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Overcoming enzymatic and absorption barriers to non-parenterally administered protein and peptide drugs

Xin Hua Zhou

Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, OK 73019, USA

Enzymatic and absorption barriers to peptide and protein drugs are discussed with particular emphasis on the approaches to overcome the barriers, including evaluation of proteinase inhibitors and penetration enhancers, application of chemical modification and the efficient use of different pharmaceutical dosage.

Key words: Enzymatic barrier; Absorption barrier; Non-parenteral administration; Protein; Peptide

Introduction

Developments in biotechnology and therapeutics have resulted in the increased use of many peptide and protein drugs [1], including the products of recombinant DNA [2,3], purified pharmacological active peptides [4] and synthesized peptides [5]. The expediency of these agents lies in the fact that they are often not toxic, have a highly specific target while only small quantities are required to obtain clinically relevant plasma levels. For systemic delivery of peptide and protein drugs, parenteral administration (i.v., i.m. or s.c.) is necessary in order to achieve therapeutic results. This is because of the drugs' susceptibility to degrading enzymes of the gastrointestinal tract, leading to poor oral bioavailability. However, associated complications such as thrombophlebitis and tissue necrosis may lead to a low patient tolerance using these routes and non-parenteral administration routes for systemic delivery of peptide and protein drugs, such as the nasal, buccal, rectal, ocular, pulmonary and vaginal, even oral routes, as needle-free alternatives, have shown promise [6]. In coordination with enzyme inhibitors or absorption enhancers, protein/peptide drugs can be delivered by non-parenteral administration into blood circulation and a sufficient bioavailability can be obtained. Thus, a nonparenteral system for the delivery of proteins/peptides and the efficient use of appropriate approaches to overcome enzymatic and absorption barriers is an attractive goal. In this review, enzymatic and absorption barriers to protein drug absorption are discussed with particular emphasis on general approaches to overcome the barriers. These include evaluation of proteinase inhibitors, penetration enhancers, chemical modification, and the efficient use of different comprehensive pharmaceutic dosages.

Barriers to nonparenteral delivery of protein/peptide drugs

Since protein and peptide drugs are recognized as foreign proteins or foreign peptides, the mammalian system possesses several extremely efficient barriers to restrict the entry of the macromolecules. These barriers may completely or

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partially obstruct the penetration of the drugs and thereby physiologically protect the safety of the mammalian body. The major barriers affecting the administration of protein/peptide drugs are the significant proteolytic activities and various epithelia at different locations.

Proteolysis barriers

Proteolytic activities are perhaps the most important barriers to the protein/peptide drugs. The reasons for it are, that in general, protein/peptide drugs have a high susceptibility to the proteolytic enzymes, and that the enzymes with high activities are widely distributed in the human body, especially between the entry point into the systemic circulation and the target site. These enzymes include exopeptidases (aminopeptidase and carboxypeptidases), endopeptidases (endopeptidase and angiotensin-converting enzyme), dipeptidases, aminotripeptidases, prolidases, prolinases, carnosinases. These enzymes and their locations are summarized in Table 1.

The proteolytic activity in any tissue presents particular complicated barriers since the activities of proteolytic enzymes are not uniform from tissue to tissue; the distribution profiles vary from one peptidase to the other. The specificity of the same peptidase may also differ for different tissues. Carnosinase from serum, for example, can readily hydrolyse homocarnosine and anserrine, whereas cellular carnosinase can not [26,29]. In order to evaluate the proteolytic barriers, several protein/peptide drugs and synthesized chemicals have been used with various tissue homogenates from different animals [8–10,30]. Hydrolysis of insulin, for example, can be monitored by using L-leucine-β-naphthylamide [9], a standard substrate of leucine aminopeptidase. This enzyme is the main cause for the degradation of insulin [31]. The analysis of the data clearly shows that the intestinal tissues are the most active and the buccal tissues the least active. Nasal and dermal including rectal tissues, have the same activity, which is slightly higher than that of buccal tissues. The rectal tissues hold an intermediate position. However, when insulin itself was used directly to explore the enzymatic barriers in different tissues, it was found that nasal tissues are the most active in hydrolysis of the compound and buccal tissues the least active [32]. Intestinal tissues have even less activity than rectal tissues. These facts can be explained in that various enzymes such as insulinase [33], glutathione insulin transhydrogenase [34], insulin-degrading enzyme [35] in nasal tissues may be involved in the hydrolysis of insulin. Particularly active is endopeptidase 24.11 which was recently identified. It utilizes the insulin B chain as substrate [20].

Generally speaking, the intestinal route presents the largest enzymatic barrier to the absorption of peptides because intestinal tissues contain relatively large amount of soluble proteins. Another reason for it is that α-chymotrypsin secreted from pancreas hydrolyses insulin rapidly in intestine [36]. On the other hand the skin presents the lowest barrier because of the enzyme protein content is small [9,10]. However, since the skin is relatively impermeable, the nasal route appears to be the best choice for delivering peptides. The proteolytic barriers in possible non-parenteral routes are summarized in Table 2.

Recently, species differences in peptidase activities were reported by Zhou and Li Wan Po [10] using L-leucine-β-naphthylamide as substrate. When the enzyme activity was adjusted for protein content, the order of the relative aminopeptidase activities of different tissue homogenates in four animal models were obtained as guinea pig > rabbit > dog > rat in intestinal tissue homogenates; guinea pig = rabbit > dog > rat in rectal tissue homogenates; guinea pig > rabbit > rat > dog in buccal; guinea pig ≥ rabbit > rat > dog in nasal tissue homogenates and guinea pig > rabbit > rat > dog in dermal tissue homogenates. Species differences were also reported by Hussain et al [37,38] using humans and rats as models for Leu-enkephalin intranasal delivery. It was found that the hydrolysis rates of the peptide to des-tyrosine metabolite (Gly-Gly-Phe-Leu) in rat nasal cavities is much higher than the rate in human nasal cavities [37]. Indeed,

TABLE 1

Proteolytic enzymes and their location in mammalians

Enzyme	Tissue	Animal	Reference
Aminoligopeptidase (EC.3.4.11.2) aminopeptidase N; aminopeptidase M; oligo-aminopeptidase; L-leucyl-β-naphthylamide hydrolase)	intestinal, nasal, rectal, vaginal, buccal and dermal	rat, rabbit, dog and ginea-pig	(7–10)
Aspartate aminopeptidase (EC.3.4.11.7) (acid aminopeptidase; aminopeptidase A)	intestinal	human	(11)
Dipeptidyl aminopeptidase IV (EC-3.4.14.5) (dipeptidyl hydrolase; post-proline dipeptidyl aminopeptidase)	intestinal	pig	(12)
γ-Glutamyltransferase (EC. 2.3.2.2) (γ-glutamyltrans peptidase)	intestinal	rat	(13)
Aminopeptidase P (EC.3.4.11.9) (prolyl aminopeptidase; x-prolyl aminopeptidase; aminoacylpeptide hydrolase)	intestinal serum pulmonary	rat human human	(14) (15) (16)
Carboxypeptidase P (EC. 3.4.17)	intestinal	rat rabbit	(17)
Angiotensin-converting enzyme (EC. 3.4.15.1) (dipeptidyl carboxypeptidase; kiniase II; peptidyl dipeptidase)	intestinal pulmonary intestinal, rectal, vaginal, buccal and nasal	human rat rat	(18) (19) (8)
Endopeptidase-24.11 (EC. 3.4.24.11) (enkephalinase; enkephalin dipeptidyl carboxypeptidase)	intestinal, nasal, ocular, rectal buccal and vaginal stomach	rat and rabbit Pig	(8) (20)
Gly-Leu dipeptidase	intestinal	human rat	(21) (22)
Asp-Lys dipeptidase	intestinal	human rat	(21) (22)
Aminotripeptidase	intestinal	rabbit	(23)
Prolidase (EC. 3.4.13.9) (imidodipeptidase; peptidase D, proline dipeptidase; aminoacyl-L-prolinehydrolase)	intestinal	pig cow	(24) (25)
Carnosinase, (aminoacyl-L-histidine hydrolase)	serum	human	(26)
Prolinase	uterus	human	(27)
Leucine aminopeptidase (EC. 3.4.11.1)	intestinal, nasal, rectal, buccal and dermal	rat, rabbit, dog and guinea-pig	(9-11)
Aminopeptidase B (EC. 3.4.11.6)	intestinal	rabbit	(11)
Cathepsin B	dermal	rat	(28)
Trypsin	dermal	rat	(28)

TABLE 2

Proteolytic barriers in possible non-parenteral routes of entry into the systemic circulation

Possible route	Proteolytic activity level	Soluble protein content (enzyme protein content)	Enzymes from other resources	Other problems
Intestinal	+++++++	+++++++	Proteinase secreted from pancreas	
Rectal	+++	+++++		
Buccal	++	+++		High drug lossing possibility, drug loading difficulty.
Nasal	++	++		
Dermal	+	+		Relatively impermeable.
Pulmonary	+?	++?		
Vaginal	++?	++?		Inconvenience for patients, also affected by menstruation

various tissue homogenates from various animals show different proteolytic activity profiles, suggesting that species differences are to be expected when comparing the same tissue from the different species using the same peptide drug. Little is known about the proteolytic barriers in different tissues of the human body and this requires further study. However, reliable data can be predicted from animal models which are, in enzyme levels, histologically similar to the human body, once the proteolytic barriers in human tissues are characterized.

Absorption barriers

Absorption barriers include clearance systems at administration sites, obstacles of the epithelial membrane to the drugs, as well as transcellular and pinocytosis processing. Various organs may have different clearance systems, but little information is available about particular transcellular and pinocytosis methods of processing of peptide/protein drugs; therefore, the focus in this part of the review is placed on the epithelial mucous membranes.

The epithelial membranes constitute a highly efficient barrier to drug absorption. These membranes universally cover the organ tissues which are used in common non-parenteral administration of peptide/protein drugs. In order to pass the membrane, the drugs have to pass through the membrane cells (transcellular transport) by means of passive or concentration-gradient diffusion, by active transport or by transport via vesicles. Alternatively, the drug has to pass through the tight junctions between cells. The transport of molecules across the membrane is dependent on several factors such as molecular weight, pH and hydrophilic characteristics of the drugs.

The pore radius of the mucosa can be considered to be a important limiting factor for the transport of peptide molecules. This was demonstrated by transport experiments across membrane with macromolecules of different sizes [39]. This also was shown with experiments using cultured endothelial cell layers [40]. The equivalent pore radius diameter of various mucous tissue appears to have a range from 4 to 8 Å [41,42]. Amino acids, dipeptides and tripeptides are therefore able to penetrate the mucous membrane while larger peptides are hindered. The pore radius of the mucosa was found to be reversibly enlarged by absorption enhancers such as sodium cholate [43,44].

Thus far, the nasal mucosa seems to have the lowest absorption barrier for administered peptides. The reasons for it are the high permeability and large vascular mucosal bed so that good absorption can be expected for molecules of up to 1 kDa. In addition, for molecules less than 10 kDa in size, sufficient quantities often can be ab-

sorbed into the systemic circulation by using special dosage forms such as degradable starch microspheres (DSM) [45,46] without the need of absorption enhancers. These forms of formulation presumably enhance peptide absorption by affecting tight junctions [47]. This aqueous channel route (tight junctions) through which even large hydrophilic molecules diffuse, may also be present in other mucosa such as buccal rectal, vaginal and ocular membranes.

The mucous barriers may also be of particular importance for peptide drugs due to the peptide-mucus interactions. Masegi [40] recently reported that, when a cultured endothelial cell culture system was used to study drug disposition characteristics in vitro, an accumulation of radioactivity on the cell layers was observed with cationic macromolecules such as ^{111}In-cBSA, ^{14}C-MMCD$_{cation}$ (T-70) (^{14}C-mitomycin-dextran conjugates). While ^{14}CMMCD$_{anion}$ (T-70) and neutral molecules such as ^{14}C-dextran displayed no significant interaction with endothelial cell layers [40]. It appears that the peptide drugs or prodrugs designed with an anionic or neutral charge have less interaction with negatively charged cell membrane surfaces. Conversely, enhancers designed with cationic charge may help to increase the bioavailability of peptide drugs by reducing the interaction between the drug and mucosal membrane surface. A good example is DEAE-dextran, a polycationic polysaccharide. This compound was demonstrated to enhance the bioavailability of intranasal insulin. The interaction between the cationic charged enhancer and mucus may lead to a change of membrane permeability of macromolecules and stimulation of pinocytic uptake [48].

Like the species differences in proteolytic activities, the species differences also exist in absorption barriers [49]. For example, Fisher reported (50) that, when the same dose of human growth hormone (0.9 IU/kg) was nasally co-administered with LPC (L-α-lysophosphatidylcholine) to rabbits (0.2% LPC) and sheep (0.171% LPC), respectively, 72.8% of bioavailability could be obtained from the rabbit model while only 2.9% of bioavailability was found in the sheep model. When a higher dose of hGH (2.93 IU/kg) was applied to rats with 0.2% of LPC, it only showed 11.8% of bioavailability. Therefore, further investigations on the relationship of absorption barrier functions between human and animal models are needed.

General approaches to bypass enzymatic and absorption barriers

In order to bypass the enzymatic and absorption barriers for the purpose of increasing the bioavailability of high-molecular-weight protein and peptide drugs, several approaches are available: (**i**) inhibition of their enzymatic degradation; (**ii**) increasing their permeability across the relevant membrane; (**iii**) improving their resistance to break down or their permeability across the membrane by structural modification; and (**iv**) by special pharmaceutic formulations prolonging their retention time with mucus at the administration site.

Inhibitors of proteolytic enzymes

For several years it has been known that protease inhibitors increase the absorption of protein drugs. Table 3 lists the different protease inhibitors which have been used to investigate the delivery of peptide and protein drugs.

Aprotinin, a bovine pancreatic kallikrein inhibitor with a molecular weight of 6500 [62], was the first protease inhibitor used for peptide/protein drug administration. It was shown to be efficient in the increase of bioavailability of injected insulin applied into a loop of the jejunum [63]. This result was later confirmed by several other investigators. However, some recent studies provide conflicting results (Table 4). Since aprotinin has a high molecular weight (M_w 6500), it is believed that this compound is not absorbed through the nasal mucosa, and abdominal skin, even while using iontophresis techniques. Thus the protease inhibitors failed to protect the hydrolysis of protein and peptide drugs, at least for AVP in the tissues [51,54]. Clearly, further studies are required to better de-

TABLE 3

Inhibitors of proteolytic enzymes used in the investigation of delivery of peptide and protein drugs

Compound	Route	Peptide studied	Animal model	Reference
Aprotonin	dermal	1-d-8-DAVP	rat	(51)
	dermal	calcitonin	rat	(28)
	dermal (s.c.)	insulin	rat	(52)
	intestinal	insulin	rat	(44)
	dermal (s.c.)	insulin	human	(53)
Soybean trypsin inhibitor	dermal	1-d-8-DAVP	rat	(54)
	nasal	1-d-8-DAVP	rat	(51)
	dermal	calcitonin	rat	
	intestinal	rhG-CSF	rat	(55)
FK-448 (chymotrypsin inhibitor)	intestinal	insulin	rat	(56)
Boroleucine Borovaline	nasal	leu-enkephalin thymopeptin	rat	(57,58)
Phosphinic acid dipeptide analogue	nasal	leu-enkephalin	rat	(59)
Puromycin	nasal	leu-enkephalin	rat	(60)
Bestatin	nasal	leu-enkephalin	rat	(57)
Amastatin	nasal	leu-enkephalin	rat	(61)
Camostat mesilate	dermal	1-d-8-DAVP	rat	(54)
	nasal	1-d-8-DAVP	rat	(51)
	dermal	calcitonin	rat	(28)
Chicken egg white trypsin inhibitor	intestinal	rhG-CSF	rat	(55)

fine the effects of aprotinin on the absorption of peptide and protein drugs.

More recently, a class of so-called transition-state analogues have been designed as potential protease inhibitors. For example, α-aminoboronic acid derivatives, such as boroleucine, have been used to stabilize peptide drugs during their intranasal absorption [57]. When these reversible inhibitors were compared with other known peptidase inhibitors, bestain and puromysin, using leucine enkephalin as substrate in rat nasal perfusate, it was found that bestatin and puromycin were much less effective than boroleucine. The amino boronic acid derivatives also show the significant inhibitory effect on thymopentin metabolism by nasal mucosal aminopeptidases [58]. Another transition-state inhibitor is a phosphinic acid dipeptide analogue which was demonstrated to be effective in stabilizing administered peptide drugs in the nasal route [59]. This compound shows a stronger potent inhibitory effect on peptidases than boroleucine does.

The protease inhibitors, such as borolecine and

TABLE 4

Summary of effects of aprotinin on protein/peptide absorption

Reference	Drug	Animal	Tissue (Route)
[1] Significantly enhanced peptide absorption			
(63)	insulin	rat	intestinal
(64)	insulin	human	s.c.
(65)	insulin	human	s.c.
(66)	insulin	human	s.c.
(44)	insulin	rat	intestinal
(53)	insulin	human	intestinal
(67)	insulin	rat	oral
[2] Limited enhancement of peptide absorption			
(52)	insulin	rat	s.c.
(28)	calcitonin	rat	transdermal
[3] Non-enhanced effect on peptide absorption			
(54)	vasopressin	rat	nasal
(51)	vasopressin	rat	transdermal
(68)	insulin	rat	rectal, nasal, buccal
(69)	insulin	rat	nasal

phosphinic acid dipeptide, may be the best choices for enhancing the bioavailability of peptide and protein drugs because of their molecular sizes and potent inhibitory effects on various proteolytic enzymes. However, the toxicity of these inhibitors used in non-parenteral administration of peptide/protein drugs must be further tested.

Absorption enhancers

In general, possible non-parenteral administration routes for delivery of peptide/protein drugs require the co-administration of absorption enhancers, due to the fact that all mucosal membranes are resistant to penetration by the majority of peptides and proteins.

A large number of absorption enhancers have been studied, particularly with respect to insulin absorption. These enhancers can be structurally divided into several groups, such as fatty acids, bile salts, enamine derivatives of phenylglycine, esters, ethers, salicylates, organic acids, glycosides, peptide lipids, amines, derivatives of fusidic acids and glycyrrhetinic acid derivatives. All of these enhancers have been reviewed by several articles [1,70].

The mechanisms of action of the peptide absorption enhancers are not clearly known, but several possibilities have been postulated. The first is increased solubility of the drugs brought about by the enhancers [71], because proteins and peptides usually form aggregates in aqueous solutions which are dissociated by the enhancer [72]. A second mechanism of the enhancer is to inhibit the proteolytic enzymes. Some enhancers such as bile salts, fusidic acid, derivatives of fusidic acid and cyclodextrins, have been shown to inhibit mucosal proteolytic activity. However, these compounds are not specific inhibitors or substrates analogues of the proteinases or peptidases, and they are not able to irreversibly or reversibly bind with the catalytic or binding sites of the enzymes. Thus, binding between protein drugs and enhancers to inhibit proteolytic activity has been shown to be a feasible mechanism [52]. The peptide or protein drug bound with enhancer presumably prevents the formation of the enzyme-substrate (enzyme-protein drug) complexes from undergoing the necessary conformational change which aligns the catalytic site on the protease with the susceptible bond of the substrate. Third, the positively charged enhancers may react with the negatively charged membrane surface, and thereby reduce the peptide drug-mucus interactions [48] resulting in an increase of the bioavailability of the drugs. The fourth possibility is that all enhancers lower the barrier function of the mucosal membrane. For example, bile salts including STDHF, have been shown to reduce the viscosity of the mucus layer adhering to all mucosal surfaces [73] and increase the pore size within the cell membrane, thereby allowing diffusion of insulin through the cells [43,71,74]. The barrier function of mucosal membrane is lowered by the enhancers also due to the capability of these compounds to increase membrane fluidity by creating disorder in the phospholipid domain in the membrane [75]. In this way, mucosal membrane components or their structural analogues such as LPG [76], LPC [48,50,76] and phospholipids [77] may play a

special role as potential enhancers, because these compounds can readily create disorder in the membrane by their highly structural similarities to the phospholipid domains of the mucosal membrane.

The main problems in clinical application of enhancers, in nonparenteral administration, are their possible toxicities. There is a direct impact on the administered mucosa, especially in chronic therapy, where repeated administration may continue for years. The use of these pharmaceutical additives usually causes surface changes in the mucosa which includes membrane protein removal, cell loss, excessive mucus discharge and ciliotoxicity [78–80]. In order to examine the toxicities, the identification of morphological change has been used as a major index to evaluate these compounds [48,76]. Beside histological changes, there are some biochemical changes which may occur locally, or systemic toxicity which may result from the absorption of the penetration enhancers themselves. Therefore, more sensitive physico-biochemical assays, such as the determinations of cholesterol, phospholipids and proteins released from mucus membrane, must be used. The measurement of the effects of enhancers on the ciliary epithelium may present an accurate estimate of the effects of the compounds on tissue pharmacology and biochemistry. Each ciliary cell has about 250–300 cilia on its surface [81], which consists of polypeptides driven by Dynein ATPase [82]. Compounds which interact with cilia polypeptides or bind to the ATP binding site of ATPase will destroy or damage the ciliary systems. Table 5 lists the effects of various nasal absorption enhancers on the ciliary epithelium [80].

Recently, cyclodextrins, one class of polysaccharides, have been suggested as potential enhancers for peptide nonparenteral absorption. Merkus et al. [88] reported that 109% of bioavailability of nasal insulin (2.0 IU/kg) could be obtained with co-administration of 5% DMβCD (dimethyl-β-cyclodextrin), as compared with 100% using intravenous insulin (0.25 IU/kg). A recent report by Irie (89) also provided promising results for this compound. It was found, however, that this compound causes the release of some components such as cholesterol, phospholipids and proteins, but the viscosity of nasal mucus layer is not reduced [89]. DMβCD also shows, to some extent, damage to the ciliary system of chicken embryo trachea [88], although the ciliostatic potency of DMβCD is much less than that found for STDHF (1%), laureth-9 (0.3%), deoxycholate (0.3%), glycocholate (1.5%) and taurocholate (1.5%) [86,90]. Therefore, the clinical use of DMβCD may be-

TABLE 5

Effect of various nasal absorption enhancers on the ciliary epithelium

Enhancer	Model	Toxic effect	Reference
1% DGC	rat nasal mucosa	slight damage to ciliary tip	(43)
0.5% DGC	rat nasal mucosa	little noticeable histological change	(83)
0.5% DDC	dog nasal mucosa	loss of the surface epithelial layer	(78)
1% lauryl-9	rat nasal mucosa	significant and complete alteration	(43)
1% lauryl-9	rat nasal mucosa	severe damage on the epithelium	(83)
1% GAHS Na_2	rat nasal mucosa	no mucosa irritation observed	(84)
DPDC	human nasal mucosa	no mucosa irritation observed	(85)
0.5% LLPDC	human nasal mucosa	irritation of mucosa	(85)
0.2% E_2+DMβCD	human nasal mucosa	minor effect on ciliary activity	(86)
1% Bile salt	sheep nasal mucosa	irreversible cell necrosis	(87)
1% DDC	sheep nasal mucosa	irreversible cell necrosis	(87)

DDC, sodium deoxycholate; DGC, sodium glycocholate; DMβCD, dimethyl-β-cyclodextrin; DPDC, didecanoyl-L-α-phosphatidylcholine; E_2, 17β-estradiol; $GAHSNa_2$, glycyrrhetinic acid hydrogen succinate; LLPDC, lauroyl-L-lysophosphatidylcholine.

come feasible after reducing its toxicity by further chemical modifications.

Chemical modification

Chemical modification can result in the denaturation of the bioactive proteins or polypeptides [36]. However, this approach is very useful for the peptides with fewer than ten amino acid residues. A modified luteinizing hormone releasing hormone (LHRH), a decapeptide, and a modified tripeptide, thyrotrophin-releasing hormone (TRH), were found to be active when given orally to rat and man [91].

Hydrogen bond potential or lipophilicity of peptides also can be changed by chemical modification. This leads often to conformational change of the peptides and thereby may increase the permeability of the drugs across cell membrane. For example, when four methyl groups were added to the peptide, acetamido-D-phenylalanyl-D-phenylalanyl-D-phenylalanyl carboxamide by methylation, it was found that the penetration rate of the peptide through Caco-2 cell membrane was significantly enhanced [92].

Some peptide drugs can be converted into prodrugs. A good example is enalapril, which was found to be orally well absorbed and metabolized to the active form, enanaprilat, in the liver. In contrast, the parent drug, enalaprilat is very poorly absorbed via the oral route [5]. The prodrug of TRH, which is derived from TRH by adding an octyloxycarbonyl group to the amino group of histidine, shows a increased permeability through human skin [93]. Since all of penetrated TRH prodrug was found to present as TRH at the receptor phase, it suggests that the prodrug, indeed, is converted into the active form by skin metabolism.

Chemical modification may also play a important role to improve the enhancing efficiency or to reduce the toxicity of the enhancers because all enhancers are small stable compounds.

Formulation approach

The formulation approach has been proposed as a potential delivery system for peptide and protein drugs for many years. In the oral route, several formulations have been explored to protect insulin from proteolysis in the gastrointestinal tract. They include water-in-oil in water emulsions, liposomes, nanoparticles, soft gelatin capsules coated with polyacrylic polymers, and spheres coated with cross-linked polymeric materials. Saffran and his group [94], for example, reported promising results using cross-linked coatings for administering insulin and other peptides orally.

The coated particles may take the form of nanocapsules. Such nanocapsules made from polyalkylcyanoacrylate, were used for delivering insulin, and a significant and prolonged hypoglycemia was observed. In recent work [95], a 50–60% decrease in glycemia was observed by the second day after oral administration of insulin nanocapsules (12.5, 25 and 50 IU/kg). This effect was maintained for 6 and 20 days with 12.5 and 50 IU/kg, respectively.

A recent study showed that a significant continuous hypoglycemic effect also can be obtained with oral microspheres of insulin mixed with protease inhibitor [67]. The dosage form was prepared by the incorporation of insulin with protease inhibitor into a polycrylic polymer (Eudragit L100). When normal and diabetic rats were orally force-fed a 20-IU/kg dose of insulin with protease inhibitor, aprotinin, or Bowman-Birk inhibitor, the continuous hypoglycemic effect lasted more than ten hours, suggesting that the bioavailability of protein or peptide drugs might be much improved if the oral formulation form can be incorporated with protease inhibitors or enhancers.

In nasal administration, the formulation approach is even more attractive. The most popular dosage form is powder or microspheres since nasal solutions and sprays provide relatively lower peptide drug availability. In a recent study by Bjork and Edman [45], insulin (0.75 and 1.7 IU/kg)-DSM preparations administered nasally as a drug powder resulted in a dose-dependent decrease in blood glucose in rats. The bioavailability of the nasal insulin was found to be 30%, whereas the administration of DSM alone or sol-

uble insulin alone produced no effect. The enhancement of the effectiveness of the nasally delivered peptide was due to the fact that the uptake of water by degradable starch microspheres and subsequent swelling produces dehydration of the epithelia cells, leading to a widening of the tight junctions and thereby facilitating paracellular transport of large hydrophilic molecules [46]. Because of their hydration properties and swelling mechanisms, dextran powder microsphere formulations appear to be more efficient in promoting absorption of insulin than viscous or gel polymer systems [96]. When DSM is combined with an enhancer, lysophophatidylcholine (LPC), the extent of absorption of insulin was improved even further [97]. This improved formulation also has been used for enhanced nasal absorption of hGH in sheep [98].

An aerosol dosage form, as used in nasal peptide administration [99], also has been employed as an inhalation aerosol for pulmonary delivery of peptides. A recent report by Adjei [100] demonstrated that leuproline, a nonapeptide analogue of LHRH, can be more efficiently absorbed in rat and healthy humans by an inhalation aerosol than intranasal aerosol. It was found that the bioavailability of the inhalation formulation ranged from 6.6 to 28% based on s.c. and 6.2 to 26% based on i.v. administration, which was significantly higher than those obtained with intranasal administration of the drug (1.8–2.9%). The increased bioavailability via the pulmonary route may be due to (**a**) higher drug permeability; (**b**) lower enzymatic hydrolysis and absorption barriers; and (**c**) smaller physical loss of drugs, as compared with that for other routes. It is still not understood, however, whether or not the pulmonary absorption of peptide and protein drugs in inhalation aerosol formulations can be further increased by co-administration of potential enhancers.

Conclusion

Peptide and protein drugs have a very important role as therapeutic agents for the control of human diseases at the present time and their role will increase in the future. Their potential usefulness depends on whether or not the enzymatic and transport barriers existing for the non-parenteral routes of delivery, can be surmounted. It is important, however, that the natural barriers remain and are only altered transiently. The studies and results reviewed suggest that use of certain enhancers and some proteolytic inhibitors, may safely improve the absorption of drugs into the systemic circulation only after the toxicity caused by the additives is eliminated. Molecular modification to make drugs more resistant to proteolysis or molecular changes of the enhancers to improve delivery is possible method for enhancement of absorption of peptide and protein drugs. Also the formulation approach is very promising. The literature reviewed suggests that some portals of entry may be preferable to others for specific peptides. Each agent must undergo careful biopharmaceutical studies in order to evaluate the effects of the enhancing additives and to discover the best specific delivery system for each agent.

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