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Is the oral route possible for peptide and protein drug delivery?

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Oral delivery of peptides and proteins remains an attractive alternative to parenteral delivery and has challenged various attempts at delivery development. Incorporation of new tools into the delivery systems that can raise membrane permeability of macromolecules is essential to attain high oral bioavailability that is acceptable in clinical applications. In developing oral protein delivery systems with high bioavailability, three practical approaches might be most helpful: (1) modification of the physicochemical properties of macromolecules; (2) addition of novel function to macromolecules; or (3) use of improved delivery carriers. Clearly, it is essential that these approaches maintain the biological activity of the proteins.

Peptides and proteins have become the drugs of choice for the treatment of numerous diseases as a result of their incredible selectivity and their ability to provide effective and potent action [1]. In general, they cause fewer side effects and have great potential to cure diseases, rather than merely treat their symptoms. A wide variety of peptide and protein drugs is now produced on a commercial scale as a result of advances in the biotechnology field [1–3]. The past decade saw an increased interest in formulating and delivering biological drugs for a range of diseases with significant unmet medical need. Unlike conventional small molecular drugs, clinical development of these types of drug will not be possible without some sort of sophisticated pharmaceutical technology.

Administering drugs orally is by far the most widely used route of administration, although it is generally not feasible for peptide and protein drugs. The main reasons for the low oral bioavailability of biologicals are presystemic enzymatic degradation and poor penetration of the intestinal membrane [4,5]. Much has been learned in the past few decades about macromolecular drug absorption from the gastrointestinal (GI) tract, including the barriers that restrict GI absorption. Various strategies have been pursued to overcome such barriers and to develop safe and effective oral delivery systems for proteins [3–5].

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The oral route for peptide and protein administration continues to present a significant challenge and represents a focus for many pharmaceutical researchers. However, we believe that only further research into delivery system**s** can make it possible for the oral route to represent a viable route of administration for peptide and protein drugs, improving convenience for, and compliance from patients who would benefit from these drugs.

Intestinal transport and issues in the oral delivery system of biologicals

Most therapeutic peptides and proteins are hydrophilic, with LogP values <0. Thus, they would not be expected to follow the transcellular route of absorption through passive diffusion [6]. The dimensions of the paracellular space lie between 10 and 30–50Å, and the paracellular route is not an option for macromolecular absorption because it is restricted to relatively small hydrophilic molecules that can fit in these spaces [7]. In the case of one of the most widely prescribed protein drugs, insulin, evidence of a paracellular route of absorption was not shown by either morphocytochemical or biochemical analyses [8]. It was demonstrated that insulin adsorbed to the apical membrane and was internalized by certain types of endocytosis [9]. Some proteins have been shown to be actively transported across the epithelial lining of the small intestine in membrane-bound vesicles after binding to cell-surface receptors or binding sites [10]. However, only a tiny fraction is

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released at the basolateral membrane and secreted into the interstitial space in an intact form. Although, interestingly, there is evidence that significant quantities of peptides and proteins (enough to demonstrate a pharmacologic effect) can be absorbed if they are protected from proteolytic enzymes in the GI tract [11,12].

To increase the oral bioavailability of biologicals, a strategy involving permeation enhancement or protease inhibitors as additives could be effective, and could provide higher reproducible bioavailability. Although such approaches can be very successful in the laboratory [3,13], they still represent a challenge for widespread acceptance by clinicians and regulatory bodies. The use of enzyme inhibitors in long-term therapy remains questionable because of possible absorption of unwanted proteins, disturbance of the digestion of nutritive proteins and stimulation of protease secretion as a result of feedback regulation [3].

A strategy for modulating tight-junction permeability to increase paracellular transport of drug molecules has been studied [14,15]. In fact, the Zonula Occludens toxin [15,16], chitosan [17], thiolated polymers [18] and Pz-peptide [19] all demonstrate a powerful capacity to increase macromolecular drug absorption. However, potentially, such a strategy is not without safety concerns. Once tight junctions have been opened, transport is enhanced not only for drugs, but also for potentially toxic or unwanted molecules present in the GI tract [13,14]. Because many biologicals are used for the treatment of chronic conditions, the long-term implications of unwanted protein absorption could represent a source of concern.

What is the most practical approach?

Even without functional modification of intestinal epithelial cell physiology or proteolytic enzyme activity, it is possible to increase bioavailability to some extent using pharmaceutical technologies. Such approaches involve protection from pepsin digestion in the stomach by enteric coating, rapid drug release in the duodenum to provide a higher concentration at the epithelial surface, temporary decrease of local pH in the small intestine, adhesion to the mucous membrane and colon targeting. These are all effective steps in increasing macromolecular drug absorption. Combination effect of these approaches has been demonstrated by the development of multifunctional smart polymers that are equipped with sharp pH-dependent drug release, Ca^{2+}-deprivation ability and mucoadhesive characteristics [20]. Smart polymer microparticles offer the advantages of decreasing the previously mentioned barriers for the absorption of biologicals. Indeed, these systems showed very high, ~10%, pharmacological availability of insulin following oral administration [21]. However, this value seems to represent the upper limit of bioavailability and it is not possible to exceed it, as long as macromolecules released in the GI tract are exposed directly to proteolytic enzymes.

Mucoadhesive delivery systems adhere to the mucous gel layer covering mucosal membranes. Such systems are expected to prolong the residence time at the local site of absorption and to increase the concentration gradient between delivery system and intestinal membrane. As a result, numerous mucoadhesive delivery systems have been proposed. The delivery systems are thought to be effective in enhancing the intestinal absorption of biologicals vulnerable to proteolytic enzymes [22,23]. Indeed, the approach can minimize presystemic metabolism of peptide and protein drugs in transit between the delivery system and the membrane through the action of luminal proteases. However, proteases in the mucus or glycocalyx and brush border membrane create strong metabolic barriers [24].

Drug absorption from the GI tract might vary according to age, diet and disease state. Therefore, it is probably wise to avoid developing protein-delivery systems when they exhibit a narrow therapeutic window, unless their low bioavailability values can be improved. For example, absorption of unexpectedly large quantities of insulin from poorly developed oral insulin-delivery systems might result in death. Therefore, it is essential to produce an oral dosage form that has reasonable, consistent and predictable bioavailability. The desirable values of pharmacologically acceptable bioavailability vary and are a matter of debate. Some pharmaceutical companies are willing to introduce protein-delivery formulations with bioavailability as low as 10%. However, for oral insulin delivery, a much higher value might be necessary. *Inter* and *intra* variation in bioavailability should be minimal.

It is clear that there will be only limited success from strategies directed at overcoming the enzymatic barrier alone. Unless one can increase the membrane permeability of biologicals, it is unlikely that high oral bioavailability can be achieved. To develop an oral delivery system with high bioavailability for biologicals it will be necessary to consider (1) modification of a physicochemical property (lipophilicity and enzyme susceptibility) of the macromolecules; (2) the addition of novel functionality (e.g. receptor recognition or cell permeability) to macromolecules; or (3) the use of a delivery carrier system. A combination of these approaches might also lead to a successful solution. Of course, it is desirable that the attempts do not affect the integrity of the tight junctions of the intestinal epithelium or other membrane bilayers while maintaining the biological activity of biologicals.

Modifying the physicochemical nature of macromolecules

GI epithelial cell membranes strictly limit the penetration of peptides and proteins. A minimum level of lipophilicity is needed for the molecules to partition into epithelial cell membrane and to be absorbed transcellularly [6]. Furthermore, in the case of biologicals, enzyme resistance is required so that a reasonable amount is absorbed. Structural modification of biologicals provides several opportunities to improve not only membrane permeability, but also proteolytic stability.

For example, the strategy of producing prodrugs [25,26] and analogs [27] of biologicals might protect them from degradation by proteases and other enzymes present in the GI tract. Lipidization, which is the covalent conjugation of a hydrophobic moiety or the noncovalent interaction with a hydrophobic compound, can increase the lipophilicity of peptide and protein molecules [28,29], whereas conjugation with polyethylene glycol (PEG) improves solubility and offers protection from enzymatic degradation [30,31]. These modifications can be used to optimize the pharmacokinetic properties of the macromolecules, but care must always be taken not to reduce their biological efficacy. To overcome the issue of low biological activity of lipidized macromolecules, a reversible lipidization technique has recently been developed to ensure the regeneration of active polypeptides from

their lipid conjugates following oral absorption [32]. Several therapeutic peptide drugs have been lipidized using this technology, and modified macromolecules exhibited an increase in epithelial absorption and GI stability.

Covalent and noncovalent drug modifications for increasing membrane permeability are currently employed by two companies, the Nobex Corporation (NC, USA) and Emisphere Technologies (Tarrytown, NY, USA). Nobex has modified recombinant human insulin at lysine-29 on the β-chain by covalently binding a hydrophilic PEG chain and a lipophilic alkyl chain. Clinical trials with type 1 and type 2 diabetic patients have demonstrated initial efficacy, but low bioavailability (estimated at 5%) continues to be a problem [33]. Emisphere's eligen^{TM} technology makes use of small hydrophobic organic compounds that interact noncovalently with macromolecules, increasing their lipophilicity and enhancing absorption [29]. Using this technology, several macromolecules have been delivered orally, safely and effectively, in humans. However, in addition to the low-bioavailability issue, another potential problem of Emisphere's strategy is the requirement of a large amount of the delivery agent in a dose [34].

Adding novel functionality to macromolecules

Potential use of endogenous cellular transport systems

Introduction of novel functionality to peptides and proteins using transport-carrier molecules that are recognized by endogenous cellular-transport systems in the GI tract might represent a more practical and safer strategy for increasing intestinal absorption of peptides and proteins. In fact, this is a method that has been undertaken by numerous investigators and companies to achieve improved bioavailability. However, no such systems are available commercially as yet. The associated transport mechanisms are membrane transporters and receptor-mediated endocytosis, recognizing and internalizing specific ligands attached to macromolecules. For example, by attaching a drug to a dipeptide that is recognized by a peptide-influx transporter, its oral absorption can be increased [35]. Efflux transporters such as P-gp might contribute significantly to the poor bioavailability of certain drugs, including peptides [36]. Therefore, treatment with P-gp inhibitors could possibly improve the oral absorption of P-gp substrates, and such a drug-delivery strategy has been recently discussed [36]. However, in general, membrane transporters are limited to transporting relatively small drugs. By contrast, receptor-mediated endocytotic systems do not seem to be limited with regard to the size of the drug transported across the cell. Receptor-recognizable ligands, such as lectins, toxins, viral haemagglutinins, invasins, transferrin, and vitamins (Vitamin B_{12} [VB_{12}], folate, riboflavin and biotin), can be tethered to a drug substance to improve the specificity of the intracellular delivery systems to specific target cells [37–39].

It has been demonstrated that receptor-mediated endocytosis of VB_{12} could be used for oral delivery of peptides and proteins [37]. Transferrin is also useful as a carrier for oral delivery of protein drugs such as insulin and granulocyte colony-stimulating factor (G-CSF). Recently, through recombinant DNA technology, Bai *et al.* [40] elegantly produced a functionally active G-CSF–transferrin fusion protein and demonstrated that its biological activity following oral administration to mice was similar to that of subcutaneous G-CSF administration. There are high expectations that this new recombinant fusion protein technology will be useful for the future development of orally effective biologicals.

Prospects for using cell-penetrating peptides (CPPs)

During the past decade, a class of short peptides, such as TAT (48–60), penetratin and oligoarginine, have been used to internalize different bioactive compounds into cells [41,42]. These CPPs were found to enable the delivery of small molecules, macromolecules, liposomes and nanoparticles into cells or tissues by chemically hybridizing with target materials. Some of the carriers, peptides and proteins delivered by CPPs are shown in Table 1.

The CPPs deliver their cargoes into the cytoplasm by directly perturbing the lipid bilayer structure of the cell membrane or by endocytosis [41,42]. With regard to the toxic effects of CPPs, it has been reported that penetratin caused very little disturbance to membranes, whereas TAT appeared to cause practically no harm to cell membranes [42]. Toxicity and undesirable side effects have not been detected in most *in vivo* applications of CPPs [42,43]. Therefore, these methods are expected to become powerful tools for overcoming the low permeability of biologicals through epithelial cell membranes, which is the greatest barrier to macromolecular oral drug delivery.

Although there is only limited information about their effects on the membrane permeability of macromolecular drugs, it has been reported that insulin transport across Caco-2 cells was dramatically increased by conjugation of insulin with TAT [44]. The CPP strategy is based on a nonspecific delivery mechanism; however, the increased targeting ability of CPPs is also proposed [45]. So far, CPP strategy research lacks sufficient numbers of *in vivo* studies that could demonstrate their therapeutic potential. More efficacy and safety data are definitely needed to develop this delivery system towards market availability.

Using particulate delivery carrier systems

Most oral delivery strategies for biologicals are based on systems equipped to protect against enzymatic degradation and provide high transfer of drugs across the epithelium mucosa. Particulate carrier systems meet these requirements. They protect fragile macromolecules against enzymatic degradation in the harsh environment of the GI tract. Certain particles can be taken up by epithelial cells or the lymphoid tissues in Peyer's patches without employing additional penetration enhancers. So far, polymeric drug delivery systems based on hydrogels, nanoparticles, microspheres, and lipid-based drug delivery systems (e.g. microemulsions, liposomes, and solid lipid nanoparticles) have been developed and employed for oral macromolecular drug delivery.

Of the particulate carrier systems, lipid-based particles generally do not entrap hydrophilic macromolecular drugs with high efficiency. In addition, they have low stability in the GI tract. Conventional liposomes and microemulsions have not met with much success in the mucosal delivery of hydrophilic macromolecular drugs. Although fusogenic liposomes that are equipped with the envelope glycoprotein of Sendai virus [46] or liposomes that are coated with a mucoadhesive polymer [47] showed significant improvement of hydrophilic macromolecular drug absorption from the intestine, overall, solid particles appear to be better than lipid-based particles for oral delivery.

TABLE 1

Examples of systems delivered by various cell-penetrating peptides (CPPs)

Systems	CPP	Application/cell type	Ref
Particulate carriers			
Magnetic nanoparticles	Tat	*In vitro*/hematopoietic and neutral progenitor cells	[58]
Gold nanoparticles	Tat	*In vitro*/HeLa cells, 3T3/NIH cells, HepG2 cells	[59]
Dendrimers	Tat	*In vitro*/NIH 3T3 MDR cells	[60]
Liposome	Tat	*In vitro*/BT20, LLC and H9C2 cells [61], *in vitro*/various cancer cells [62], *in vitro*/tumour and dendritic cells [63]	[61–63]
Liposome	Penetratin	*In vitro*/various cancer cells	[62]
Liposome	Antennapedia	*In vitro*/tumour and dendritic cells	[63]
Peptides and proteins			
Beta-galactosidase, Rnase A, Horseradish peroxidase, Domain III of pseudomonas exotoxin A	Tat	*In vitro*/HeLa cells, *in vivo*/BALB/c mice	[64]
Peptide derived from the VHL tumour suppressor	Tat	*In vitro*/RCC786-O cells, *in vivo*/Harlan Sprague Dawley nude mice	[65]
p53	11 Poly-arginine peptides	*In vitro*/cancer cells	[66]
Insulin	Tat	*In vitro* permeation/Caco-2 cells	[44]
p16-derived synthetic peptide	Antennapedia	*In vivo*/BALB/c nu/nu mice	[67]
Antibody	Tat	*In vitro*/3T3-L1 cells	[68]
Green fluorescent protein	hCT(9–32)	*In vitro*/bovine nasal mucosa	[69]

Oral nanoscale carriers

Peyer's patches are follicles of lymphoid tissue covered by a specialized epithelium containing M cells [48]. These M cells are responsible for particle uptake, and surface charge and size of particles are the important factors governing the uptake of particulates by the M cells [49]. In general, nanoscale dimensions favour transport of particles across the mucosal epithelium. Desai *et al*. demonstrated that 100 nm poly(lactic-*co*-glycolic acid) (PLGA) particles diffused throughout the submucosal layers, whereas 10 μm particles were predominantly localized on the epithelial lining of the tissues [50]. Taken together, nanoscale carriers composed of biocompatible polymers are thought to be promising for the development of an oral delivery system for macromolecules. Representative nanoscale oral polymer carriers employed for oral peptide and protein drug delivery are shown in Table 2. Indeed, these nanocarriers show pharmacological effects of the incorporated biologicals following oral administration *in vivo*. The potential of chitosan nanoparticles for oral peptide administration has been recently reported by several researchers, as shown in Table 2. Insulin-loaded chitosan nanoparticles administered orally to diabetic rats reduced their glucose levels to a normal range for more than several hours [51,52].

However, chitosan-coated nanoparticles clearly reduced the transepithelial resistance of a Caco-2 cell monolayer [17,53]. Therefore, their potential use for clinical applications is questionable. To target the epithelium and provide a greater uptake of particles, the surface of carriers has been linked to ligands. Nanoparticles with VB_{12} or lectins attached to their surface have been

TABLE 2

Oral nanoparticle (NP) drug carrier[a]

Carrier	Drug	Size (nm)	Animal	Outcome	Ref
Poly(isobutylcyanoacrylate) NP	Insulin	220	STZ-induced diabetic rat	Long-lasting strong hypoglycemic response	[70]
Chitosan NP	Insulin	250–400	Alloxan-induced diabetic rat	Pharmacological availability[b] was 14.9%	[51]
Chitosan NP	Insulin	269, 339	STZ-induced diabetic rat	Pharmacological availability[b] was 3.2–5.1%	[52]
Chitosan-coated lipid NP	sCT	537.0	Rat	Long-lasting hypocalcemic response	[17]
Chitosan-coated PLGA NP	Elcatonin	650	Rat	Long-lasting hypocalcemic response	[71]
PLGA NP	sCT	171.9–315.1	Rat	Bioavailability of sCT was ~0.4%	[72]
Poly(*N*-isopropyl acrylamide) NP	sCT	148–895	Rat	Hypocalcemic response	[73]
Nanocubicle	Insulin	220	STZ-induced diabetic rat	Strong hypoglycemic effect	[74]
Acrylic-based copolymer NP	Insulin	200 (pH 2)–2000 (pH 6)	STZ-induced diabetic rat	Significant reduction of serum glucose	[75]

[a] Abbreviations: PLGA, poly(lactic-*co*-glycolic acid); sCT, salmon calcitonin; STZ, streptozotocin.
[b] Pharmacological availability of peroral chitosan-insulin nanoparticles was determined based on the extent of hypoglycemic response relative to subcutaneous [51] or peritoneal [52] insulin injection.

shown to be absorbed through the receptor-mediated endocytosis pathway [37]. However, as Florence pointed out, we should consider issues in relation to nanoparticle ligand–receptor interactions, such as the possibility of the hydrolytic loss of ligands and particle aggregation preventing access to the ligands [54].

In recent years, polymeric micelles have received growing attention as functional nanomaterials [55]. Polymeric micelles are formed through the self-assembly of amphiphilic block copolymers in an aqueous environment. They have a nanoscopic, core/shell structure in which the hydrophobic core acts as a microreservoir for the encapsulation of hydrophobic drugs. Among them, polyion complex micelles can entrap biomacromolecules such as enzymes and DNA, and might attain increased stability against various environmental factors by the micellar structure [56]. Recently, it has been shown that the polymer micelles cross the intestinal barrier after oral administration [57], therefore, the polymeric micellar systems might also be useful for the oral delivery of macromolecules.

Several issues remain to be resolved for the success of nanoparticle-mediated delivery of biologicals: the low-incorporation efficiency of hydrophilic drugs; precise control of drug release; and avoidance of particle aggregation. These problems must be solved before we can attain an efficient and reliable uptake via the oral route that allows a therapeutic response. In addition, the possible accumulation of nondegradable particles in tissues might lead to harmful effects. Even for degradable particles, the use of unreasonably high quantities of the carrier can lead to problems of carrier toxicity. The fate of the carrier systems in the body should be clarified. As suggested by Hamman *et al.* [5], reproducible absorption in humans should first be proven to ensure the feasibility of carrier systems providing clinically useful delivery. Indeed, the relevance of animal studies to humans has been raised by Florence [54].

Conclusions

It is thought that a prerequisite for the successful delivery of oral peptides and proteins is the maximization of the absorptive cellular intestinal uptake and stabilization of the biologicals at all stages before they reach their target. To develop and improve oral delivery systems with such properties, the focus should be on the development of superior materials and delivery carriers for oral bioactive macromolecular drug delivery systems. The oral route for peptide and protein drug delivery might be possible in the near future using innovative delivery systems. Although considerable efforts have been already made to develop oral delivery systems of macromolecules, extensive *in vivo* studies with these delivery systems have not been publicly reported. Therefore, development of improved oral delivery devices for peptides and proteins will require continuous comparison of the *in vitro* and cellular studies with *in vivo* studies.

References

1 Frokjaer, S. and Otzen, D.D. (2005) Protein drug stability: a formulation challenge. *Nat. Rev. Drug Discov.* 4, 298–306
2 Torchilin, V.P. and Lukyanov, A.N. (2003) Peptide and protein drug delivery to and into tumors: challenges and solutions. *Drug Discov. Today* 8, 259–266
3 Shah, R.B. *et al.* (2002) Oral delivery of proteins: Progress and prognostication. *Crit. Rev. Ther. Drug Carrier Syst.* 19, 135–169
4 Mahato, R.I. *et al.* (2003) Emerging trends in oral delivery of peptide and proteins. *Crit. Rev. Ther. Drug Carrier Syst.* 20, 153–214
5 Hamman, J.H. *et al.* (2005) Oral delivery of peptide drugs. *BioDrugs* 19, 165–177
6 Camenisch, G. *et al.* (1998) Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. *Eur. J. Pharm. Sci.* 6, 317–324
7 Rubas, W. *et al.* (1996) Flux measurements across Caco-2 monolayers may predict transport in human large intestinal tissue. *J. Pharm. Sci.* 85, 165–169
8 Bendayan, M. *et al.* (1994) Biochemical and morpho-cytochemical evidence for the intestinal absorption of insulin in control and diabetic rats. Comparison between the effectiveness of duodenal and colon mucosa. *Diabetologia* 37, 119–126
9 Agarwal, V. and Khan, M.A. (2001) Current status of the oral delivery of insulin. *Pharm. Technol.* 10, 76–90
10 Bastian, S.E.P. *et al.* (1999) Transport of IGF-I across epithelial cell monolayers. *J. Endocrinol.* 162, 361–369
11 Morishita, M. *et al.* (1993) Site-dependent effect of aprotinin, sodium caprate, Na2EDTA and sodium glycocholate on intestinal absorption of insulin. *Biol. Pharm. Bull.* 16, 68–72
12 Tozaki, H. *et al.* (1998) Use of protease inhibitors to improve calcitonin absorption from the small and large intestine in rats. *J. Pharm. Pharmacol.* 50, 913–920
13 Ward, P.D. *et al.* (2000) Enhancing paracellular permeability by modulating epithelial tight junctions. *Pharm. Sci. Technol. Today* 3, 346–358
14 Salamat-Miller, N. and Johnston, T.P. (2005) Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium. *Int. J. Pharm.* 294, 201–216
15 Salama, N.N. *et al.* (2006) Tight junction modulation and its relationship to drug delivery. *Adv. Drug Deliv. Rev.* 58, 15–28
16 Fasano, A. (1998) Novel approaches for oral delivery of macromolecules. *J. Pharm. Sci.* 87, 1351–1356
17 Prego, C. *et al.* (2005) Transmucosal macromolecular drug delivery. *J. Control. Release* 101, 151–162
18 Bernkop-Schnurch, A. (2005) Thiomers: a new generation of mucoadhesive polymers. *Adv. Drug Deliv. Rev.* 57, 1569–1582
19 Yen, W.-C. and Lee, V.H.L. (1995) Penetration enhancement effect of Pz-peptide, a paracellularly transported peptide, in rabbit intestinal segments and Caco-2 cell monolayers. *J. Control. Release* 36, 25–37
20 Morishita, M. *et al.* (2006) Oral insulin delivery systems based on complexation polymer hydrogels. *J. Drug Del. Sci. Tech.* 16, 19–24
21 Morishita, M. *et al.* (2006) Novel oral insulin delivery systems based on complexation polymer hydrogels: Single and multiple administration studies in type 1 and 2 diabetic rats. *J. Control. Release* 110, 587–594
22 Junginger, H.E. (1990) Bioadhesive polymer systems for peptide delivery. *Acta Pharm. Technol.* 36, 110–126
23 Bernkop-Schnurch, A. and Clausen, A.E. (2002) Biomembrane permeability of peptides: Strategies to improve their mucosal uptake. *Mini Rev. Med. Chem.* 2, 295–305
24 Aoki, Y. *et al.* (2005) Region-dependent role of the mucous/glycocalyx layers in insulin permeation across rat small intestinal membrane. *Pharm. Res.* 22, 1854–1862
25 Gangwar, S. *et al.* (1997) Prodrug strategies to enhance the intestinal absorption of peptides. *Drug Discov. Today* 2, 148–155
26 Mizuma, T. *et al.* (2000) Intestinal transport and metabolism of glucose-conjugated kyotorphin and cyclic kyotorphin: metabolic degradation is crucial to intestinal absorption of peptide drugs. *Biochim. Biophys. Acta* 1475, 90–98
27 Hichens, M. (1983) A comparison of thyrotropin-releasing hormone with analogs: influence of disposition upon pharmacology. *Drug Metab. Rev.* 14, 77–98
28 Hashimoto, M. *et al.* (1989) Synthesis of palmitoyl derivatives of insulin and their biological activities. *Pharm. Res.* 6, 171–176
29 Goldberg, M. and Gomez-Orellana, I. (2003) Challenges for the oral delivery of macromolecules. *Nat. Rev. Drug Discov.* 2, 289–295
30 Calceti, P. *et al.* (2004) Development and *in vivo* evaluation of an oral insulin-PEG delivery system. *Eur. J. Pharm. Sci.* 22, 315–323
31 Basu, A. *et al.* (2006) Structure-function engineering of interferon-beta-1b for improving stability, solubility, potency, immunogenicity, and pharmacokinetic properties by site-selective mono-PEGylation. *Bioconjugate Chem.* 17, 618–630
32 Wang, J. *et al.* (2003) Reversible lipidization for the oral delivery of salmon calcitonin. *J. Control. Release* 26, 369–380
33 Kipnes, M. *et al.* (2003) Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with type 2 diabetes. *Diabetes Care* 26, 421–426

34 Kidron, M. *et al.* (2004) A novel per-oral insulin formulation: proof of concept study in non-diabetic subjects. *Diabetic Med.* 21, 354–357
35 Han, H.-K. and Amidon, G.L. (2000) Targeted prodrug design to optimize drug delivery. *AAPS PharmSci* 2, E6
36 Varma, M.V.S. *et al.* (2003) P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. *Pharmacol. Res.* 48, 347–359
37 Russell-Jones, G.J. (2004) Use of targeting agents to increase uptake and localization of drugs to the intestinal epithelium. *J. Drug Target.* 12, 113–123
38 Hwa Kim, S. (2005) Folate receptor mediated intracellular protein delivery using PLL-PEG-FOL conjugate. *J. Control. Release* 103, 625–634
39 Lim, C.J. and Shen, W.C. (2005) Comparison of monomeric and oligomeric transferrin as potential carrier in oral delivery of protein drugs. *J. Control. Release* 106, 273–286
40 Bai, Y. *et al.* (2005) Recombinant granulocyte colony-stimulating factor-transferrin fusion protein as an oral myelopoietic agent. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7292–7296
41 Trehin, R. and Merkle, H.P. (2004) Chances and pitfalls of cell penetrating peptides for cellular drug delivery. *Eur. J. Pharm. Biopharm.* 58, 209–223
42 Zorko, M. and Langel, U. (2005) Cell-penetrating peptides: mechanism and kinetics of cargo delivery. *Adv. Drug Deliv. Rev.* 57, 529–545
43 Schwarze, S.R. *et al.* (1999) *In vivo* protein transduction: delivery of a biologically active protein. *Science* 285, 1569–1572
44 Liang, J.F. and Yang, V.C. (2005) Insulin-cell penetrating peptide hybrids with improved intestinal absorption efficiency. *Biochem. Biophys. Res. Commun.* 335, 734–738
45 Vives, E. (2005) Present and future of cell-penetrating peptide mediated delivery systems: ''is the Trojan horse too wild to go only to Troy?'' *J. Control. Release* 109, 77–85
46 Goto, T. *et al.* (2006) Novel mucosal insulin delivery systems based on fusogenic liposomes. *Pharm. Res.* 23, 384–391
47 Takeuchi, H. *et al.* (2003) Mucoadhesive properties of carbopol or chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats. *J. Control. Release* 86, 235–242
48 Brayden, D.J. *et al.* (2005) Keynote review: Intestinal Peyer's patch M cells and oral vaccine targeting. *Drug Discov. Today* 10, 1145–1157
49 Shakweh, M. *et al.* (2005) Poly (lactide-co-glycolide) particles of different physicochemical properties and their uptake by peyer's patches in mice. *Eur. J. Pharm. Biopharm.* 61, 1–13
50 Desai, M.P. *et al.* (1997) The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. *Pharm. Res.* 14, 1568–1573
51 Pan, Y. *et al.* (2002) Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin *in vivo*. *Int. J. Pharm.* 249, 139–147
52 Ma, Z. *et al.* (2005) Pharmacological activity of peroral chitosan-insulin nanoparticles in diabetic rats. *Int. J. Pharm.* 293, 271–280
53 Garcia-fuentes, M. *et al.* (2005) A comparative study of the potential of solid triglyceride nanostructures coated with chitosan or poly(ethylene glycol) as carriers for oral calcitonin delivery. *Eur. J. Pharm. Sci.* 25, 133–143
54 Florence, A.T. (2004) Issues in oral nanoparticle drug carrier uptake and targeting. *J. Drug Target.* 12, 65–70
55 Aliabadi, H.M. and Lavasanifar, A. (2006) Polymeric micelles for drug delivery. *Expert Opin. Drug Deliv.* 3, 139–162
56 Yuan, X. *et al.* (2005) Stabilization of lysozyme-incorporated polyion complex micelles by the omega-end derivatization of poly(ethylene glycol)-poly(alpha,beta-aspartic acid) block copolymers with hydrophobic groups. *Langmuir* 21, 2668–2674
57 Mathot, F. *et al.* (2006) Intestinal uptake and biodistribution of novel polymeric micelles after oral administration. *J. Control. Release* 111, 47–55
58 Lewin, M. *et al.* (2000) Tat peptide-derivatized magnetic nanoparticles allow *in vivo* tracking and recovery of progenitor cells. *Nat. Biotechnol.* 18, 410–414
59 Tkachenko, A.G. *et al.* (2004) Cellular trajectories of peptide-modified gold particle complexes: comparison of nuclear localization signals and peptide transduction domains. *Bioconjug. Chem.* 15, 482–490
60 Kang, H. *et al.* (2005) Tat-conjugated PAMAM dendrimers as delivery agents for antisense and siRNA oligonucleotides. *Pharm. Res.* 22, 2099–2106
61 Torchilin, V.P. *et al.* (2001) Tat peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8786–8791
62 Tseng, Y.L. *et al.* (2002) Translocation of liposomes into cancer cells by cell-penetrating peptides penetratin and tat: a kinetic and efficacy study. *Mol. Pharmacol.* 62, 864–872
63 Marty, C. *et al.* (2004) Enhanced heparan sulfate proteoglycan-mediated uptake of cell-penetrating peptide-modified liposomes. *Cell. Mol. Life Sci.* 61, 1785–1794
64 Fawell, S. *et al.* (1994) Tat-mediated delivery of heterologous proteins into cells. *Proc. Natl. Acad. Sci. U. S. A.* 91, 664–668
65 Datta, K. *et al.* (2001) The 104-123 amino acid sequence of the beta-domain of von Hippel-Lindau gene product is sufficient to inhibit renal tumor growth and invasion. *Cancer Res.* 61, 1768–1775
66 Takenobu, T. *et al.* (2002) Development of p53 protein transduction therapy using membrane-permeable peptides and the application to oral cancer cells. *Mol. Cancer Ther.* 1, 1043–1049
67 Hosotani, R. *et al.* (2002) Trojan p16 peptide suppresses pancreatic cancer growth and prolongs survival in mice. *Clin. Cancer Res.* 8, 1271–1276
68 Mie, M. *et al.* (2003) Intracellular delivery of antibodies using TAT fusion protein A. *Biochem. Biophys. Res. Commun.* 310, 730–734
69 Machova, Z. *et al.* (2002) Cellular internalization of enhanced green fluorescent protein ligated to a human calcitonin-based carrier peptide. *Chem. Biochem.* 3, 672–677
70 Damge, C. *et al.* (1988) New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier. *Diabetes* 37, 246–251
71 Kawashima, Y. *et al.* (2000) Mucoadhesive DL-lactide/glycolide copolymer nanospheres coated with chitosan to improve oral delivery of elcatonin. *Pharm. Dev. Technol.* 5, 77–85
72 Sang Yoo, H. and Park, T.G. (2004) Biodegradable nanoparticles containing protein-fatty acid complexes for oral delivery of salmon calcitonin. *J. Pharm. Sci.* 93, 488–495
73 Sakuma, S. *et al.* (2002) Optimized chemical structure of nanoparticles as carriers for oral delivery of salmon calcitonin. *Int. J. Pharm.* 239, 185–195
74 Chung, H. *et al.* (2002) Self-assembled 'nanocubicle' as a carrier for peroral insulin delivery. *Diabetologia* 45, 448–451
75 Foss, A.C. *et al.* (2004) Development of acrylic-based copolymers for oral insulin delivery. *Eur. J. Pharm. Biopharm.* 57, 163–169