Peroral Route: An Opportunity for Protein and Peptide Drug Delivery†

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I. Introduction

The better understanding of endogenous proteins, peptides, and peptidergic molecules and their role in various body functions and pathological conditions in last few decades has resulted in realization of the enormous therapeutic potential of proteins and peptides (PPs). As a consequence, a variety of new PP drugs have been developed which offer the advantages of being very potent and specific therapeutic agents.[1] Initially, use of PPs as pharmaceuticals was severely limited, as they were difficult to produce and were isolated from animal sources. These PP products obtained from animals differed from functional molecules present in the human body, and their use as therapeutic agents raised concerns with regard to their immunogenic potential.[2,3] As a result of intensive research efforts in both academic and industrial laboratories, recombinant DNA, protein engineering, and tissue culture techniques can now be used to obtain PPs, on a commercial scale, which resemble endogenous molecules and thus provoke fewer or minimal immunological responses. Additionally, due to advances in analytical separation technology, recombinant proteins can now be purified to unprecedented levels.[4] Today, PPs along with informational macromolecules normally produced by the body including endorphins, enkephalins, leutinizing hormone releasing hormone, and interferons form an increasingly important class of therapeutic agents. Table 1 lists PP products introduced in the market over the past few years.[5−8]

Though the initial problems related to obtaining nonimmunogenic PP drugs in purer form at commercial scales have been overcome to quite some extent,[9] their formulation and optimum delivery still remain as the biggest challenges to pharmaceutical scientists. Use of PPs as therapeutic agents is limited due to lack of an effective route and method of delivery. Various critical issues associated with PP delivery that have drawn the attention of formulation scientists include the following. (i) PPs are high molecular weight biopolymers which serve as enzymes, structural elements, hormones, or immunoglobulins and are involved in several biological activities. However, due to their large molecular weight and size, they show poor permeability characteristics through various mucosal surfaces and biological membranes.[10−12] (ii) Many PP drugs are efficacious, in large part because of their tertiary structure. The tertiary structure can be lost under various physical and chemical environments, resulting in their denaturation or degradation with consequent loss in biological activity, hence, making these molecules inherently unstable.[8,13,14] (iii) Many PPs have very short biological half-lives in vivo due to their rapid clearance in liver and other body tissues by proteolytic enzymes.[15−17] (iv) As PP drugs have very specific actions and are highly potent, precise clinical dosing is of utmost importance.[18]

The most important consideration when designing an effective delivery system for any drug is that of achieving a predictable and reproducible absorption into systemic circulation with high bioavailability. In the case of PP drugs, an interplay of poor permeability characteristics, luminal, brush border, and cytosolic metabolism, and hepatic clearance mechanisms results in their poor bioavailability from oral

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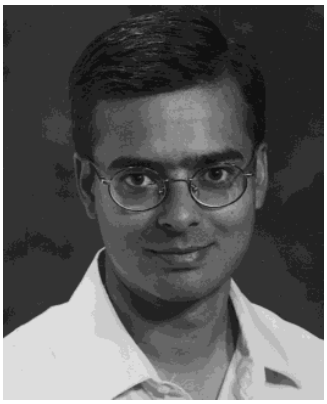
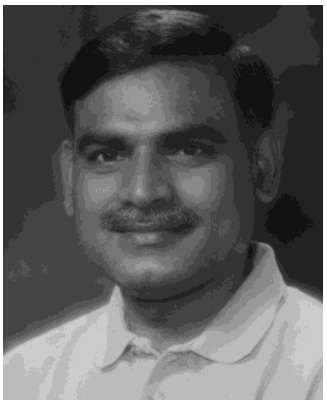


Anurag Sood was born in 1972. He received his B.Pharm and M.Pharm degrees from Dr. Hari Singh Gour Vishwavidyalaya, Sagar, India. After finishing his Master's degree, he joined Dr. Reddy's Research Foundation, Hyderabad, India, as Trainee Pharmacologist, where he was a part of team responsible for in vivo characterization of potential drug candidates for pharmacokinetics and metabolism. Presently, he is a research scholar at National Institute of Pharmaceutical Education and Research (NIPER), India. He has been a recipient of a Junior Research Fellowship (1994–96) from the University Grants Commission (UGC), India, and a Senior Research Fellowship (1998 onward) from the Council of Scientific and Industrial Research (CSIR), India. His research activities include design, development, and evaluation of conventional and novel *peroral*-controlled release drug delivery systems. Various drug delivery systems, drug release kinetics and mechanisms, and analytical method development and validation are the topics of his interest. He has 13 publications to his credit and has presented his research at various national and international conferences. He is a recipient of the G. P. Nair Award of the Indian Drugs Manufacturer's Association (IDMA) for securing first rank in his B.Pharm degree course at University. He is fond of music and loves listening to Hindi and Urdu Ghazals during his leisure time.



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and nonoral mucosal routes.[19] Hence, at present these drugs are usually administered by parenteral route. However, inherent short half-lives of PPs and almost warranted chronic therapy requirements in a majority of cases make their repetitive dosing necessary. Frequent injections, oscillating blood drug concentrations, and low patient acceptability make even the simple parenteral administration of these drugs problematic. This has prompted researchers to develop new delivery systems which can effectively deliver this important class of drugs.[20−30] Although there have been reports of successful delivery of various PP therapeutics across non-*peroral* mucosal routes,[31,32] *peroral* route continues to be the most intensively investigated route for PP administration. This interest in the *peroral* route, despite enormous barriers to drug delivery that exist in the gastrointestinal tract (GIT), can be very well appreciated from obvious advantages such as ease of administration, large patient acceptability, etc. Potential cost savings to the health care industry further augment the advantages of *peroral* systems in terms of patient compliance and acceptability, since *peroral* formulations do not require sophisticated sterile manufacturing facilities or the direct involvement of health care professionals. There have been efforts to circumvent the gastrointestinal (GI) absorption barriers to PP drugs since the 1920s, when insulin was used first as a therapeutic protein, however only with a limited success.[33−38] After the success of *peroral* cyclosporin formulations,[39−41] the efforts in this field have further intensified. There are a plethora of attempts and reports wherein the use of different approaches for *peroral* PP delivery has been investigated. The purpose of the present review is to examine recent developments in *peroral* PP drug delivery. Various barriers to PP drug absorption have been discussed in brief with attention particularly focused on drug delivery approaches that have been used or are being developed to overcome these barriers. The reports of successful improvement of *peroral* bioavailability of PPs and mechanisms involved therein are emphasized the most.

II. Barriers to Peroral Delivery of PP Drugs

The *peroral* route poses significant challenges for PP drug delivery. The barriers to PP absorption from GIT are primarily chemical, enzymatic, as well as penetration related. Acid-induced hydrolysis in the stomach, enzymatic degradation throughout the GIT by several proteolytic enzymes, bacterial fermentation in the colon, and physical barriers to absorption are traditionally believed to prevent the *peroral* delivery of PPs (Table 2). However, the nature of these barriers has now been expanded to include intracellular metabolism by cytochrome P450−3A4 as well as apically polarized efflux mediated by ATP-dependent P-glycoproteins.[42−44] Although, P-glycoprotein-mediated efflux systems are most commonly observed in tumor cells, they are also present in normal intestinal cells and act to reduce the intra-

Table 1. PP Drug Products Approved in the United States over the Last Few Years

product name	protein/peptide	company
Actimmune	Interferon gamma-1b	InterMune Pharmaceuticals
Activase	Alteplase recombinant	Genentech
Adagen	Pegademase bovine	Enzon
Alferone N	Interferon alfa-n3	Interferon Sciences
Avonex	Interferon beta-1a	Biogen
BeneFIX	Recombinant human factor IX	Genetics Institute
Betaserone	Interferon beta	Chiron/Berlex
BioTropin	Human growth hormone	Bio-Technology General
Bioclate	Recombinant antihemophilic factor	Centeon
CEA-Scan	Technetium-99m-arcitumomab	Immunomedics
Cerezyme	Recombinant glucocerebrosidase	Genzyme
Comvax	Recombinant vaccine	Merck
Crofab	Crotalidae polyvalent immune Fab (ovine)	Protherics
Enbrel	Recombinant soluble receptor	Immunex
Engerix-B	Hepatitis B vaccine recombinant	SmithKline Beecham
EPOGEN	Epoetin alfa	Amgen
Follistim	Recombinant follicle-stimulating hormone	Organon
GenoTropin	Somatropin	Pharmacia & Upjohn
Geref	Human growth hormone releasing factor	Serono Laboratories
Gkucagen	Recombinant glucagons	Novo Nordisk
Gonal-F	Recombinant human follicle stimulating hormone	Serono Laboratories
Helixate	Recombinant antihemophilic factor	Centeon
Herceptin	Anti-breast cancer MAb[3]	Genentech
Humalog	Insulin lispro	Eli Lilly
Humate-P	Antihemophilic factor	Centeon
Humatrope	Somatropin	Eli Lilly
Humulin	Human insulin (recombinant DNA origin)	Eli Lilly
Infergen	Interferon alfacon-1	Amgen
Intron	Interferon alfa-2b	Schering-Plough
KoGENate	Recombinant anti hemophilic factor	Bayer Corporation
Leukine	GM-colony stimulating factor	Immunex
LYMErix	Recombinant OspA	SmithKline Beecham
MYOBLOC	Botulinum toxin type B	Elan
MyoScint	Imiciromab pentetate, Mab	Centocor
Nabi-HB	Hepatitis B immune globulin (human)	Nabi
Neumega	Oprelvekin, Mab	Genetics Institute
NEUPOGEN	Filgrastim	Amgen
Norditropin	Somatropin	Novo Nordisk
Novolin	Recombinant insulin	Novo Nordisk
Nutropin AQ	Somatropin	Genetech
Nutropin Depot		
Nutropin	Somatropin	Genentech
OncoScint	Satumomab pendetide, Mab	Cytogen
Oncospar	PEG-L-asparaginase	Enzon
Ontak	Denileukin diftitox	Ligand Pharmaceuticals
Orthoclone OKT 3	Muromonab-CD3, Mab	Ortho Biotech
PEG-Intron	Peginterferon alfa-2b	Schering Corporation
Prevnar	Diphtheria CRM197 Protein	Lederle
Procrit	Epoetin alfa	Ortho Biotech
Proleukin	Interleukin-2	Chiron
ProstaScint	Capromab pentitate, Mab	Cytogen
Protropin	Somatrem	Genentech
Pulmozyme	Recombinant dornase alfa	Genentech
Rebetron	Ribavirin/interferon alfa-2b combination	Schering-Plough
Recombinate	Recombinant anti hemophilic factor	Baxter Healthcare
RECOMBIVAX HB	Recombinant hepatitis B vaccine	Merck
ReFacto	Recombinant antihemophilic factor	Genetics Institute
Refludan	Lepuridin	Aventis
Regranex	Becaplermin	Ortho-McNeil
Remicade	Infliximab, Mab	Centocor
ReoPro	Abciximab, anti-platelet Mab	Centocor/Eli Lilly
Retavase	Reteplase	Centocor
Rituxan	Ritiximab, Mab	Genentech
Roferone−A	Recombinant interferon alfa-2a	Hoffmann-La Roche
Saizen	Somatropin	Serono laboratories
Serostim	Somatropin	Serono Laboratories
Simulect	Basiliximab, Mab	Novartis
Synagis	Palivizumab, Mab	MedImmune
Thymoglobulin	Thymocyte globulin, polyclonal antibody	SangStat
Thyrogen	Thyrotropin alfa	Genzyme
TNKase	Tenecteplase	Genentech
Verluma	Nofetumomab, MAB	DuPont Merck
Wellferone	Interferon alfa-n1	Glaxo Wellcome
Zenapax	Daclizumab, Mab	Hoffman-La Roche

Table 2. Various *Peroral* Absorption Barriers and Their Bearing on PP Drug Absorption from GIT

barrier nature	location and description	effect on PP drug absorption
chemical	acidic environment in stomach (pH 1.2−3.0) and alkaline environment in intestine (pH 6.5−8.0)	pH-induced oxidation, deamidation, or hydrolysis
enzymatic	luminally secreted, membrane-bound, and cytosolic proteolytic enzymes throughout the length of GI tract	proteolytic degradation in lumen and during absorption through enterocytes
	microbial flora present in colon	breakdown PP as part of their metabolic activity
physical	unstirred aqueous boundary layer and viscous mucus layer covering the surface of GI epithelial cell lining	decreased diffusion to reach absorptive epithelial cell membrane
	lipid bilayer of epithelial cell membrane	inhibits absorption of PP drugs that are hydrophilic and charged through the cell (transcellular transport)
	intercellular spaces (mean pore radii of 0.8, 0.3, and 0.3 nm in duodenum, ileum, and colon, respectively) gated by closely fitting tight junctions (TJ) on apical side of epithelial cells	TJ prevent passage of PP macromolecules through the intercellular spaces (paracellular transport)
	p-glycoprotein present on epithelial cell membrane	promote apically polarized efflux to remove permeated drug molecules

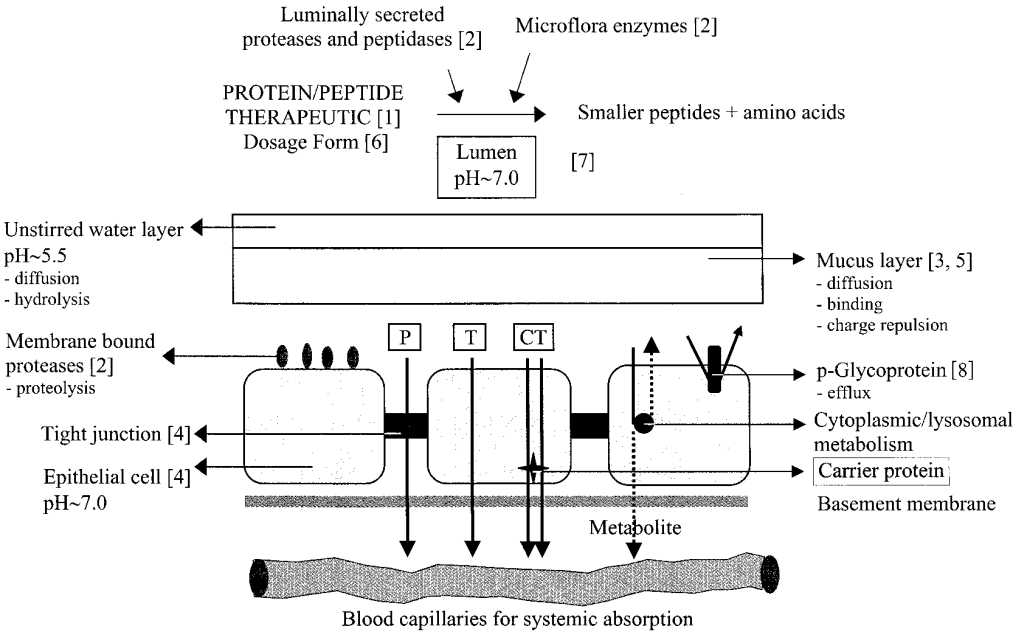

Figure 1. Diagrammatic representation of different barriers to protein and peptide drug absorption from the intestinal tract. Shaded square text boxes show the pathways for drug absorption: P, paracellular; T, transcellular; CT, carrier-mediated transport. Target sites for different absorption enhanceement strategies are indicated by numerals in paranthesis: 1, prodrugs/analogues; 2, protease inhibitors; 3, mucolytic agents; 4, paracellular and transcellular absorption enhancers; 5, mucoadesive polymers; 6, dosage form modifications; 7, pH modulation to enzymatic activity minima 8, p-glycoprotein inhibitors.

cellular accumulation or the transcellular flux of a wide variety of drugs, including peptides.[45,46] Figure 1 shows an overall view of the various barriers to PP drug absorption from *peroral* route and various targets for enhancing their absorption. A brief description of these barriers has been provided individually at appropriate places in the subsequent sections.

Traditional drug candidates also encounter similar barriers, but PP drugs seem to be highly susceptible to all these factors, and the options available to pharmaceutical scientists are very limited. The synthetic chemistry approaches that are often successful in ameliorating one or more of the barriers and resulting in efficacious in vivo absorption of traditional, small organic molecules have proved to be of little value in the case of PPs due to their much more complex chemistry. Various approaches that have been taken to overcome barriers with reference to poor bioavailability of PP drugs from *peroral* route are enumerated as follows and have been described later in the review: (i) Chemical modification of the protein or peptide lead compound—prodrug/analogue approach; (ii) Use of absorption enhancers such as surfactants, bile salts, or calcium chelators; (iii) Use of enzyme inhibitors to lower the proteolytic activity; (iv) Designing a drug delivery system which is targeted to a part of the gut where proteolytic activity is relatively low so as to protect PPs from luminal proteolytic degradation and release the drug at the most favorable site for absorption.

A. Prodrug/Analogue Approach

Prodrug or analogue development has probably remained one of the most favored approaches in solving many drug delivery related problems. The most recent example of insulin LysPro, although for parenteral administration, has demonstrated the

possibility of modifying biopharmaceutic as well as pharmacokinetic characteristics of PP drugs by using a prodrug/analogue approach. LysPro, a human insulin analogue produced by inverting the native sequence Pro^{B28}, Lys^{B29} in the c-terminal of the B-chain of human insulin,[47] was developed by Eli Lilly and Company and approved for clinical use in 1996. The sequence inversion results in reduced self-association properties of LysPro, making it more readily monomeric,[48] and consequently LysPro exhibits different pharmacokinetic properties from soluble insulin on subcutaneous administration (rapid onset, higher and earlier peak plasma concentrations with shorter duration of action).[49,50] There are a number of other insulin analogues that are presently under different phases of investigations for increasing its stability and/or modifying its onset and duration of activity.[51,52] In context to the scope of present review, the prodrug/analogue approach can be defined as conversion of PPs into derivatives (prodrugs or analogues) by means of incorporation of sufficient modifications so as to engender oral activity.[53−58] Hydrophilic nature and charge of PP drugs are because of the polar and ionizable functional groups (including terminal amino and carboxyl groups) in the molecules. The presence of amide bonds at different positions, free N-terminal amino groups, and free C-terminal carboxyl groups make them susceptible to endopeptidases-, aminopeptidases-, and carboxypeptidases-mediated degradation, respectively. Thus, chemical modification, such as masking or blocking polar amide bonds and terminal amino and carboxyl groups, primarily brings about an alteration in the physicochemical properties of drugs such as lipophilicity, hydrogen-bonding capacity, charge, molecular size, solubility, configuration, isoelectric point, chemical stability, etc., which are known to affect their membrane permeability, enzyme liability, and affinity to carrier systems.[59,60] Various structural features of peptides that influence their passive diffusion, carrier-mediated transport, and efflux mechanisms have been recently reviewed by Wang et al.[59] and Pauletti et al.[61] The lipophilicity of various drugs, as expressed in terms of logP (logarithm value of octanol−water partition coefficient) or logD (logarithm value of octanol−pH 7.4 buffer partition coefficient), can be correlated with cell membrane permeability.[62] The generalization is that within a homologous series, drug absorption increases as lipophilicity rises and is maintained at a plateau for a few units of logP after which there may be a steady decrease, giving a parabolic relation. However, in the case of PP drugs, logP or logD values may not always correlate well with drug permeability.[63] In a study with a series of six model peptides, prepared from D-phenylalanine and glycine, Conradi et al. observed that the permeability of peptides across Caco-2 cell monolayers was inversely related to the number of hydrogen-bonding groups in the structure as these hydrogen bonds must be broken for the solute to transfer into the interior of cell membrane.[64] They showed that although addition of amino acid with a large hydrocarbon chain (phenylalanine) to the peptidic chain resulted in increased lipophilicity of modified peptides, their permeability was affected adversely. The effect was explained to be due to introduction of very polar amide bonds, capable of forming strong hydrogen-bonding interactions with water, in the peptide chain with the addition of hydrophobic amino acid residue. In another study with a tetrapeptide, Conradi et al. showed that methylation of amide nitrogens resulted in a substantial increase in transport across the Caco-2 cell monolayer but without any significant change in the octanol−water partition coefficient, suggesting that a reduction in the overall hydrogen-bonding potential is more important than an increase in lipophilicity.[65] Similarly, Saitoh and Aungst showed that lipophilicity and charge of DMP-728 (a potent GP IIb/IIIa receptor antagonist) prodrugs did not influence intestinal permeability determined in vitro using rat jejunum in diffusion cells; instead, N-methyl-substituted analogues exhibited 2-fold greater jejunal permeability than DMP-728.[66] However, these observations were not always consistent with the hypothesis that reducing the hydrogen-bonding capacity of peptides can increase permeability and suggested that this could be because of confounding influence of secretory transport by P-glycoprotein. Additionally, there are a number of reports where an increase in lipophilicity, as indicated by partition coefficient values of PP molecules by means of chemical modification, has been shown to improve their membrane permeability.[53,67]

As explained earlier, PP molecules harbor more than one polar and ionizable group that contributes to the total charge and polarity of molecules and/or serves as a site for enzymatic attacks. A chemical modification at one site may not always be sufficient to significantly improve permeability characteristics and/or reduce liability to enzymatic degradation in vivo, especially when there are multiple enzymes involved in degradation at different sites. In such instances, various strategies have been tried which allow simultaneous masking of more than one functional group. Borchardt, Wang, Pauletti, and coworkers[59,68−75] described preparation of cyclic prodrugs which allow for simultaneous masking of an amino and a carboxyl group of peptide drug. These cyclic prodrug systems can be prepared by using acyloxyalkoxy-, phenolpropionic acid- or coumarine-based prodrug moieties (Table 3). Wang et al.[59] explained that cyclization of linear peptides by using these prodrug moieties results in significantly altered physicochemical properties (due to derivatization of carboxyl and amino groups into ester and amide, respectively), altered effective size and shape along with restricted conformational freedom of the cyclic peptide, which consequently reduces the charge on peptide and promotes intramolecular hydrogen bonding within the peptide molecule rather than intermolecular hydrogen bonding between peptide functional groups and solvent. These prodrugs have reduced susceptibility to peptidase metabolism; however, they are esterase sensitive and release the parent peptide under esterase activity. To achieve similar results, chemical modifications at two or three functional groups in the PP molecules have also been

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