REVIEW ARTICLE

Recent trends in protein and peptide drug delivery systems

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With the discovery of insulin in 1922, identification and commercialization of potential protein and peptide drugs have been increased. Since then, research and development to improve the means of delivering protein therapeutics to patients has begun. The research efforts have followed two basic pathways: One path focused on noninvasive means of delivering proteins to the body and the second path has been primarily aimed at increasing the biological half-life of the therapeutic molecules. The search for approaches that provide formulations that are stable, bioavailable, readily manufacturable, and acceptable to the patient, has led to major advances in the development of nasal and controlled release technology, applicable to every protein or peptide. In several limited cases, sustained delivery of peptides and proteins has employed the use of polymeric carriers. More successes have been achieved by chemical modification using amino acid substitutions, protein pegylation or glycosylation to improve the pharmacodynamic properties of certain macromolecules and various delivery systems have been developed like the prolease technology, nano-particulate and microparticulate delivery systems, and the mucoadhesive delivery of peptides. The needle and syringe remain the primary means of protein delivery. Major hurdles remain in order to overcome the combined natural barriers of drug permeability, drug stability, pharmacokinetics, and pharmacodynamics of protein therapeutics. In our present review we have tried to compile some recent advances in protein and peptide drug delivery systems.

Key words: *Drug delivery, peptide, protein*

INTRODUCTION

Proteins and peptides

Proteins and peptides are the biopolymers which yield two or more amino acids on hydrolysis. Peptides and polypeptides are the principal components of the protoplasm of cells and are high molecular weight compounds consisting of alpha amino acids connected together by peptide linkages.[1-3] Proteins may have thousands of amino acid residues. Although the terms "protein" and "polypeptide" are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000 and those called proteins have higher molecular weights[4] [Table 1]. Molecular sizes of proteins are greater than those in traditional pharmaceuticals, and they have secondary and tertiary structures, which make them very susceptible to physical and chemical degradation. Molecular weight and size greatly influence the diffusion of drugs through the epithelial layer. Several authors have investigated the effects of molecular weight upon the oral absorption of various hydrophilic compounds.[5-7] One of the challenges in working with peptide therapeutics is their small size, which typically equates to a short circulating life. It is a fact that the lower the molecular weight of the peptide, the shorter the lifespan is. Thus, a natural peptide with a molecular weight of less than 4 KD would travel microns in seconds to minutes before it is degraded, while proteins, such as cytokines and growth factors with a molecular weight of 16 to 35 KD, would travel meters in minutes or hours before clearance, and plasma proteins with a molecular weight of more than 50 KD would travel kilometers over a period of weeks before clearance. Moreover, such molecules exhibit low solubility or poor stability, leading to short shelf lives.[4] As a result, macromolecule therapeutics often quickly

Table 1: List of some protein and peptide drugs

Drugs	Chemical formulae	Mol. mass
Peginterferon α-2a	$C_{860}H_{1353}N_{227}O_{255}S_9$	19241 g/mol
Peginterferon α-2b	$C_{860}H_{1353}N_{229}O_{255}S_9$	19269.1 g/mol
Pegfilgrastim	$C_{845}H_{1343}N_{223}O_{243}S_9$	18802.8 g/mol
Doxorubicin	$C_{27}H_{29}NO_{11}$	543.52 g/mol
Insulin	$C_{257}H_{383}N_{65}O_{77}S_6$	5801.6 g/mol
Exenatide	$C_{184}H_{282}N_{50}O_{60}S$	4186.6 g/mol

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lose their effectiveness or require frequent dosing. Proteins easily get denatured by heat or by agitation and are therefore kept at refrigerated temperatures, along with stabilizing agents for long-term storage. These factors impact not only the cost of therapy, but also patient acceptance and compliance, thus affecting their therapeutic usefulness.[8]

Most of therapeutic proteins and peptide-based drugs are administered by the parenteral route, that is, via an injection. The obvious downside of this delivery method is patient acceptance and compliance, limiting most macromolecule development to indications in which the need to use invasive administration routes are not outweighed by the associated expenses or inconvenience.

To alter amino acid sequences and chemistries, to reduce degradation by enzymes, and antigenic side effects, protein and peptide drugs were incorporated into polymeric particles, that is, liposomes, to prolong their action, or fused to immunoglobulins or albumin to improve their half-life.[9-11] These methods have some limitations as in the case of liposomes, which rapidly enter the liver, spleen, reticuloendothelial systems, and kidneys, and can cause some adverse effects.[12-17]

Protein and peptides when delivered orally would not achieve therapeutically acceptable bioavailability because of the enzymatic barriers, the intestinal epithelial and vascular endothelial barriers, which typically digest them with the help of the GI system. Therapeutic quantities of most macromolecules are able to pass through the skin and mucous membranes with the help of penetration enhancers or penetration-enhancing techniques, such as detergents or electric impulses, increasing the likelihood of irritation or other side effects. Penetration enhancers are compounds that are added to increase the absorption of the solute across the biological membranes. Use of surfactants decreases the self-association and absorption of the protein on the hydrophobic interface of the delivery matrix. They increase the penetration and stability of protein and peptide formulations.

Novel drug delivery technologies, such as, engineering molecules with advanced PEGylation,[18-20] Pulmonary Delivery, Nasal Delivery, and Transdermal Delivery, offer exciting alternatives to improve the viability of potential protein and peptide drug candidates, improve drug performance, and increase patient compliance through more convenient modes of dosing and administration.[21]

ADVANCED PEGYLATION

Poly Ethylene Glycol (PEG) is non-toxic and has been approved by the FDA for use in foods, cosmetics, and pharmaceuticals. PEG polymers can be linear or branched in shape, and can be engineered in a variety of molecular weights. Studies on PEG solution show that each ethylene glycol subunit is tightly associated with two or three water molecules. A binding process with water makes PEGylated compounds function as though they were 5 to 10 times larger than a corresponding soluble protein of similar molecular weight. Further, the PEG polymer with associated water molecules is very mobile, and acts like a shield to protect the attached drug from enzyme degradation and interactions with cell surface proteins, and provides increased size to prevent rapid renal filtration and clearance.[22]

Advanced PEGylation, which involves modification of protein, peptide, or non-peptide (drug or therapeutic protein) by attaching with specific PEG polymer chains, is a proven method for enhancing the potentials of peptides and proteins as therapeutic agents. The advantages of advanced PEGylation for therapeutic molecules can include enhanced bioavailability, decreased dosing frequency, due to prolonged residence in the body, a decreased degradation by metabolic enzymes, optimized pharmacokinetics, increased efficacy, improved safety profile, a reduction or elimination of protein immunogenicity, improved drug solubility, and stability to hydrophobic drugs and proteins.[23]

Prodrugs are also prepared by this advanced PEGylation technique. During biotransformation, active drugs are released by degradation of more complex molecules (prodrugs) under suitable physiological conditions, providing an efficient method of drug delivery.

The advanced PEGylation technology also offered new opportunities for creating viable peptides and protein drugs by site-specific PEGylation. For example, coupling certain PEG reagents to protein thiol groups on cysteines may offer advantages, as cysteines are typically less abundant in proteins than other polymer attachment sites, such as amino groups, resulting in more selective PEGylation of the target protein.[24] Greater selectivity allows greater control over the resulting PEG-conjugate in both the number of attachment sites and the position of the attachment, by reducing the likelihood of protein deactivation upon conjugation. In addition to minimizing loss of biological activity, site-specific PEGylation can also reduce immunogenicity. Thiol groups may be naturally occurring or the biomolecule may be modified or engineered to contain a thiol group suitable for conjugation.[25,26]

SUCCESSFUL COMMERCIAL PEG DRUGS

Two approved PEGylated interferon alfa products are now available for treatment of hepatitis C: Peginterferon alfa-2a (PEGASYS® –Hoffman-La Roche) and peginterferon alfa-2b (PEG-INTRON® – Schering-Plough/Enzon) used for the treatment of chronic hepatitis C. [Table 1]. PEGylated interferons are typically administered weekly, only as opposed to three times per week for non-PEGylated drugs. Moreover they maintain a more constant level of interferon in the blood. The superior performance of these products has led the National Institute of Health to declare PEG-interferon to be the standard of treatment for hepatitis C. Another highly successful PEG drug in the marketplace is Neulasta® (pegfilgrastim) from Amgen

[Figure 1], which is the second generation of Amgen's highly successful Neupogen® product. Neulasta is the recombinant methionyl human granulocyte colony stimulating factor for severe cancer chemotherapy-induced neutropenia. It has a longer biological half-life and increased bioavailability over Neupogen, allowing for significantly reduced dosing frequency to once per chemotherapy cycle. Doxil® (Sequus) is another PEGylated liposome containing doxorubicin [Figure 2] for the treatment of cancer.[27]

PULMONARY DRUG DELIVERY SYSTEM

The primary mode for administering macromolecule drugs for systemic diseases is the invasive method of drug delivery such as infusions and injections, and these methods are the least desirable by the practitioners. Pulmonary drug delivery is the key to obtaining effective, non-systemic delivery alternative to injections and is held as a method to directly target disorders of the lung, which could not be treated by using oral medications (i.e., those that require macromolecule drug therapies, insulin), and which offers many advantages over oral, intranasal, and transdermal alternatives.[28]

The drugs can be administered by the pulmonary route, through aerosol delivery systems for the administration of compounds to treat pulmonary diseases, such as asthma.[29] Devices such as jet or ultrasonic nebulizers, metered-dose inhalers (MDI), and dry powder inhalers (NBI)[30-33] are used. Metered-dose inhalers are the most frequently used aerosol delivery systems, whereas, dry powder inhalers are designed to deliver drug/excipient powder to the lungs. These inhalers are typically used to deliver bronchodilators or corticosteroids, very effective for delivery of the drugs to the upper airways. Of late, add-a-device also called as spacers have been added, to be used with MDI's in order to remove some of the non-respirable particles, by impaction on their walls and valves.[34]

Pulmonary drug delivery is most commonly used in the case of asthma, but recent advancement in technologies by particle engineering and formulation methods to manage particle size, morphology, uniformity, chemical stability, and dispersibility are used to manufacture drug powders for inhalation, which have created exciting opportunities to expand the applications for pulmonary delivery to many therapeutic molecules, including proteins and peptides.

Pulmonary bioavailability largely depends on the physical properties of the delivered protein and it is not the same for all peptide and protein drugs. Insulin liposomes are one of the recent advances in the controlled release of aerosol preparation.[28] Hypoglycemic effects have been significantly enhanced by the intratracheal delivery of insulin liposomes (dipalmitoylphosphatidyl choline:cholesterol, 7:2).

The development of new macromolecule drugs that can treat diseases that were previously either not treatable or only partially treatable has led to renewed interest in noninvasive drug delivery technology. Many new agents are now under investigation for pulmonary delivery, both for targeted lung as well as systemic delivery. These include growth hormones (for growth hormone deficiencies), -1 antitrypsin (for emphysema and cystic fibrosis), interferons (for multiple sclerosis and hepatitis B and C), and para thyroid harmone (PTH) and other peptides (for osteoporosis). Inhalation delivery methods may also be applied to gene therapy via tissue targeting and organ targeting, as well as vaccines. Recent advances in particle technology and formulation have opened the doors to more targeted treatment of lung disorders by treating the lung directly.[26]

SUCCESSFUL PULMONARY DRUGS

Inhaled versions of systemically delivered macromolecule therapeutics in development have shown considerable promise. Pulmonary insulin, in particular, is now in the late stages of human clinical testing, with the most advanced version, Exubera®#174. Nektar in conjunction with Pfizer began dosing the first diabetic patients for the phase III clinical

Figure 1: Chemical structure of Pegfilgrastim

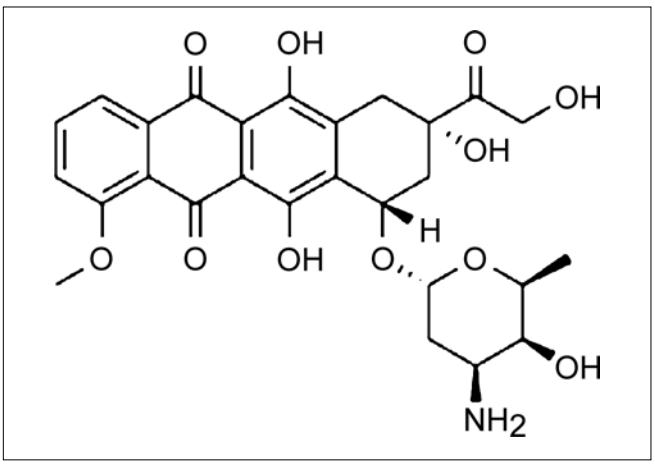

Figure 2: Chemical structure of doxorubicin

trial for inhalable insulin Exubera®. Clinical data for Exubera have indicated its ability to provide glycemic control as well as insulin [Figure 3] injections and better glycemic control than combinations of oral diabetic agents.[35-39] Additionally, preference studies with diabetic patients have underscored their desire for such an alternative to injections.[40-42] In addition to Exubera, other inhaled insulin products are being developed by Aradigm/Novo Nordisk, and Alkermes/Lilly alkermes has designed an inhalation technology (AIR), which would enable an efficient delivery of a dry powder of small molecules of peptide and protein drug particles, deep into the lungs.[43]

DEVICES UNDER INVESTIGATION

A number of companies are in clinical trials of novel pulmonary delivery systems, such as Inhale Therapeutic Systems, Alkermes, Aradigm, AeroGen, Alliance, Battelle, Delsys, Elan, Nastech, Sheffield, and Vectura.[44] Each has a device/formulation combination that works to deliver small particles or aerosolized liquid droplets deep within the lung. Inhale Therapeutic Systems, for example, has developed a device that uses a dry powder approach, and has partnered with several drug companies to investigate the use of its delivery device with their drugs. A notable example is their joint development with Pfizer on Exubera™, an inhaled insulin product. This product is in late-stage trials, and has shown good results, although some questions with regard to lung function and insulin antibodies came up during the studies. In collaboration with Amylin Pharmaceuticals, Inc., Alkermes has developed a once-a-week Medisorb® formulation of BYETTA® (exenatide) [Figure 4] for the treatment of type 2 diabetes known as exenatide LAR. In collaboration with Eli Lilly and Company, Alkermes is using the AIR® (Advanced Inhalation Research) pulmonary drug delivery technology to develop inhaled formulations of insulin and recombinant parathyroid hormone.[43,45] The companies continue to gather data in multicenter trials for eventual FDA submission. The BD Company has developed a novel device for the delivery and active dispersion of a dry powder, which can be used for either nasal or pulmonary delivery. The SoloVent™- BD's Dry Powder Nasal Delivery Device (Naso-pulmonary delivery platform) is an inconspicuous, pocket-sized, unit-dose, disposable system, which utilizes a proprietary pressure capsule for dispersing an active medicament for delivery without priming steps or prior preparation. The device delivers emitted doses of 96 to 98%, depending on the drug formulation. The pulmonary version has been demonstrated to be a more efficient delivery system than many commercial dry powder pulmonary devices, requiring less of the drug to deliver a given dose deep into the lungs.[18-20,46]

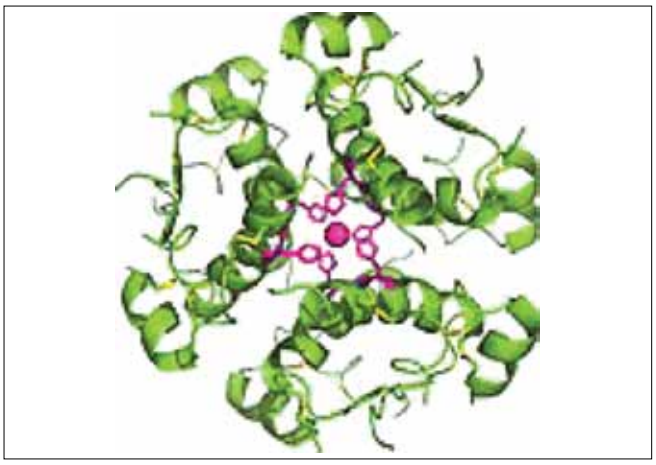

Figure 3: Chemical structure of insulin

DEVELOPMENT OF TRANSDERMAL TECHNOLOGY FOR DELIVERY OF THERAPEUTIC PROTEINS AND PEPTIDES

Transdermal delivery of therapeutic agents has been used successfully for several decades. It has been in the frontline of research with a focus on the development of noninvasive methods, for the systemic administration of peptide and protein therapeutics, generated by the biotechnology

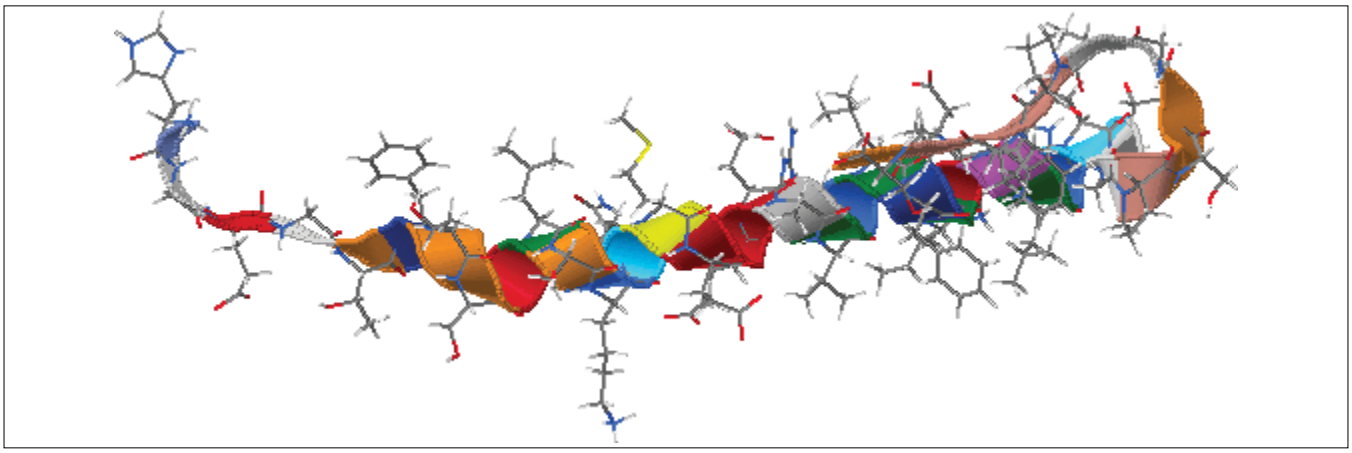

Figure 4: Chemical structure of exenatide

revolution. Numerous approaches have been suggested for overcoming the skin's formidable barrier function; although, certain strategies simply act on the drug formulation or transiently increase skin permeability, others are designed to remove the outermost skin layer.[47-49] Transdermal route for the delivery of proteins and peptides is particularly attractive for a variety of reasons as all other routes (i.e., nasal, buccal, oral, rectal, vaginal, and pulmonary) exhibit enzymatic activity and degradation due to gastrointestinal tract and the hepatic first pass metabolism, whereas, skin contains aminopeptidases, which exhibit less enzymatic activity. This means that the bioavailability of the peptide drug delivered is increased.[50] Transdermal systems for hormone replacement therapy, smoking cessation, and pain management are well accepted; however, there have been challenges in expanding the use of this technology to the delivery of peptides, proteins, and other macromolecules. These biopharmaceuticals have to face the challenge that they cannot permeate the skin's outer stratum corneum layer at levels or rates that achieve significant therapeutic effect. Although mechanical abrasion and chemical enhancers increase drug permeation, their effects on the skin are inherent, the rate-controlling properties are difficult to control, and they may irritate the skin.[50-52]

Macroflux® transdermal patch technology has been developed to deliver biopharmaceutical drugs in a controlled, reproducible manner that optimizes the bioavailability and efficacy without significant discomfort to the patient. It offers marked advantages over other transdermal delivery systems for the efficient delivery of peptides, proteins, and other therapeutic macromolecules. Dose delivery is controlled by the patch size and drug loading on the microprojections. The system is minimally invasive and well tolerated. It is convenient for the users and provides controlled, consistent dosing. Drug-coated Macroflux® microprojections penetrate the skin and deliver the drug into the epidermal layer for rapid dissolution and absorption that yield high drug utilization and bioavailability.

Macroflux® technology incorporates a thin titanium microprojection array affixed to a polymeric adhesive back. The array has an area of up to 8 cm^2 and contains as many as 320 microprojections per cm^2 with individual microprojection lengths of <200 μm. Macroflux® arrays can be up to 5 or 10 cm^2 in area. The design and length of the microprojections as well as their density are chosen specifically for each particular application, to optimize delivery and tolerability.[53]

Three types of Macroflux® integrated systems have been designed and tested in preclinical studies. These include: (a) Dry-Coated Macroflux® systems for bolus or short duration administration that consist of a drug or vaccine-coated microprojection array adhered to a flexible polymeric adhesive backing, (b) D-TRANS® Macroflux® systems for extended passive delivery that consist of a microprojection array coupled with a drug reservoir, and (c) E-TRANS Macroflux® systems for pulsatile or on-demand delivery that include a microprojection array coupled with an electrotransport system.

In short-term, placebo-wearing studies in humans, the Macroflux® patch has been well tolerated, with little or no erythema at the application site. Although microprojection penetration into the skin is not normally visible, application of methylene blue disclosing dye, after removal of the patch, shows an exact replica of the Macroflux® microprojection array pattern. The dye penetration outlines the new pathways that are open for drug delivery. Transepidermal water-loss measurements indicate that these pathways close within one hour after patch removal. In clinical studies, to date, there has been no evidence of skin infection following wearing and removal of the Macroflux® patch. In addition, for product convenience and stability benefits, the system provides rapid and efficient drug delivery, beyond the existing injectable products.

Macroflux® patch technology has several distinguishing features. A patch application system has been developed to ensure consistent dosing and ease of use. Drug coating on the microprojection array allows for rapid, direct delivery of high-molecular-weight drugs through the skin barrier layer, optimizing bioavailability, and efficient drug utilization.

In many traditional patch technologies, only a small percentage of the drug is actually delivered from the patch reservoir into the skin. In the current environment of cost containment and disposal risks, this is undesirable, particularly for the more expensive, potent biopharmaceuticals. In order to maximize the efficiency of drug incorporation into the patch and to ensure the precision of drug transport to the skin, a coating process has been developed that applies the drug formulation just on the tips of the Macroflux® microprojections.

Scientists at ALZA have also demonstrated that intracutaneous Macroflux® delivery of a 45-kDa protein antigen provided a better vaccine response than an equivalent dose delivered by intramuscular or subcutaneous injection, in preclinical studies. In addition, Macroflux® transdermal technology provided system-controlled and sustained delivery of an antisense oligodeoxy- nucleotide, 7 kDa, achieving a delivery of 15 mg, over a 24-hour period, from a 2 cm^2 patch.[54-56]

CONCLUSION

With the discovery of insulin, identification and commercialization of potential protein and peptide drugs have increased and so has the research. Delivering protein and peptide drugs via the oral route is a challenging task. The needle and syringe remain the primary means of protein delivery. Major hurdles remain in order to overcome the combined natural barriers of drug permeability, drug stability, pharmacokinetics, and pharmacodynamics of protein therapeutics. Considerable progress has been made

and work has been carried out over the past few years in delivering proteins and peptides to the body. Many other routes of administration have been explored and tested. As each delivery system has its own pros and cons, these systems can improve patient compliance and bioavailability when compared with the conventional delivery systems.

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