REVIEW

Department of Pharmaceutics[1], Bharati Vidyapeeth Deemed University, Poona College of Pharmacy and Research Centre, Pune; Department of Pharmacology[2], R. C. Patel College of Pharmacy, Shirpur, Maharashtra State, India

Permeation enhancers in the transmucosal delivery of macromolecules

S. Sharma[1], J. Kulkarni[2], A. P. Pawar[1]

Received October 5, 2005, accepted January 16, 2006

Prof. Dr. A. P. Pawar, Department of Pharmaceutics, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy and Research Centre, Erandawane, Pune-411038, Maharashtra State, India
p_atmaram@rediffmail.com

Pharmazie 61: 495–504 (2006)

The present article presents a compilation of information regarding various chemical permeation enhancers useful for transmucosal delivery of macromolecules. In the recent past, biotechnology has provided a great number of macromolecules for treatment of various disorders. With the rise in importance of macromolecules, especially proteins and peptides, an enormous volume of research on various novel routes of drug delivery has been carried out. Inspite of its giving the highest and fastest bioavailability, the parenteral route is not a preferred option, due to its inconvenience and the noncompliance of patients. Mucosal surfaces are the most common and convenient routes for delivering drugs to the body. However, macromolecular drugs such as peptides and proteins are unable to overcome the mucosal barriers and/or are degraded before reaching the blood stream. Transmucosal drug delivery with various bioavailability enhancers is receiving increasing attention as a possible alternative to parenteral delivery of macromolecules. Among the various bioavailability enhancers, chemical permeation enhancers have been most studied. Permeation enhancers reversibly modulate the permeability of the barrier layer in favor of drug absorption. Newer permeation enhancers like zonula occludin toxin, poly-L-arginine, chitosan derivatives etc have shown a significant increase in drug absorption through transmucosal routes without serious damage to the barrier layer. In particular delivery of macromolecules via the nasal and pulmonary routes using newer permeation enhancers has emerged as a possible alternative to the parenteral delivery of macromolecules.

1. Introduction

In the last few decades a variety of transmucosal routes have been studied for the improved delivery of pharmaceutically active agents, especially macromolecules. These studies have been driven mainly by the inconvenience and noncompliance of patients associated with parenteral routes, the most widely used route for macromolecules. Transmucosal routes have the major disadvantage of low bioavailability compared to parenteral routes. Low bioavailability leads to low plasma levels and increased inter- and intra-subject variability. To overcome this, a variety of approaches have been tried; among these, the use of chemical permeation enhancers has been the most studied.

Permeation enhancers are substances that increase the membrane permeation rate of a co-administered drug. They are formulation additives that modify the barrier properties of the absorbing cell layer and thus improve trans-membrane flux (Ganem-Quintanar 1997). Permeation enhancers reversibly increase the permeability of the mucosal epithelium to allow the unhampered transport of the drug to the blood circulation and/or lymph system. Co-administration of permeation enhancers with drugs such as large hydrophilic macromolecules like peptides, proteins, certain antibiotics, insulin, heparin etc. is usually necessary for better absorption. Insulin is one of the classic examples of a macromolecular drug. Most patients suffering from insulin dependent diabetes mellitus need to self-administer at least two injections of insulin per day. This reduces the patient's compliance and also leads to lipoatrophy or lipohypertrophy at the injection sites.

Many articles have already been published (Aungst et al. 1996; Fix 1996; Nishihata and Rytting 1997; Aungst 2000; Kaur and Smitha 2000; Veuillez et al. 2001; Remon 2002; Nicolazzo 2005) on specific permeation enhancers acting on a particular drug delivery route, but this article offers a compilation of information regarding different classes of permeation enhancers, their mechanisms of action, and their importance in different transmucosal drug delivery systems including recent advances in the field of permeation enhancers.

2. Classes of permeation enhancers

The ideal properties which a permeation enhancer should have are as follows:

– Compatibility with drug and other formulation excipients.

Table 1: Classification of permeation enhancers

Class	Examples
Bile salts	Sodium glycocholate, sodium deoxycholate, sodium taurocholate, sodium fusidate, sodium glycodeoxycholate, sodium taurodihydrofusidate.
Surfactants	Sodium lauryl sulfate, Brij®-35, lysophosphatidylcholine, dioctyl sodium sulfosuccinate, laurenth-9, polysorbate-80, polyethyleneglycol-8-laurate, glyceryl monolaurate.
Fatty acids and derivatives	Sorbitan laurate, sodium caprate, sucrose palmitate, lauroyl choline, sodium myristate, palmitoyl carnitine.
Glycerides	Phospholipids, monohexanoin, medium chain glycerides.
Chelators	Ethylene diamine tetraacetate (EDTA), disodium EDTA.
Salicylates	Salicylic acid, sodium methoxysalicylate, aspirin.
Polymers	Chitosan, polycarbophil, sodium carboxymethylcellulose and their derivatives.
Others	Azone®, cyclodextrins, benzalkonium chloride, phenothiazines, nitric acid donors, menthol.
Newer agents	Zonula occluden toxin, poly-l-arginines, soybean derivative glucosides, citicholine, α-acid derivatives.

- Immediate permeation-enhancing action after administration of formulation.
- Rapidly reversible effect on barrier layer.
- Reduction of barrier functions of the absorbing surface in one direction only.
- Pharmacologically inert, nontoxic and inexpensive.

Various classes of permeation enhancers along respective examples of each (Aungst et al. 1996; Nishihata and Rytting 1997; Aungst 2000; Veuillez et al. 2001; Remon 2002) are shown in Table 1.

3. Mechanisms of action

3.1. Increase in the paracellular permeability of the barrier layer

Hydrophilic drugs mostly permeate through the paracellular pathway and hence it has been widely studied and elucidated in the recent past. Various junctions, such as tight junctions, adherens junctions, desmosomes and gap junctions, modulate paracellular permeability. Tight junctions are the apical most surface organelles and possess a complex molecular structure. Adherens junctions are primarily responsible for cell-cell adhesion, desmosomes bind cells to one another and gap junctions are porous channels for communication between cells (Lutz and Siahaan 1997). Tight junctions display minimal porosity which allows only small molecules (<11 Å) and ions to cross via this paracellular route. Large molecules are not able to follow this route in normal physiology. In the case of most of the mucosal routes viz. intestinal, rectal and nasal (except sub-oral) routes, the increase in paracellular permeability of the barrier layer is due to loosening of the tight junctions. In the case of the sub-oral route where tight junctions are almost absent, disruption of the intercellular lipid structure increases the paracellular permeability of the barrier layer (Veuillez et al. 2001).

Tight junctions appear as multiple strands of fibrils forming a continuous circumferential seal around cells in freeze fracture replicas (Godfrey et al. 1992; Godfrey 1997). Four integral membrane proteins have been localized to the tight junction: occludin, claudins, junctional adhesion molecule (JAM), and the coxsackie and adenoviral serotype 2/5 receptor (CAR) (Furuse et al. 1993, 1998; Martin-Padura et al. 1998; Furuse et al. 1999; Ozaki et al. 1999; Cohen et al. 2001). JAM and CAR are members of the immunoglobulin gene super-family containing single transmembrane spanning domains. They not only serve as adhesion molecules, but also as receptors for reovirus and adenovirus (Bergelson et al. 1997; Barton et al. 2001). CAR also mediates homotypic intercellular interactions. In polarized epithelial cells, CAR is closely associated with the tight junction, where it contributes to the barrier to paracellular flow of solutes and macromolecules (Coyne and Bergelson 2005).

Occludin and claudin have four transmembrane spanning domains that interact through a series of homophilic and heterophilic interactions to regulate the permoselectivity of the junction. These integral membrane proteins are linked to a series of peripheral cytoplasmic plaque proteins (adaptors) located adjacent to the junction that not only anchor the integral membrane proteins to cytoplasmic and cytoskeletal elements, but also recruit regulatory and signaling molecules to the junction (Johnson 2005).

More than 23 species of claudins have been identified, each with distinct tissue distributions (Tsukita and Furuse 1998, 1999; Tsukita et al. 2001; Yu 2003a). This suggests that the profile of claudins within epithelia from different sites may determine the permeability properties of their tight junctions to ions and solutes. Several studies have established a role for claudins in mediating the charge and solute selectivity of the junction. Expression of molecular chimeras of claudins 2 and 4 and mutations of amino acids adjacent to conserved regions of the first extracellular loop of claudin-15 from acidic to basic residues have been shown to reverse the paracellular charge selectivity from cationic to anionic (Colegio et al. 2002, 2003).

3.2. Increase in the fluidity of membrane lipid bilayers

The transcellular pathway is the most widely accepted route for absorption of lipophilic drugs (Squier and Lesch 1988; Hoogstraate and Bodde 1993). A transient increase in the fluidity of the membrane lipids or proteins may be thought of as a nontoxic effect but extraction of membrane lipids or proteins may be viewed as a toxic effect. Transcellular transport (Lutz and Siahaan 1997) can be broadly divided into

- Passive diffusion,
- Passive diffusion that is modified by an efflux pump (Yeh and Smith 2001),
- Transport by an active transporter (Hunter 1993).

3.3. Changes in mucus rheology

The mucus layer acts as a barrier to drug permeation. A decrease of the viscosity and/or elasticity of the mucus is the mechanism of action of many permeation enhancers such as bile salts (Lee et al. 1991).

3.4. Increase in the thermodynamic activity of the drug

This is affected by vehicle composition, influencing drug solubility, micellization and by formation of ion-pairs between enhancer and drug (Coutel-Ergos et al. 1992; Ganem et al. 1996).

Table 2: Considerations for delivery of macromolecules through various transmucosal routes

Consideration	Route						
	Buccal/ sublingual	Intestinal	Rectal	Vaginal	Ocular	Pulmonary	Nasal
First pass metabolism	O	H	M	O	O	O	O
Enzymatic degradation	L/M	H	L/M	L	L	L/M	L/M
Absorptive surface area	L	H	L	L	VL	M/H	L
Vascularity	M/H	L	M	M	M	H	M/H
Permeability	M/H	L	L	L	M	H	H
Residence time	L	H	M/H	M/H	VL	L/M	L/M
Sensitivity	L	L	L	L/M	H	H	M/H
Onset	Moderate/Fast	Slow/Moderate	Slow	Slow/Moderate	Moderate/Fast	Fast	Fast
Patient compliance	H	H	L	L	L/M	M	M
Other	–	–	–	Menstrual cycle variations	Fast precorneal clearance	–	–

O – Nil, VL – Very low, L – Low, M – Moderate, H – High

4. Permeation enhancement via different mucosal routes

Major considerations for delivery of macromolecules via various transmucosal routes are listed in Table 2 (Aungst et al. 1996; Fix 1996; Nishihata and Rytting 1997; Aungst 2000; Kaur and Smitha 2000; Veuillez et al. 2001; Remon 2002). The effect of permeation enhancers in various transmucosal drug delivery routes is described below.

4.1. Sublingual and buccal mucosa

The buccal and sublingual routes offer many advantages such as high perfusion with blood vessels, avoidance of degradation in the gastrointestinal (GI) tract, avoidance of hepatic first-pass metabolism and high patient compliance. Absorption through the oral cavity occurs mainly through the sublingual and buccal mucosa because both of these are non-keratinized. On the other hand the palatal and gingival mucosas are keratinized and hence are less permeable. There are clear differences between the oral mucosal membrane and the epithelial membranes of the intestine, nasal cavity and rectum. Tight junctions typically interconnect cells of these tissues, but are almost absent in the oral mucosa. In the oral mucosa, intercellular spaces are filled with lipids extruded from the membrane coating granules. These lipids are organized into lamellae and constitute the principal barrier against molecular diffusion (Ganem-Quintanar et al. 1997). A common theme regarding permeation enhancement through the buccal and sublingual routes is to increase paracellular permeability by enhancing the lipid fluidity of intercellular lipids (Veuillez et al. 2001). Recently, Nicolazzo et al. (2005) reviewed the mechanism of buccal permeation enhancement by bile salts, surfactants and fatty acids, concluding that these agents result in, (a) extraction but not disruption of intercellular lipids, (b) an increase in the partition of drugs into the buccal epithelium and (c) interaction with epithelial protein domains.

Among the permeation enhancers, bile salts are widely used for the buccal route. Fatty acids and surfactants are important other examples. Bile salts are physiological surfactants having a lipid solubilizing role in the intestine. They are thought to act by micellization of lipids, from both the intercellular domain and the cell membranes. Hoogstraate et al. (1996) investigated the buccal delivery of fluorescein isothiocyanate dextran 4400 (FD4) *in vivo* in pigs and used glycodeoxycholate as a permeation enhancer. They reported a concentration dependent rise in absolute bioavailability of FD4 (from $1.8 \pm 0.5\%$ to $12.7 \pm 2.0\%$). Histological and IR spectroscopy studies on the transbuccal delivery of morphine sulfate with sodium glycodeoxycholate showed that the interaction of bile salts with epithelial lipids and the enhancement in absorption was concentration dependent (Şenel et al. 1997). The enhancing effects of dihydroxy bile salts, sodium glycodeoxycholate, sodium taurodeoxycholate, trihydroxy bile salts, sodium glycocholate and sodium taurocholate have been investigated on many drugs. Bile salts are thought to act by modifying the cell membrane integrity in such a way that the intracellular domain is opened up. Studies on the safety of bile salts using mucosal irritation and histological studies of buccal mucosa as criteria have shown that bile salts are relatively safe at optimum concentration but long-term studies are required for their clinical application (Şenel and Hincal 2001).

Jasti et al. (2000), reported enhancement in the absorption of an antisense oligonucleotide, ISIS3082, through the buccal route by using sodium glycocholate. Antisense oligonucleotides are prone to degradation and hence they can be administered only systemically and even then in a modified form. In the buccal mucosa, solubilization of lipids in the intercellular space by sodium glycocholate increases the diffusivity of hydrophilic compounds like ISIS 3082 and acyclovir.

Among surfactants, both cationic and anionic surfactants are more potent enhancers than non-ionic compounds but are also considered to be more toxic (Siegel and Gordon 1985, 1986). Surfactants act by protein denaturation or by swelling of tissue and extraction of lipid components.

The effects of fatty acids on the absorption of drugs depend on many factors like the presence and positioning of double bonds, isomer type (*cis* or *trans*), ionization state, chain length and the degree of branching. The exact mechanism of action of fatty acids in transbuccal delivery has yet to be confirmed, but except for oleic acid it is thought that inclusion of a fatty acid in a membrane may change van der Waals interaction between the hydrocarbon chains. Fatty acids may also form bonds between their carboxyl groups and neighboring moieties (Ganem-Quintanar et al. 1997). Oleic acid dissolves considerable amounts of cholesterol which acts as a membrane stabilizer (Siegel and Gordon 1985). It has been shown that azone primarily enhances the transport of lipophilic drugs across the keratinized oral mucosa. Hadgraft et al. (1996) have suggested that azone forms an ion-pair with anionic drugs thereby promoting their penetration.

Among the newer permeation enhancers, cyclodextrins, chitosan and menthol have been studied in the recent past for the buccal route. Chitosan significantly affects penetration via the buccal, nasal, and intestinal routes (Junginger et al. 2000). The enhancing effect of menthol on buccal permeation of a hydrophilic nucleoside analog, dideoxycytidine, has been reported. It is thought to increase the partition coefficient of a drug by facilitating the transcellular pathway in a concentration independent manner (Shojaei et al. 1999).

4.2. Intestinal mucosa

The oral route is the most preferred route, but because of the minimal bioavailability of many macromolecules, the use of permeation enhancers is required. Commonly used intestinal permeation enhancers are bile salts, nonionic surfactants, fatty acids, glycerides, chelating agents and some newer agents like mucoadhesive polymers, nitric oxide donors, zona occluden toxins, etc. Some enhancers show selective absorption in some parts of the gastrointestinal tract or in a selective pH range (e.g. chitosan shows a pH dependent absorption enhancing effect).

A large number of permeation enhancers have been evaluated for the intestinal route by *in vitro* and animal studies. A few representative studies are summarized in Table 3 (Alexander and Fix 1988; Lundin et al. 1997; Duizer et al. 1998; Leone-Bay et al. 1998; Lindmark et al. 1998; Clausen and Bernkop-Schnurch 2000; Prasad et al. 2003; Raiman et al. 2003).

At higher concentrations, bile salts increase the absorption of drugs through intestinal membrane disruption, caused by the solubilization of phospholipids. Bile salts also enhance drug absorption at low concentrations by formation of reverse micelles and calcium complexation without membrane disruption (Feldman and Gibaldi 1969; Anderberg et al. 1992). Uchiyama et al. (1996) have shown that sodium glycocholate, sodium taurocholate and *N*-lauryl-β-D-maltopyranoside (each at 20 mM concentration) promote absorption of phenol red with low toxicity. They also reported that sodium deoxycholate and mixed micelles are effective permeation enhancers but cause considerable release of proteins and phospholipids.

The size and structure of the alkyl chain and the polar group affect the absorption enhancing activity of nonionic surfactants. Sodium lauryl sulfate is an effective enhancer (Remon 2002). Among the non-ionic surfactants, polyoxyethylene fatty acid ethers and analogous lauryl esters are some of the more effective (Sekine et al. 1985). Anderberg et al. (1992) reported that surfactants (polysorbate 80, polyoxyl 40 hydrogenated castor oil etc.) demonstrated a concentration dependent effect on the permeability of hydrophilic markers.

Among the fatty acids, medium chain fatty acids like capric acid and lauric acid are more effective and nontoxic. Fatty acids are thought to act by paracellular and transcellular routes. Caprate activates phospholipase-C and increases intracellular calcium levels resulting in contraction of actin microfilaments and dilation of the tight junctions. A detailed mechanism for the absorption-enhancing effect of sodium caprate is shown in the Figure (Tomita et al. 1995; Hayashi et al. 1999).

Acylcarnitines are fatty acid esters of carnitine. Palmitoyl-DL-carnitine chloride significantly increases the duodenal and rectal absorption of a somatostatin analog in rats (Fix et al. 1986). Recently, Shah et al. (2004) have reported on the cytotoxicity of orally used permeation enhancers, and found Tween 80®, tetradecylmaltoside and sodium-dodecyl-maltoside to be highly toxic, and EDTA and cyclodextrins safe for short-term use only.

Over the last decade, both weakly cross-linked polyacrylic acid derivatives and chitosan derivatives have been studied extensively (Junginger et al. 2000). Polyacrylic acid derivatives act by enhancing paracellular permeability, inhibition of proteolytic enzymes and mucoadhesive properties. Chitosan derivatives interact reversibly with components of the tight junctions, leading to widening of the paracellular route (Hayashi et al. 1999). *N*-Trimethyl chitosan chloride is the chitosan derivative that has been most studied and has shown better results compared to chitosan and other derivatives (Kotzé et al. 1997). Among other polymers, sodium carboxymethylcellulose and thiolated derivatives have been reported to enhance the absorption of sodium fluorescein and bacitracin. Cysteine conjugates of chitosan, sodium carboxymethylcellulose and polycarbophil increase the paracellular permeability of drugs. Cysteine conjugates are more effective than the parent poly-

Table 3: Overview of representative studies describing selected intestinal permeation enhancers

Permeation enhancer	Barrier layer	Active molecule	Conc. of enhancer	Mechanism
Capric acid	Caco-2 cell layer	↑ Sodium fluorescein, ↓ TEER*	≥5 mM	Paracellular and transcellular.
Choline ester salts	Rat duodenum	↑ Cefoxitin	≥0.05%	–
Palmitoyl carnitine	Caco-2 cell layer	↑ Mannitol and PEG*** 4000, ↓ TEER*	≥0.4 mM	Paracellular
Sodium lauryl sulfate	Caco-2 cell layer	↑ Mannitol and PEG® 4000, ↓ TEER*	<0.58 mM	–
Monohexanoin	Rat jejunum	↑ Desmopressin	9 : 1 Ratio of enhancer to drug	Paracellular
EGTA**	Caco-2 cell layer	↑ Clondronate	2.5 mM	Paracellular
D-α-tocopheryl PEG*** 1000 succinate	Rat ileum	↑ Vancomycin	50% v/v 12.5%v/v	Paracellular, P-glycoprotein inhibition
Thiolated polycarbophil	Guinea pig intestine	↑ Sodium fluorescein	0.5% w/v	Paracellular
Acylated non-α-amino acids	Rat or monkey intestine	↑ Heparin sodium USP	–	Transcellular

* TEER: Trans-epithelial electrical resistance
** EGTA: Ethylene glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid
*** PEG: Polyethylene glycol

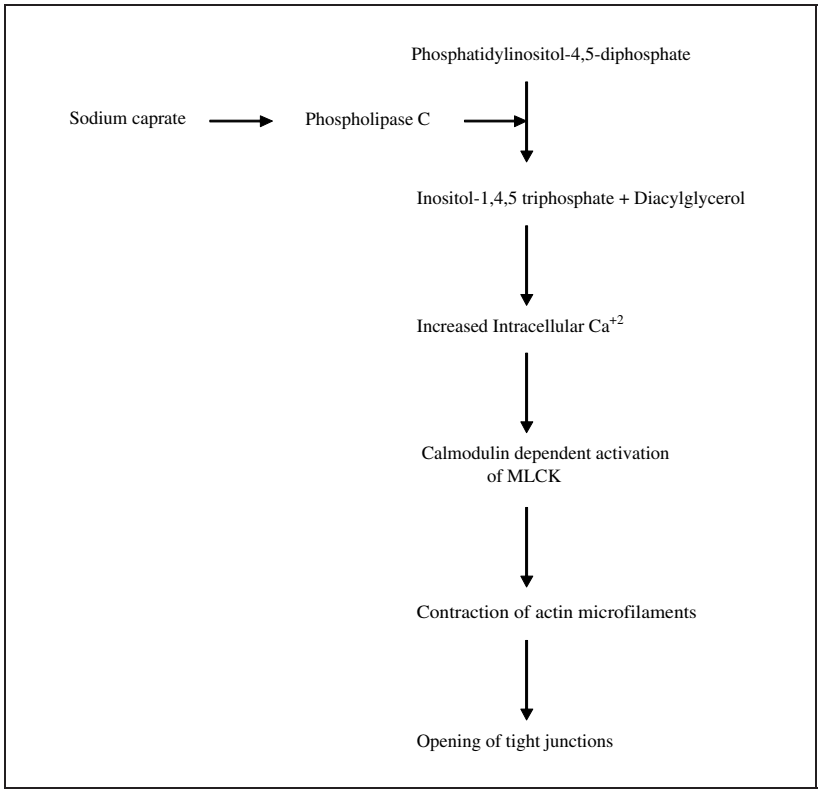

Fig.:
Permeation enhancing mechanism of sodium caprate
MLCK – Myosin Light Chain Kinase

mers because these polymers act by alteration of occludin and increase opening of intercellular junctions via phosphorylation of tyrosine residues by protein kinases. Dephosphorylation of these tyrosine groups is mediated by protein tyrosine phosphatase, which requires cysteine-215 for its activity. Cysteine conjugates of the above mentioned polymers form a disulphide bond with cysteine-215 and prevent dephosphorylation of tyrosine groups; hence they increase paracellular permeability more effectively (Clausen and Bernkop-Schnürch 2001).

Recently there have been some interesting reports in the field of intestinal permeation enhancers. Demirbas and Stavchansky (2003) have reported the enhancing effect of citicholine (an essential intermediate in the biosynthetic pathway of structural phospholipids in the cell membrane) on mannitol absorption. It was postulated that citicholine might have the potential to modify the effective permeability by changing the structural phospholipids of the cell membrane but the study shows that it increases absorption through the paracellular pathway by modulating tight junctions. Cox et al. (2001) reported that a protein prepared from *Vibrio cholerae* and known as *Zonula occludens* toxin is capable of reversibly opening tight junctions between intestinal cells by binding to a specific receptor on the luminal surface. *Zonula occludens* toxin (Zot) derived from *Vibrio cholerae* interacts with a specific intestinal epithelial surface receptor, with subsequent activation of a complex intracellular cascade of events that regulate tight junction permeability. Zot localizes in the bacterial outer membrane with subsequent cleavage and secretion of a C-terminal fragment in the host intestinal milieu. This toxin mimics the effect of a related endogenous modulator of intestinal tight junctions. The mammalian zot analogue, zonulin, has been identified as inducing tight junction disassembly in non-human primate intestinal epithelia (Miyoshi and Takai 2005). Micro-organisms induce zonulin secretion and increase small-intestinal permeability (Asmar et al. 2002). Comparison of amino acids between the Zot active fragment and zonulin confirms the presence of an octapeptide receptor-binding domain toward the N-terminus of the processed Zot (Miyoshi and Takai 2005).

Bioavailability of insulin via the oral route is very low even in the presence of permeation enhancers. An oral form of insulin has been an elusive goal for many investigators. The oral permeation enhancers such as bile salts have not been successful for oral delivery of insulin because of their non-specificity. *Zonula occludens* toxin enhances oral insulin absorption by acting specifically on the actin filaments, resulting in an increase in the permeability of tight junctions. When 10 IU of insulin were co-administered with 5 μg of *Zonula occludens* toxin protein, a significant decrease in blood glucose level was observed in rabbits (Carino and Mathiowitz 1999; Cox et al. 2001). Another study reported that small molecular weight alpha acid derivatives are able to enhance intestinal absorption of human growth hormone in the rabbit. This effect was specific for human growth hormone and enhanced absorption was due to an association of enhancer and human growth hormone that is more transportable across intestinal tissue (Mlynek 2000). Nitric oxide donors (*S*-nitroso-*N*-acetyl-DL-penicillamine and NOC7) have enhanced the absorption of FD-4 with very little mucosal damage (Numata et al. 2000).

4.3. Rectal mucosa

The surface area of the rectal surface is smaller compared with the small intestine (due to absence of microvilli and smaller size), but the rectum provides an efficient drug absorption site due to its rich blood supply, long residence time, lower concentration of digestive enzymes, less drug spreading and partial avoidance of presystemic metabolism. A large number of studies on rectal permeation en-

hancement have been done. Ampicillin suppositories incorporating sodium caprylate as a permeation enhancer were confirmed clinically to be an effective formulation. Important classes of permeation enhancers which are known to enhance rectal absorption are salicylates, fatty acids, enamine derivatives, chelators, phenothiazines, nonsteroidal anti-inflammatory drugs and bile salts. Newer agents include nitric oxide donors.

Enamines, salicylates and fatty acids enhance rectal absorption of drugs by both transcellular and paracellular routes. Enamines are prone to rapid hydrolysis at neutral and acidic pH and hence are more useful in prodrug development rather than as permeation enhancers (Nishihata and Rytting 1997).

Salicylates like 5-methoxy salicylic acid and nonsteroidal anti-inflammatory drugs have shown a concentration dependent permeation enhancing action. The mechanism of action of salicylates and nonsteroidal anti-inflammatory drugs is not completely understood. It is proposed that at high concentrations they act directly on cell surface calcium or decrease cytosolic calcium to induce inhibition of calmodulin action (Takahata et al. 1986; Nishihata and Rytting 1997). The gene expression regulated synthesis of calmodulin in the cell and its binding to extracellular calcium ions at the tight junction site causes closing of tight junctions (Nishihata et al. 1988).

Insulin delivery via the portal vein is important in normalizing both blood glucose and insulin levels in the postprandial state. Rectal delivery of insulin results in partly portal insulin delivery, because of the three veins coming out of the rectum, one goes through the portal route (Nishihata and Rytting 1997). Insulin suppositories with salicylates, enamines and other permeation enhancers have been investigated. The rectal route is thought to control postprandial hyperglycemia in diabetic patients in a more natural manner compared to conventional parenteral insulin therapy.

Nishimura et al. (1985) reported that the enhancing action of fatty acids is dependent on the partition coefficient. The optimal partition coefficient was calculated at $\log P = 4.2$. It has been proposed that fatty acids perturb membrane structural integrity by becoming incorporated into the plasma membrane (Muranishi et al. 1981). Tomita et al. (1988) added that pertubation by fatty acids occurs in both the lipid fraction and protein fraction. Sasaki et al. (2003) reported a comparison of the absorption of glycyrrhizin from oral, rectal and nasal routes in rats with the aid of sodium caprate. They reported that absorption from the rectal route was greater than from the oral route. Bile salts have been studied for the rectal delivery of antibiotics e.g. ampicillin, but their hemolytic activity is a serious side effect (Motihoro 1983).

Phenothiazines at concentrations from 1 to 100 mM act as calmodulin antagonists. The maximum action of prometazine and perfenazine was observed at concentrations of 5–50 mM (Suzuka et al. 1987). Nishihata and Rytting (1997) proposed that suppression of the adjuvant action of prometazine and perfenazine at concentrations above 100 mM may be due to their own Na^+-K^+-ATPase inhibition at high concentrations. Nitric oxide donors enhance the absorption of fluorescein isothiocyanate dextrans (FDs) not only from the small intestine but also from the rectum. NOC7 increased the absorption of FD-4 in the rat rectum with less toxicity than sodium caprylate (Numata et al. 2000). Another nitric oxide donor, *S*-nitroso-*N*-acetyl-DL-penicillamine, enhanced FD-4 absorption in the rabbit rectum with little cytotoxicity (Ramamurthi and Lewis 1997). Nitric oxide donors are assumed to act by the paracellular route. NOC7 can undergo spontaneous degradation when placed in buffer solution, thus releasing nitric oxide, while *S*-nitroso-*N*-acetyl-DL-penicillamine releases nitric oxide through enzymatic and nonenzymatic cleavage of the S−NO bond (Kowaluk and Lewis 1990). Nitric oxide and/or its donors are thought to increase blood flow to the site of absorption and also cause dilation of tight junctions.

4.4. Vaginal mucosa

The transvaginal route offers many advantages for systemic medication, viz. avoidance of first pass metabolism, minimization of proteolytic degradation and possibility of prolonged absorption, but as yet this route has been relatively little studied in terms of permeation enhancers. Nonionic surfactants, e.g. bile salts, glycerides and acids, are the permeation enhancers which have been most studied in relation to the vaginal route (D'Augustine et al. 2002). Laurenth-9, lysophosphatidyl choline and palmitoylcarnitine chloride were found to be highly effective vaginal permeation enhancers, but all induced epithelial damage (Richardson et al. 1989). Organic acids, such as citric acid, succinic acid, tartaric acid etc., have shown enhancement in vaginal absorption of a potent leutinizing hormone releasing hormone analogue (leuprolide) in rats. The acidifying and chelating effects of the acids may result in a potent enhancement of vaginal absorption of leuprolide (Okada et al. 1983). α-Cyclodextrin has been reported to facilitate vaginal absorption of leuprorelin in rats by approximately six-fold (Chien 1991). Bijl et al. (2002) reported enhancement of the absorption of cyclosporine through the human vaginal mucosa by benzalkonium chloride. A comparative *in vitro* permeability study of human vaginal, small intestinal and colonic mucosa has shown that the vaginal mucosa is relatively more permeable for many therapeutic agents, viz. arecoline, 17β-estradiol and arecaidine (Bijl and Eyk 2003). Recently, a chitosan derivative, 5-methyl-pyrrolidinone chitosan, has been reported to be an efficient vaginal and buccal permeation enhancer for acyclovir. 5-Methyl-pyrrolidinone chitosan has been found to be more effective than chitosan hydrochloride and partially reacylated chitosan (Sandri et al. 2004). In spite of all these studies fluctuations in vaginal absorption resulting from variation in serum estrogen levels is a major hindrance to the use of this route.

4.5. Ocular mucosa

The ocular route provides an efficient non-invasive means of systemic drug delivery because of avoidance of first pass metabolism and rich blood supply. Systemic absorption of an ocular instilled drug takes place from the nasal mucosa and conjuctival mucosa. Permeation enhancers, which increase drug absorption through the ocular route, include calcium chelators, fatty acids, bile salts, preservatives and surfactants like alkyl glycosides. Recently azone and some cyclodextrins have shown positive results as ocular permeation enhancers (Tang-Liu et al. 1994).

Surfactants act by both transcellular and paracellular pathways. At low concentrations surfactants are incorporated into the lipid bilayer, forming polar defects in the membrane. When the lipid bilayer is saturated, mixed micelles begin to form, resulting in the removal of phospholipids from the cell membranes and hence leading to membrane solubilization (Kaur and Smitha 2000). Ahsan et al.

(2003a) reported sucrose cocoate (a component of cosmetic preparations) to be an ocular permeation enhancer for calcitonin and insulin in anesthetized male Sprague-Dawley rats. Calcium chelators like ethylene diamine tetraacetate have been reported to loosen the tight junctions between the superficial epithelial cells, thus facilitating paracellular transport (Volk and Geiger 1986; Hochman and Artursson 1994). The calcium depletion does not act directly on the tight junctions, but rather induces many changes in the cells including (Citi 1992):

- Disruption of actin filaments,
- Disruption of adherent junctions,
- Diminished cell adhesion of actin filaments,
- Activation of protein kinases.

Purified quillaja saponin has been reported to enhance ocular absorption of insulin in rats (Lee et al. 2002). Insulin because of its high molecular weight is one of the most challenging drugs to deliver via the ocular route. Some examples of permeation enhancers that have been studied for ocular delivery of insulin are Brij 78, Brij 99, fusidic acid, alkylglycosides and sorbic acid (Sasaki et al. 1993; Morgan and Huntzicker 1996). Hirai et al. (1981) suggested that the use of polyoxyethylene ether type enhancers with Hydrophilic-Lipophilic-Balance (HLB) between 8 and 14 would give optimal enhancement of insulin absorption.

The ocular route is the site of choice for the localized delivery of ophthalmologically active drugs. But a lot of work remains to be done to explore this route for the systemic delivery of drugs. Extensive precorneal clearance and the limited dosage volume that can be accommodated by the precorneal cavity, necessitate the use of permeation enhancers. But toxicity studies on ocular permeation enhancers have shown that many of these compounds are harmful (Monti et al. 2002).

4.6. Pulmonary mucosa

In recent years, pulmonary delivery has emerged as a promising non-invasive route for macromolecules. This is because of the large surface area of the alveolar epithelium and the short length of the air-blood pathway (O'Hagan and Illum 1990). Research in the last decade shows that the pulmonary and nasal routes could be possible alternatives for the parenteral administration of insulin.

The pulmonary route gives a lower bioavailability compared to the parenteral route and hence permeation enhancers have been tried, e.g. bile salts (e.g., sodium glycocholate), phospholipids, cyclodextrins (e.g., hydroxypropyl-β-cyclodextrins), citric acid, surfactants especially alkyl glycosides (e.g., tetradecylmaltoside, *N*-lauryl-β-D-maltopyranoside etc.) (Morita et al. 1993; Yamamoto et al. 1994, 1996; Ahsan et al. 2003b). Sodium caprate is effective in enhancing the bioavailability of phenol red and isothiocynate labeled dextrans but not of calcitonin and insulin (Yamamoto et al. 1994). Salicylates and calcium chelators (e.g. ethylene diamine tetraacetate) are less effective. Sodium glycocholate and *N*-lauryl-β-D-maltopyranoside are effective in enhancing pulmonary absorption of macromolecules. *N*-lauryl-β-D-maltopyranoside shows a permeation enhancing effect for insulin at the optimal concentration of 5 mM (Morita et al. 1993). Possible mechanisms that could be involved in enhanced absorption are: (i) alteration of the mucus layer (ii) protection against enzymatic degradation (iii) dissociation of high order protein multimers into lower order oligomers or monomers (iv) increased paracellular absorption due to opening of the tight junctions between epithelial cells and (v) extraction and subsequent solubilization of membrane phospholipids and proteins by forming micelles, thereby facilitating transcellular passage of exogenous protein molecules (Gordon et al. 1985; Hersey and Jackson 1987). But bile salts may not be suitable for chronic use as they may erode the epithelial surface. A different concept of using liposomes and phospholipids has been investigated for the systemic absorption of proteins after intratracheal delivery. Absorption enhancement by liposomes may be attributed to the presence of surfactants on the alveolar surface. Lung surfactants consist primarily of dipalmitoyl phosphotidyl choline and low amounts of surfactant protein molecules A, B and C, which undergo rapid recycling. Addition of exogenous liposome molecules hastens the surfactant recycling process in the alveolar cells leading to enhanced uptake of the protein molecule into the systemic circulation (Hussain et al. 2004).

Cyclodextrins are relatively new permeation enhancers and show an effect on hydrophilic macromolecules through the pulmonary, ophthalmic and nasal routes (Shimpi et al. 2005). β-Cyclodextrins in particular have been found to be more useful in animal studies (Merkus et al. 1992; Ahsan et al. 2003b). There have been reports that methylated β-cyclodextrins increase transcellular as well as paracellular movement of peptide drugs. Cyclodextrins may have a direct disruptive effect on the alveolar epithelial membrane as evidenced by the extraction of membrane lipids and proteins. Complexation with lipophilic penetrants, inhibition of proteolytic enzyme activity or alteration of the physicochemical properties of a protein in the formulation such as altering its solubility, drug partition coefficient or formation of weak ionic interactions with the drug are other possible effects of cyclodextrins. However, the molecules of many peptides and proteins are too hydrophilic and bulky to be included in the cyclodextrin cavity; thus their interaction with cyclodextrin may be local, and accessible only to hydrophobic side chains (Cooper 1992; Horsky and Pitha 1994; Beslow et al. 1998). Such interactions may alter the three-dimensional conformation of proteins and thus alter their physicochemical parameters (Hussain et al. 2004). Interspecies differences exist in the permeation enhancing efficiency of cyclodextrins, and so more studies on human beings are required to estimate their usefulness (Merkus et al. 1992).

4.7. Nasal mucosa

Intranasal administration of systemic drugs has attracted much attention over the last two decades. Lipophilic and macromolecular drugs require excipients like permeation enhancers for adequate nasal absorption. Most of the permeation enhancers have associated problems of irritation or membrane damage (Behl et al. 1998). Permeation enhancers that are thought to be relatively safe are bile salts, surfactants, phospholipids and few cationic compounds (Meezan and Pillon 1997; Bechgaard et al 1999; Merkus et al. 1999; Natsume et al. 1999). Gordon et al. (1985) reported on eight different bile salts which increased insulin absorption in humans. Also, conjugation of a taurine or glycine group to the free acid of deoxycholate appeared to decrease irritation without decreasing bioavailability. Bile salts such as sodium glycocholate promote insulin absorption by retarding insulin degradation by leucine aminopeptidase, a proteolytic enzyme, in addition to other mechanisms (Quan et al. 1998).

Alkyl glycosides are nonionic surfactants capable of enhancing glucagon or insulin absorption through the nasal, ocular or pulmonary routes (Meezan and Pillon 1997). The mechanism is not entirely clear but Ahsan et al. (2003b) proposed internalization via endocytotic vesicles. Alkylglycosides such as tetradecylmaltoside or dodecylmaltoside administered with insulin via the nasal, ocular or inhalational routes have shown significant reductions in blood glucose levels in rats (Meezan and Pillon 1997).

Among cationic compounds, chitosan is the most important permeation enhancer. Sinswat and Tengamnuay (2003) reported that chitosan has a better nasal permeation enhancing effect on absorption of salmon calcitonin compared with two β-cyclodextrins (dimethyl β-cyclodextrin and hydroxylpropyl β-cyclodextrin). In another study, Yang et al. (2004) reported that dimethyl β-cyclodextrin was more efficacious than hydroxypropyl β-cyclodextrin in enhancing the absorption of low molecular weight heparins both *in vivo* and *in vitro*. Hamman et al. (2002) reported the effects of the degree of quaternization of *N*-trimethyl chitosan chloride on the enhancement of mannitol absorption through rat nasal epithelia at pH of 6.20 and 7.40. All the *N*-trimethyl chitosan chloride polymers studied (12–59% quaternization) increased the nasal absorption of [^{14}C]-mannitol significantly at pH 6.20, but polymers with higher degrees of quaternization (36%) were also able to increase mannitol absorption at pH 7.40. This can probably be explained by steric effects caused by the attached methyl groups and changes in the flexibility of the *N*-trimethyl chitosan chloride molecules with an increase in the degree of quaternization above an optimum value for absorption enhancement in a neutral environment. Among other cationic compounds, poly-L-arginines are quite safe and efficient in enhancing absorption of FD-4 through the nasal route (Natsume et al. 1999).

Soybean derived sterylglucoside and β-D-glucoside have shown an absorption enhancing effect on the nasal absorption of verapamil in rabbits. It is thought that they increase mucus fluidity and enhance both transcellular and paracellular absorption. Sterylglucoside may increase the freedom of motion of lipids in the mucosal membrane and facilitate calcium flux from extracellular sources, causing increase in intracellular calcium concentration and the opening of tight junctions. Another explanation is that sterylglucoside may act as an ionophore and create holes as reverse micelles. The absorption of verapamil via the nasal route from a powder formulation containing sterylglucoside is comparable to that via intravenous injection (Maitani et al. 2000).

5. Challenges and future perspectives

A few intestinal and rectal commercial formulations containing permeation enhancers are already available because of significant advances in the field of permeation enhancers. But many challenges remain to be dealt with:

- Most of the permeation enhancers so far investigated are nonspecific and hence they increase permeation not only of drugs but also of toxins and other undesirable substances. Thus, a search for specific permeation enhancers is essential.
- Many of the permeation enhancers cause mucosal irritation or other toxic effects. Understanding the complete mechanism of action may help in solving this problem. Permeation enhancers of natural (e.g. piperine) or semi-synthetic origin should be investigated extensively because these are usually less prone to cause toxicity.
- Most of the studies using permeation enhancers have been done either with *in vitro* models or in animals but there are very few *in vivo* human trials. So more human trials or the use of simulated human models is essential.

Information about the different transmucosal routes and the permeation enhancers available for each route would be useful to researchers in search of optimum delivery systems for macromolecules.

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