protein formulation then pH 7.8 would be the ideal pH, but if the aim is to prepare a formulation with improved aerosol characteristics then pH 3.6 would be ideal, but I would suggest the use of pH 7.8 because the stability of the protein is far more important.

Parathyroid hormone (1–34, PTH), which is used for the treatment of osteoporosis (Coldrons et al., 2003) by stimulating new cortical and trabecular bone formation, has been successfully formulated as a DPI. Coldrons et al. showed that PTH dry powders presented high emitted dose and fine particles in vitro with limited dependence on airflow rate, and that the peptide incorporated was intact following formulation. Inhalation of the PTH powders resulted in high absolute bioavailability in rats without acute inflammatory response. They were also able to demonstrate that albumin as an excipient could markedly decrease systemic absorption through binding.

Cetrorelix acetate, a decapeptide used for the controlled ovarian super stimulation for assisted reproductive technique in a 0.25 mg dose (Lizio et al., 2000) has been formulated as a DPI and the role of particle engineering in relation to formulation and de-aggregation in the development of a dry powder formulations for inhalation of cetrorelix studied (Zijlstra et al., 2004). It was showed that it is possible for a stable peptide to be micronized by milling, spray drying and spray freeze-drying without degradation of the protein. It was also found that the possibility of micronization technique producing particles suitable for use in an inhalation depended on the type of formulation and process used.

Hygroscopic growth, the basic problem presently facing the formulation of proteins/peptides as a DPI has been addressed by Kuo et al. (2002). They were able to study the effects of different hygroscopic growth inhibitors (HGI) on dry powder aerosol performance and bioavailability. It was discovered that polymers or compounds like maltodextrin and hydro-ethyl starch, with low moisture absorptivity could be used as HGIs for protein dry powders. They were able to establish that the emitted dose

(ED) at high humidity conditions (32 °C/95–99.5%, RH) was less affected with HGI-containing formulations and that based on the lung model used, as %RH increases, the relative change in alveolar deposition for HGI formulation is less than that for non-HGI formulation, which will translate to a higher bioavailability for HGI-containing formulation. This is because dispersed particles grow larger at high relative humidity since they absorb moisture. HGIs with their low moisture adsorptivity properties will reduce the rate at which the formulation absorbs moisture and so reduce the structural changes that normally occur in their absence.

5. Use of MDIs for pulmonary delivery of protein/peptides

Not as much work has been performed on the use of MDIs for delivery of proteins possibly because of the fear of instability in the hydrofluoroalkane (HFA) propellants currently used for MDIs. HFAs are being used as alternative to the ozone depleting chlorofluorocarbons (CFCs) because they are more environmentally friendly (Purewal, 1998). Since the introduction of the HFAs in the mid nineties, various studies have been performed on the possibilities of formulating proteins as MDIs.

The use of MDIs for insulin delivery was first proposed in the 1950s (Thiel, 1996). However, this was not pursued at the time because the hypoglycaemic response in animals was considered too variable. Thus, it was not until much later that the subject was reopened (Brown, 1996). By then, it had been established that a polymeric particulate carrier system could offer controlled release characteristics as well as protection from chemical degradation of an entrapped molecule (Thanoo et al., 1992). Williams et al. (1998) investigated the use of chitosan, a biodegradable polymer, as a potential carrier for therapeutic proteins, peptides and plasmid DNA for administration to the lungs from a pressurised metered dose inhaler. It was discovered that through the use of different cross-linking agents and additives, the physico-chemical properties of chitosan microspheres were modified to improve compatibility in an MDI delivery system. Furthermore, the densities of the non-cross-linked and the glutaraldehyde cross-linked chitosan microspheres closely matched that of liquid P134a (HFA propellant).

An increase in the median particle size and polydispersity after exposure to P134a was found for all types of chitosan microspheres tested except for those crossed-linked with glutaraldehyde. This event was found to be due to the presence of water in P134a which hydrated and plasticized the chitosan microspheres causing aggregation during storage of the MDI formulations. It was, therefore, concluded that both the non-cross-linked and the glutaraldehyde cross-linked chitosan microspheres were found to be potential candidates for carrying biotherapeutic compounds to the lungs via an MDI system due to their compatibility with P134a and their physico-chemical characteristics.

The main concern in the formulation of protein as MDIs is the maintenance of the conformational stability of the protein in the HFA propellants, as the biological activity of such protein will depend on their conformational structures. The conformational stability of proteins in P134a and P227 has been analysed by Fourier Transform Raman Spectroscopy (FT-RS). It was established that FT-RS provided molecular structural information on the peptide backbone, disulfide bonds and C–C stretching vibrations of hen egg lysozyme, enabling the secondary conformation of proteins in HFA propellants to be determined and the structural integrity of lysozyme was maintained in both propellants (Quinn et al., 1999). This shows there is a big potential for the formulation of protein MDIs without altering their conformational structures.

Raman spectroscopy was used by Quinn et al. because Raman analysis requires essentially no sample preparation when compared with other spectroscopic techniques. Furthermore, Raman studies are not hampered by variations in sample morphology. Under physiological conditions, the polypeptide chain of a protein is folded into a specific conformation defined as the native state. This is the functional state of the protein in vivo. Since water is a weak Raman scatterer, the interference from water seen in the infrared studies, is minimised. This presents a great advantage for biological samples because it allows biomolecules to be studied in their native (aqueous state) and also allows the comparison of the conformational states of protein in different environments.

Raman spectroscopy also allows analysis of HFA propellant systems in glass and polyethylene tetraphthalate (PET) vials without requiring the need for high-pressure cell facilities (Quinn et al., 1999).

The stability of protein in HFA based MDIs has been established by various authors. Oliver et al. (2000) were able to show that the biological activity of DNase I was maintained after particle size reduction and subsequent storage in HFA propellant at −18 °C for 6 months.

Furthermore, Brown et al. (2002) successfully processed adenosine deaminase (ADA) to target respirable range of 1–3 μm with retention of biological activity. This ADA was also established to be stable for at least 6 months in an HFA suspension MDI when stored at 4 °C/50%RH or 25 °C/60%RH conditions.

Kos Pharmaceuticals has successfully completed a 4-week; phase IIa human clinical trial evaluating inhaled insulin therapy for type 2 diabetic patients. The results of this trial showed that inhaled insulin formulation is comparable to the market leading injectable insulin in controlling blood glucose levels, while also reducing blood lipids such as low-density lipoprotein cholesterol and triglycerides. Kos inhaled insulin had also demonstrated bioavailability of up to 23% in previous phase I trial (Benfait, 2004).

6. Problems facing pulmonary delivery of proteins/peptides and possible solutions

The delivery of proteins/peptides for systemic activity via the pulmonary route looks quite promising but there are some limitations facing this route and various studies have been performed with a view to finding lasting solutions to these limitations.

The following limitations have been identified by various authors: short duration of action due to rapid clearance of drugs

deposited in the peripheral airways (Shiavone et al., 2003), immunogenicity (Byron, 1990), lack of precise dosing (Davis, 1999) and chemical instability of the protein (Byron, 1990).

6.1. Short duration of action

Although rapid drug absorption is a major advantage of pulmonary route, it also represents a limitation for the delivery of drugs as the relatively short duration of clinical effects may mean multiple daily dosing. Various efforts have been made to tackle this problem and these include, the use of biodegradable polymers and microcrystallization of the protein molecules.

Shiavone et al. (2003) looked at the formulation feasibility of drug loaded poly-lactic glycolic acid (PLGA) microspheres for pulmonary delivery and concluded that under some optimization of the processing conditions, drug-loaded PLGA microsphere formulations may be prepared that exhibit performance suitable for sustained release via pulmonary delivery.

Also, PEGylation of protein has been used (Leach et al., 2003) to provide prolonged systemic activity via the pulmonary route. The advantages of binding protein to PEG also include increased bioavailability, increased plasma half lives, decreased immunogenicity, reduced proteolysis and enhanced solubility and stability (Banga, 2003). The main problem with the use of biodegradable polymer is the accumulation of the polymer in the lung (Patton et al., 1999) and the loss of protein activity during the preparation of the microspheres (Sanchez et al., 1999).

Recently, a unique insulin microcrystallization process using a seed zone method has been developed and a patent has been filed (Kwon and Kim, 2002, 2004a). Insulin in acetate buffer conditions can form a stable zone (pH 10.5 ± 0.5) characterised by an excess of seeds. Upon the induction of super saturating conditions, the seeds grow into microcrystals suitable for pulmonary delivery (Kwon et al., 2004b). Kwon et al. (2004b) were also able to prepare a microcrystalline insulin aerosol using the method stated above and after intratracheal inhalation in rats; the microcrystal suspension significantly reduced the blood glucose level over 7 h. This shows that insulin microcrystals show promise as a sustained release formulation and as a suitable therapeutic form of pulmonary delivery.

6.2. Chemical stability

Proteins and peptides have been shown to produce reduced biological activities after aerosolization (Byron, 1990).

The use of polymers like PEG has been shown to help maintain the chemical stability of proteins after formulation (Thanoo et al., 1992).

Other methods such as the use of sugars like lactose have also been used.

6.3. Immunogenicity

Local, pulmonary cell mediated immunity induced by parenteral immunization, has been shown to exist on the mucosal surface after antigenic challenge by aerosol inhalation (Miyamoto et al., 1971). However, Bukowski et al. (2002) have been able to show that attaching PEG to a protein could reduce the chances of immunogenic responses occurring after inhalation.

6.4. Lack of precise dosing

The ability to deliver large quantities of drug selectively to the lung and particularly to the deep lung presents problems (Davis, 1999). Scintigraphic data obtained in man suggest that with conventional MDI systems and dry powder inhalers most of the drug does not reach the lung at all (Newman, 1993). Some of the dose may be left in the device, some may be left in the spacer system (if used), but majority impacts at the back of the throat and is then swallowed (Davis, 1999). For example, for a typical DPI, it is expected that approximately only 10–20% of the dose reaches the lung and normally only half of this reaches the peripheral region. For a peptide molecule, it is estimated that approximately only half of this dose, that reaches the alveolar region, will be absorbed. This will indicate that for a conventional pulmonary delivery system, the probable bioavailability, with reference to the original dose, will be relatively small (10% or less; Davis, 1999).

However, some of the more recent concept in delivery, in the form of DPIs and MDIs, should permit larger quantities of drug to be delivered into the lung and more importantly into the deep lung with greater dosing precision (Davis, 1999).

7. Discussion and conclusion

Pulmonary drug delivery looks a promising alternative to injection for the systemic delivery of therapeutic proteins and peptides. This route is non-invasive and so helps patient compliance. Various studies by different research laboratories and pharmaceutical companies have proved that proteins and

Table 1
List of proteins/peptides that could feasibly be delivered via the lungs

Protein/peptide	Indications
Interferon-alpha	Hepatitis, cancer
Interferon-beta	Multiple sclerosis
Interferon-gamma	Chronic granulomatous disease
Interleukin 2	Renal cancer
Interleukin 6	Thrombocytopenia
Interleukin 11	Thrombocytopenia
Interleukin 12	T-cell disorders
Interleukin 1 receptor agonist	Rheumatoid arthritis
Macrophage and granulocyte colony stimulating factor	Infections due to cancer
Erythropoietin	Anaemia
Calcitonin	Paget's disease, osteoporosis
Parathyroid hormone	Osteoporosis
Insulin	Type 1/11 diabetes
Amylin	Type 1 diabetes
Growth hormone	Wasting

The delivery of proteins/peptides via the lung is an area that many studies have been performed and there is still room for more work to be done especially in the area of retaining the biological activity of the protein, post-formulation and delivery.

peptides can indeed be formulated as aerosols but the best aerosol for a particular protein will depend on many factors such as stability of the protein in the delivery medium, cost, compatibility with the excipients used and convenience for the patients. Also the choice of particle engineering technique and potential excipients used are also important factors to be considered when formulating a protein for pulmonary delivery.

Presently, pulmonary delivery of proteins such as insulin, calcitonin, growth hormones and parathyroid hormone is being investigated (Agu et al., 2001).

Inhaled calcitonin was found to have 66% of the bioactivity and 28% of the bioavailability of injected calcitonin (Banga, 2003). Inhaled insulin is currently under review by the regulatory authorities in the USA and Europe.

Table 1 shows some of proteins/peptides which could feasibly be delivered via the lungs (Byron and Patton, 1994).

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References

Adjei, A.L., Gaupta, P.K. (Eds.), 1997. Inhalation Delivery of Delivery of Therapeutic Peptides and Proteins. Dekker, New York.

Agu, R.U., Ugwoke, M.I., Armand, M., Kinget, R., Verbeke, N., 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2, 198–209.

Bagger, Y., Tanko, L.B., Alexandersen, P., Karsdal, M.A., Olson, M., Mindeholm, L., Azria, M., Christiansen, C., 2005. Oral salmon calcitonin induced suppression of urinary collagen type 11 degradation in post menopausal women: a new potential treatment of osteoarthritis. Bone 33, 425–430.

Banga, A.K., 2003. Delivery of protein therapeutics. Business Briefing: Pharmatech., 198–201.

Benfait, C., 2004. Kos Reports Achievement of New Research and Development Milestones. Kos Press Release, August 26, 2004.

Brown, A.R., 1996. Propellant driven aerosols of proteins. Aerosol Sci. Technol. 24, 45–56.

Brown, B.A.S., Thatcher, M.L., Smith, K.L., Johnson, P.R., Podgorski, J.J., 2002. The Stability of a Particle Size Reduced Therapeutic Protein in an HFA MDI Formulation. RDD IIX.

Bukowski, R.M., Tendler, C., Cutler, D., Rose, E., Loughlin, M.M., Statkevich, P., 2002. Treating cancer with PEG-intron: pharmacokinetic profile and dosing guidelines for an improved interferon-alpha-2b formulation. Cancer 95, 389–396.

Byron, P.R., 1990. Determinants of drug and polypeptide bio-availability from aerosols delivered to the lung. Adv. Drug Deliv. Rev. 5, 107–132.

Byron, P., Patton, J.S., 1994. J. Aerosol Med. 7, 49–75.

Chan, H.K., Clark, A., Gondal, M.M., Hsu, C., 1997. Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. Pharm. Res. 14, 431–437.

Cipolla, D.C., Clark, A.R., Chan, H.K., Gonda, I., Shire, S.J., 1994. Assessment of aerosol delivery systems for recombinant human deoxyribonuclease STP. Pharm. Sci. 4, 50–62.

Clark, A., Lalor C.B., Aldous, B., Kuo, M., 2001. Hygroscopic Growth Inhibitor for the Delivery of Active Drug, U.S. Patent Application.

Coldrons, V., Vanderbist, F., Vanbeeck, R.K., Arras, M., Lison, D., Preat, V., Vanbever, R., 2003. Systemic delivery of parathyroid hormone (1–34) using inhalation dry powders in rats. J. Pharm. Sci. 92, 938–948.

Crommelin, D., van Winden, E., Mekking, A., 2002. Delivery of pharmaceutical proteins. In: Aulton, M.E. (Ed.), Pharmaceutics, The Science of Dosage Form Design. Churchill Livingstone, Edinburg, pp. 544–553.

Cullander, C., Guy, R.H., 1992. Routes of delivery: case studies. 6. Transdermal delivery of peptides and proteins. Adv. Drug Deliv. Rev. 8, 291–329.

Davis, S.S., 1999. Delivery of peptide and non-peptide drugs through the respiratory tract. PSTT 2, 450–456.

Dunkley, M., Tuckwell, J., Vernon-Harcourt, E., Shirgaonkar, S., 2004. Aerosolization apparatus with air inlet shield. World Intellectual Property Organisation, Patent WO 2004/091705 Al.

Edman, P., Bjork, E., 1992. Routes of delivery: case studies. 1. Nasal delivery of peptide drugs. Adv. Drug Deliv. 8, 165–177.

Gloger, O., Withohn, K., Muller, B.W., 2002. Stabilization of aviscumine during freeze drying by different polysaccharides. In: Proceedings of the Fourth World Meeting ADRITELF/APGI/APV, Florence, pp. 607–608.

Harris, D., Liaw, J.H., Robinson, J.R., 1992. Routes of delivery: case studies. 7. Ocular delivery of peptide and protein drugs. Adv. Drug Rev. 8, 331–339.

Hilsted, J., Madsbad, S., Hivdberg, A., Rasmussen, M.H., Krarup, T., Ipsen, H., Hansen, B., Pedersen, M., Djurup, R., Oxenboll, B., 1995. Intranasal insulin therapy: the clinical realities. Diabetologia 38, 680–684.

Ho, N.F.H., Barsuhn, C.L., Burton, P.S., Merckle, H.P., 1992. Routes of delivery: case studies. 3. Mechanistic insights to buccal delivery of proteinaceous substances. Adv. Drug Deliv. Rev. 8, 197–235.

Hoegen, P.V., 2001. Synthetic biomimetic supra molecular biovector (SMBV) particles for nasal vaccine delivery. Adv. Drug Deliv. Rev. 51, 113–125.

Hollinger, M.A., 1985. Respiratory Pharmacology and Toxicology. W.B. Saunders, Philadelphia, pp. 1–20.

Ip, A., Arkawa, T., Silvers, H., Ransome, C., Niven, R., 1995. Solubility of recombinant consensus interferonto air jet and ultrasonic nebulization. J. Pharm. Sci. 84, 1210–1214.

Kuo, M.C., Tep, V., Yang, B., Lalor, C.B., Kadrichi, N., Lechuga-Ballesteros, D., Clark, A., 2002. Effect of hygroscopic growth inhibitors on dry powder aerosol performance and bioavailability. In: Presented at AAPS Annual Meeting and Exposition, Toronto, Ont., Canada, November 10–14, 2002.

Kwon, J.H., Kim, C.W., 2002. Process Development for Insulin Microcrystal Production. Korea Patent 0327597.

Kwon, J.H., Kim, C.W., 2004a. A novel insulin microcrystal preparation using a seed zone method. J. Cryst. Growth 263, 536–543.

Kwon, J.H., Lee, B.H., Lee, J.J., Kim, C.W., 2004b. Insulin microcrystal suspension as a long acting formulation for pulmonary delivery. Eur. J. Pharm. Sci. 22, 107–116.

Langer, M., Witthohn, K., Berlin, J., Mockel, B., Eck, J., Zinke, H., Lentzen, H., 2002. New analytical methods to describe the quality of the active substance manufactured under GMP. In: Proceedings of the Fourth World Meeting ADRITELF/APGI/APV, Florence, pp. 337–338.

Leach, C.L., Davidson, P.J., Hasselquist, B.E., Boudreau, R.J., 2000. Deposition comparison of CFC–fluticasone, CFC–beclomethasone, and HFA–beclomethasone MDIs in healthy subjects. Am. J. Respir. Crit. Care Med. 161, A34.

Leach, C.L., Patton, J.S., Perkins, K.M., Kuo, M., Bueche, B., Guo, L., Bentley, M.D., 2003. PEG-insulin delivered by the pulmonary route provides prolonged systemic activity compared with insulin alone. In: Paper Presented at 2002 AAPS Meeting and Exposition, Toronto, Ont., Canada, November 10–14, 2002.

Lizio, R., Klenner, T., Borchard, G., Romeis, P., Sarlikiotis, A.W., Reissmann, T., Lehr, C.M., 2000. Systemic delivery of the GnRH antagonist cetrorelix by intratrcheal instillation in anaesthetized rats. Eur. J. Pharm. Sci. 9, 253–258.

Lucas, P., Anderson, K., Staniforth, J.N., 1998. Protein deposition from dry powder inhalers: Fine particle multiplets as performance modifiers. Pharm. Res. 15, 562–569.

Maa, Y.F., Nguyen, P.A., Andya, J.D., Dasovich, N., Sweeny, T.D., Shire, S.J., Hsu, C.C., 1998. Effect of spray drying and subsequent processing conditions on residual moisture content and physical/biochemical stability of protein inhalation powders. Pharm. Res. 15, 768–775.

Maa, Y.F., Nguyen, P.A., Sweny, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying VS spray freeze-drying. Pharm. Res. 16, 249–254.

Manning, M.C., Patel, K., Borchardt, R.T., 1989. Stability of proteins. Pharm. Res. 6, 903–917.

Mercer, T.T., Goddard, R.F., Flores, R.L., 1968. Output characteristics of three ultrasonic nebulizers. Ann. Allergy 26, 18–27.

Miyamoto, T., Kabe, J., Noda, M., Kobayashi, N., Miura, K., 1971. Physiologic and pathologic respiratory changes in delayed type hypersensitivity reactions in guinea pigs. Am. Rev. Respir. Dis. 103, 509–514.

Newman, S.P., 1993. Crit. Rev. Drug Carrier Syst. 10, 65–109.

Niven, R. (Ed.), 1997. Dry powder formulations for inhalation. Adv. Drug Deliv. Rev. 26, 1–67 (special issue).

Oliver, M.J., McKenzie, L., Graffiths, W.D., Morgan, G.R., O'Kelly, N., 2000. Initial Assessment of a Protein Formulated in Pressurized MDIs for Pulmonary Delivery. RDD VII.

Patton, J.S., Bukar, J., Nagarajan, S., 1999. Inhaled insulin. Adv. Drug Deliv. Rev. 35, 235–247.

Phipps, P.R., Gonda, I., 1990. Droplets produced by medical nebulizers: some factors affecting their size and solute concentration. Chest 97, 1327–1332.

Purewal, T.S., 1998. Formulation of metered dose inhalers. In: Purewal, T.S., Grant, D.J.W. (Eds.), Metered Dose Inhaler Technology. Interpharm, Illinois, pp. 9–68.

Quinn, E.A., Forbes, R.T., Williams, A.C., Oliver, M.J., McKenzie, L., Purewal, T., 1999. Protein conformational stability in the hydrofluoroalkane propellants tetrafluoroethane and heptafluoropropane analysed by Fourier Transform Raman Spectroscopy. Int. J. Pharm. 186, 31–41.

Sanchez, A., Villamayor, B., Mclver, J., Alonso, M.J., 1999. Formulation strategies for stabilization of tetanus toxoid in poly (lactide-co-glycolide) microspheres. Int. J. Pharm. 85, 255–266.

Shen, W.C., Wan, J., Ekrami, H., 1992. Means to enhance penetration. 3. Enhancement of polypeptide and protein absorption by macromolecular carriers via endocytosis and trnacytosis. Adv. Drug Deliv. Rev. 8, 93–113.

Shiavone, H., Wist, A., Tzannis, S.T., 2003. Formulation feasibility of drug-loaded PLGA microspheres for pulmonary delivery. In: A Paper Presented at the 14th Congress of the International Society for Aerosols in Medicine, Baltimore, MD, June 14–18, 2003.

Smith, P.L., Wall, D.A., Gochoco, C.H., Willson, G., 1992. Routes of delivery: case studies. 5. Oral absorption of peptides and proteins. Adv. Drug Deliv. Rev. 8, 253–290.

Steckel, H., Eskander, F., Witthohn, K., 2003. The effect of formulation variable on the stability of nebulized aviscumine. Int. J. Pharm. 257, 181–194.

Taylor, K., 2002. Pulmonary drug delivery. In: Aulton, M.E. (Ed.), Pharmaceutics, The Science of Dosage Form Design. Churchill Livingstone, Edinburgh, pp. 473–488.

Taylor, K., McCallion, O., 1997. Ultrasonic nebulizers for pulmonary drug delivery. Int. J. Pharm. 153, 93–104.

Taylor, K., Venthoye, G., Chawla, A., 1992. Pentamidine isothionate delivery from jet nebulizers. Int. J. Pharm. 85, 203–208.

Thanoo, B.C., Sunny, M.C., Jayakrishnan, A., 1992. Cross-linked chitosan microspheres: preparation and evaluation as a matrix for controlled release of pharmaceuticals. J. Pharm. Pharmacol. 44, 283–286.

Thiel, C.G., 1996. From Susie's Question to CFC Free: An Inventor's Perspective on Forty Years of MDI Development and Regulation. RDD V, pp. 115–123.

Timsina, M.P., Martin, G.P., Marriott, C., Ganderton, D., Yianneskis, M., 1994. Drug delivery to the respiratory tract using dry powder inhalers. Int. J. Pharm. 101, 1–13.

Tobyn, M., Staniforth, J.N., Morton, D., Harmer, Q., Newton, M.E., 2004. Active and intelligent inhaler device development. Int. J. Pharm. 277, 31–37.

Williams, W., Roberto, O., Barron, M.K., Alonso, M.J., Remunam-Lopez, C., 1998. Investigation of a pressurized metered dose inhaler system containing chitosan 174, 209–222.

Witthohn, K., Mockel, B., Hausmann, I., Gloger, O., Lentzen, H., 2002. Pre-formulation studies with thermodynamic protein, aviscumine as prerequisite for clinical trials. In: Proceedings of the Fourth World Meeting ADRITELF/APGI/APV, Florence, pp. 871–872.

Yang, B., Lesikar, D., Tan, M.M., Ramachandran, S., Stevenson, C.L., 2002. Formulation of human growth hormone for pulmonary delivery. In: Presented at the 2002 AAPS Annual Meeting and Exposition, Toronto, Ont., Canada, November 10–14, 2002.

Zheng, J.Y., Fulu, M., 2006. Decrease of genital organ weights and plasma testosterone in rat following oral administration of Leuprolide microemulsion. Int. J. Pharm. 307, 209–215.

Zijlstra, G.S., Hinrichs, W.L.J., de Boer, A.H., Frijlink, H.W., 2004. The role of particle engineering in relation to formulation and de-agglomeration principle in the development of a dry powder formulation for inhalation of cetrorelix. Eur. J. Pharm. Sci. 23, 139–149.