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Review Article

NONINVASIVE DELIVERY OF PROTEIN AND PEPTIDE DRUGS: A REVIEW

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ABSTRACT

Till recent, injections remained the most common route for administration of protein and peptide drugs because of their poor bioavailability in the other routes. Because it is generally recognized that injection based delivery is a major impediment to the commercial success of therapeutic proteins and peptides, research in both academia and industry continues to focus on ways to overcome this problem. Possible non-parenteral administration routes for delivery of peptide and protein drugs include oral, nasal, ocular, transdermal, rectal, colonic, and vaginal route. The large surface area associated with most of these routes makes them attractive targets for drug delivery. While non-invasive administration by these routes is considered a more logical and achievable option for local treatment regimens, systemic delivery of proteins and peptides is significantly more challenging. In spite of effort made on the development of drugs for these routes, most of the successes fail to address how the technology will be transformed to a commercial product. The only notable exceptions have been the successful commercialization of nasal formulations for systemic delivery of a limited number of therapeutic peptides, and recent regulatory approvals of both pulmonary and buccal delivery systems for systemic delivery of insulin and an oral formulation of a small peptide analog, cyclosporine, have been commercialized. The present review aims to discuss the potential non-invasive routes of protein and peptide drug delivery. The factors which will affect drug transport and the bioavailability of proteins administered through these routes is also emphasized.

Keywords: Protein and peptide, Non-invasive, Oral, Nasal, Rectal, Transdermal, Colonic, Vaginal.

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INTRODUCTION

In recent years, proteins and peptides have emerged as promising therapeutic agents. The pharmaceutical challenges posed by these systems include (a) selection of a suitable route of drug delivery and (b) formulation of these bioengineered drugs. The most common route of protein and peptide drug delivery is the parenteral route. However, this route is associated with pain at the site of administration, resulting in poor patient compliance, and the formulation needs to be sterile. Drugs administered by the gastrointestinal route are subjected to acid hydrolysis and extensive gut and/or hepatic first-pass metabolism. Thus, these protein drugs may exhibit poor oral bioavailability for which, non-invasive routes such as mucosal, transdermal, nasal, ocular, pulmonary, rectal, vaginal, buccal and sublingual, offer effective alternatives for systemic drug delivery.

Potential mechanisms of transport across cells include passive paracellular, passive transcellular, facilitated transcellular, active carrier-mediated, and transcytosis. While the cellular barrier for each noninvasive delivery route is more complex than what is depicted (Fig. 1).

ORAL DELIVERY

Oral route is preferred over any other route because of good patient compliance and acceptance. This is the most preferred route for chronic ailments. Designing and formulating protein and peptide drug delivery system for gastrointestinal route is a challenge because of the unfavorable conditions posed by it such as pre-systemic enzymatic degradation and poor membrane permeability.

Barriers for oral absorption

The barrier that prevents the entry of protein or peptide drugs into the systemic circulation is intestinal epithelial tight junctions. The major problems involved in oral delivery of proteins can be given as denaturation of proteins due to acidic environment in stomach, degradation of proteins in stomach and intestine due to proteolytic enzymes, intestinal wall which is impermeable to macromolecules, mucin barrier which is formed by mucus that is secreted by goblet cells, intestinal transit time [1,2].

Enzymatic barriers

Most of the proteins are known not to be absorbed in humans as intact forms. They are usually broken down into amino acids or di- and tripeptides first in the gastrointestinal tract (GIT). The four peptidases secreted by the pancreas, i.e., trypsin, chymotrypsin, carboxypeptidase, elastase, convert proteins, and polypeptides to oligopeptides [3].

Luminal degradation of proteins is up to 20% of the total degradation in small intestine the rest of the degradation occurs on contact with the brush border membrane or after entry into the cell. Brush border peptidases such as amino oligopeptidase, aminopeptidase, and dipeptidyl aminopeptidase then breakdown the oligopeptides to amino acids (up to 70%) and di- and tripeptides (up to 30%) [3,4].

The above-mentioned enzymatic barriers must be overcome to improve oral absorption of protein and peptide drugs from the GIT. This may be possible to achieve to some extent by using enzyme inhibitors or by chemical modification or by other approaches has been given in Table 1. GI absorption of peptides and peptide-like drugs is given in Table 2.

Bioavailability

Captopril, lisinopril, and enalapril have good oral bioavailability due to their low molecular weight and their ability to inhibit tissue carboxyl peptidases [11]. Cyclosporine is a cyclic peptide with a number of methylated amino acid residues and is resistant to hydrolysis, and therefore, has good bioavailability [12]. Some protease inhibitors and absorption enhancers have been coadministered with peptide drugs to enhance their oral absorption. A good example is oral arginine vasopressin. To produce a 50% reduction in urine flow, in the rat, an oral dose of about 3500 pmol was required. When the drug was

Table 1: Approaches to increase the oral bioavailability

Approach	Example	Outcome	References
Absorption enhancers	Detergents, bile salts, fatty acids, chitosans, acylcarnitine, alkanoyl choline, N-acetylated α-amino acids, N-acetylated non-α-amino acids	Temporarily disturb of intestinal barrier to improve the permeation	[5]
Enzyme inhibitors	Trypsin, chymotrypsin, carboxypeptidases, aprotinin, pancreatic inhibitors Or kunitz inhibitors, FK-448, camostat mesylate, etc.	Protects from degradation by enzymes in stomach and intestine	[1]
Chemical modification			
Amino acid modification	Conjugation with polymer PEG or ligands like transferring	Affects receptor binding capacity and decreases rate of clearance from systemic circulation	[1]
Lipidization	1, 3-dilpalmitoylglycerol	Benefit to transcellular passive or active absorption	[5]
Muco adhesive polymer systems	Types: Anionic, cationic, nonionic, amphoteric, thiomres, dendrimers, synthetic glycol polymers etc., ex: Chitosan	Longer transit time and, decreases diffusion barriers	[1]
Formulation vehicles			
Emulsions	S/O/W emulsion (surfactant-insulin complex is dispersed into oil phase)	Protect drug from chemical and enzymatic breakdown in lumen	
Microspheres	Microspheres of poly(methacrylic-g-ethylene glycol)	Protects over the influence of the pH variability through the stomach to intestine	[5]
Nanoparticles	Polystyrene, chitosan, PLA-PGA	Less sensitive to enzymatic degradation	[6]
Liposomes	PEG-coated liposomes and mucin containing liposomes	Resistance against digestion by bile salts and stability in GIT	

PEG: Polyethylene glycol, GIT: Gastrointestinal tract, PLA: Poly (lactic acid), PGA: Poly (glycol acid)

Table 2: GI absorption of peptides and peptide-like drugs

Compound	Extent of absorption (%)	References
Dietary di- and tripeptides	5-50	[7]
Aminocephalosporins	>50	[8]
Enalapril	>50	[9]
Dietary tetrapeptides	≈5	[10]
TRH analogs	≈5	[10]
Enkephalins	<2	[10]
Bradykinin	<2	[10]

GI: Gastrointestinal, TRH: Thyrotropin-releasing hormone

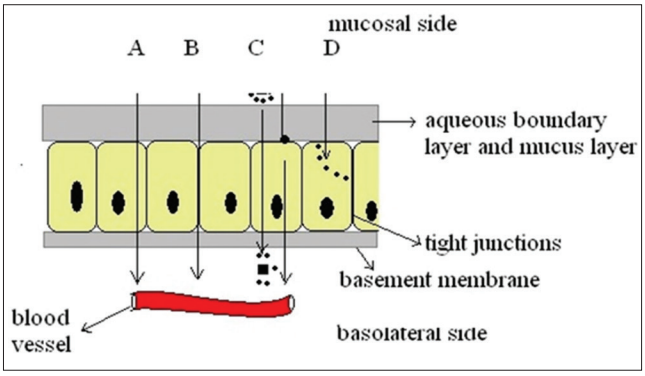

Fig. 1: A schematic illustration of potential mechanisms of transport across the cellular barrier in noninvasive routes of drug delivery systems. (a) Passive transcellular, (b) passive paracellular, (c) facilitated transcellular or active carrier mediated, (d) transcytosis

coadministered with aprotinin, a protease inhibitor, the oral dose was reduced to 1000 pmol [13].

NASAL DELIVERY

Barriers for nasal absorption

The greater permeability of nasal mucosa is attributed to its large surface area which provides a rapid onset of therapeutic action. The limitations associated with the oral route are overcome by low-metabolic environment of nose and this will duplicate the advantages of intravenous administration. Targeting the central nervous system by bypassing blood–brain barrier is the main interesting advantage of nasal drug delivery [14]. The drugs absorbed through the olfactory epithelium are reported to enter in olfactory neurons and supporting cells and subsequently into the brain, which reduced not only the systemic toxicity of centrally acting drugs but also enhanced therapeutic efficacy [15]. This route received great attention as a possible route for vaccination [16]. In spite of large number of advantages, bioavailability of nasal dosage form is limited by various factors.

Nasal mucosa

The mode of transportation of drug molecule across the mucus barrier is mainly based on diffusion governed by factors such as molecular weight of drug, viscosity of mucus, surface charge, drug-mucus interaction, etc. [17]. Small unionized molecules readily cross the mucosal barrier. Sialic acid is a constituent of mucus which contains anionic carboxylic group, which repels the anionic molecule and hence it reduces the transport of such drugs [18].

Nasal epithelium

The nasal epithelium consists of loosely packed cells with high permeability and vasculature. Passive diffusion, carrier mediated transport, and transcytosis are the transportation mechanisms, through which nasal absorption is achieved. However, nasal absorption was hindered by efflux transporters such as glycoprotein [19]. Through the nasal mucosa, low-molecular lipophilic compounds permeate readily. For example, the bioavailability of nasal absorption of ondansetron was comparable with intravenous route in rats [20]. This study revealed complete and rapid absorption of drugs through nasal epithelium. In case of nasal cavity, slightly acidic pH, sterile environment, and low enzymatic activity offers higher bioavailability of certain substances such as propranolol, metoclopramide, nifedipine, apomorphine, and midazolam than oral drug delivery [19]. But for high-molecular compounds such as desmopressin, insulin, human growth hormones the nasal epithelium acts as a barrier.

Mucociliary clearance

Nasal mucociliary clearance is the most important physiological barrier, which reduces the nasal residential time of drugs and/or dosage forms.

Residential time of the drug from the nasal dosage form influences the bioavailability in the nasal cavity. The mucociliary clearance system transports the mucus layer that covers the nasal epithelium toward the nasopharynx by ciliary movement. The protective defense mechanism to the body against any noxious materials inhaled in the respiratory tract is mucociliary clearance [21]. The mucociliary movement changes with the drug were extensively studied [22]. Antihistaminic drugs, beta blockers, general anesthetics, cocaine, etc., arrest the mucociliary clearance [23-26]. Between the mucociliary clearance rate reduction and drug concentration, a correlation has been established [27]. Nasal residence time decreases with increase in mucociliary clearance by the drugs such as cholinergics, beta-adrenergic agonists, and surfactants [28]. Thereby, it reduces the bioavailability of drugs [29]. Various approaches for nasal delivery have been given in Table 3.

Drug metabolism

Even though, nasal route provides low-metabolic environment, metabolism of proteins and peptide molecules in nasal cavity is a major barrier for bioavailability [35]. The main enzymatic barrier present in the nasal mucosa is cytochrome P450 enzymes. Cytochrome P450 is present in both respiratory and olfactory mucosa, thus reducing both nose to blood and nose to brain transport of drugs [36]. Low bioavailability of protein and peptide drugs is obvious by the presence of various proteolytic enzymes such as exopeptidase (mono and diamino peptidase) and endopeptidase (serine, cysteine, and aspartic peptidase) [37].

OCULAR DELIVERY

Ocular route is mainly used for the treatment of ocular inflammation, corneal wounds, and glaucoma. The administration of biopharmaceuticals through eye is complicated by the normal processes of blinking, tearing and drainage from the eye which wash out the drugs which are administered.

Drug absorption

Structure of the cornea and conjunctiva

The cornea is composed of three major layers: Two boundary cellular layers, the epithelium, the endothelium and the stroma (a thick connective tissue) in between. The corneal epithelium is a non-keratinized stratified squamous epithelium, 5-6 cell layers in thickness (Fig. 2).

The conjunctiva consists of a stratified squamous epithelium, overlying a loose, highly vascular connective tissue, the substantia propria [38]. The conjunctival epithelium is continuous with the corneal epithelium at the corneoscleral limbus. However, the two differ somewhat, with the conjunctival epithelium being thicker and possessing mucus-secreting goblet cells. A drug administered topically on the percorneal area comes into contact with the corneal and conjunctival epithelia followed by absorption by ocular tissue. This absorbed drug enters into the systemic circulation by various competing processes such as (1) absorption by the cornea: Cornea is avascular and the drug

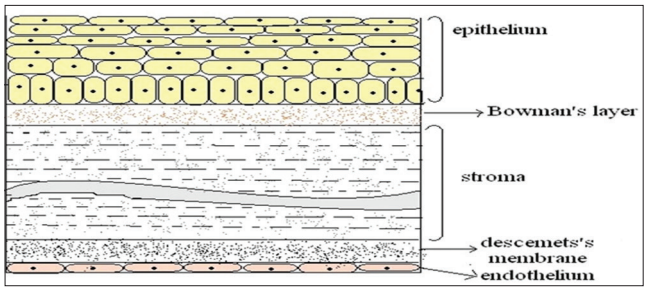

Fig. 2: Diagrammatic representation of cross section of cornea

Table 3: Approaches for nasal delivery

Approach	Description	References
Mucoadhesive	Ideal choice of delivery system increases the nasal residence time. Also close contact of drug delivery system to nasal mucosa increases the extent of absorption, e.g.: 98% bioavailability was achieved for apomorphine by mucoadhesive polymers such as polyacrylic acid, carbopol, and CMC	[30]
Increased nasal blood flow	By increasing the concentration gradient for passive diffusion of peptide drugs with an increase in nasal blood flow, an enhancement in nasal peptide absorption was achieved Nasal blood flow can be increased by vasoactive agents like histamine, prostaglandin E1 and beta-adrenergic agonists	[31]
Particulate drug delivery		
Microspheres	Absorb water into the sphere matrix, resulting in swelling and gel formation, this increase the residential time of the drug in the mucosa Materials used to construct microspheres include starch, dextran, albumin, hyaluronic acid, carbopol, chitosan, etc. e.g. Dextran used for insulin, octreotide, nicotine Degradable starch microspheres for insulin, gentamicin, human growth hormone metoclopramide, cynacobalamin, and desmopressin	[32]
Liposome	The amphiphilic nature of liposomes is well characterized for favorable permeation of drugs through biological membranes The comparative pharmacokinetics in rats showed high permeability of liposome pretreated with permeation enhancer than solution form containing the same quantity of permeation enhancer	[33]
Supercritical fluid technology	Infusion of drug carriers with bioactive materials prepared by techniques using supercritical fluid include RESS, GAS recrystallization ASES or PCA Drug carriers used include ammonium glycyrrhizate, polyacrylic acid, cross-linked polyacrylic acid, polyethylene oxide, and chitosan. e.g., insulin powder formulations prepared using CO_2 infusion. Three-fold increases in bioavailability of insulin by CO_2 infusion compared to freeze-dried powders were achieved.	[34]
pH modification	At isoelectric point proteins and peptides usually have low solubility; by adjusting pH far away from the isoelectric point its solubility can be increased e.g.: DDAVP exhibits good solubility at pH 4.0	[21]

DDAVP (Desmopressin): 1-deamino (-8-D-arginine)-vasopressin, CMC: Critical micelle concentration, RESS: Rapid Expansion of supercritical solutions, GAS: Gas anti-solvent, SES: Aerosol solvent extraction system, PCA: Precipitation with compressed fluid anti-solvent

absorbed by it is distributed largely to the adjacent ocular tissues. (2) absorption by the conjunctiva: Since the conjunctival tissue (substantia propria) is highly vascularized, most of the drug enters into the systemic circulation. This can also be attributed to the fact that the surface area of conjunctiva is larger than that of the cornea. (3) Drug is lost from the percorneal region due to tear turnover by the nasolacrimal secretions into the nasal passages. Drug that is cleared by these fluids is available for absorption by the nasal mucosa [39]. (4) Enzymatic degradation: The transport of administered protein and peptide drugs across ocular barriers is mainly limited by proteinases such as neutral protease and aminopeptidase. Approaches for ocular delivery have been given in Table 4.

TRANSDERMAL DELIVERY

Transdermal drug delivery provides lot of advantages due to large surface area which can enhance the flux of the drugs with the use of appropriate enhancers. The stratum corneum the major barrier. It also avoids first pass metabolism. This route high patient compliant route and any time the drug action can be terminated [43].

Barrier for transdermal delivery

Skin also serves as an extremely effective permeability barrier, a property conferred by the thin (15 mm) outermost layer of epidermis known as the stratum corneum (SC). As a result, transdermal transport by passive diffusion is limited. The transdermal route for systemic delivery of proteins and peptides has been hindered by the extremely low permeability of skin to high molecular weight and typically hydrophilic molecules and to achieve therapeutically useful systemic drug levels; permeation needs to be enhanced [44].

Skin barrier function

SC and routes of passive permeation: The remarkable barrier function of the skin is primarily located in the SC, the thin, outermost layer of the epidermis [45]. The SC consists of several layers of protein-filled corneocytes (i.e., terminally differentiated keratinocytes) embedded in an extracellular lipid matrix. Passive permeation across the SC is believed to occur primarily through the intercellular lipid pathway (Fig. 3a) which constitutes the only continuous phase through the SC, appendageal transport through hair follicles and sweat glands is another potential route, these structures offering "shunt" pathways across the continuity of the SC (Fig. 3b). Visualization of appendageal transport has been accomplished both for passive diffusion and percutaneous transport enhanced by one means or another (e.g., iontophoresis) [46,47]. A third possible route across the SC is the transcellular path (Fig. 3c).

Approaches for transdermal delivery

Several approaches have been explored that provide additional driving force in the form of electrical (iontophoresis) [48] or ultrasound (sonophoresis) [49] energies, structural perturbation of SC (e.g., electroporation, thermal microporation, and microneedles) [50] penetration enhancers [51,52] or a combination of these strategies.

Iontophoresis

To a few square centimeters of skin a small amount of physiologically accepted current is applied to drive drug molecules into and across the skin. Iontophoretic delivery is achieved by 2 ways of electrorepulsion and electroosmosis [44].

Electrorepulsion

Delivery of charged molecule across skin is possible due to repulsion between same charges when a charged molecule is placed under an electrode of the same polarity.

Electroosmosis

Under the influence of electric current, net flow of water from anode to cathode occurs which is called electroosmosis. Since skin is negatively charged, transport of positively charged drugs is possible.

Iontophoretic delivery of the luteinizing hormone-releasing hormone analog leuprolide has been demonstrated in clinical studies in human subjects [53-55] and showed an increase in luteinizing hormone levels comparable to those following a subcutaneous injection.

Electro poration

To permeabilize the skin by application of high voltage electrical (100-1000 V) pulses for a shorter period is called electroporation. By creating new aqueous pathways and enlargement of existing pathway electroporation will lead to transient changes in SC. Electrophoretic movement, enhanced diffusion across the permeabilized skin and electrodiffusion to a small extent are the mechanism of actions.

Sonophoresis

The use of low-frequency ultrasound in the range of 1-3 MHZ for delivery of molecules across the skin is called sonophoresis. Piezoelectric crystal, which is made of lead zirconate is the main component of equipment, will convert the electrical energy into mechanical energy giving raise to acoustic waves or ultrasounds [56].

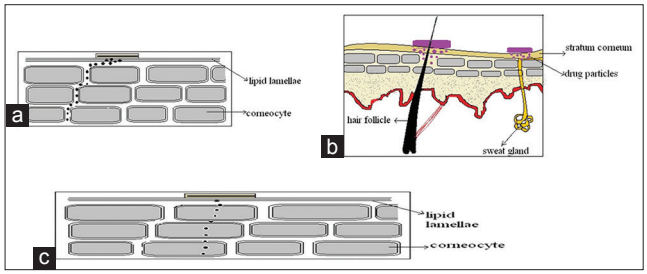

Fig. 3: Routes of passive permeation across the stratum corneum. (a) Intercellular lipid pathway; (b) appendageal transport; (c) transcellular path

Table 4: Approaches for ocular delivery

Approach	Description	References
Cyclodextrin complexes	Cyclodextrin complexes resulted in higher bioavailability compared to conventional eye drops. And do not interrupt the biological membrane like other permeation enhancers e.g., Cyclosporine	[40]
ECT	Entrapment of immunologically isolated cells or proteins with hallow fibers or microcapsules before administration enables the controlled, continuous, long-term delivery of therapeutic proteins directly to posterior region	[41]
siRNA therapy	Delivery of siRNA duplexes to cells using POD, allowed for silencing of transgene expression by >50%. Upon ocular delivery POD rapidly entered neural retina and localized to RPE, photoreceptors and ganglion cells	[42]

siRNA: Small interfering RNA, POD: Novel peptide for ocular delivery, ECT: Encapsulated cell technology

Formulation approaches

A variety of chemical penetration enhancers with or without protease inhibitors or colloidal vehicles (liposomes) have been investigated for their potential to enhance the skin permeability of peptides and proteins, but these approaches have only been limited to animal models or *in vitro* or *in vivo* models of human skin and have not progressed to human clinical studies. A notable exception is the use of so-called transfersomes.

Transferosomes

Ultraflexible liposomes, containing a mixture of soybean phosphatidylcholine and sodiumcholate, which are thought to reversibly create intercellular hydrophilic pathways through the skin to facilitate transport [57,58]. Insulin-loaded transfersomes applied epicutaneously to human volunteers have been shown to result in a glucodynamic response. With a single application of transfersomes to patients with Type I diabetes, normoglycemia has been maintained for 16 hrs. This approach has also been successfully demonstrated with other polypeptides such as interferons a, b, and g, calcitonin and superoxide dismutase in preclinical experiments [59].

COLONIC DELIVERY

The colon has received considerable attention as a possible delivery site for protein and peptide drug delivery compared to other possible oral routes because enzyme activities are significantly lower when compared to small intestine, the residence time in colon is longer, the bacteria present in colon secrete many enzymes which act as triggers for colon-specific drug delivery and the microflora degradation mechanism has been used as a tool for the site-targeted delivery of peptide and protein drugs.

Barrier for colonic absorption

Colonic absorption of protein and peptide drugs administered orally are seriously restrained by diffusional barriers (unstirred layers and mucosal permeability) as in the case of small intestine but, more importantly the inhibition is due to metabolism which occurs in the lumen, brush broder and at the cytosol level [60].

Colonic absorption

The lack of organic nutrient transporters may limit the potential for drug design with respect to carrier-mediated transport across the colon. The active transport pathways of the colon have been reviewed [61]. The transmucosal and membrane potential differences may be significant in the absorption of ionized or ionizable drugs [62]. Colon offers less barrier to macromolecules than small intestine, therefore, it is suitable for both protein and peptide absorption. The bulk water absorption in this region of the intestine provides scope for solvent drag and possibly improved drag and possibly improved drug absorption [63].

Approaches for colonic delivery

Peptide and protein drugs are coated with azoaromatic groups to form an impermeable film to protect them from digestion in the stomach and small intestine. When the polymer coated peptide and protein drugs reach the colon, the colonic bacteria cleaves the azo bonds and breaks the polymer film, releasing the drugs into the lumen of the colon for absorption. This polymeric system was demonstrated to protect and deliver orally administered insulin and vasopressin in rats [13]. Other approaches for colonic delivery have been given in Table 5.

Drug metabolism

Drug metabolism in the colon is caused by the host enzymes in the epithelial cells or by the microbial enzymes in the gut flora. Metabolic activities in the wall of the colon can be attributed to enzymes such as cytochrome P450, esterases, amidases, and various transferases [67]. The colonic mucosa resembles the small intestinal mucosa with respect to the spectrum of metabolizing enzymes. However, the total metabolic capacity of the colonic wall is inferior, because the mucosal mass in the lower part of the intestine is several times smaller than that in the upper part.

VAGINAL DELIVERY

Vaginal administration of peptide and protein drugs which are used specifically for the treatment of female-related conditions is a favorable alternative to parenteral administration.

Vaginal barrier for absorption

The vaginal wall consists of three main layers: An outer fibrous layer, a middle muscular layer, and an epithelial layer. The vaginal epithelium is a stratified, squamous epithelium which rests on a lamina propria. The surface area of the vagina is increased by numerous folds in the epithelium and by microridges covering the epithelial cell surface [68].

In common with other mucosal routes, drugs administered vaginally will be transported across the vaginal membrane by a number of different mechanisms (Fig. 4):

i. By diffusion through the cell due to a concentration gradient (transcellular route);

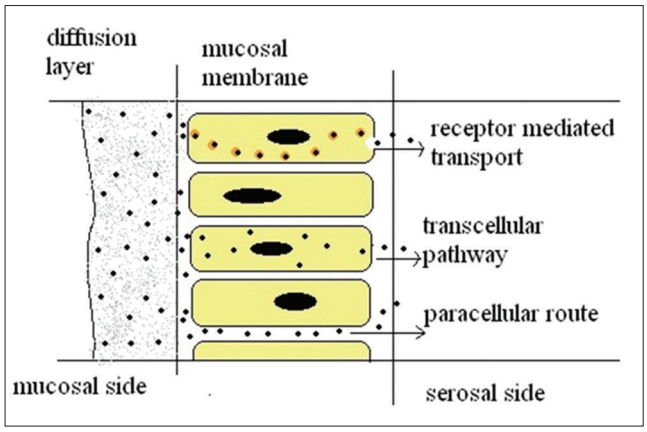

Fig. 4: Schematic representation of vaginal membrane as transport barrier

Table 5: Approaches for colonic delivery

Polymeric approach	Mechanism	Polymers used	References
Enteric coating polymers	Dissolve in the pH range 4.8-7.0	Eudragit	[64]
Timed release polymers	Retard the release of drug long enough to reach large intestine	HPMC, polysaccharides like pectin and calcium pectinate	[65]
Biodegradable azo polymers	Azo reduction causes degradation of polymer	Copolymers of styrene and hydroxyl-ethylmethacrylate cross-linked with divinilazobenzene derivative	
Saccharidic polymers	Increase in the porosity of the film as a result of enzymatic degradation	Mono, di, or oligosaccharides are treated with synthetic polymers e.g.: galactomannans+eudragit, dextran hydrogels crosslinked with diisocynate	[66]

HPMC: Hydroxypropyl methylcellulose

ii. By a vesicular or receptor-mediated transport mechanism; or
iii. By diffusion between the cells through the tight junctions (intercellular route).

Advantages

Despite traditionally being a site for the delivery of locally acting drugs, the vagina has great potential for the systemic absorption of drugs due to its large surface area, rich blood supply and permeability to a wide range of compounds, including peptides and proteins [69]. Avoidance of first pass metabolism, gastrointestinal effects, side effects at GIT. Steroids used in hormone replacement therapy or for contraception have been administered vaginally to reduce the possibility of hepatic side-effects associated with oral route [70]. Another advantage is the possible self-insertion and removal of the dosage form [71].

Disadvantages

The main disadvantages include the gender specificity, personal hygiene, local irritation, sexual intercourse, and cultural sensitivity.

Factors affecting the vaginal absorption of drugs

Menstrual cyclic changes in the thickness and porosity of the vaginal epithelium may affect the vaginal absorption of drugs. The volume, viscosity and pH of vaginal fluids may also affect the vaginal absorption of drugs. As a drug must be in solution before it can be absorbed, the presence of a film of moisture on the vaginal epithelium is an advantage. However, the presence of thick cervical mucus may present a barrier to drug absorption and copious vaginal secretions may cause the removal of a vaginal dosage form and therefore reduce absorption. Finally, the pH of the vaginal fluid may also affect vaginal absorption [71].

Drug absorption

Research on the intravaginal delivery of peptides and proteins has focused on insulin and gonadotropin-releasing hormones (GnRH) and their absorption into the systemic circulation. Insulin and thyroid-stimulating hormone have been shown to achieve some absorption from the vagina of rats and rabbits but with a high dependency on the menstrual cycle [72]. Hydrophilic molecules, such as insulin and GnRH, may be absorbed through intercellular channels, hence absorption would be greater when the epithelium is thinner [73,74]. As commonly observed with protein and peptide research, an enhancer is often needed to assist in absorption.

Initial work with leuprolide found greater potency in rats via vaginal administration over rectal, nasal, and oral administration [75]. Enhancement of absorption by organic acids (citric, succinic, tartaric, and glycocholic) increased bioavailability by 20%.

The vaginal absorption of oestradiol and progesterone in postmenopausal women was found to be significantly higher in those with thin, atrophic vaginal epithelia [76].

Approaches for vaginal administration

Hydrogel slabs

The vaginal slabs are produced from PEG-hexane triol diisocyanate hydrogel and form a tridimensional lattice which swells when exposed to water and in this way it can be loaded with the drug. After drying, the drug is trapped in the hydrogel matrix in a near dry state which results in increased stability of the drug. The hydrogel swells and drug are released, after vaginal administration [76].

Microbicidal gel

Microbicidal gel containing monoclonal human antibodies is used for topical immunization, for protecting genital skin and epithelia from HIV and sexually transmitted infections pathogens [69].

Mucoadhesive delivery systems

Polycarbophil, hydroxypropylcellulose, and polyacrylic acid are the bio adhesive polymers employed for intravaginal formulations. Hyaluronic acid-based intravaginal delivery of calcitonin, a polypeptide used in the treatment of postmenopausal osteoporosis, have shown promise for intravaginal administration of drugs for systemic effect [69].

RECTAL ROUTE

Rectal delivery of peptide and protein drugs is another very active area of research.

Advantages of rectal protein and peptide drug delivery

Low levels of protease activity, particularly of pancreatic origin; avoidance of first pass metabolism; potential for absorption into the lymphatic system, due to large pore radii: Retention of large volumes (10-25 ml); potential for time controlled release; constant environment aids reproducible absorption; large surface area, potentiated by using spreading/foaming agents [77,78].

Barriers for rectal absorption

Physical barrier includes the glycocalix, apical membrane, cell body, basolateral membrane, and tight junctions.

Enzymatic barrier: Various enzymes including peptidases which are present in the lumen, rectal wall and intestinal tissue [79].

Approaches for rectal delivery

Use of absorption enhancers

Due to poor membrane permeability and metabolism by the peptidases, poor bioavailability for proteins by rectal route is observed which can be overcome by use of absorption enhancers. The effect of absorption enhancers includes, increase in membrane fluidity, expansion of the dimension of the intercellular space, Solubilization of the mucosal membrane, increase in water flux, and reduction of the viscosity of the mucus layer adhering to all mucosal surfaces. The various absorption enhancers include sodium taurodihydrofusidate, sodium 5-methoxy salicylate, enamine derivatives, and sodium caprate.

Use of protease inhibitors

As mentioned earlier the absorption of protein drugs is limited by proteases, hence protease inhibitors are used, which includes aprotinin, trypsin inhibitors, bacitracin, puromycin, bestatin, and bile salts [80].

Drug absorption

Although extensive villi and microvilli are not present in the rectum tissue, sufficient surface area is present to allow absorption of readily permeable drugs. The lack of motility in the rectum, as opposed to extensive motility in the small intestine, provides an additional advantage in terms of maintaining maximum concentration gradients at the absorptive surface. Together with a limited fluid volume in the lower colon, typically 2-3 ml of inert mucous fluid in the absence of fecal material, the static environment of the rectum and lower colon provides an area for maintaining significantly higher drug concentrations than is readily achievable in the small intestine.

Significant rectal absorption of growth hormone has also been demonstrated with the help of absorption enhancing agents. The apical membranes of the small intestine epithelial cell layer express high levels of membrane-associated or membrane-bound enzymes, such as peptidases and saccharidases, which are not present in high amounts on the apical surfaces of epithelial cells in the rectal cavity. This absence of membrane surface metabolic potential affords advantages when delivering drugs susceptible to enzymatic degradation [81-84].

An overview of non-invasive protein and peptide drug delivery is given Table 6, and the available marketed formulations are given in Table 7.

Table 6: An overview of non-invasive protein and peptide drug delivery

Route	Enzymatic activity level	Barriers	Transport mechanism	Examples of drugs
Oral	+++++++	Enzymes, rapid post-absorptive clearance, physical instabilities such as adsorption and aggregation	Transcellular, paracellular, receptor mediated, carrier-mediated, transcytosis	Aminocephalosporine, cyclosporine, enalapril
Colonic		Absence of villi and micro villi, higher concentration of anaerobic bacteria	Transmucosal and membrane potential differences	Tissue necrosis factor, proleukin, y-interferon, epidermal growth factor
Nasal	++	Mucus secretions, mucociliary clearance, extent of absorption varies with mucus secretions, rhinitis	Passive diffusion, carrier-mediated transport and transcytosis	Desmopressin, oxytocin, buserelin
Ocular		Enzymatic degradation by neutral protease and aminopeptidase, nasolacrimal secretions	Paracellular and transcellular transport	Enkephalins, epidermal growth factor, mesodermal growth factor
Transdermal	+	Lipophilicity of stratum corneum, low permeation for proteins due to large molecular weight and hydrophilicity	Intercellular lipid pathway; appendageal transport; transcellular path	Insulin, salson calcitonin, LHRH, leuprolide
Vaginal	++	Menstrual cyclic changes in thickness and porosity of the vaginal epithelium, presence of thick cervical mucus	Paracellular, transcellular, receptor mediated	Leucine enkephalin, salmon calcitonin, recombinant Human relaxin
Rectal	+++	Extensive villi and microvilli are not present, intercellular junctional complexes are tighter	Transmucosal and membrane potential differences	Tetragastrin, pentagastrin

LHRH: Luteinizing hormone-releasing hormone

Table 7: Brands names of marketed protein or peptide drugs

Oral	Nasal	Ocular	Transdermal
Drug: Captopril Brand: Capoten	Drug: Calcitonin-salmon Brand: Miacalcin, Fortical	Drug: Cyclosporine Brand: Restasis	Drug: Insulin Brand: Dermisonic U-Strips

CONCLUSION

An increasing number of peptide and protein drugs are likely to be introduced into therapeutics in the forthcoming years. The desire to overcome the necessity for injection based delivery of protein and peptide pharmaceuticals has fueled intense research efforts exploring the feasibility of non-invasive administration. Armed with a greater understanding of the intricate structural, physiological and biochemical characteristics of each non-invasive route, scientists have begun to devise promising technologies to overcome the significant barriers to effective absorption into systemic circulation. Leading the success among non-invasive routes are several nasal delivery systems incorporating highly potent peptides that have already received regulatory approval and are commercially available. While tremendous progress has been made in the area of non-invasive delivery, significant drawbacks of current technologies limit their applicability to a broad range of protein and peptide pharmaceuticals. Continuing research may eventually result in a breakthrough technology that effectively overcomes the various barriers associated with noninvasive delivery of proteins and peptides, and simultaneously enables commercialization of cost-effective and patient-friendly biopharmaceutical products.

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