Noninvasive Routes of Proteins and Peptides Drug Delivery

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Recent advances in the field of pharmaceutical biotechnology have led to the formulation of many protein and peptide-based drugs for therapeutic and clinical application. The route of administration has a significant impact on the therapeutic outcome of a drug. The needle and syringe is a well established choice of protein and peptide delivery which has some drawback related to patient and to formulation such as pain, cost, sterility etc. Thus, the noninvasive routes which were of minor importance as parts of drug delivery in the past have assumed added importance in protein and peptide drug delivery and these include nasal, ophthalmic, buccal, vaginal, transdermal and pulmonary routes. The pharmaceutical scientists have some approaches to develop the formulations for protein and peptide delivery by noninvasive routes. But, due to the physiochemical instability and enzymatic barrier of proteins and peptides there are several hurdle to develop suitable formulation. So there is need of penetration enhancers, enzyme inhibitors and suitable vehicles for noninvasive delivery to increase the bioavailability. In this review, the aim is to focus on the approaches to formulation of protein and peptide based drug administration by noninvasive route.

Key words: Enzymes, insulin, noninvasive route, nanoparticles, protein and peptide

Over 125 biotechnology-based drugs are already available, and the US market for advanced drug delivery systems is currently estimated to be $75 billion (10% of total pharma sales), being expected to reach $121 billion by 2010 total sale of pharma (12% pharma sales)[1,2].

The route of administration has a significant impact on the therapeutic outcome of a drug[3,4]. Recent advances in pharmaceutical biotechnology, by virtue of the biophysical and biochemical properties, have made specific route of delivery as well as the design of the delivery system. Thus, routes which were of minor importance as parts of drug delivery in the past have assumed added importance in protein and delivery and these include nasal, ophthalmic, buccal, rectal, intrauterine, vaginal, transdermal and pulmonary routes[5-14].

Pharmaceutical biotechnological formulations comprises the preservation their stability, therapeutic effectiveness during their acceptable shelf-life and also storage condition and transportation and till the delivery.

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The oral, nasal and pulmonary route have been the primary non-invasive routes of protein delivery investigated to research in this field to despite the observation that bioavailability of the peptide and proteins have been proven to be very low in most of the non-invasive routes tested. Due to the high cost of these complex molecules also may limit the number of protein drug that would be economically feasible to deliver via these noninvasive routes[15].

General consideration in formulation design of protein drugs:

For all kinds of systemic delivery except intravenous, one of the first barriers for absorption is the permeation across a cell layer. Being charged, large and hydrophilic, proteins are notoriously poor permeators (poor bioavailability). Therefore, it is often necessary to add enhancers to the protein formulation. In formulating dosage forms of protein and peptides drugs, an intelligent selection of additives which enhance their absorption across membranes and their stability is very significant.

The noninvasive routes which are used for the development of such drugs are the GI Tract including colon, intramucosal routes such as sublingual, nasal

and pulmonary routes. Specially, protein transduction domains (PTDs) and cell-penetrating peptide (CPPs) have been used as enhancers, e.g. transdermal delivery.

The additives for such formulation include polymer carriers, penetration enhancers, protease inhibitors etc. some substances like mucoadhesive polycarbophils have been reported to inhibit trypic digestion of protein and thus improve oral absorption of compounds such as demopressin.

The physical size of the protein drugs and their susceptibility to degradation are key determinants of their delivery route. Noninvasive delivery of proteins would be very desirable, and there have been interesting efforts to develop oral protein formulation similar to the ones cited earlier with regard to insulin. Unfortunately, these efforts have been hampered by the low bioavailability[16].

Oral route of delivery:

Delivering therapeutically active protein and peptides by the oral route has been a challenge and a goal for many decades. The oral route is unsuitable for the systemic delivery of therapeutic peptides and proteins because of the potential degradation by the strongly acid environment in the stomach and by the proteolytic enzymes in the intestinal tract, as well as presystemic elimination in the liver. For such drugs to be absorbed through the gastrointestinal tract, they must be protected from enzyme and must traverse through the luminal barriers into the blood stream in an unchanged form. Currently only two peptide and protein based drugs (Interferon alpha and human growth hormone) that can be given orally are known to be in clinical developed[17].

The use of enhancers for oral delivery of proteins is common. Reversible opening of tight junctions with paracellular permeability enhancers (PPEs) is possible using peptide enhancers[18]. Peptides are perhaps better subjects than large molecules of proteins for oral administration[19]. Mucoadhesive delivery systems adhere to the mucous gel layer covering mucosal membranes. Such systems are expected to prolong the residence time at the local site of absorption and to increase the concentration gradient between delivery system and intestinal membrane. As a result mucoadhesive delivery systems are thought to be effective in enhancing the intestinal absorption of peptides and proteins[20,21].

Adjutants like water-oil-emulsions, surfactants, mixed bile salts micelles etc, all of which lower the surface resistivity of the apical membrane, may also enhance absorption of macromolecules. Sodium glycocolates and poly-ethylene-9-dodecyl enhance permeation of insulin. Alpha and beta human interferon are better absorbed in large intestines of rats when given with apricot kernel oil and polyethylene glycol in a citrated buffer solution. One formulation type employs encapsulation into particles containing, for example, chitosan or thiomers to increase the adhesion to the intestinal mucus[19-24].

The use of enzyme inhibitors in long-term therapy remains questionable because of possible absorption of unwanted proteins, disturbance of the digestion of nutritive proteins and stimulation of protease secretion as a result of feedback regulation[25].

A strategy for modulating tight-junction permeability to increase paracellular transport of drug molecules has been studied[26,27]. In fact, the Zonula Occludens toxin[17], chitosan[28], thiolated polymers[29] and Pz-peptide[30], all demonstrate a powerful capacity to increase macromolecular drug absorption. However, potentially, such a strategy is not without safety concerns. Once tight junctions have been opened, transport is enhanced not only for drugs, but also for potentially toxic or unwanted molecules present in the GI tract[26,31]. Because many biologicals are used for the treatment of chronic conditions, the long-term implications of unwanted protein absorption could represent a source of concern. Following approaches have been used as potential means to enhance the oral delivery of peptide-based pharmaceuticals.

Molecular weight and size:

Molecular weight and size influence the diffusion of drugs through the epithelial layer, the very large molecule have lower diffusivities and only small molecules (<75-100 Dalton) appear to cross the barriers rapidly[14]. However permeability falls of markedly as the molecular size increases. Several authors have investigated the effects of the molecular weight upon oral absorption of various hydrophilic compounds[32-34].

Entrapment in liposomes:

Moufti *et al*.[35] were able to produce a 50% reduction in blood glucose level in normal rats by insulin-containing liposomes. Dobre *et al*. illustrated a

lowering of blood glucose level in normal rats following oral administration of insulin entrapment in phosphatidycholin (PC) and cholesterol (CH) liposomes[36].

Oral nanoscale carriers:

Insulin-loaded chitosan nanoparticles administered orally to diabetic rats reduced their glucose levels to a normal range for more than several hours[37,38]. Nanocapsules fabricated from biodegradable poly (isobutycyanoacrylate) were also evaluated as the oral delivery system for insulin to enhance the systemic bioavailability and therapeutic efficacy[39].

Modifying the physicochemical nature of macromolecules:

Structural modification of biologicals provides several opportunities to improve not only membrane permeability, but also proteolytic stability. The strategy of producing prodrugs[40,41] and analogs[42] of biological might protect them from degradation by proteases and other enzymes present in the GI tract. Lipidization, which is the covalent conjugation of a hydrophobic moiety or the noncovalent interaction with a hydrophobic compound, can increase the lipophilicity of peptide and protein molecules[43,44], whereas conjugation with polyethylene glycol (PEG) improves solubility and offers protection from enzymatic degradation[45,46]. These modifications can be used to optimize the pharmacokinetic properties of the macromolecules, but care must always be taken not to reduce their biological efficacy.

Nasal routes route of delivery:

A drug delivery route that is less technologically demanding than pulmonary delivery is nasal delivery. By virtue of relatively rapid drug absorption, possible bypassing of presystemic clearance and relative ease of administration, delivery of drug by the nasal route offers an attractive alternative for administering systemically active drugs. Simple nasal drops or a nasal spray, nasal gel can be used, and for particulate nasal delivery the particle size is not as important. A special aspect of nasal delivery is the possibility of achieving delivery transsynaptically directly into the brain using nanoparticles[47]. The nasal epithelium suited for permeation has an area of approximately 150 cm^2, and this will limit the dose range given by this route. Higher bio-availabilities can be obtained with more advanced delivery systems, especially by adding enhancers that modulate the permeability of the epithelium[48,49].

Pharmaceutical drugs as well as endogenous hormones such as luteinizing-hormone-releasing hormone LHRH, thyrotropin-releasing hormone (TRH)[50,51], vasopressin[52], calcitonin, oxytocin[53], ACTH[54], glucagon, insulin[55,56], interferons[57], and enkephalins[58], have been shown to be absorbed nasally in animal and human. The studies of the nasal delivery of a number of peptide-based pharmaceuticals demonstrated that systemic bioavailability can be improved by nasal route.

For hydrophilic peptide and protein which furthermore can be degraded in the nasal cavity by peptidase and absorption considerably smaller for peptide calcitonin and insulin bioavailability of the order less than 1% has been reported[56].

In order to overcome the barrier to nasal absorption of these molecules, two main approaches have been utilized, modification of permeability of nasal membrane by employment of absorption enhancer, such as surfactants, bile salts, cyclodextrins, phospholipids, and fatty acids, and use of the mucoadhesive system such as bioadhesive, liquid formulation (e.g. chitosan) microsphere powder and liquid gelling, formulation that decreases the mucociliary clearance of the drug formulation and thereby increase contact time between the drug and site of the absorption[49].

Morimoto *et al*. have improved that nasal bioavailability of calcitonin and insulin by means of formulation employing corbopol 941 (corboxy polymethylene, polymer of acrylic acid cross linked with allyl sucrose) and carboxymethyl cellulose (CMC)[57].

Pulmonary route of delivery:

Pulmonary administration is an attractive route of proteins and peptides than other alternative routes of administration. The lungs offer a large surface area for drug absorption, of approximately 80-140 m^2. The alveolar epithelium is very thin (approximately 0.1–0.5 mm thick), thereby permitting rapid drug absorption. The alveoli can be effectively targeted for drug absorption by delivering the drug as an aerosol, with a mass median aerodynamic diameter of less than 5 μm. Furthermore, the first-pass metabolism of the GIT is avoided. Although metabolic enzymes are found in the lungs, the metabolic activities and pathways may differ from those observed in the GIT, and this makes the pulmonary administration of many

peptides and proteins very promising[58]. Bioavailability of pulmonary insulin in clinical trials has been estimated to be ~10% compared with subcutaneous injection[59]. The pulmonary route, through aerosol delivery systems is for the administration of drugs molecules to treat pulmonary diseases, such as asthma[60]. Devices such as jet or ultrasonic nebulizers, metered-dose inhalers (MDI), and dry powder inhalers are used. MDIs 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. These are very effective for delivery of the drugs to the upper airways by the device called as spacers have been added, to be used with MDIs in order to remove some of the non-respirable particles, by impaction on their walls and valves[61-63].

A number of companies have products in clinical trials of novel pulmonary delivery systems, such as Inhale Therapeutic Systems, Alkermes, AeroGen, Alliance, Battelle, Delsys, Elan, Nastech, Sheffield, and Vectura, while Pfizer. Nektar Therapeutics and Sanofi-Aventis marketed a pulmonary insulin product, It was formulated with sodium citrate (dehydrate), mannitol, glycine and sodium hydroxide in 1-5 micron particles, with a bioavailability of 10% relative to s.c. administration (Exubera also included a higher level of insulin than s.c. administration of a convention insulin). Other insulin formulations have been under development latterly, ranging from dry crystal to coated particles to liquid droplets.

The absorption chemical enhancers, which increase the permeability of drugs through the epithelial membranes without causing any tissue damage, are especially useful for the delivery of peptide and protein drugs[12,64]. The surfactants, bile salts and fatty acids have been evaluated as absorption enhancers and, although most of them exhibit permeation-enhancing effects, they also produce membrane damage. Polyoxyethylene (PE) oleyl ether also showed good enhancing ability for the peptide. However, sorbitan trioleate, PE sorbitan monooleate and PE sorbitan trioleate only showed moderate enhancement while the enhancing effects of glycerol trioleate, ethyl oleate, oleyl alcohol, palmitic acid and stearic acid were relatively low. The mechanisms of absorption promoting effects of these agents in various mucous membranes have been reported[65].

In contrast, liposomes are very promising pulmonary absorption enhancers for peptide and protein drugs because they consist of biogenic phospholipids and are biocompatible, biodegradable and non-immunogenic. The use of liposomes has been suggested to provide sustained pulmonary release for various drugs. However, liposomes and phospholipids have also been investigated for the systemic absorption of different proteins after intratracheal delivery. In recent study[66] interleukin 2 (IL-2) liposomes were administered as aerosol to individuals with immune deficiency. Patient acceptance, safety, toxicity and immune effects of IL-2 liposomes were studied. No significant changes in chest X-ray or pulmonary function tests were noticed following administration of the liposomes.

Various protease inhibitors, surfactants, lipids, polymers and agents from other classes have been tested for their efficacy in improving the systemic availability of protein and macromolecular drugs after pulmonary administration.

Nafamostat mesilate, which has been studied as an absorption enhancer for insulin, strongly inhibits a variety of proteases such as trypsin, plasmin and kallikarin[67]. The amount of absorption enhancement will typically depend on what enzyme the protease inhibitor inhibits (i.e. serine inhibitor, amino peptidase inhibitor, etc.) as well as the specific vulnerabilities of the peptides which are being delivered. Some of the various protease inhibitors investigated have include nafamostat mesilate, bacitracin, soybean trypsin inhibitor, chymostatin, potato carboxy peptidase inhibitor (PCPI), phosphoramidon, foroxymithin, amastatin, aprotonin, Tos-Phe-chloromethylketone, 3,4-dichloroisocumarin, trans-epoxysuccinyl-leucylamido (4-guanido) butane[68].

Buccal route of delivery:

The buccal mucoadhesive formulations are to be an alternative to the conventional oral small amount of medicaments as they can be readily attached to the buccal cavity retained for a longer period of time and removed at any time. The epithelium of the mouth is accessible with small surface area approximately 100 cm^2 Buccal adhesive drug delivery systems using matrix tablets, films, layered systems, discs, microspheres, ointments and hydrogel systems have been studied and reported by several research groups. However, limited studies exist on novel devices that

are superior to those of conventional buccal adhesive systems for the delivery of therapeutic agents through buccal mucosa[69].

A number of formulation and processing factors can influence properties and release properties of the buccal adhesive system. The formulations designed for buccal administration should contain the following functional agents: Mucoadhesive agents, to maintain an intimate and prolonged contact of the formulation with the absorption site; penetration enhancers, to improve drug permeation across mucosa (transmucosal delivery) or into deepest layers of the epithelium (mucosal delivery); and enzyme inhibitors, to eventually protect the drug from the degradation by means of mucosal enzymes[70].

There are numerous important considerations that including biocompatibility (the drug/device and device/environment interfaces), reliability, durability; environmental stability, accuracy, delivery scalability and permeability which are to be considered while developing such formulations. While biocompatibility is always an important consideration, other considerations vary in importance depending on the device application. Bioadhesive formulations designed for buccal application should exhibit suitable rheological and mechanical properties, including pseudoplastic or plastic flow with thixotrophy, ease of application, good spreadability, appropriate hardness, and prolonged residence time in the oral cavity. These properties may affect the ultimate performance of the preparations and their acceptance by patients.

The buccal mucosa represents a potentially important site for controlled delivery of macromolecular therapeutic agents, such as peptides and protein drugs with some unique advantages such as the avoidance of hepatic first-pass metabolism, acidity and protease activity encountered in the gastrointestinal tract. Another interesting advantage is its tolerance (in comparison with the nasal mucosa and skin) to potential sensitizers.[71]

Vaginal route of delivery:

The vagina as a route of drug delivery has been known since ancient time. The anatomical position, the rich blood supply and the large surface area of the vagina predestines it as an application site for systemic drug delivery. In recent year numerous studies prove that this has a good permeability for macromocules and can be a potential route for systemic delivery to a wide range of compounds including peptides and proteins[72,73].

The main advantages of vaginal drug delivery over conventional drug delivery are the ability to by-pass first pass metabolism, ease of administration and high permeability for low molecular weight drugs. However, several drawbacks, including cultural sensitivity, personal hygiene, gender specificity, local irritation and influence of sexual intercourse, need to be addressed during the design of a vaginal formulation.

The vaginal route offers a favourable alternative to the parenteral route for some drugs such as bromocriptine[74,75], oxytocin[76], misoprostol[77], calcitonin[78], LHRH agonists, human growth hormone[79] and insulin.

For systemic delivery, insulin suspended in a poly(acrylic acid) gel base was observed to facilitate the rate of vaginal absorption in diabetic rats and rabbits. Plasma insulin reached a peak and hypoglycaemic effects were observed[80].

In recent years, there have been several reports of successful immunization with DNA vaccines administered via various mucosal routes including the vaginal route[81-82]. A recent study demonstrates the formulation and application of plasmid DNA vaccine to mucosal inductive tissues, including the vagina. The female genital tract has the capacity to produce humoral and cellular immune responses against locally encountered antigens[83]. Intravaginal delivery of cholera vaccine showed a greater mucosal response in female genital tract compared to oral administration of the vaccine[84].

Transdermal drug delivery:

The advantages of transdermal drug delivery (TDD) have therapeutic benefits such as sustained delivery of drugs to provide a steady plasma profile, particularly for drugs with short half-lives, and hence reduced systemic side effects; reducing the typical dosing schedule to once daily or even once weekly, hence generating the potential for improved patient compliance; and avoidance of the first-pass metabolism effect for drugs with poor oral bioavailability. Additionally, TDD represents a convenient, patient-friendly option for drug delivery

with the potential for flexibility, easily allowing dose changes according to patient needs and the capacity for self-regulation of dosing by the patient. The noninvasive delivery of TDD makes it accessible to a wide range of patient populations and a highly acceptable option for drug dosing.

The transdermal route has attracted interest as a promising way to advance the delivery of peptides and to minimize side effects as well as first-pass metabolism. The skin, being the body's largest organ, also represents an important target site for drug administration aside from its physiological functions. To reach therapeutic concentrations in different skin layers or the systemic circulation, the main challenge is to overcome the stratum corneum, which represents the uppermost skin barrier. To impede the flux of toxins into the body and minimize water loss, the barrier has a very low permeability for foreign molecules. As mentioned above, transdermal delivery of therapeutic peptides is very difficult because of the physicochemical properties (polarity, size) of the molecules[85,86].

Iontophoresis represents a non-invasive technique for a controlled administration of peptides with low molecular weight. Greater drug amounts can be delivered in a shorter time induced by an electric field, which increases the drug's mobility. Usually, only a few milliamperes of current are applied to a few square centimetres of skin. To enhance the permeation of large peptides, Pillai and Panchagnula used iontophoresis in combination with fatty acids[86]. The results of the *in vitro* investigations showed a synergistic flux enhancement of skin pretreated with fatty acids and iontophoresis compared to passive diffusion in pretreated skin and iontophoresis alone[87].

Banga *et al*. was investigated and studied the hydrogel-based iontotherapeutic devices as the drug reservoir matrix for peptide-based pharmaceuticals, and the iontophoresis release and transdermal delivery of three model peptides, insulin, calcitonin, and vasopressin. The experimental results shown that permeability coefficients for these peptides across the hairless rat skin were evaluated using the hydrogel formulations prepared from polyacrylamide, p-HEMA, and carbopol. A rank order of vasopressin>calcitonin>insulin was obtained in accordance with the order of molecular size[88].

Another noninvasive technique undergoing commercial experimentation is the use of low-frequency ultrasound in a method called sonophoresis. Cavitations' seems to be the main effect which enables the improved permeation and delivery of macromolecules such as peptide and proteins[89].

During recent years various scientist have developed method for facilitating transdermal delivery of insulin. Chemical enhancers based on biphasic lipid system or flexible lecithin vesicles-containing insulin showed hypoglycaemic effect in experimental animals. Additional approach to facilitate transdermal delivery of insulin include altering skin characteristics by physical tools such as iontophoresis, sonophoresis, electroporation and photomechanical treatment[90,91]. Combination of chemical enhancers and iontophoresis also showed facilitated transdermal delivery of insulin[92]. Recent novel techniques using ultradeformable carriers (transfersomes) and $CaCO_3$-nanoparticles encapsulating insulin may also serve as efficient insulin delivery systems[93].

The ocular route of drug delivery:

The ocular route may also be used for the systemic delivery of peptides and protein based pharmaceutical drugs. The ocular route is the choice for the localized delivery of ophthalmologically active peptide and protein for the treatment of ocular disease in the pharmaceutical dosage form of solutions, suspensions and ointment. Ocular drug delivery is one of the most interesting and challenging endeavours faced by the pharmaceutical researcher because of the critical and pharmacokinetic ally specific environment that exists in the eye[94-96].

Several peptide and protein pharmaceuticals, such as enkephalins, thyrotropin releasing hormone, LHRH, glucagon, and insulin[97-99], were reportedly absorbed to some extent into the blood stream of the albino rabbit. The topical application of insulin may be beneficial in preventing diabetic retinopathy or potentially lessening its severity. Transscleral delivery of insulin to the retina and choroid would be of considerable therapeutic value as the hydrogel-based transcutaneous iontophoresis could deliver biologically active insulin, calcitonin, and vasopressin across hairless rat skin. Yamamoto *et al*.[100] studied the systemic delivery of topically applied [D-ala2]-met-enkephalinamide and insulin, respectively, in fully awake rabbits and reported the attainment of peak plasma concentration within 15-20 min following a solution instillation, with a bioavailability of around 36% achieved for the enkephalin analog and less than 1% for insulin. The systemic bioavailability of insulin

was improved to 4-13% with the incorporation of absorption promoters, such as bile salts (the sodium salt of glycocholic, taurochlolic, and deoxycholic acid) and non-ionic surfactants (polyoxyethylene-9-lauryl ether)[101].

Johnson *et al*. studied the use of a novel peptide for ocular delivery (POD) with protein transduction properties, for delivery of small and large molecules across the plasma membrane at the local site. POD enters cells within 5 min in a temperature dependent manner and directly compact and deliver plasmid DNA, achieving transgene expression in >50% of human embryonic retinoblasts. Delivery of small interfering RNA (siRNA) duplexes to cells using POD, allowed for silencing of transgene expression by >50%. POD could also be used to deliver quantum dots *in vitro* and *in vivo* experiments. Upon ocular delivery, POD rapidly entered neural retina and localized to retinal pigment epithelium (RPE), photoreceptor, and ganglion cells. Additionally, POD was able to enter corneal epithelium, sclera, choroid, and the dura of the optic nerve via topical application. POD also functions as a bacteriostatic, a useful property for a carrier of molecules to post mitotic neural ocular tissues[102].

Some of the general approaches that have been found useful in enhancing the ocular absorption of ocular absorption of organic–based pharmaceuticals, such as the use of nanoparticles, liposomes, gels, ocular inserts, bioadhesive, or surfactants[103,104] may also improve the ocular delivery of peptide-based pharmaceuticals.

CONCLUSION

Currently, recent progresses in pharmaceutical biotechnology, many protein or peptide-based drugs have been or are being developed. The noninvasive route is easy way to administrate them, but due to physiochemical and enzymatic barriers, they have to be administered parenterally. To improve the patient's compliance and life, many researchers have been working on development of protein and peptide noninvasive route delivery formulation, such as tablet, aerosol, MDI, gel, cream etc. The formulation needs to have newer technology/excipients such as penetration enhancers, polymers, enzyme inhibitors, etc. In the future many protein and peptide formulations will be available to the patients for better therapeutic response, life style and safety over the parenteral formulations. The formulation would be available at a low price in pharmaceutical market.

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